WHAT IS NEW IN
GASTROENTEROLOGY AND HEPATOLOGY

Editors:
Ioan Sporea
Alina Popescu

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Edited by

Ioan Sporea

&

Alina Popescu

Department of Gastroenterology and Hepatology
"Victor Babeş" University of Medicine and Pharmacy
Timişoara
Romania

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Editors:Ioan Sporea, Alina Popescu

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FOREWORD

In this book, professors Ioan Sporea and Alina Popescu from the Medical University of Timişoara have put together important contributions to highlight the enormous progress that has been made in gastroenterology and hepatology in recent years. With an outstanding faculty, the state-of-the-art in diagnosis, management, and treatment of digestive diseases is illustrated. In 33 chapters relating to the most interesting and pressing issues in the field, the reader is informed about current optimal practice and standards of care in gastroenterology and hepatology. The spectrum of subjects goes from pulmonary manifestations of gastroesophageal reflux disease and management of Barrett‘s oesophagus to neuroendocrine tumours and hepatocellular carcinoma. Screening for the most common tumour in the digestive tract, colon cancer, and postpolypectomy care to prevent interval cancer are as important as is the management of liver disease caused by viral hepatitis. The very modern times in our field are represented by the use of telemedicine in hepatology and artificial intelligence to improve diagnostic accuracy in endoscopy. Many high-quality endoscopic pictures and sonographic images highlight the remarkable technical progress with these techniques. When I was a fellow in gastroenterology many years ago, the only thing we had at patient conferences was radiographs after administering barium sulphate: barium swallow, upper GI-series, as we called it, small bowel follow-through, and barium enema. Look also at the capsule endoscopy in this book, and you will see that we have come a long way! Congratulations to the editors and authors for demonstrating this progress so impressively!

Guenter J. Krejs
Medical University of Graz
Austria

PREFACE

Knowledge in medicine is a very dynamic process due to the continuing progress in this field. New developments influence research, but also the clinical practice. Hence the continuous need for improvement in the field in which we work is required. Gastroenterology and hepatology, as part of internal medicine, are very dynamic fields of medicine, with numerous innovations, in the last 20-30 years at least. Starting with clinical medicine and continuing with endoscopy, interventional endoscopy or ultrasound and ending with precision medicine, with proteomics or metabolomics, the future of medicine seems to be here.

This book aims to bring to the readers' attention the latest advances in gastroenterology and hepatology. The book offers a variety of topics in the field of gastroenterology and hepatology, approached in a structured, clear and comprehensive fashion, but also with practical applications. The invited authors are the best in this field, all members of a Society older than 60 years (Romanian Society of Gastroenterology and Hepatology). The book's was designed in such a way that every invited author must contribute with his/her best topic in the field of gastro/hepato!

Topics such as eosinophilic esophagitis, bariatric surgery, Barrett esophagus, neuroendocrine tumors, inflammatory bowel diseases, intestinal microbiota, videocapsule endoscopy, endoscopic ultrasound, *etc.*, in the field of gastroenterology, as well as liver elastography, alcoholic liver diseases and non-alcoholic fatty liver disease, HBV and HCV chronic liver diseases, contrast enhanced ultrasound (CEUS), *etc.* in the field of hepatology, recommend this book to all those interested in these fields, either specialists, or researchers or fellows in training and even students. The hot topics of precision medicine, artificial intelligence, the "omics" cascade, telemedicine are also included in this book.

In the end, after finishing the book, we hope that you have enjoyed the time spent reading what is new and "hot" in the field of gastroenterology and hepatology. If you liked it, please recommend this e-book to a friend!

Ioan Sporea

&

Alina Popescu
Department of Gastroenterology and Hepatology
"Victor Babeş" University of Medicine and Pharmacy
Timişoara
Romania

List of Contributors

Acalovschi Monica	Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, Cluj-Napoca, Romania
Balaban Daniel Vasile	"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest, Romania
Bancu Ligia	1st Department of Internal Medicine, UMFST " George Emil Palade" Târgu Mureş, Romania
Bărboi Oana-Bogdana	Institute of Gastroenterology and Hepatology, University of Medicine and Pharmacy Grigore T.Popa, Iași, Romania
Băţagă Simona	GE Palade University of Medicine, Pharmacy, Science and Technology Târgu-Mureş,Emergency Hospital, Târgu-Mureş, Romania
Bende Felix	Department of Gastroenterology and Hepatology, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania
Brisc Ciprian	University of Oradea, Faculty of Medicine and Pharmacy, Oradea, Romania Clinical County Emergency Hospital Oradea, Gastroenterology Clinic, Oradea, Romania
Cijevschi Cristina Prelipcean	"Grigore T. Popa" University of Medicine and Pharmacy, "Sf. Spiridon" Emergency Hospital, Institute of Gastroenterology and Hepatology, Iasi, Romania
Constantinescu Codruța	Research Center in Gastroenterology and Hepatology, University of Medicine and Pharmacy Craiova, Romania
Constantinescu Gabriel	Department 5, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania Department of Gastroenterology, Clinical Emergency Hospital of Bucharest, Bucharest, Romania
Crisan Dana	University of Medicine and Pharmacy Cluj-Napoca, Romania
Dănilă Mirela	Department of Gastroenterology and Hepatology, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania
Diculescu Mircea	Fundeni Clinical Institute Buchares, University of Medicine and Pharmacy, "Carol Davila" Bucharest, Romania
Dobru Daniela E.	Department of Gastroenterology, GE Palade University of Medicine, Pharmacy, Science and Technology, Târgu-Mureş, Romania
Drug Vasile-Liviu	Institute of Gastroenterology and Hepatology, University of Medicine and Pharmacy Grigore T.Popa, Iași, Romania
Dumitraşcu Dan L.	2nd Department of Internal Medicine, Iuliu Haţieganu University of Medicine and Pharmacy Cluj-Napoca, Romania
Dumitru Eugen	Ovidius University of Constanta, Romania Gastroenterology Clinic, Clinic Emergency Hospital of Constanta, Romania Center for Research And Development of the Morphological and Genetic Studies of MalignantPathology (CEDMOG), Romania

Fierbinţeanu Carmen Braticevici	Internal Medicine II and Gastroenterology, University Hospital Bucharest, "Carol Davila" University of Medicine and Pharmacy Bucharest, Romania Department of Gastroenterology, University Hospital Bucharest, Romania
Gheonea Dan Ionuţ	Department of Gastroenterology, University of Medicine and Pharmacy of Craiova, Romania
Ghiuchici Ana Maria	Department of Gastroenterology and Hepatology, Victor Babeş, University of Medicine and Pharmacy Timişoara, Romania
Gîrleanu Irina	"Grigore T. Popa" University of Medicine and Pharmacy Iaşi, Institute of Gastroenterology and Hepatology, Iasi, Romania
Goldiş Adrian	Department of Gastroenterology and Hepatology, "Victor Babeş" University of Medicine and Pharmacy Timişoara, Romania
Grigorescu Mircea	University of Medicine and Pharmacy Cluj-Napoca, Romania
Ilie Mădălina	Department 5, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania Department of Gastroenterology, Clinical Emergency Hospital of Bucharest, Bucharest, Romania
Istratescu Doina	Department of Gastroenterology and Hepatology, Fundeni Clinical Institute Bucharest, Romania
Jinga Mariana	"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest, Romania
Kurniawan Timothy	University of Oradea, Faculty of Medicine and Pharmacy, Romania
Manuc Mircea	Department of Gastroenterology and Hepatology, Fundeni Clinical Institute Bucharest, Romania
Mateescu Radu Bogdan	University of Medicine and Pharmacy Carol Davila, Faculty of Medicine, Bucharest, Romania Gastroenterology Department, Colentina Clinical Hospital, Bucharest, Romania
Mihai Cătălina	"Grigore T. Popa" University of Medicine and Pharmacy, "Sf. Spiridon" Emergency Hospital, Institute of Gastroenterology and Hepatology, Iasi, Romania
Miutescu Bogdan	Department of Gastroenterology and Hepatology, "Victor Babeş" University of Medicine and Pharmacy Timişoara, Romania
Miutescu Eftimie	Department of Gastroenterology, Faculty of Medicine, "Vasile Goldiş" Western University of Arad, Romania
Moga Tudor	Department of Gastroenterology and Hepatology, "Victor Babeş" University of Medicine and Pharmacy Timişoara, Romania
Moldoveanu Alexandru	Internal Medicine II and Gastroenterolog, University Hospital Bucharest, "Carol Davila" University of Medicine and Pharmacy Bucharest, Romania Department of Gastroenterology, University Hospital Bucharest, Romania
Nenu Iuliana	Institute for Gastroenterology and Hepatology "O.Fodor", University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania

Negreanu Lucian	Internal Medicine I-Gastroenterology Department, Emergency University Hospital of Bucharest & UMF Carol Davila Bucharest, Romania
Oancea Carmen Nicoleta	Department of Analytical Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacyof Craiova, Romania
Popescu Alina	Department of Gastroenterology and Hepatology, University of Medicine and Pharmacy Timişoara, Romania
Porr Paul J.	Polisano Med Life Clinics, Sibiu, Romania
Rogoveanu Ion	Department of Gastroenterology, University of Medicine and Pharmacy of Craiova, Romania
Săftoiu Adrian	Research Center in Gastroenterology and Hepatology, University of Medicine and Pharmacy Craiova, Romania
Seicean Andrada	Regional Institute of Gastroenterology and Hepatology Cluj-Napoca, University of Medicine and Pharmacy "Iuliu Haţieganu" Cluj-Napoca, Romania
Seicean Radu	First Surgical Clinic, Cluj-Napoca, University of Medicine and Pharmacy, "Iuliu Haţieganu" Cluj-Napoca, Romania
Şirli Roxana	Department of Gastroenterology and Hepatology, "Victor Babeş" University of Medicine and Pharmacy Timişoara, Romania
Spârchez Zeno	Institute for Gastroenterology and Hepatology "O.Fodor", University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania
Sporea Ioan	Department of Gastroenterology and Hepatology, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania
Stroie Tudor	Fundeni Clinical Institute Buchares, University of Medicine and Pharmacy, "Carol Davila" Bucharest, Romania
Ţanco Mihaela	Ovidius University Constanţa, Faculty of Medicine, Romania Gastroenterology Clinic, Constanţa County Clinical Emergency Hospital "Sf. Apostol Andrei", Constanţa, Romania
Tanţău Marcel	Department of Gastroenterology, 3th Medical Clinic, Cluj-Napoca, Romania
Tanţău Alina Ioana	Department of Gastroenterology, 4th Medical Clinic, "Iuliu Hatieganu" University of Medicineand Pharmacy, Cluj-Napoca, Cluj Napoca, Romania
Tocia Cristina	Ovidius University of Constanta, Romania Gastroenterology Clinic, Clinic Emergency Hospital of Constanta, Romania
Tofolean Ioan Tiberiu	Ovidius University Constanţa, Faculty of Medicine, Romania Gastroenterology Clinic, Constanţa County Clinical Emergency Hospital "Sf. Apostol Andrei", Constanţa, Romania
Trifan Anca	"Grigore T. Popa" University of Medicine and Pharmacy Iaşi, Institute of Gastroenterology and Hepatology, Romania
Ungureanu Bogdan Silviu	Department of Gastroenterology, University of Medicine and Pharmacy of Craiova, Romania

Voiosu Theodor Alexandru

University of Medicine and Pharmacy Carol Davila, Faculty of Medicine, Bucharest, Romania
Gastroenterology Department, Colentina Clinical Hospital, Bucharest, Romania

CHAPTER 1

What's New in Extra-digestive Gastroesophageal Reflux Disease?

Vasile-Liviu Drug[1,*] and **Oana-Bogdana Bărboi**[1]

[1] *Institute of Gastroenterology and Hepatology, University of Medicine and Pharmacy, Grigore T. Popa Iași, Romania*

Abstract: Gastroesophageal reflux disease (GERD) is a highly prevalent complex chronic condition. The most extensive prospective and multicenter cohort study conducted in Europe has estimated that one-third of the patients with GERD may exhibit extra-esophageal symptoms. The Montreal Consensus recognized chronic cough, chronic laryngitis, bronchial asthma and tooth erosions as extra-digestive manifestations of GERD. The experts also considered that manifestations such as recurrent otitis media, idiopathic pulmonary fibrosis, sinusitis or pharyngitis are likely to be associated with GERD.

The traditional techniques used in the diagnosis of typical GERD are less useful for the diagnosis of extra-digestive GERD. No single testing methodology exists to definitively identify reflux as the etiology for the suspected extra-esophageal symptoms. The PPI trial is the first diagnostic but also a therapeutic step, while evaluation through esophageal impedance-pH monitoring currently represents the gold-standard for diagnosis.

Despite extensive work, extra-digestive GERD remains incompletely understood.

Keywords: Extra-digestive manifestations, Esophageal impedance-pH monitor ing, Gastroesophageal reflux disease, Proton pomp inhibitors.

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a chronic complex clinical condition, which is also recurrent, multi-factorial, with a risk of complications and significant morbidity. It has become undoubtedly one of the most commonly diagnosed diseases by the gastroenterologists in specialized ambulatory care, being one of the most common diseases of modern civilization.

[*] **Corresponding author Vasile-Liviu Drug:** Institute of Gastroenterology and Hepatology, University of Medicine and Pharmacy, Grigore T. Popa Iași, Romania; Tel: +40745589065; E-mails: vasidrug@email.com, vasidrug@gmail.com and vasile.drug@umfiasi.ro

Ioan Sporea and Alina Popescu (Eds.)

GERD is defined by 2006 Montreal Consensus as a condition that develops when the gastric content is refluxing into the esophagus and causes troublesome symptoms and/or complications [1]. Typical GERD is characterized by esophageal symptoms such as regurgitation and heartburn, but in some categories of patients, extra-esophageal manifestations are recognized as a form of GERD.

GERD with extra-digestive manifestations continues to represent a controversial issue in terms of epidemiology, diagnosis and treatment, for both gastroenterologists and ear-nose-throat (ENT) surgeons, pneumologists and dentists. Despite several papers published regarding this subject, extra-digestive GERD still remains incompletely understood [1].

The Montreal Consensus recognized chronic cough, chronic laryngitis, bronchial asthma and tooth erosions as extra-digestive manifestations of GERD. The experts also considered that manifestations such as recurrent otitis media, idiopathic pulmonary fibrosis, sinusitis or pharyngitis are likely to be associated with GERD [1, 2].

Epidemiological studies report different prevalence data, based on different methodology and heterogeneous study's design. Moreover, the prevalence of extra-digestive GERD is hard to establish due to the difficulty of confirming the diagnosis. Thus, the diagnosis of GERD-related extra-esophageal manifestations requires a good collaboration between specialists, to exclude other causes [3].

The most extensive prospective, multicenter cohort study conducted in Europe has estimated that one-third of patients with GERD may present extra-esophageal symptoms [4]. Chest pain (14.5%), chronic cough (13%), laryngeal manifestations (10.4%) and bronchial asthma (4.8%), were the commonest conditions associated with GERD. Another large study on extra-digestive GERD conducted in the US showed that non-cardiac chest pain (23.1%) and the respiratory symptoms (pneumonia 23.6%, bronchitis 14.0%, asthma 9.3%) were the most frequent manifestations recorded, followed by ENT symptoms (hoarseness 14.8%, globus sensation 7.0%) [5]. In Romania, there are very few studies on the epidemiology of GERD with extra-digestive manifestations. Angelescu *et al.* [6] reported a prevalence of 31.1% for extra-digestive manifestations in patients with GERD. Dental erosions were found in 76.3% of patients, non-cardiac chest pain in 55.5% of patients, while chronic cough was identified in 44.5% of patients and chronic laryngitis in 22.7% of GERD patients.

There are two possible main mechanisms involved in the pathophysiology of extra-digestive GERD: the direct mechanism – the direct injury of the esophageal and laryngopharyngeal mucosa due to gastro-duodenal contents, with or without

airway microaspiration (the reflux theory) and the indirect mechanism–vagal-mediated tracheobronchial reflex, caused by acidification of the distal esophagus (the reflex theory). The reflux of gastroduodenal contents into the esophagus and hypopharynx may be classified as: high reflux (reflux crosses the esophagus and causes ENT or respiratory manifestations, either by direct pharyngo-laryngeal stimulation or aspiration) or distally reflux (occurs by reflex mechanism) [7].

Majority of the papers published on extra-digestive GERD are related to ENT and respiratory manifestations.

The diagnosis of reflux disease and the establishment of a clear relationship between reflux and extra-esophageal symptoms have proven to be very challenging. This is difficult to achieve because typical GERD symptomatology may be lacking in these patients. The presence of classical GERD symptoms in a patient with extra-digestive manifestations may suggest the diagnosis of GERD, but does not establish a certain causal relationship.

Unfortunately, the diagnostic methods currently available in clinical practice have serious limitations. The traditional techniques used in the diagnosis of typical GERD are less useful for the diagnosis of extra-digestive GERD. No single testing methodology exists to identify reflux definitively as the etiology for the suspected extra-esophageal symptoms. An association between clinical presentation, diagnostic test results and response to therapy is needed in order to determine if the reflux is the cause for the extra-esophageal manifestations or not [3].

When extra-digestive reflux is suspected in a patient who also experiences heartburn and/or regurgitation, most guidelines recommend the therapeutic test with double-dose proton pump inhibitors (PPI) for a period of at least 3 months, as long as there are no warning signs [8, 9]. If the therapeutic test is positive (amelioration or disappearance of digestive and extra-digestive symptoms), most likely GERD is the etiopathogenic substrate for the extra-digestive manifestation. Non-responsive PPI patients should be further investigated to confirm or refute the diagnosis of GERD. However, there are also other authors who recommend abandoning this diagnostic test as studies showed a sensitivity and a specificity of 54-92% and 67-86%, respectively [10]. Unlike other GERD diagnostic methods, the therapeutic test is relatively simple, non-invasive and cost-effective.

Upper digestive endoscopy (UDE) has long been the main diagnostic test in GERD. Nowadays endoscopy is recommended when alarm signs are present, in non-responders to PPI patients, in patients with long-lasting extra-esophageal symptoms or screening in patients with high risk for developing complications,

like adenocarcinoma. These are Caucasian men over 50 years old, obese, with symptoms duration of more than 5 years [2].

Data available on the endoscopic evaluation in patients with GERD who have exclusively extra-digestive symptoms is very limited. Although there is great variability between studies, most of them show a prevalence of reflux esophagitis in these patients ranging from 10 to 25% [11]. Therefore, UDE is not a cost-effective diagnostic method. The presence of erosive esophagitis in patients with extra-digestive manifestations does not necessarily establish the diagnosis of GERD; UDE visualizes the lesions, but cannot be a proof for the involvement of GERD in extra-digestive manifestation. However, in combination with typical reflux symptoms, C or D Los Angeles esophagitis or Barrett's esophagus are highly suggestive of the diagnosis for GERD. The opposite is also true: the absence of esophageal lesions does not exclude the diagnosis of GERD [10].

Ambulatory esophageal pH/pH- impedance monitoring is generally considered to provide the most objective evidence for pathologic reflux and it is the only test that can assess temporal relationships between reflux events and symptoms. The main recommendations for esophageal pH monitoring of the American Gastroenterology Association are: uncertain diagnosis of GERD, patients with reflux symptoms and non-erosive disease, for preoperative evaluation in patients undergoing anti-reflux endoscopic or surgical therapy, PPI-refractory patients and suspected extra-digestive GERD [2]. Testing should be done off PPI therapy in patients with a low probability of baseline reflux (in patients without the previously documented or suspected GERD), in order to identify moderate to severe reflux at baseline and on PPI in patients with a high probability of baseline reflux (those with previous esophagitis, Barrett’s esophagus or abnormal pH) [12]. The identification of pathological reflux by ambulatory pH/impedance monitoring suggests a high likelihood of GERD, whereas a negative test should direct the diagnosis to a non-GERD etiology [12].

Mucosal impedance (MI) is a novel technology that assesses the epithelial integrity of the esophagus by measuring the changes in the mucosal current conduction. It seems that it is a useful diagnostic tool for GERD, as previous studies showed. However, MI is not widely used [13]. A recent study showed that MI could also detect reflux in patients with extra-digestive GERD [14].

The detection and quantification of pepsin in the saliva is a relatively recent method that has become very popular lately and can be useful for the diagnosis of GERD. This noninvasive tool turned out to be promising in the detection of typical GERD. Its potential role in the diagnosis of extra-digestive GERD is still to be determined, as contradictory data have been published so far [13].

The treatment of GERD, irrespective of the clinical features (typical or atypical), has three major goals: to achieve relief in symptoms, to heal esophageal lesions and to prevent recurrence and complications. These therapeutic goals could be obtained by diet and lifestyle modifications, medical therapy, antireflux endoscopic or surgical procedures. Although diet is recommended all over the world as a cornerstone in GERD treatment, high-quality data lacks regarding its efficacy, especially in patients with extra-digestive manifestations [12]. It seems that measures such as bed elevation and lose weight can be effective in treating esophageal acid exposure. The mainstay of GERD therapy is still represented by PPI, with the remark that in extra-digestive GERD a double-dose PPI is needed for a longer period of time (3-4 or even 6 months) [12]. Although the endoscopic treatment, as a minim-invasive alternative to surgical therapy, was very promising, studies that followed demonstrated the opposite. Nowadays only two techniques (transoral fundoplication and radiofrequency technique) continue to be successfully used in some medical centers for controlling extra-digestive GERD symptoms. Regarding surgical treatment, its efficacy is lower for extra-esophageal GERD, comparing to classic GERD and it is, in general, discouraged. However, it may be considered useful in patients with objective evidence of refractory GERD [12].

Chronic Laryngitis

Reflux laryngitis, also known as laryngopharyngeal reflux (LPR), supra-esophageal reflux disease or atypical gastroesophageal reflux disease, is an extra-digestive manifestation of GERD due to gastric reflux in the esophagus/ pharynx/larynx axis.

The prevalence of reflux laryngitis is difficult to be determined due to the different clinical forms of LPR and also to the lack of consensus on the diagnosis. It is estimated that 4-10% of patients who are referred to an ENT department have also GERD. Moreover, 50% of patients with dysphonia actually have LPR. Nowadays, the prevalence of GERD and LPR has significantly increased [3]. Using a statistical model in the analysis of 17 studies, El-Serag [15] showed that the average rate of increase in LPR prevalence since 1976 was 4% per year ($P < 0.0001$). People with professions associated with intense use of voice, such as teachers, aerobics instructors or professional singers have a higher risk of developing LPR [16, 17].

Although the hypothesis that there is a cause-effect relationship between GERD and chronic laryngitis has been strengthened by past studies, diagnosis and treatment, remain controversial. The larynx is about 100 times more sensitive than the esophagus and carries a higher risk of injury due to its anatomical position

near the digestive tract. In addition, the larynx does not possess the intrinsic and extrinsic defense mechanisms of the esophagus. Thus, only a minimal amount of gastric reflux is sufficient to cause lesions of the larynx [18]. The vocal cords are covered by stratified squamous epithelium, creating a barrier against reflux. Erickson and Sivasankar [18] conducted a study using trans-epithelial electrical resistance to test the permeability of the vocal cord epithelium. They found that exposure of the vocal cords to the reflux causes decreased epithelial resistance, making them more susceptible to injury.

The diagnosis of reflux laryngitis continues to remain a difficult task. A complete anamnesis and accurate clinical exam are required. The symptoms of LPR are diverse and there is a risk that many clinicians will not link them to GERD, especially in the absence of classic GERD manifestations. The most common clinical manifestations of LPR are: hoarseness, chronic cough, sore or burning throat, the sensation of a lump in the throat, repetitive throat clearing, excessive phlegm and voice fatigue. These complaints are non-specific for LPR and may also occur due to allergens, smoke or other irritant agents, postnasal drip syndrome or vocal effort [3]. Thus, clinicians should not rely only on these symptoms in establishing the diagnosis of LPR. It is important to note that patients with LPR do not usually have the typical symptoms of reflux, which may delay the correct diagnosis [3].

There is a series of controversies in the literature regarding the most accurate method to establish the diagnosis of LPR. Laryngoscopy is a technique considered essential by ENT doctors for LPR diagnosis. Branski *et al.* [19] showed that laryngoscopy is operator dependent in interpreting the same laryngeal signs and this is why the diagnosis of LPR can be wrong. The most common ENT lesions that may occur in LPR are laryngeal edema and erythema, especially in its posterior region (arytenoids and inter-arytenoids region). Ulcer and ventricular obliteration, vocal cord polyps or granuloma, subglottic or posterior glottic stenosis, leukoplakia or lymphoid hyperplasia have been less frequent in LPR [20]. Yet, these features lack specificity and are highly operator-dependent parameters. Furthermore, these signs of laryngeal irritation are present in over 80% of healthy controls [21]. To increase the accuracy of the diagnosis, Belafsky *et al.* created a reflux score (Reflux Finding Score - RFS), based on the presence and severity of lesions discovered at the laryngo-scopic examination [22]. Nevertheless, even this score proved to have several limitations, as it was demonstrated by two recent studies that showed that LPR findings could vary according to the type of reflux and patient features [23, 24].

Although the role of empirical PPI test for the diagnosis of LPR is controversial, many clinicians continue to use it as a good diagnostic tool for LPR [25]. Most

researchers consider the test positive if LPR symptoms are disappearing after 3 months of double-dose PPI, without taking into consideration the evolution of laryngeal signs [3, 25]. The advantages of this method are cost-effectiveness, easy administration and good sensitivity. However, it has several limitations regarding the lack of specificity. That is why the current recommendation is that the PPI therapeutic test be used in patients who also associate typical symptoms [2].

UDE has a low diagnostic yield in the context of suspected LPR, as erosive esophagitis is observed only at 1/3 of patients with symptomatic GERD and even fewer after PPI treatment. Moreover, the presence of esophagitis does not establish, with certainty, the diagnosis of reflux laryngitis [2].

The use of ambulatory multichannel intraluminal impedance–pH monitoring (MII-pH) and pharyngeal pH monitoring in order to diagnose LPR is debatable. Still, MII-pH is considered the most reliable tool to diagnose acid or nonacid reflux and gas, liquid or mixed reflux. Addition of impedance testing to classical pH monitoring improves the diagnostic sensitivity (70%-80%) and the false-negative rate (20%-50%) [26]. However, Bartoli *et al.* showed that less than 40% of patients with suspected LPR have an abnormal pH-impedance study, emphasizing the inconvenience caused by the lack of specific signs and symptoms of LPR [27]. Hypopharyngeal and proximal esophageal pH monitoring have a sensitivity of 40% and 55%, respectively [3].

A noninvasive diagnostic tool that showed to be promising in the identification of LPR is the detection of pepsin in the saliva [27]. Several studies have used pepsin as a diagnostic marker of GERD, as this enzyme is produced in the stomach and its presence above the upper esophageal sphincter is an evidence of GERD. However, there is no consensus on the value of the concentration of pepsin in saliva that is clinically relevant. A recent meta-analysis showed a sensitivity and a specificity of salivary pepsin detection of 64% and 68%, respectively [28]. Future research is needed to establish the value of this method in the diagnosis of LPR.

Bronchial Asthma

The association of GERD and asthma has drawn the researchers' attention over time. It also continue to be a debated subject to elucidate the physiopathological mechanisms involved in this two-way relationship. The estimated prevalence of reflux-induced asthma varies in different studies from 30 to 90% [29]. The ProGERD study showed that 4.8% of GERD patients have bronchial asthma [4], while silent GERD was identified with high prevalence in difficult-to-manage asthmatic patients [3]. Esophageal pH monitoring studies show a prevalence of GERD in asthmatic patients similar to the symptom based prevalence reported by studies (32-84%) [28].

A cause-effect relationship is still difficult to establish because each clinical condition can induce the other. Potential mechanisms through GERD that causes air obstruction in asthmatic patients include the vagal reflex, bronchial hyperreactivity and microaspiration of gastric content. The presence of acid in the esophagus stimulates vagal receptors, causing bronchoconstriction and neurogenic inflammation through non-adrenergic non-cholinergic fibers, mediated by nitric oxide and increases airway resistance by 10% [3].

GERD precipitates and aggravates the symptoms, representing one of the causes of difficult-to-control bronchial asthma. GERD-induced asthma should be considered in patients with adult onset of bronchial asthma, in patients with nocturnal symptoms, in those with dyspnea preceded by heartburn or regurgitation or in whom respiratory symptoms worse after meals (especially after heavy meals and alcohol consumption) and in asthmatic patients who do not respond to optimal anti asthmatic therapy [3]. In asthma-induced GERD, several precipitating factors were described: lung hyperinflation, cough or asthma therapy (theophylline, beta-agonists or corticosteroids). A high proportion of asthma patients have silent nocturnal GERD, because during sleep the usual protective responses are lacking. The nocturnal symptoms that wake patients not only affect the quality of sleep, but also have a negative impact on daytime activity, which leads to decreased productivity in the workplace. GERD is currently a public health problem, as it implies periods of absenteeism and the use of expensive, often unnecessary investigations and treatments, which burdens the budget of the medical system and generates a substantial negative economic effect [3].

Similar to chronic laryngitis, the diagnosis of GERD-related asthma is still difficult, especially because the classic GERD symptoms are often lacking and diagnosis tools like UDE, PPI therapeutic test and esophageal pH-impedance are less specific and sensitive [3]. Although esophageal pH-impedance is nowadays considered the most accurate diagnostic method for GERD, in GERD-induced asthma, it does not have good predictive value (sensibility and specificity of 66%) [30].

Though there are no randomized controlled trials to demonstrate that lifestyle and dietary changes are significantly improving GERD-induced asthma, these are still recommended in clinical practice. The inconsistent data related to the efficacy of acid suppression therapy in patients with GERD and asthma may be due to different endpoints, different methodologies, small samples, and absence of a control group. Thus, some studies aimed to measure respiratory parameters with a role in quantifying the respiratory function control under anti reflux treatment (such as FEV1), while others were based on questionnaires, subjective assessment and the need for anti-asthmatic medication [31].

The efficacy of the PPI in controlling GERD-related asthma symptoms is still controversial. Several studies showed an improvement of respiratory symptoms and lung function, while others did not manage to demonstrate this effect [3]. However, the current recommendation for treating patients with GERD-induced asthma is an initial empirical trial with double-dose PPI for at least 3-4 months [2, 3].

Contradictory results were also obtained for anti-reflux surgery. Kaufman *et al.* [32] demonstrated an improvement in respiratory symptoms in approximately 70% of cases. Da Silva and colleagues [33] showed that Nissen fundoplication is effective not only for typical GERD, but also for controlling asthma manifestations, reducing drug use and improving the quality of life. In contrast, other studies have found no benefit of Nissen fundoplication in improving the pulmonary symptoms, respiratory parameters or quality of life [3]. A study that compared the effectiveness of Nissen fundoplication with that of the Stretta procedure in patients with GERD and severe asthma pointed out that both techniques are effective in controlling digestive and extra-digestive symptoms (asthma and ENT manifestations), with better results for the surgical technique [34].

Chronic Cough

Chronic cough induced by GERD is defined as an unproductive cough lasting for more than 8 weeks, which occurs especially after meals [often about 10 minutes and after eating foods that lower LES pressure (*e.g.* mint, chocolate)], in the supine position or posing from supine to orthostatic position, in non-smoker patients with normal chest X-rays and who do not consume angiotensin converting enzyme inhibitors [35]. GERD is one of the three leading causes of an unexplained chronic cough, along with asthma and postnasal drip syndrome [7]. Chronic cough induced by GERD affects 11-25% of the adult population [4]. It is difficult to appreciate the real prevalence of reflux-induced cough, as the cough may have several etiological substrates in the same patient. Studies using pH monitoring showed a higher prevalence (around 40%) for GERD-induced chronic cough, due to the silent clinical reflux [3].

It seems that the vagal mediated reflex mechanism plays a more important role in the production of chronic cough than reflux microaspiration [7]. However, GERD can trigger cough, but also increased intra-abdominal pressure during the cough may cause reflux, creating a self-perpetuating circle. There are three possible situations regarding the cough-GERD relationship: cough precedes reflux, reflux precedes cough, or cough-reflux-cough sequence [36].

Therefore, chronic cough caused by GERD should be suspected in patients that don't exhibit other causes.. These causes may be: respiratory diseases (asthma, chronic bronchitis, obstructive sleep apnea) or ENT diseases (posterior nasal drip syndrome, rhinosinusitis), the use of angiotensin converting enzyme inhibitors and environmental irritants or smoking. Typical reflux symptoms are absent in 75% of cases and chronic cough may be the only manifestation of GERD [3]. Other extra-digestive symptoms may accompany the reflux cough, such as dysphonia, globus or odynophagia. That is the reason why patients with chronic cough, ENT and respiratory manifestations usually are first referred to ENT and pulmonology specialists and much later to gastroenterologists.

Similar to previous extra-digestive manifestations, the usual tests used to detect typical GERD in chronic cough patients are less useful. Three months of empirical treatment with double dose PPI in these patients remains the first step for diagnosis. Baldi *et al.* [37] demonstrated that the administration of PPI twice a day for 4 weeks is an effective criterion for selecting patients who will respond to subsequent standard PPI therapy. Although, American Gastroenterological Association Guidelines recommend 24-h pH monitoring before the PPI trial in patients with suspected GERD-induced cough and an absence of typical esophageal findings [2]. Baldi *et al.* [37] showed that 53% of patients with chronic cough have pathological reflux at pH-monitoring, while Patterson and Murat [38] identified a tiny percentage of only 1% of cough episodes associated with hypo-pharyngeal reflux.

In patients with documented GERD who have not responded to PPI therapy, impedance-pH monitoring under PPI is recommended to detect non-acid reflux as a cause of chronic cough. A temporal relationship between GERD and cough has been demonstrated in 70% of cases, using impedance-pH in combination with an acoustic cough monitoring device. The study of Sifrim and colleagues [39] using impedance-pH monitoring and the symptom-association probability parameter (SAP) on patients with reflux-induced chronic cough allowed the precise determination of the temporal association between cough and episodes of weak acid reflux.

The treatment of chronic cough induced by GERD has been studied in several clinical trials, but many had important limitations, such as the small number of patients and inconsistent and varied results. A number of uncontrolled studies have shown that anti reflux therapy relieves reflux cough in 75-100% of cases [3, 40]. In contrast, many studies have failed to show a significant improvement in chronic cough after the PPI treatment, which would suggest a possible role of non-acid reflux in the pathophysiology of chronic cough [41, 42].

Despite these, PPIs are the commonest treatment used in clinical practice when GERD-induced chronic cough is suspected.

In patients with GERD and chronic cough non-responsive to anti-reflux drug therapy, surgical treatment (Niessen's fundoplication) looks promising [3, 43]. Allen and Anvari [43] followed 527 patients with chronic cough treated for 5 years and noticed a decrease in the improvement of symptoms over the years. Also, in cases with refractory to standard treatment, an alternative, even suboptimal, would be the use of Baclofen. Unfortunately, data showed that 40% of patients are resistant to Baclofen [44].

Dental Erosions

Dental erosions secondary to reflux represent changes produced by the loss of dental structure through a physico-chemical process, due to gastric acidic content reflux associated with bacterial activity [1]. If the prevalence of dental erosions in the general population is estimated between 5 and 16%, in patients with GERD it varies in a very wide range between 5 and 78.9% [45]. The latest data reported in our country identified a percentage of 57.7% of dental erosions in patients with reflux disease [46].

Dental erosions are produced by the destruction of tooth enamel (from both lingual and palatal face of the tooth) due to acid reflux, with a critical threshold of pH <5.5. An added factor is the reduction of salivary secretion. Saliva can protect the teeth and oral cavity through the ability to dilute and buffer. Patients with reduced salivary secretion are 5 times more prone to develop dental erosions [47]. Therefore, the examination of the oral cavity is mandatory in patients with GERD.

Dentists must be aware of the association between the two conditions. Acid reflux first affects the palatal surface of the upper incisors (due to the fact that they are protected by the major salivary glands and the tongue maintains contact with the acid content) and then, if the reflux is continued further, the occlusal surfaces of the other teeth are affected. The lingual surface is eroded only if the acid acts for a longer period of time. In the early stages, the loss of tooth structure is not significant and repair can be done easily, however, in more severe cases complications can occur (such as exposure of the pulp, destruction of the entire crown or even dysfunction of the temporomandibular joint), and situations may arise in which a complex rehabilitation is needed.

The early diagnosis and initiation of anti-reflux therapy stop the erosion of tooth enamel and prevent further destruction [3]. In suspected dental erosion due to GERD, the diagnostic protocol should start with a PPI test, followed by UDE and esophageal pH monitoring. Barlett *et al.* [48] observed that 60% of patients with

dental erosions had pathological acid reflux at the pH monitoring. Wilder-Smith *et al.* [49] conducted a large cohort study of patients with dental erosions and investigated by impedance-pH monitoring and found that the majority (73%) reported reflux symptoms less frequently than once a week and 69% of patients had pathological aspects at impedance-pH evaluation, suggesting that in a large proportion of patients with tooth erosion silent GERD may occur.

The treatment of GERD with oral manifestations is starting with the elimination of the main cause. Thus, a number of studies have suggested that the administration of gastric acid suppressants is effective in stopping the progression of dental erosion. Preventive measures such as stimulating salivary secretion or the use of substitutes that effectively neutralize the effect of intrinsic and extrinsic acids, a proper diet, strengthening the integrity of the tooth surface (using metal ions or phosphate fluids) and optimal oral hygiene are required [3, 49]. Current guidelines recommend therapy with PPI twice a day in patients with suspected GERD-related oral manifestations [2]. There are no published studies regarding the effect of anti-reflux surgical therapy on GERD-related dental erosions.

CONCLUSIONS

Extra-digestive GERD remains a controversial topic in terms of its epidemiology, diagnosis and treatment. It still represents a diagnostic and therapeutic challenge for gastroenterologists and physicians of other specialties (pneumology, ENT, dentistry). The PPI trial is the first diagnostic, but also therapeutic step, while evaluation through esophageal impedance-pH monitoring currently represents the gold-standard.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author confirms that this chapter contents have no conflict of interest.

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CHAPTER 2

Optical Diagnosis in Barrett Esophagus and Related Neoplasia

Daniela E. Dobru[1,*]

[1] *Department of Gastroenterology, GE Palade University of Medicine, Pharmacy, Science and Technology Târgu-Mureş, Romania*

Abstract: The detection of high grade dysplasia and esophageal adenocarcinoma with improved survival rates is the aim of optical diagnosis in BE. Advanced imaging technologies improve the characterization of dysplastic BE by mucosal visualization and enhancement of the fine structural and microvascular details (mucosal and vascular pattern) and may guide targeted biopsies for the detection of dysplasia during surveillance of patients with previously non-dysplastic BE.

Keywords: Barrett esophagus, Dysplasia, Esophageal adenocarcinoma, Optical diagnosis.

INTRODUCTION

Barrett's esophagus (BE) is a well-known pre-malignant lesion of esophageal adenocarcinoma (EAC). Even though there is an increased risk of developing EAC in patients with BE, the absolute risk remains low [1, 2]. However BE is found in the majority of patients with EAC, but only 5% of the patients with EAC had a prior diagnosis of BE [3], showing that unfortunately most cancers are diagnosed outside of surveillance programs.

Surveillance of patients with confirmed BE is recommended by all guidelines and largely applied. The Seattle protocol, consisting of target biopsies of visible lesions and four-quadrant forceps biopsies at every 2 cm, is accepted as the standard for surveillance in BE, although difficulties resulting from this procedure are well known by endoscopists.

* **Corresponding author Daniela E. Dobru:** Department of Gastroenterology, GE Palade University of Medicine, Pharmacy, Science and Technology Târgu-Mureş, 38 Gh. Marinescu Str., 540139 Târgu-Mureş, Romania;, Tel: +40 744775889, Fax: +40 265266171; E-mail: danidobru@gmail.com

Ioan Sporea and Alina Popescu (Eds.)

The recognition of dysplastic BE offers the possibility to intervene at an early stage of EAC with improving the survival rate and reducing mortality. The efforts should be undertaken to better identify the patients at risk of developing EAC.

The Rationale of Optical Diagnosis in BE

Optical diagnosis in BE endeavors to enhance survival outcomes by catching high-grade dysplasia and esophageal adenocarcinoma. Surveillance in patients with previously non-dysplastic BE can now be expanded to include targeted biopsies, that could uncover dysplasia [4 - 6]. Furthermore, this new technology allows the practitioner to examine visually the fine mucosal and vascular details of BE with dysplasia [4 - 6]. Real time optical diagnosis allows taking therapeutic decisions if dysplastic lesions are diagnosed.

Optical diagnosis in BE is a complex, time consuming procedure that requires training and expertise and a continuous contact with an expert high-volume center.

Pre-adoption Requirement to Start Optical Diagnosis in BE

• Quality measures: To ensure a basic standard of endoscopic quality in optical diagnosis, it is recommended that the ESGE (European Society of Gastroenterological Endoscopy) and UEG (United European Gastroenterology) key performance measures for upper gastrointestinal tract endoscopy to be adopted. In accordance with this, the best practice is an inspection time of at least 1 minute/cm of the circumferential extent of Barrett's epithelium, in order to inspect and describe the mucosal and vascular pattern.
• The size and extent of Barrett epithelium have to be done by using Prague C& M criteria, which assess the circumference (C) and maximum (M) extent of the Barrett epithelium endoscopically visualized, above the gastroesophageal junction. Barrett islands have to be reported separately.
• High definition - white light endoscopy (HD-WLE) equipment has become a routine part of the practice of most endoscopists and should be a "must", when the optical diagnosis of BE is addressed.
• There are two ESGE-required instruction modules for endoscopists wishing to perform optical diagnosis in BE patients for the purposes of early detection of neoplasia:
-BORN ["Barrett's Esophagus-Related Neoplasia" (BORN)] for high- definition white-light endoscopy or Chedgy instruction in utilizing acetic acid for chromoendoscopy [6].
ESGE recommends the use of validated classification systems to support the use of optical diagnosis with advanced endoscopic imaging and chromoendoscopy.
Stages of Optical Diagnosis in BE.

Fig. (1). Stages of optical diagnosis in BE.

Optical diagnosis in BE starts with the inspection of the entire Barrett epithelium. In order to detect any abnormality which might be in the dysplastic areas or even cancer, is mandatory to be aware and compare the appearance of these modifications with the normal view of Barret epithelium.

The *regular endoscopic view of Barret epithelium* is shown in Fig. (**2**) and the followings are the most common characteristics:

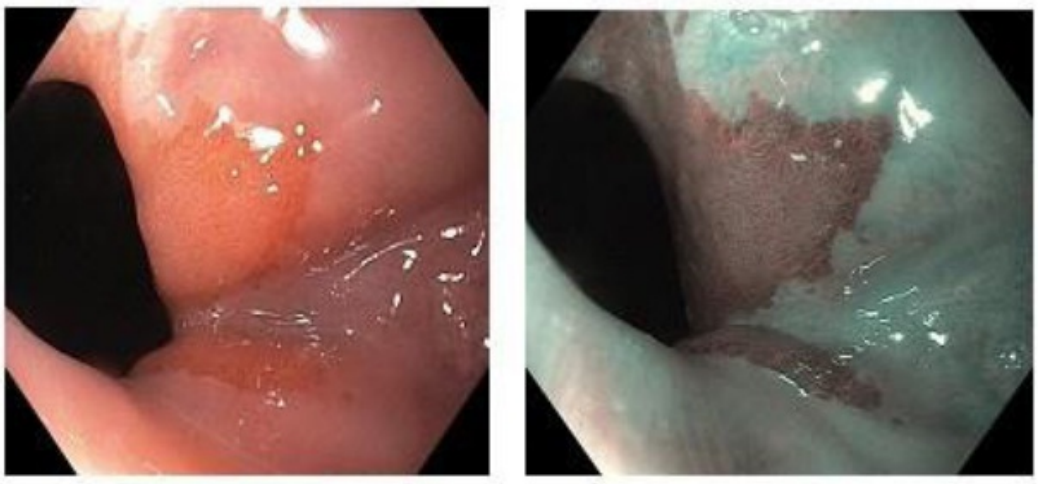


Fig. (2). Non-displastic Barrett epithelium: HD-WLE and NBI (narrow band image).

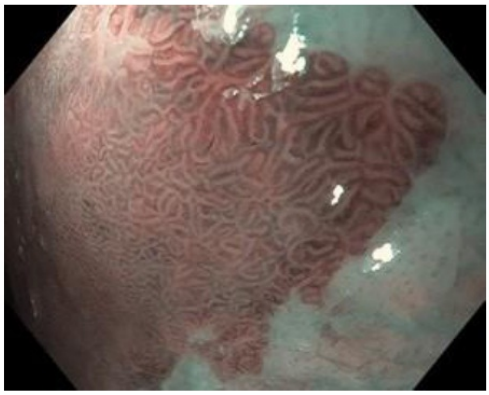


Fig. (3). Non-displastic Barrett epithelium: NBI + Near focus mode: regular mucosal and vascular pattern.

- Regular mucosal pattern: Circular, ridged/villous, or tubular patterns
- Regular vascular pattern: Blood vessels situated regularly along or between mucosal ridges and/or showing normal, long, branching patterns

Characterisation of Barrett epithelium is the second step of optical diagnosis and advanced endoscopic imaging technologies improve the characterization of dysplastic BE by mucosal visualization and enhancement of the fine structural and microvascular details.

This step of optical diagnosis has to be done using one of the three validated classification systems:

1. BING classification for NBI

2. BLINC classification for BLI

1. PREDICT classification for chromoendoscopy using acetic acid.

An international association of experts developed and subsequently validated the so-called Barrett's International NBI group (BING) classification framework for detection of dysplastic BE using near-focus NBI. This framework's accuracy is over 90% and showed a large degree of high interobserver agreement [7].

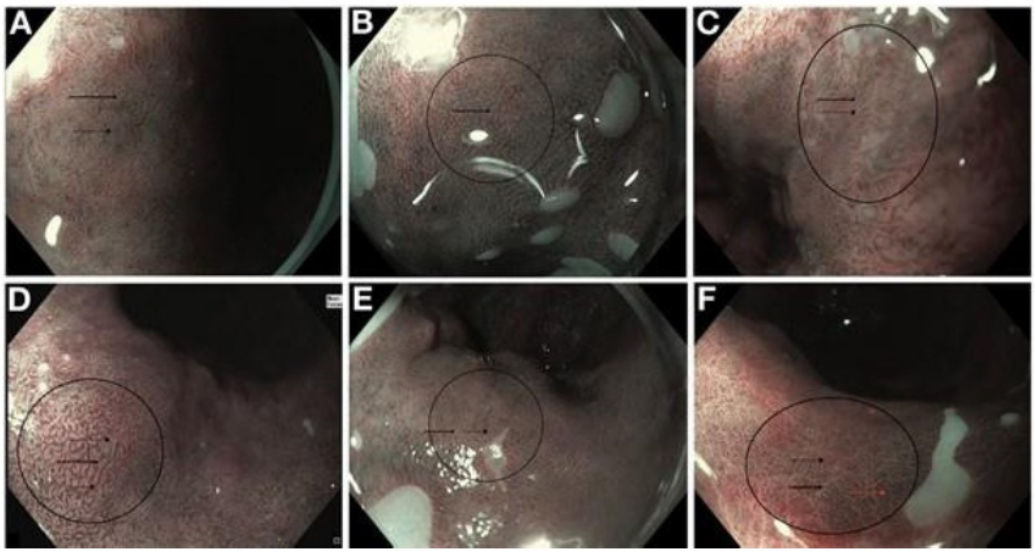


Fig. (4). BING classification: High-resolution images of non-dysplastic BE using NBI.

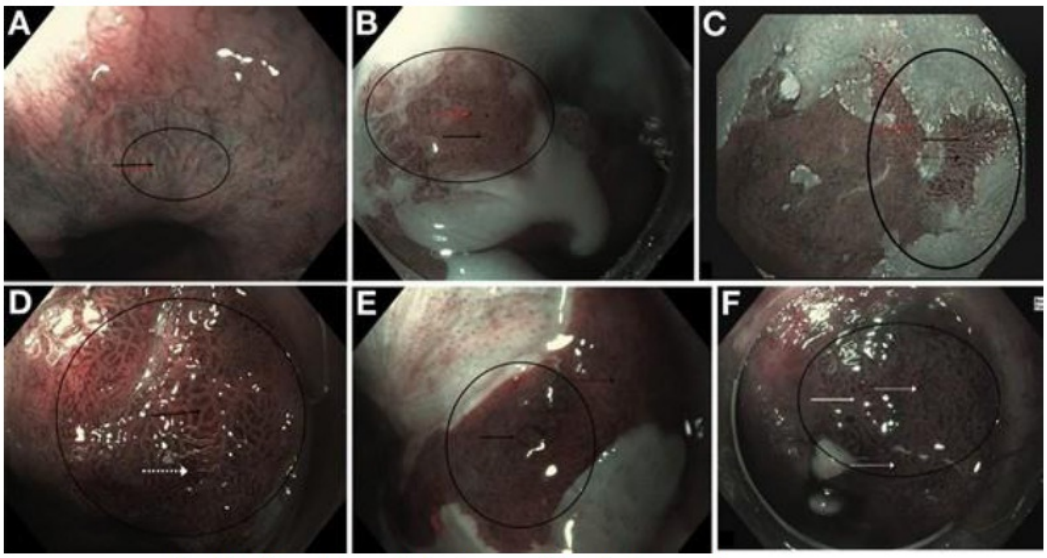


Fig. (5). BING classification: High-resolution images of dysplastic BE using NBI.

In daily practice, for simplification of the procedure, the morphologic characteristics of mucosal and vascular pattern are recommended to be classified in regular (non-dysplastic Barret), irregular or absent (dysplastic Barrett) (Figs. **6** and **7**).

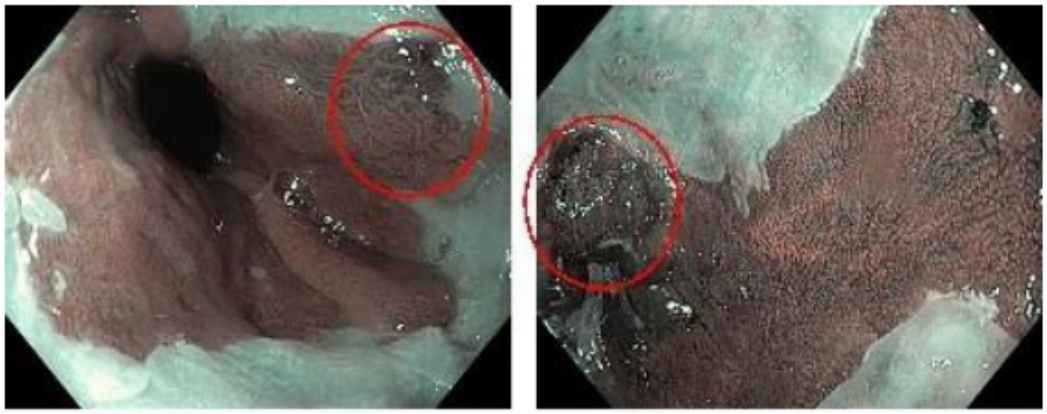


Fig. (6). Iregular mucoasal and vascular pattern – HGD Barrett.

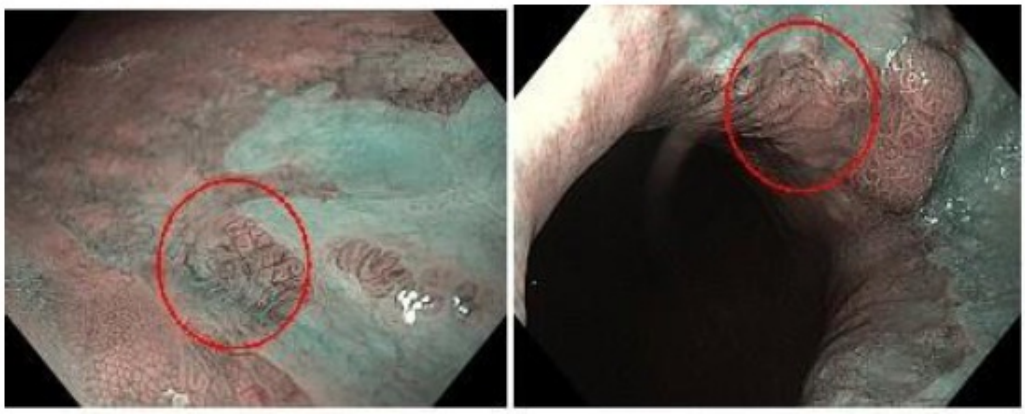


Fig. (7). Iregular mucoasal and vascular pattern – HGD Barrett.

Acetic acid chromoendoscopy is an old, simple and cheap technique that has been proven to highlight neoplastic areas during characterisation and delineation of Barrett epithelium (Fig. **8**). PREDICT (Portsmouth acetic acid classification) is a validated classification framework in the diagnosis of Barrett's neoplasia using acetic acid chromoendoscopy. Its key benefits are an increase in sensitivity from 79% to 98% ($P<0.001$) and an increase in NPV from 80% to 97% ($P <0.001$) The most important endoscopic sign of dysplastic areas is an early loss of acetowhitening [8].

Anecdotally, the new BLI visualization technology is beneficial for endoscopists wishing to diagnose Barrett's neoplasia. However, previously there has not been sufficient evidence to support this.

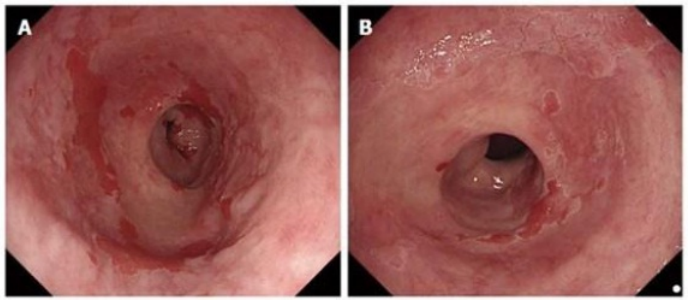


Fig. (8). A: Barrett's epithelium with HDWL; **B:** Same patient with dysplasia only visible post acetic acid with an early loss of acetowhitening sign.

The BLI New Classification, BLINC classification (Fig. **9**), for the characterization of neoplastic and non-neoplastic BE is based on color, pits, and vessels [6, 8]. When BLINC classification was used, the overall sensitivity, specificity and accuracy of neoplasia identification were 96%, 94.4%, and 95.2%, respectively [8].

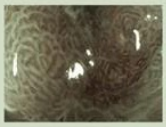
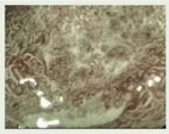
		Non Neoplastic	Neoplastic
Color		No focal darkness	Focal darkness
			
Mucosal pits (crypts)	**Type**	Circular, tubular, gyriform	Amorphous, circular, tubular, gyriform
	Distribution	Regular	Irregular
	Density	Normal	Increased
			
Vessels	**Type**	Non dilated, non branching	Dilated, branching
	Distribution	Pericryptal	Non cryptal
	Density	Normal	Increased or focal loss
			

Fig. (9). BLINC classification for non-neoplastic and neoplastic Barrett esophagus.

CONCLUSIONS

The detection of subtle Barrett's neoplasia *via* surveillance endoscopy may not always be straightforward and the Seattle biopsy protocol can often miss focal neoplasia.

Recent advances in the endoscopic imaging as well as the development of validated classifications may lead to improved detection of dysplastic BE and related neoplazia. Beside the pre-adoption requirement necessary to start optical diagnosis in BE, which was presented earlier, the instruction necessary to attain proficiency in optical diagnosis is perhaps the most important issue.

Before performing the optical diagnosis on at-risk patients, an endoscopist should first complete either the BORN or the Chedgy training course as well as onsite instruction with an experienced practitioner of optical diagnosis in BE. The learning curve is steep and training is time-consuming – however, it is necessary. Fortunately, the BORN training course is now available at no cost on several portals: www.iwgco.net, www.ueg.eu, or www.best-academia.eu. It has also received accreditation for Continuing Medical Education.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author confirms that this chapter contents have no conflict of interest.

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Declared none.

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CHAPTER 3

Eosinophilic Gastrointestinal Disorders

Dan L. Dumitraşcu[1,*] and **Andrei V. Pop**[1]

[1] *2nd Department of Internal Medicine, Iuliu Haţieganu University of Medicine and Pharmacy Cluj-Napoca, Romania*

Abstract: Eosinophilic infiltration of the gut occurs unusually and its clinical relevance was only recently recognized. The medical conditions with eosinophilic infiltration are commonly named eosinophilic gastrointestinal disorders [EGID]. EGID is described as a gastrointestinal tract disorder with functional and morphological abnormalities due to a dense infiltration of eosinophils in the gastrointestinal wall. The cause could be an allergic reaction due to varied allergens, food or the environment. EGID is including eosinophilic esophagitis [EoE], eosinophilic gastroenteritis [EGE], and eosinophilic colitis [EC].

EGIDs pathophysiology is not yet fully understood, but histopathology is characterized by degranulation and an excessive number of eosinophils. A role in the pathophysiology of EGIDs is played by a hypersensitive reaction. Diagnosing EGIDs is quite challenging. It can be described as a combination of eosinophilic invasion of one or more organs from the GI tract with non-specific GI symptoms. The gold standard for EGIDs diagnosis is the histology of gastrointestinal mucosal biopsy, an overabundance of eosinophils being the principal diagnostic criterion without a known cause.

The treatment for EGID is not well defined yet, because of the limited prospective controlled studies performed. The treatment is an empiric one and is administrated according to the severity of the symptoms and it is represented by diet, corticosteroids, and steroid agents.

Keywords: Eosinophilic colitis, Eosinophilic esophagitis, Eosinophilic gastroenteritis, Eosinophilic gastrointestinal disorders, Hypersensitive reaction.

INTRODUCTION

The largest surface in the body, with an important number of immune cells, is represented by the gastrointestinal (GI) tract, an organ system that fulfills many important roles, including the oral tolerance and absorption of nutrients [1]. Eosinophils are normally present in most parts of the gastrointestinal tract, they

[*] **Corresponding author Dan L. Dumitrascu:** 2nd Department of Internal Medicine, Iuliu Haţieganu University of Medicine and Pharmacy Cluj-Napoca, 4 Clinicilor Str., 400006 Cluj-Napoca, Romania; Tel: +40-722-756475;, Fax: +40-264-593355; E-mail: ddumitrascu@umfcluj.ro

Ioan Sporea and Alina Popescu (Eds.)

may be quite numerous, except at the esophagus level, where they are not present in physiological states [2, 3]. Eosinophils can be present in chronic diseases that appear and disappear, from months to years as well as in any inflammatory condition present for days to weeks. A raised number of eosinophils are also found in auto-immune gastritis, gastroesophageal reflux disease, inflammatory bowel disease, radiation enteritis, collagen vascular disease, neoplasm and many other disorders, and even in the absence of a specific disease which is not quite common. They can be distinguished by the presence of many neutrophils and an association of inflammation. Most of these entities show a mix, between-neutrophil-rich, inflammation and other features, which allow their distinction [2]. Eosinophilic infiltration of the gut, uncommonly appears even in the lack of aforementioned cause. The disorder with eosinophilic infiltration is commonly named eosinophilic gastrointestinal disorders (EGID), formed by eosinophilic esophagitis (EoE), eosinophilic gastroenteritis (EGE), eosinophilic colitis (EC) [3, 4].

EGID is described as a gastrointestinal tract with functional and morphological abnormalities, due to a dense infiltration of eosinophils in the gastrointestinal tissue. The cause could be an allergic reaction due to varied allergens, food or the environment [5].

EGIDs is present at both sexes, more common at males (3:2), is present at different ages, including children. The Caucasian population is most affected, but it can affect all races and ethnic backgrounds [3, 6]. EGIDs are rare disorders, EoE is the most common disorder from EGID, with a prevalence of 10-57 per 100,000, compared to those found outside of the esophagus like EC, EGE or EG which are 2.1-5.1 cases per 100,000 [7].

EOSINOPHILIC ESOPHAGITIS (EOE)

Introduction

A great interest in the last years has been raised by EoE, which is an immune-mediated disease, that is characterized by infiltration of eosinophils at the esophagus level, causing esophageal dysfunction. Now EoE represents an important differential diagnosis when patient symptoms are gastroesophageal reflux, dysphagia and food impaction [8, 9]. One of the strongest risk factors for developing EoE is the gender, male being the most affected gender showing 3:1 male to female predominance [8, 10].

Epidemiology

For the first time a patient with EoE was mentioned in a report in 1978 [11], and it

was accepted as a distinct clinical entity in the early 1990s [12, 13]. Although, initially it was a rare case report, but in the last few years, its prevalence and incidence have considerably expanded, and it became a common condition found in any gastroenterology clinic or emergency room [14]. The expansion of the cases of EoE could have two explanations, one is a real increase of the incidence, and the other could be due to an improved recognition [15].

The incidence in European countries is between 2.1-7.4 cases per 100,000 inhabitants [16, 17], the prevalence is between 13.8-44.6 cases per 100,000 inhabitants [18, 19]. EoE can affect any age, from patients at 1 year old, to patients at 98 years old [20], with a higher prevalence in adults [14], and the highest prevalence at 30-40 years of age [21].

The incidence and prevalence of EGID is increasing due to endoscopic detection, although in the United States it is still categorized as a rare disease with < 200,000 people affected.

The approximate prevalence is 1 to 2,000, with 150,000 cases in the US and more than 1 billion $ being the estimated expenditures for EoE, from a total of 18,1 billion $ spent annually for esophageal disorders. This represents a colossal number for a rare disease, being comparable with the cost for more common diseases [22, 23]. One of the modifications that appear is the esophageal remodeling, with functional damage and stricture development, being progressive in its natural course. The duration of untreated disease are associated with the prevalence of esophageal strictures [24, 25].

Pathophysiology

The mechanism of EoE has still not been entirely clarified, due to the fact that is a new disease described in the literature, but in the last few years, there has been a progression in the understanding of EoE pathophysiology [26, 27]. It was described as a genetic predisposition, where a patient with a food allergy, with GERD, a possible disturbance of the microbiota or epithelial barrier favors allergens to infiltrate the epithelium and trigger the receptors and the inflammatory cells, like eosinophils to activate [28]. Due to its response to dietary therapy, EoE is correlated with food antigen-driven hypersensitivity, being triggered by different foods like milk, combined sometimes with eggs, soy or wheat and resulting in 15% of cases serious IgE mediated reactions like anaphylaxis or mild reactions as urticaria. It was described that almost half of the patients with EoE, diagnose positive for food antigens from serum testing and to skin prick testing [29, 30]. Also EoE patients present aeroallergen hypersensitivity or a known history of respiratory allergy [31].

In the recent literature, some studies have shown that EoE is linked to some genetic susceptibility. In the esophagus, squamous epithelial cells have a high expression for an intercellular protease for a gene called Calpain-14 (CAPN14), implicated in cytoskeletal dynamics, gene expression, cell-cycle progression and located in an epigenetic hotspot controlled by the cytokine and Il-13 [32]. The 2 - type immune response is induced by TSLP which is produced by the esophageal epithelial cells. Allergic diseases such as allergic rhinitis, asthma and EoE are being correlated with genetic variants of 5q22 encoding TSLP. Autoimmune conditions like Crohn's disease, ulcerative colitis, celiac disease, or rheumatoid arthritis are present in approximately 1/3 of patients with EoE. An increased risk for both non-allergic, immune-mediated disease and EoE was described in a recent study for a common locus 16p13, a region that encodes for genes expressed in the esophageal epithelial cells and immune cells [33, 34]. A higher risk for EoE is due to PPIs and overly hygienic environment, antibiotics and cesarean delivery that influence the host,s microbiome balance [21].

For a patient with a genetic susceptibility and an injured epithelial barrier, microbiota antigens and food infiltrate the epithelium and determine a Th2 type immune response in the esophagus, which has a key role in the pathophysiology of EoE, demonstrated by the presence in the esophageal mucosa of Th2 type cytokines [especially Il5 and Il-13] of patients with EoE [5, 35, 36]. Il-13 and Il-5 are both increasing eosinophils, Il-13 raise eotaxin-3 production by esophageal epithelial cells, which determines secondary eosinophil accumulation and IL-5 determines eosinophil productions. These eosinophils are the ones responsible for epithelial barrier breakage and injure neurons due to the release of granular proteins, and also altering motor and sensory function of the esophagus, increasing periostin and TGF-β, which stimulate fibrosis and establish tissue remodeling over time [21].

Clinical Manifestations

EoE can be present at any age, clinical manifestation varying by age, described with digestive symptoms correlated with respiratory (asthma, rhinoconjunctivitis) and allergic manifestations [28]. In young children, feeding difficulties, failure to thrive, gagging on solid foods, irritability, vomiting, and regurgitations can lead to failure to thrive and alteration of general status. Older children complain of nausea, epigastric abdominal pain, vomiting, food impaction and dysphagia [27, 37, 38]. In adults the most common symptom is dysphagia. Other common symptoms are food impaction, chest pain and heartburn (Table **1**) [39].

EoE has symptoms that resemble very much with GERD [40]. EoE cannot be predicted by any symptoms, but dysphagia seems to appear much more frequently

in positive patients. There is a median delay of 6.5 years from the first symptom to EoE recognition, and a median delay of 1 year from the prime presentation at the hospital to the effective diagnosis [21]. Adult EoE patients have frequently confronted with atopy. Patients with EoE have higher a prevalence of asthma and allergic rhinitis, compared with the general population. Also, environmental allergies are much more common in patients with EoE, symptoms as eczema and IgE-mediated food allergy (*e.g.* urticaria, anaphylaxis). Half of EoE patients have a family history of the atopic disorder [37, 41].

Table 1. Characteristics of EoE in children and adults.

-	**Adults**	**Children**
Presenting Symptoms	Dysphagia	Feeding difficulties
-	Food impaction	Failure to thrive
-	Chest pain	Gagging on solid foods
-	Heartburn	Vomiting
-	-	Food impaction
-	-	Dysphagia

Diagnosis

EoE was defined, in the latest guideline, as a "clinicopathologic condition that was immune or antigen driven" [42]. The EoE is clinically characterized by the symptoms of esophageal dysfunction, but the diagnosis is definitively histological (Table **2**). The esophageal biopsy is mandatory, at least 6 biopsy samples are necessary, from proximal and distal halves of the esophagus. At least 15 eosinophils per high power field (eos/hpf), approximately 60 eosinophils/mm^2 are required for positive diagnosis (Fig. **1**) [8, 21, 42].

Table 2. Diagnosis of EoE.

Endoscopic finding	Rings Strictures Mucosal ulceration Edema Linear furrows Plaques multiple white papules Small caliber-esophagus
Histological findings	At least 15 eos/hpf 60 eosinophils/mm^2
Barium swallow	Stenosis Sub-stenosis Rings Length of esophagus

eos/hpf - eosinophils per high power field.

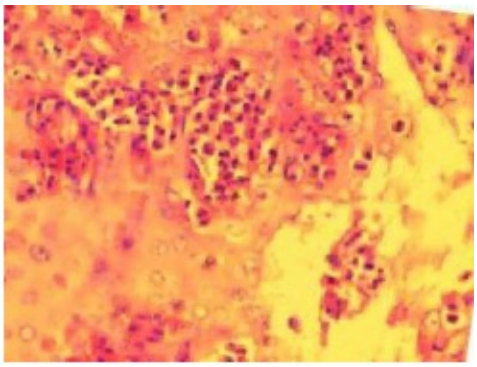

Fig. (1). Histological presentation of EoE (Courtesy of the authors).

Once EoE is suspected, an endoscopy should proceed [27]. Characteristic endoscopic findings associated with EoE are represented by rings, strictures, mucosal ulceration, linear furrows, plaques multiple white papules and small caliber-esophagus (Fig. **2**) [43, 44]. Hirano *et al*. proposed a novel endoscopic reference score (EREFS) for grading EoE, being an acronym for Exudates (plaques or white spots), Rings (concentric rings, fixed), Edema (decreased vascular markings), Furrows (longitudinal lines) and Strictures [45].

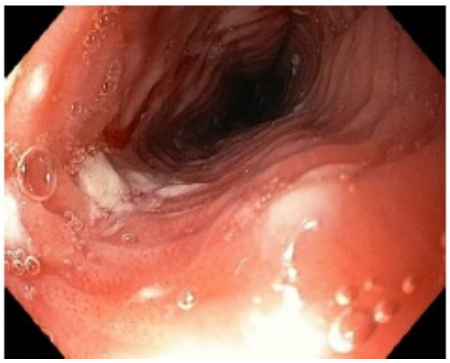

Fig. (2). Endoscopic presentation of EoE (Courtesy Prof. Simona Bataga).

Complementary information to endoscopy is provided by barium swallow. Before performing a dilation procedure, it is essential to perform a barium swallow, which can detect stenosis or sub-stenosis, rings and showing the length body. A good way to evaluate the treatment response is to measure the maximum esophageal diameter [46, 47].

Also peripheral blood samples should be analyzed. More than half of the patients having peripheral blood eosinophilia described a relationship between the number of eosinophils in the peripheral blood and the eosinophils that infiltrate the

esophagus. Patients with EoE and allergies have a higher number of blood eosinophils than patients with allergies and without EoE [21].

Treatment

The management of EoE is multidisciplinary, and gastroenterologists, allergists, dieticians, and nurses should be part of the process [12]. The management of EoE can be achieved by the three "D's": drugs, diet, and dilation (Table **3**) [48].

Table 3. Treatment of EoE.

Drugs	PPIs for 8 weeks Topical corticosteroids *Budesonide 1-2mg twice daily* *Fluticasone 440-880 microgram twice daily*
Diet	Six-food elimination diet Elemental diet
Endoscopic Dilation	Savary dilator Balloon dilation

Drugs

At this moment, the first step is a trial of 8 weeks of PPIs. If the patients do not respond, the next step would be diet, or topical corticosteroids. For patients with severe symptoms, it is preferred to administrate topical corticosteroids, and the same for patients with low adherence to the diet. Patients who develop fibrostenotic abnormalities are proposed for endoscopic dilation [8].

Proton-pump Inhibitors

Patients with EoE responding to PPIs (PPI-REE) are also included, in the spectrum of EoE, since the last guideline [8]. PPIs are the most effective drugs for the treatment of reflux disease, due to their anti-secretory properties [49], but PPIs also display several other activities, independent of their antisecretory activity. The most relevant PPIs's properties in the treatment of EoE are the anti-inflammatory and the mucosal protective ones. The mechanisms of anti-inflammatory properties are represented by an influence on the gut microbiota, antioxidant activity and effects on inflammatory epithelial and endothelial cells [50]. PPIs have mucosal protective activity, partially restoring mucosal integrity.

The integrity of the esophageal mucosa is impaired both in patients with EoE or PPI-REE, the trans-epithelial transport of allergens or small molecules being allowed. PPIs reduced transe-pithelial electrical resistance and dilates intercellular spaces, but only in patients with PPI-REE. This normalization is similar to that realized in patients with GERD [51, 52].

Corticosteroids

Serious reflux symptoms encountered in patients with EOE, which were not receptive to probiotics or PPIs were treated more than 20 years ago with oral corticosteroids. A histological and clinical improvement was seen in patients after 4 weeks of receiving methylprednisolone, but the use of oral corticosteroids is limited by the high frequency of adverse effects, over 40% [53, 54].

Nowadays the mainstay of EoE therapy is topical corticosteroids. There was a demonstrated efficacy of swallowed topical steroids in symptomatic patients and histological remission in EoE [8, 55]. One of the adverse effects of topical corticosteroids is esophageal asymptomatic candidiasis [56]. In the treatment of EoE, different types of steroids designed for airway distribution are used, and patients are instructed to swallow them (puffs from inhalers, oro-dispersible tablets, suspensions or viscous slurry). The longer the contact between the steroid and esophageal mucosa, the better results they have, an example is swallowed puffs *versus* viscous formulations, when should be preferred viscous formulations, because studies show that they result in a higher reduction in eosinophils number [8, 57]. To induce remission, Budesonide (1-2 mg twice daily) or fluticasone (440 to 880 microgram twicea day) can be used. There are still no guidelines regarding doses or duration of the treatment with steroids in EoE, but using topical corticosteroids in long-term therapy was noticed to be an effective way in maintaining remission [8].

Diet

The first step of the EoE treatment is represented by dietary elimination, along with swallowed topical steroids and PPIs therapy [8]. The three types of dietary therapies are used- including elemental diet, empiric elimination diet, and allergy testing–directed elimination diet [58].

An elemental diet is highly efficacious as it reduces eosinophilic inflammation and induces clinical remission, but it is costly and difficult to adhere to due to the unpleasant taste, avoiding all table food, social limitation. Using exclusively an amino acid-based formula, there is a total elimination of food allergens [59, 60]. Allergy testing–directed diets are less efficacious [61], specific food allergens are searched and removed from the diet, using prick testing. Nowadays, for adults, this type of approach is not recommended [62]. The recommended approach is empiric elimination dietary therapy. The most used is a six-food elimination diet, a simultaneous exclusion of most common allergens associated with EoE: milk protein, wheat, eggs, soy, nuts, and seafood, for 6-8 weeks [63]. EOE can be histopathological and endoscopically improved, and also the symptomatology can be ameliorated significantly by a six-food elimination diet [64]. Endoscopy should

be performed after reintroducing each group of foods separately, in response to any observational 6-week diet. The purpose is to remove completely all the triggers food that induces inflammation, providing a personalized therapy for maintenance after each individual reintroduction [65].

Endoscopic Dilation

The tissue remodeling is made by the chronic inflammation of the esophageal mucosa, with a change in the esophageal caliber and fibrosis. Stricture formation is pointed out by fixed rings and narrow caliber diameter, symptoms for a long time and patient's age are the risk factors for esophageal strictures. Food impaction appear at an esophageal diameter < 17 mm, and dysphagia at <13 mm [21]. For patients with dysphagia that are unresponsive to anti-inflammatory treatment, patients with food impaction or severe strictures, dilatation should be taken into consideration as a therapy for fibrostenotic EoE [66, 67]. Esophageal dilation can be performed with either a Savary dilator or a balloon. The length and the tightness of strictures determine what kind of method should be used, although both devices being useful. For a short stricture (1-2 cm), use a balloon dilator, and for a long stricture in a narrowed esophagus a Savary dilator is an ideal one [68]. In 95% of EoE patients who have narrow caliber esophagus or strictures, symptoms are improved after endoscopic dilatation, which shows that a big contribution to the symptomatology of EoE is represented by tissue remodeling [69].

EOSINOPHILIC GASTROENTERITIS (EGE) AND EOSINOPHILIC COLITIS (EC)

Introduction

Eosinophilic gastroenteritis is a rare, heterogeneous disorder, with diverse gastrointestinal symptomatology, depending on the specific layer and site of the gastrointestinal tract. Most of the cases involve the stomach or proximal small bowel. EGE is described as 50 or more eosinophils per high power field, manifested with abnormal gastrointestinal symptoms, like abdominal pain and eosinophilic invasion in one or more parts of GI. The stomach is the organ most commonly affected in this [70].

Epidemiology

EGE was first described in 1937 by Kaijser [71]. The incidence is around 1 in 100,000 individuals and the prevalence is between 5-8 cases per 100,000 [72, 73]. The EG appears more often between the thirties and forties, but it can affect any age from infant to elderly, it affects predominantly male sex, with a ratio of 1,25:1

male: female ratio [74]. Up to 70% of patients have a family history of atopic disorders like eczema, asthma, or hay fever [75]. Eosinophilic colitis has a prevalence of 2-3/100,000 in the USA, being a rare disorder, but it is present in 0,1% of the colonoscopy examinations performed for diarrhea [76].

Pathophysiology

Eosinophilic colitis and eosinophilic gastroenteritis pathogenesis are not yet fully understood, but histopathology is characterized by degranulation and an excessive number of eosinophils [39, 72]. A role in the pathophysiology of EGE and EC is played by a hypersensitive reaction [70]. It can be considered a non-IgE dependent Th2 type allergic disease. As with EoE, the triggers and aggravating factors are supposed to be the food allergens [36]. Eosinophils are located in the GI tract, in the lamina propria, excepting in the esophagus. The physiological role of eosinophils is to protect the tissue against parasitic infections, not to cause inflammation or damage to the tissue. In eosinophilic enteritis, eosinophils are the ones that cause gastrointestinal lesions being activated by allergens, nonspecific tissue damage or infections. Activating the eosinophils will determine degranulation, which leads to the secretion of IgE cytotoxicity of cationic proteins. Also, activated eosinophilic determine the release of some proinflammatory cytokines like IL-5, IL-4 and IL-13 and RANTES enroll immune cells to the inflammation spot and amplifying the local inflammatory response. The development of eosinophilic inflation of the intestinal mucosa is supposed to be concerned with the IgE mediated response and also the delayed Th2 adaptive response, but the mechanism is still unknown [77].

Clinical Manifestations

In EGE and EC, the clinical presentation is widely variable depending on the layer of GI wall involved and the site of the GI tract, with a diversity of symptoms. A history of atopic conditions has been noted in patients with manifestation as allergic rhinitis, asthma, and allergy to food, medicine or pollen.

Klein *et al*. suggested a classification of EGE, based on the depth of eosinophilic infiltration into the GI wall and clinical manifestations into three different types: predominantly mucosal pattern, predominantly muscular pattern and predominantly serosal pattern [74]. The patients with mucosal pattern have symptoms like vomiting and nausea, anemia and iron deficiency, protein-losing enteropathy, malnutrition and weight loss [78]. This is the most common presentation [74]. Muscular disease, the second most common presentation, can be correlated with intestinal obstruction [mainly jejunal obstruction].

Serosal disease, the rarest presentation, has an improved response to corticosteroid therapy and is correlated with bloating and ascites and possibly a better response to corticosteroid therapy [74, 78].

EC is also classified into 3 types, with variable clinical presentation. The most common form of EC is the mucosal one, which is correlated with mucosal injury, protein losing enteropathy, diarrhea and malabsorption. Less frequent is the transmural disease with severe manifestations like colonic wall thickening, acute intestinal obstruction [intussusception or cecal volvulus], and sometimes even perforation. The rarest form is represented by the serosal disease, with eosinophils in 95% of cases majority cell type, manifested with ascites, but having a good prognosis [76, 79].

Diagnosis

Diagnosing EC or EGE is quite challenging. It is about a combination of eosinophilic invasion of one or more organs from the GI tract and non-specific GI symptoms [78, 79]. The "gold standard" for diagnosis of EC or EGE is the histology of gastrointestinal mucosal biopsy, an overabundance of eosinophils without a known cause being the principal diagnostic criterion. To have a diagnostic EGE, a number of 30 eosinophils per high-power field was suggested and different numbers for colon parts: 50 eosinophils per right colon, 35 transverse colons and 25 per left colon to put the diagnostic of EC [72]. Ulcerated mucosal surfaces, mucosal erythema, nodular appearance and edema are the endoscopic findings, but about half of the patients have a normal-appearing mucosa at endoscopy [78]. Most of the radiological findings of the patients with EC or EGE are normal. In the mucosal type a local fold thickening is a common manifestation, but it can appear as polyps, luminal narrowing, and and ulceration. In the muscular pattern dysmotility, obstruction, rigidity or stenosis are the patient's clinical manifestations [74, 80].

Treatment

The treatment for EGID is not well defined yet, because of the limited prospective controlled studies performed. The treatment is an empiric one and is administrated according to the severity of the symptoms [3].

Diet

Diet therapy is the first therapeutic approach for EGE and EC [79]. Patients with EoE, elimination and elemental diets have improved clinical manifestations and reduced mucosal eosinophils. Similar management can be applied in the EGE and EC [3].

Corticosteroids

The first line therapy in EC is corticosteroids therapy, every time the dietary one does not bring the expected results, or it is impractical [79]. The mechanism of corticosteroids is the inhibition of eosinophilic growth factors IL-5, IL-3 and GM-CSF. An absence of a positive response after corticosteroid therapy should reconsider the diagnosis because EGID is sensitive to these [3]. The main therapeutic corticosteroids used are budesonide, prednisone and fluticasone [74]. The doses that induce remission for oral prednisone are 20-40 mg per day for 2 weeks, in most of the patients [79]. Budesonide, is an alternative systemic drugs, that has local action, but with almost the same efficiency as prednisone, and shows an improvement in the clinical manifestations, reduces tissue eosinophilia and the protein loss in the terminal ileum and cecum [72].

Steroid-sparing Agents

Mesalazine has been studied, and seems to be effective in some cases of EC [79]. Patients with EGE with the mucosal and serosal disease have received cromolyn sodium which is a mast cell stabilizer, alone or with steroids, with some success; but without any notable effects in treating EC patients [3, 79]. Other mast cell stabilizers are ketotifen and sodium cromoglycate [73].

A selective, competitive leukotriene receptor antagonist is montelukast which is considered to be quite effective and safe steroid-sparing in the therapy of EC and EGE [74, 79]. In a study with 9 patients with eosinophilic gastroenteritis, omalizumab, which is an anti-IgE agent, has improved symptoms and reduced gastroduodenal eosinophils number [72]. CCR3 is a novel antibody directed against, an eotaxin receptor expressed by eosinophils. It has been shown to decrease diarrhea and eosinophilic inflammation in experimental eosinophilic gastroenteritis on mice [72].

CONCLUSIONS

Eosinophilic gastrointestinal disorders are increasing in daily practice, mainly eosinophilic esophagitis. Early recognition and then treatment is necessary to reduce the symptoms in this category of patients.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author confirms that this chapter contents have no conflict of interest.

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CHAPTER 4

Postoperative Digestive Complications of Bariatric Surgery

Andrada Seicean[1,*] and **Radu Seicean**[2]

[1] *Regional Institute of Gastroenterology and Hepatology Cluj-Napoca, University of Medicine and Pharmacy "Iuliu Haţieganu" Cluj-Napoca, Romania*

[2] *First Surgical Clinic, Cluj-Napoca, University of Medicine and Pharmacy "Iuliu Haţieganu" Cluj-Napoca, Romania*

Abstract: Digestive complications of bariatric surgery are quite rare, especially those which are sever in nature, with a lower rate aftersleeve gastrectomy compared to Roux-en-Y gastric bypass. This chapter discusses the bleeding, anastomotic leaks, stenosis and ulceration, gastroesophageal reflux, bowel transit dysfunction, gallstones and complications related to adjustable gastric banding and other after bariatric surgeries.

Keywords: Bariatric surgery, Complications, Endoscopic treatment, Endoscopy, Gastric bypass, Obesity, Sleeve gastrectomy.

INTRODUCTION

Bariatric surgery is known for reducing the risks of medical and/or metabolic complications related to obesity such as diabetes and cardiovascular diseases or even cancers. The main surgical procedures include sleeve gastrectomy (SG), Roux-en-Y gastric bypass (RYGB) or mini-gastric bypass, laparoscopic adjustable gastric banding (LAGB) and single anastomosis duodeno-ileal bypass with sleeve gastrectomy (SADI-S).

In the last seven years, in the USA,over 200,000 bariatric surgeries were performed, out of which61 percent sleeve gastrectomy, 17 percent gastric bypass, 1 percent gastric band, and 0.8 percent biliopancreatic diversion with duodenal switch. The remaining 15 percent were revisional procedures [1].

[*] **Corresponding author Andrada Seicean:** Regional Institute of Gastroenterology and Hepatology Cluj-Napoca, University of Medicine and Pharmacy "Iuliu Haţieganu" Cluj-Napoca 19-21Croitorilor Str., 400162 Cluj-Napoca, Romania; Tel: +40-264-433427, Fax: +40-264-431758; E-mail: andradaseicean@yahoo.com

Ioan Sporea and Alina Popescu (Eds.)

Operating on obese patients is challenging, because of the anatomic and physiologic characteristics and comorbidities of obese patients. Adverse intraoperative complications (1 - 5% of cases) [2, 3] are serious, like myocardial infarction or pulmonary embolus. The postoperative severe complications are rarely seen, compared to open surgery (3.37% *vs*. 7.42%; $p<0.0001$) and they are more frequent in the case of RYGB (3.3%) than in SG or adjustable gastric banding (1%) [4].

Compared to the LAGB, SG has a higher rate of anastomotic/staple line leaks, fluid/electrolyte/nutrition problems, strictures, infection/fevers, pulmonary embolism, bleeding and events not otherwise specified. Compared to LRYGB, SG has a lower, but comparable rate of nearly all postoperative bariatric specific occurrences requiring readmission, reoperation or an intervention, except for a lower rate of stricture, intestinal obstruction, and anastomotic ulcer [5].

If the obese patients present already a significant comorbidity, like cirrhosis, the proportion of postoperative complications is higher. A meta-analysis on 18 studies and 471 patients with obesity and liver cirrhosis showed that the rate of complications was 22% (lower for SG of 10% compared to RYGB of 31%) with 0.08% intraoperative complications and 4.62% 90-days related mortality [6].

Table 1. The main complications related to bariatric surgery.

-	**Intraoperative Complications**	**Perioperative Immediate Complications**	**Postoperative Delayed Complications**
Cardiovascular	Myocardial infarction Deep vein thrombosis Pulmonary embolism		-
Digestive	Laparoscopic access related injuries Splenic or hepatic injuries Portal vein injury Bowel ischemia	Bleeding Stenosis Leaks Anastomosis ulcerations GERD	Stenosis Leaks Anastomosis ulcerations GERD Bowel transit dysfunction Internal hernias with bowel obstruction Gastric banding -slippage and erosion Gallstones
Other	Comorbidities related	Wound complications	Malnutrition Hypoglycemia Weight regain Recurrent port-site infection

Bleeding occurs usually during the first hours after surgery, although this is quite rare (1-4%) and occurs at the level of the staples line, in case of SG or at the level of gastro-jejunal anastomosis in case of RYGB. The diagnosis is based on tachycardia, oliguria and falling hemoglobin level and it can be produced into the

GI tract or peritoneum, usually at the level of staples or suture line. Endoscopy with haemoclips or bipolar coagulation isuseful, but OTSC (Over-The-Sco- e-Clip) or large volume injection can be applied, although the risk of anastomotic stenosis increases with this technique. In case of failure, full thickness re-suturing with monofilament sutures is used. The PPI (proton pump inhibitor) highdose intravenously is needed for decreasing the gastric acidity.

Stenosis of the level of anastomosis, signalized by dysphagia, may occur at the level of proximal staple line in 4% of the cases with GS, and in 3-28% of cases with RYG [7]. Also, functional stenosis after GS can be identified: type 1 due to the twist of the gastric tube with the endoscopic appearance of an anti-reflux valve, while type 2 is owing to a spiral course of gastric stapling that winds around the stomach [8]. Treatment with several endoscopic balloon dilatation sessions should start 3-4 weeks after surgery, until the luminal diameter is 12-15 mm.

Anastomotic leaks are the most fearful complications and they occur usually in the first postoperative week or even after discharge. Up to 90% of SG leaks occur at the esophago-gastric junction [9] and rarely at the distal part of the staple line. Therefore, the patient must be followed-up carefully in the first 30 days after the operation. The rate of leaks varies between 0.8% to 6% [10 - 13]. The risk of leaks is higher in the case of RYGB (1.6%), than in case of SG (0.8%) [13]. The risks for fistula in case of SG are perigastric hematoma and/or twisting of the distal part of gastric remnant on 48h CT scan [14]. After SADI-S, some patients may develop a leak from the duodeno-ileal anastomosis or within the gastric tube. They occur usually in the proximal portion of the anastomosis because this region is exposed to high pressure with ingested liquids, gastric juice, bile and saliva in the most proximal portion of the staple line and alsothere is a relative obstruction in the mid body portion of the stomach as the narrow gastric sleeve traverses the incisura angularis.

They can be classified according to the time of occurrence as acute (<7 days), early (within 1 to 6 weeks), late (within 6 to 12 weeks) and chronic (> 12 weeks). The risk factors are advanced age, BMI>50, male gender, revisional surgery and obstructive sleep apnea.

Acute leaks are associated with severe abdominal pain or peritonitis, because of lack of time for localization. They are related to the misfiring of a stapler or inadequate suture technique.

In case of a delayed leak, the generalized peritonitis arerare, usually, they present an intraabdominal abscess localized by the omentum of neighboring organs and the patients present fever and pain irradiating in the shoulder. More likely the

cause of such leaks is ischemia at the suture line. The systemic inflammatory response syndrome (SIRS) with sustained tachycardia >120bpm impose the differential diagnosis with pulmonary embolism, but in case of leaks, the high CRP (C-Reactive Protein) is present. The leaks aredetected during upper GI endoscopy, CT scan with oral and intravenous contrast and administration of blue methylene for highlighting the fistula through the percutaneous drain.

To lower the risk of barotrauma to the surgical suture during upper endoscopy, CO_2 insufflation should be used. The combination of endoscopy and radiological fluoroscopy is a reasonable option in case of fistula. The fistula should be intubated with a catheter, and a water-soluble contrast medium should be applied in order to visualize the fistula's tract, with characterization of the length and course of the fistula. If computed tomography (CT) and endoscopy are unable to detect a highly suspected post-operative complication, diagnostic laparoscopy should be considered, especially in the acutely ill patient. The findings as the differential diagnosis could be ischaemic bowel loop, internal hernias, non-contained perforations, gastric outlet obstruction [15]. After diagnosis and during treatment contrast enhanced CT scan should be performed regularly to follow-up the evolution of the leaks.

Endoscopic treatment is based on drainage of collections situated in immediate proximity to the GI lumen internally by internal endo-vacuum therapy or by laparoscopic or radiological drainage, if the collections are situated more distally. After drainage, the fistula is closed with OTSC, while larger defects are covered with self- expandable stents. The stents straighten the angle created by SG at the level of incisura angularis, facilitating the closure of the fistula (Fig. **1**).

A meta-analysis showed an overall success rate of 72.8%, with a migration rate of 28.2% for CSEMS (self-expandable metallic stent) [16]. The use of specifically designed stents longer and with a larger diameter were maintained in place for 4-6 weeks, with a 78% success rate, which increased to 94%, when other endoscopic procedures were added. However, perforation occurred in one case and migration was also considered as a severe adverse event, especially in stents placed distally from pylorus [17].

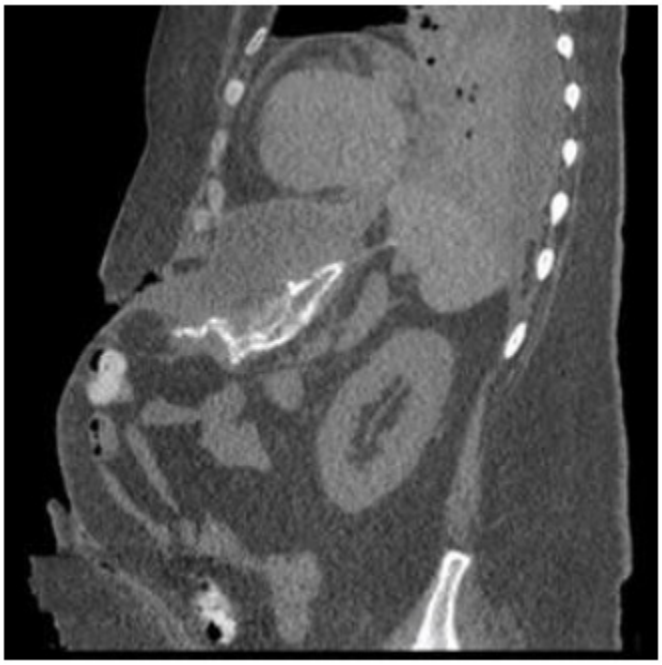

Fig. (1). The CT scan view of a metallic stent placed for a fistula after sleeve gastrectomy.

Sometimes, gastro-pleural or gastro-bronchial fistulas may occur, more frequently managed surgically, either by thoracic or digestive approach, after endoscopic treatment is attempted, with a high rate of morbidity, and with more failures in case of use of thoracic surgery as initial treatment [18]. SG with omentopexy and a modified cyanoacrylate sealant is a promising technique to diminish the rate of fistulas [19].

Anastomotic ulceration at the level of gastro-jejunal anastomosis occurs in 2-18% of RYGB in several weeks [20] and they are related to post-operative ischaemia, the presence of sutures and staples [21], the use of non-steroidal anti-inflammatory drug, gastric hypersecretion, and smoking or Helicobacter pylori infection. The clinical manifestations are nausea, epigastric pain, lack of appetite and rarely hematemesis. The treatment based on proton pump inhibitors is efficient. The suture material present at the ulcer level should be removed (cut or tear), with forceps or with endoscopic scissors.

Gastroesophageal Reflux

Usually, the improvement of GERD is noticed after bariatric surgery because of the accelerated emptying of the antrum and decreasing of the acid producing gastric mucosa. Also,the intraabdominal pressure is decreased, the angle of His is

modified immediately post SG and the pressure of the lower sphincter is decreased. However, when the pouch is too large or a concurrently hiatal hernia is not treated, the GERD symptoms can be worsened. The pre-operative GERD could change the plan of surgery in favor of Roux-en-Y gastric bypass although a similar consensus does not exist in favor of SG if a gastric/duodenal pathology is detected pre-operatively in a planned RYGB. When GERD may occur for the first time after RYGB, this means the presence of a gastro-gastric fistula.

A large study including 12,000 SG and 8,000 RYGB showed that SG is associated with a 1.87 risk of GERD compared to RYGB [22], meanwhile, 9-27% experienced de novo GERD symptoms [23, 24]. However, esophagitis is present in 6-63% of patients, especially in the male, with previous hiatal hernia or GERD symptoms [25]. Revisions with minimal by-pass is the solution, especially in early interventions, with GERD improvement in 75-96% [26].

Nissen Sleeve gastrectomy, a new procedure in the bariatric armamentarium, was proposed to minimize the rate of postoperative gastro esophageal reflux disease. This operation is designed to protect the staple line of the angle of His, with an acceptable early postoperative complication rate (10%) [27]. A comparison between SADI-S and OAGB-MGB (one anastomosis bypass or mini gastric bypass) favored SADI-S in terms of GERD alleviation, although the difference was not significant [28].

Bowel Transit Dysfunction

Diarrhea may occur postoperatively in the case of RYGB or biliopancreatic diversion with duodenal switch [29]. The mechanism is related to bacterial overgrowth or impaired fat absorption, in considerably shortened small bowel. Colonoscopy for ruling out an inflammatory bowel disease is helpful. Symptoms related to irritable bowel disease doubled 2 years after RYGB and strong predictors were symptoms present before surgery and fibromyalgia [30].

Dumping syndrome results from patients inability to regulate gastric emptying of simple carbohydrates or other osmotic loads in RYGB. The patient presents sweating, dizziness, palpitations, abdominal pain, nausea, vomiting, and/or diarrhea. The diet tailored for avoiding intestinal hyper-osmolarity, leads to disappearing of symtoms within 1 year.

Small bowel obstruction is rare and can occur years after the operation. In the first weeks, this can be caused by a trapped loop of bowel at the laparoscopic port site, or within a non-repaired umbilical or incisional hernia. Later on, an internal hernia can occur with an incidence of 3% to 16% after RYGB and it is manifested with abdominal pain. There arethree possible locations. The commonest is the

opening of the transverse mesocolon, through which the Roux limb is brought to become anastomosed to the gastric pouch (67%). The second location is underneath the edge of the mesentery of the biliopancreatic limb at the jejunojejunostomy site (21%). The third location is the space between the transverse mesocolon and Roux limb mesentery (Petersen's space – 7.5%) [31, 32]. Even if these defects are closed at the time of surgery, when the weight is lost and the mesentery becomes thinner, it is possible for the defect to open up months or years later. Internal hernias are commoner after laparoscopic gastric bypass than in open operations, presumably because there are fewer adhesions after laparoscopic surgery [33]. The diagnosis is made on CT scan images and the re-intervention with the cutting of the adhesions and decompression of the dilated loop of the biliopancreatic limb (especially in case of the duodenal switch when is a closed-loop obstruction) should be done for avoiding intestinal ischemia.

Slippage or erosions of the adjustable gastric banding may be done in the proximal or distal direction. The clinical presentation might be rapid onset of regurgitations or dysphagia, but usually,theseare asymptomatic, with regain of their weight. The presence of an air-fluid level above the gastric band on X_Ray highly suggests band slippage. The diagnosis is established during endoscopy, with the band still held in place by a tissue bridge of variable size (Fig. **2**).

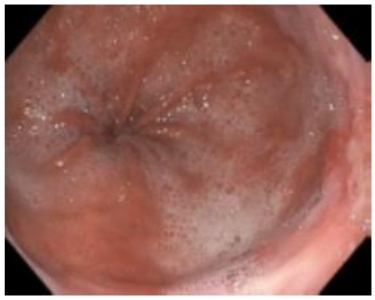

Fig. (2). The endoscopic aspect of a distal slippage of a adjustable gastric banding.

As there is a risk of gastric necrosis, the port system needs to be emptied for tissue release. The most severe problem in such cases is transmural migration, which can occur in 7% of patients [34, 35] (Fig. **3**).

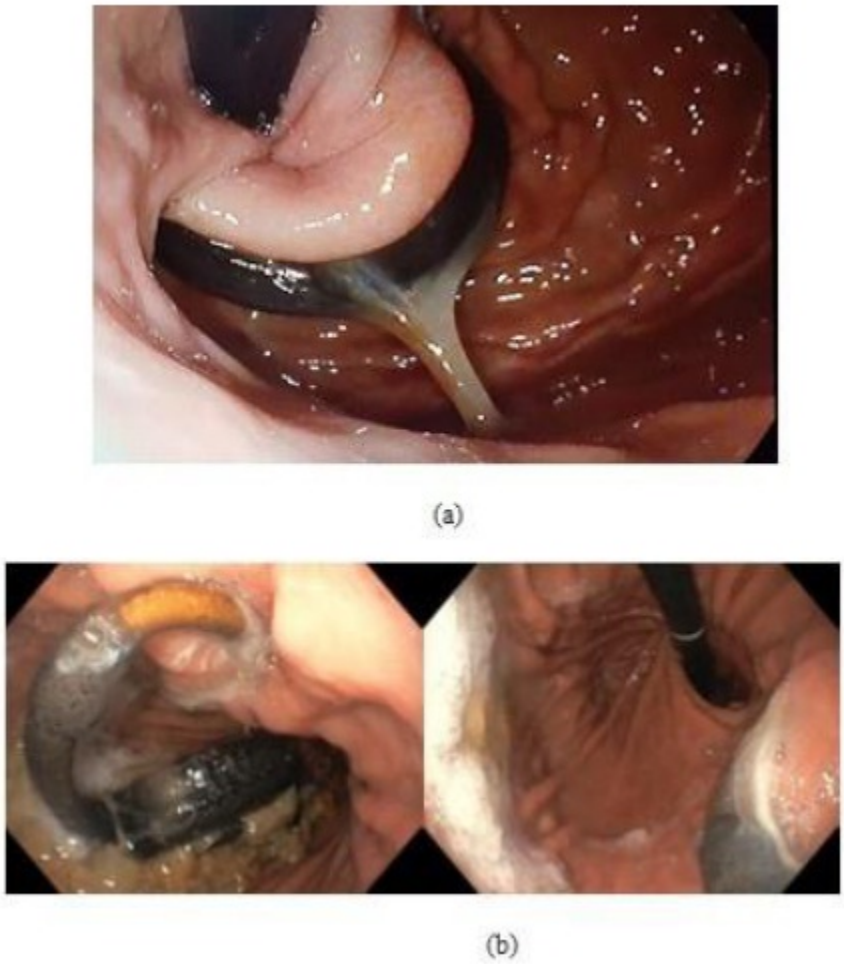

Fig. (3). The endoscopic view of a gastric banding eroded partially **(a)** and completely **(b)** the gastric wall.

Although the endoscopic attempt has been done, the patients has to be treated surgically, for a correct dissection of the dense adhesions and scar that have sealed off the band from the peritoneal cavity. The reconstructive options are to do a simple gastro-gastrostomy or to convert to a Roux-Y gastric bypass.

Gallstones are common in bariatric patients, due to obesity and rapid weight loss. Up to 30% of patients can develop gallstones, 12–18 months after a gastric bypass [36]. Also, there are reports on the high rate of emergency cholecystectomy in 50% of the patients, who developed gallstones after RYGB [37]. The incidence rate of biliary complications after bariatric surgery was 5.54 cases/1,000 patient year in a meta-analysis and concomitant cholecystectomy increases the risk for postoperative complications and operative time [38]. However, prophylactic

cholecystectomy is not indicated, patients should be carefully followed with attention for biliary complications, because cholecystectomy performed after bariatric surgery is associated with a higher risk for complications and reoperations [38]. The risk for biliary acute pancreatitis seems higher in vertical SG than in RYGB [39]. The access tothe common bile duct with sphincterotomy after RYGB poses a technical challenge. Laparoscopic assisted ERCP [40], deep enteroscopy-assisted ERCP [41] (60-70% success rate) or combined EUS access of the jejunal limb through a lumen apposing metal stent, followed by standard ERCP (over 90% success rate) [42] represent alternatives for accessing the common bile duct, depending on the work-flow and expertise of each center.

CONCLUSION

Digestive complications in the post-bariatric surgery population can be a challenging problem. Although rare, they can be severe and life threatening. The close follow-up of the patient postoperatively and regular consultations of these patients can recognize the complications and find the best option for solving them.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author confirms that this chapter contents have no conflict of interest.

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CHAPTER 5

What is New in Gastro-Entero-Pancreatic Neuroendocrine Tumors

Adrian Săftoiu[1,*] and **Codruța Constantinescu**[1]

[1] *Research Center in Gastroenterology and Hepatology, University of Medicine and Pharmacy Craiova, Romania*

Abstract: Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are a group of heterogeneous malignancies that can occur anywhere in the digestive system, with a growing incidence over the past decade. For proper diagnosis and management, the grading and histological diagnosis have been revised recently. Thus, the WHO grading criteria have been updated in 2017 as well as the TNM staging for pancreatic NETs in 2018. To establish a correct diagnosis, a multimodal approach is required, including various biomarkers, endoscopic tumor biopsy and tumor imaging. Over the past decades, improved diagnostic techniques including endoscopic ultrasound and somatostatin receptor fusion imaging have gained ground and have assisted treatment decision making. Regarding the treatment strategy, the management implies taking into account the tumor stage and degree of tumor differentiation, as well as tumour growth and spread. Novel therapies such as molecular-targeted agents, tryptophan hydroxylase inhibitor and peptide receptor radionuclide therapy were recently approved by FDA, improving the prognosis for advanced GEP-NETs.

Keywords: Carcinoids, Diagnosis, Follow-up, Gastroentero pancreatic neuroendocrine tumor, GEP-NETs, Update.

INTRODUCTION

Neuroendocrine tumors (NETs) are a group of tumors originating from neuroendocrine cells, with various anatomic locations, such as gastrointestinal (GI) tract, pancreas, lungs, thymus and endocrine glands [1, 2]. Gastro-ente--pancreatic (GEP) NETs can occur anywhere in the digestive system, the GI tract representing the most common site for this type of tumor. Over the last decade, the incidence of GEP NETs increased, due to improved diagnostic techniques, which resulted in higher detection rate of gastric and rectal NETs. However, they

[*] **Corresponding author Adrian Săftoiu:** Research Center of Gastroenterology and Hepatology Craiova, University of Medicine and Pharmacy Craiova, 2 Petru Rareș Str., 200349 Craiova, Romania; Tel: +40 744 823355; Fax: +40 251 310287; E-mail: adriansaftoiu@gmail.com

Ioan Sporea and Alina Popescu (Eds.)

are still considered rare tumors, accounting for 2% of all GI tumors. In general, the tumors are sporadic, but a variable number of NETs can also be encountered in some genetic syndromes such as multiple endocrine neoplasia (MEN) type 1, von Hippel-Lindau disease, von Recklinghausen disease (neurofibromatosis type 1) and tuberous sclerosis [1].

NETs have a wide variety of clinical presentations, depending on the type of hormone hypersecretion (see below). Furthermore, a constellation of symptoms, which are classically known as carcinoid syndrome (CS) has been described. Most often, CS occurs in primary tumors in the distal small intestine or proximal colon and is usually due to metastatic disease, especially liver metastases. The symptoms may vary, depending on the release of vasoactive compounds, but the most common presenting features include flushing, diarrhea and intermittent abdominal pain [2]. Non-functioning tumors, accounting for about of 60-70% of GEP NETs, may be undetected for years, most of them being *incidentally* diagnosed.

Grading and Staging

The classification of neuroendocrine neoplasms (NENs) arising in the GEP system was firstly published by the World Health Organization (WHO) in 2000 and it was later updated in 2004, 2010 and 2017 [3]. The current classfication is based on a combination of mitotic count and Ki-67 proliferation index, categorizing NETs as grade 1 to grade 3 in the latest update. In addition, the nomenclature for MANEC was changed to mixed endocrine non-endocrine neoplasm (MiNEN) in order to adress the issue that not all MiNENs are high-grade malignant carcinomas [3] (Table **1**).

Table 1. The 2017 WHO classification for pancreatic neuroendocrine neoplasms.

2017 WHO classification	Mitoses/10 HPF	Ki-67 index %
Well-differentiated NENs		
NET grade 1	< 2	< 3
NET grade 2	2-20	3-20
NET grade 3	> 20	> 20
Poorly-differentiated NENs		
NEC grade 3 − Small cell type − Large cell type	> 20	> 20
MiNEN		

Regarding the pathological staging of NETs, TNM staging systems are currently developed for the following tumor sites: pancreas, gastric, duodenum/ampulla/ proximal jejunum, lower jejunum and ileum, appendix, colon and rectum. The 8th edition of the American Joint Committee on Cancer (AJCC) staging system has updated the T staging for pancreatic NETs (Pan-NETs), specifically T3, to be consistent with the European Neuroendocrine Tumor Society (ENETS) system for well-differentiated NETs, as the previous edition might have been a source of confusion for the clinicians (Table **2**) [4].

Table 2. The differences between the AJCC and ENETS staging systems for pancreatic NETs.

T Stage	7th Eition AJCC	8th Edition AJCC	ENETS
T1	Confined to pancreas, < 2 cm	Limited to pancreas, < 2 cm	Confined to pancreas, < 2 cm
T2	Confined to pancreas, > 2 cm	Limited to pancreas, 2–4 cm	Confined to pancreas, 2–4 cm
T3	Peripancreatic spread, without major vascular invasion	Limited to pancreas, > 4 cm, or tumor invading the duodenum or common bile duct	Confined to pancreas, > 4 cm, or invades duodenum or bile duct
T4	Tumor involves coeliac axis or superior mesenteric artery	Invading adjacent organs or the wall of large vessels	Invading adjacent organs or major vessels

Diagnosis

In general, the diagnosis of NETs is often incidental and usually delayed for several years, but patients suspected of GEP NETs should undergo initially a clinical evaluation (medical and family history, physical examination). The clinical assessment should exclude any cancer syndromes and also guide the appropriate diagnostic and therapeutic procedures [5, 6].

The diagnostic algorithm comprises a combination of blood and urine markers and various imaging modalities, such as conventional imaging (ultrasound, CT, MRI) and endoscopy (gastroscopy and/or colonoscopy), including endoscopic ultrasound (EUS), as well as functional imaging, with a combination of somatostatin receptor scintigraphy (SRS) and cross-sectional imaging using single photon emission CT (SPECT) in addition to CT (SPECT-CT). A minimal initial workup consists of a multimodality approach: a site-specific endoscopic assessment with tumor biopsy and computer tomography (CT) or magnetic resonance imaging (MRI). Over the last decade, functional imaging techniques, especially ^{68}Ga-DOTA-Phe^{1}-Tyr^{3}-Octreotide (^{68}Ga-DOTATOC) PET-CT or ^{99m}Tc-EDDA/HYNIC-Tyr^{3}-Octreotide (Tektrotyd) SPECT-CT, have been found

to be highly effective for staging, treatment decision making and for assessing secondary tumors [7, 8].

Regarding other diagnostic tools, authors debate if baseline tests should mandatorily include biochemical measurements such as chromogranin A (CgA) and neuron-specific enolase (NSE). As a general rule for NETs, CgA is most often negative in localized tumors, it can occur in other conditions as well and can give false positive results in patients with proton-pump inhibitor (PPI) therapy [4].

Workup

Gastric NETs

For the initial assessment of this tumor site, measurement of gastric pH and serum gastrin level are recommended in order to differentiate the subtypes of gastric NETs. Furthermore, measurement of anti-parietal cell antibodies or anti-intrinsic factor antibodies should be considered for type I and plasma or urinary 5-hydroxyindoleacetic acid (5-HIAA) and CgA for type III gastric carcinoids. Another reliable biochemical indicator of gastric NETs is pancreastatin, but its utility in a clinical setting still needs to be validated in future trials. For the final diagnosis, esophagogastroduodenoscopy (EGD) with multiple biopsies is essential (Fig. **1a-d**), whilst EUS, CT or MRI are effective for preoperative evaluation [1-5].

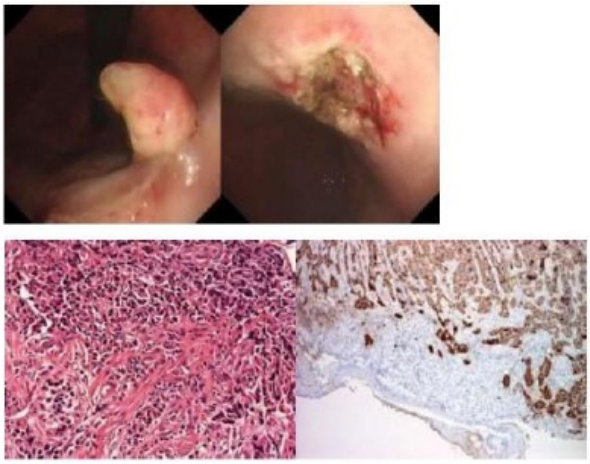

Fig. (1). Polypoid gastric carcinoid **(a)** resected after saline plus adrenaline 1: 10,000 injection, with the placement of a ligation band at the base **(b)** Argon plasma coagulation was used to coagulate the margins of the resection defect. Pathology showing a well-differentiated NET grade 1 (col. HE, 50x) **(c)** with positive chromogranin depicted in tumor cells, with clear resection margin (50x) **(d)** (courtesy of Dr. Claudia Georgescu).

NETs of the Duodenum, Jejunum, Ileum, Appendix

For the clinical staging, the workup consists of biochemical markers (CgA, plasma or urinary 5-HIAA, pancreastatin and neurokinin A). For duodenal NETs, endoscopy with biopsy is needed for diagnosis confirmation, whilst EUS can be used for staging the tumor in terms of tumor depth (T stage) and regional lymphadenopathy (N stage). Moreover, for terminal ileal NETs, colonoscopy with ileocecal valve intubation can be used to identify and biopsy for the tumor and chest CT scans, triple-phase contrast-enhanced CT of the abdomen and pelvis, as well as abdominal MRI for the localization of NET metastases. ^{68}Ga-labeled octreopeptide PET-CT or ^{99m}Tc-Tektrotyd SPECT-CT can also be used for detecting primary and/or metastatic tumors [1 - 5].

NETs of Colon and Rectum

For the initial assessment of colonic and rectal NETs, the biochemical evaluation should include CgA, plasma or urinary 5-HIAA and any other biochemical markers clinically indicated by symptoms of hormone secretion. For this site, pancreastatin and NKA utility has not been demonstrated. For establishing a diagnosis, patients should undergo colonoscopy with biopsy of polyps or submucosal nodules (Fig. **2a-c**), as well as tattoo for localization during subsequent colonoscopy/ laparoscopy. For staging, transrectal ultrasound (TRUS) should be considered that can discriminate between T1/T2 tumors and T3/T4 tumors. Also, both, pelvic MRI and triple-phase CT of the abdomen should be performed. Nuclear imaging can be an alternative to detect any metastases [1 - 5].

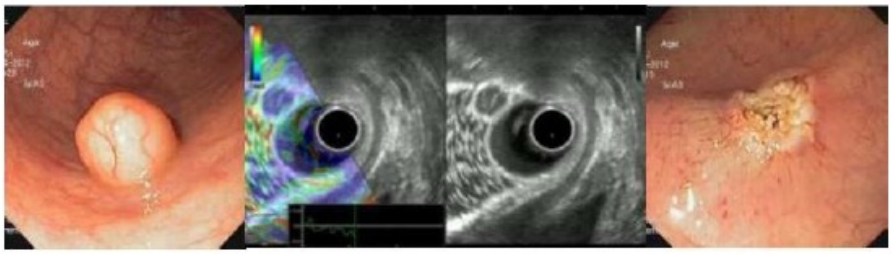

Fig. (2). Small rectal submucosal NET **(a)**, considered limited to the submucosa based on EUS imaging and depicted as hard during EUS elastography **(b)**. The small tumor was resected after submucosal injection of saline plus adrenaline 1:10 000 **(c)**.

Pancreatic NETs

Unless it is an incidental finding (Fig. **3a and b**), the initial workup for pancreatic NETs (pNETs) should consider the functional status of the tumor. Functional pNETs can exhibit characteristic syndromes depending on the type of hormone secreted and thus directing the workup to specific biochemical markers:

plasma insulin, proinsulin and C-peptide for insulinomas, fasting serum gastrin for Zollinger-Elison (gastrinomas) (Fig. **4a and b**), plasma vasoactive intestinal peptide levels for VIPomas, plasma glucagon levels for glucagonoma and other series hormones as clinically indicated. Non-functional pNETs are usually asymptomatic, but can determine weight loss, abdominal pain and jaundice and in this clinical setting it is recommended to determine serum levels of CgA, NSE, pancreatic polypeptide and/or pancreastatin. If the clinical or family history is positive for genetic syndromes, genetic testing should be strongly considered.

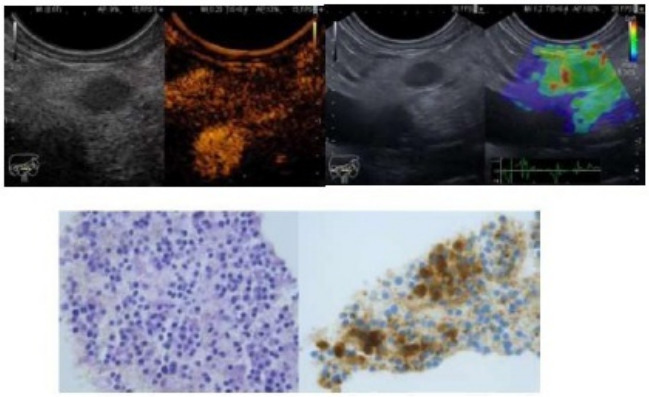

Fig. (3). Incidental EUS finding of a small pNET (7 mm), visualized as an enhanced mass during contrast-enhanced EUS **(a)**, soft during EUS elastography **(b)**. Pathology exam depicted a well-differentiated neuroendocrine tumor (col. HE, 200x) **(c)**, G1 (Ki-67<2%), with positive chromogranin (400x) **(d)** (courtesy of Dr. Nona Bejinariu).

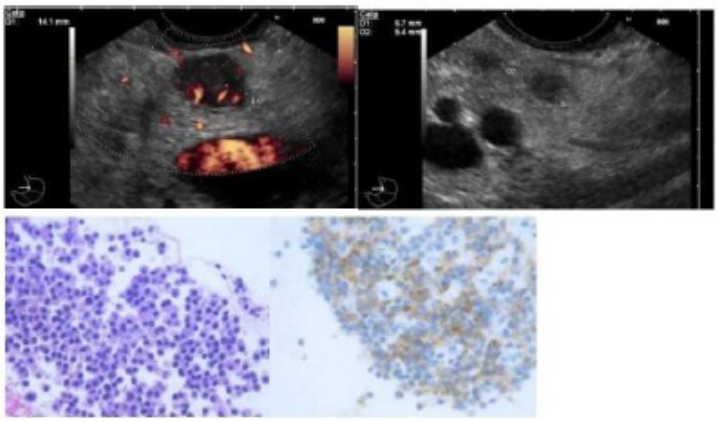

Fig. (4). Small pNET (gastrinoma) in a MEN-1 patient, visualized as a 20 mm mass at the level of the pancreatic body, with intense power Doppler signals inside **(a)**, with multiple other diminutive lesions of ~5mm disseminated through the pancreatic parenchyma **(b)**. Pathology showing a well differentiated neuroendocrine tumor (col. HE, 400x) **(c)**, G1 (Ki67<2%), with positive chromogranin (400x) **(d)**, both in the primary lesion but also secondary in the satellite lesions (courtesy of Dr. Nona Bejinariu).

To confirm the diagnosis, a EUS-guided fine needle aspiration biopsy should be performed, preferably with "histological" needles, followed by microhistological and immunohistochemical analysis of the obtained tissue cores. To evaluate the primary tumor expansion and for TNM staging, workup should include contrast-enhanced ultrasound (CEUS) (Fig. **5a and b**), multiphasic CT or MRI and additionally, SRS and EUS (Fig. **6a and b**). Promising imaging techniques with great potential for correct evaluation (including initial staging and follow- up), especially for rare functional pNETs, are either PET-CT with ^{68}Ga-labeled somatostatin analogs or ^{99m}Tc-Tekrotyd SPECT-CT [1 - 6] (Fig. **7**).

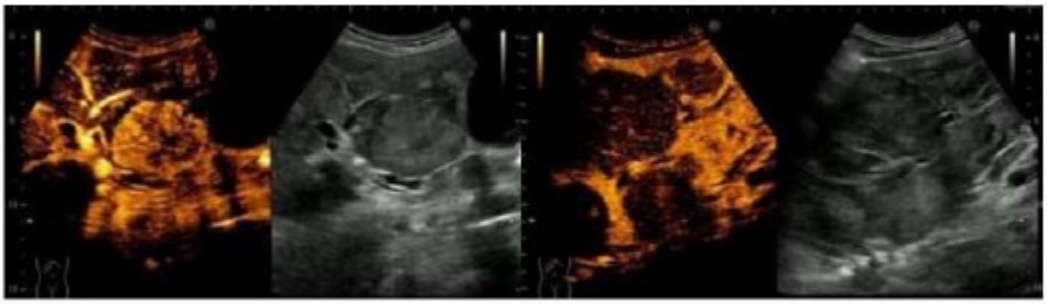

Fig. (5). Pancreatic head NET with multiple liver mets depicted as hyper-enhanced in the arterial phase **(a)**, with washout in the venous phase **(b)**.

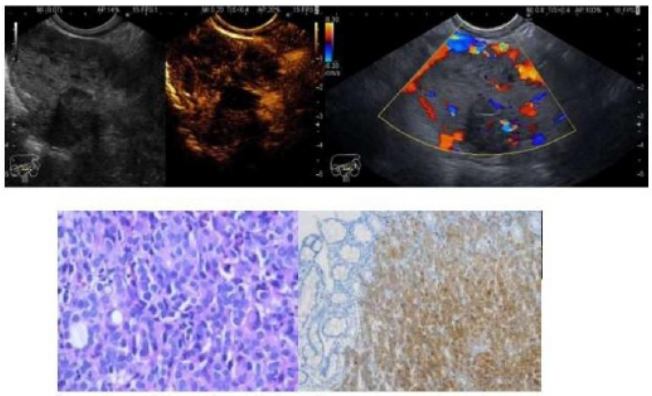

Fig. (6). Advanced pancreatic neuroendocrine tumor (pNET) with multiple livers and lymph node mets, one of the lymph nodes being depicted as mixt hyper- and hypo-enhanced in the arterial phase **(a)**, with intense color Doppler signals **(b)**. Pathology showing a typical appearance of neuroendocrine tumor (col. HE, 400x) **(c)**, G3 (Ki67>80%), with positive chromogranin and synaptophysin (200x) **(d)**, both in the primary lesion but also in the secondary lesions (courtesy of Dr. Nona Bejinariu).

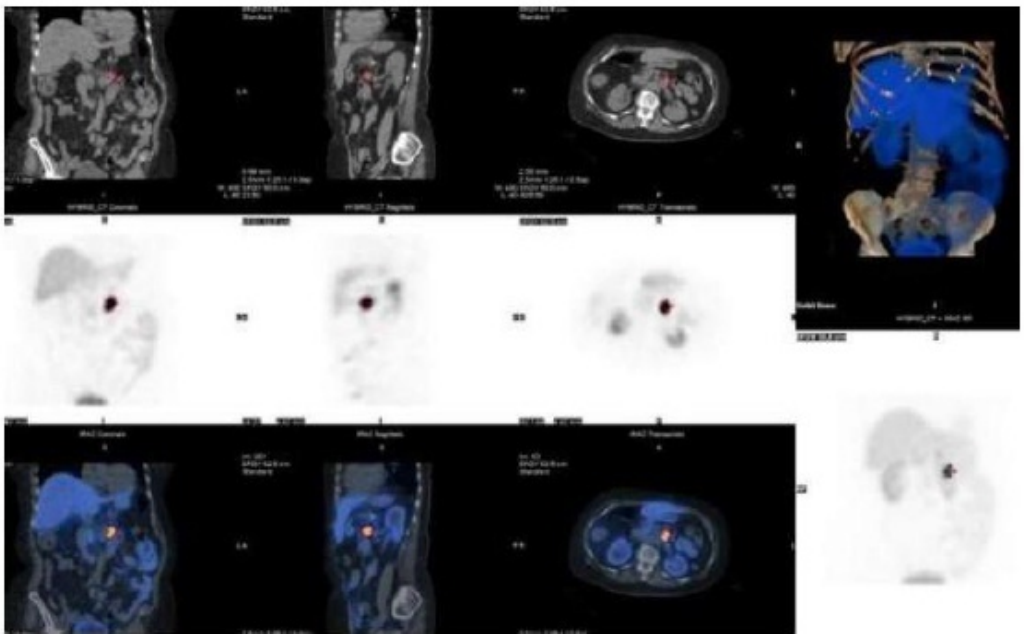

Fig. (7). Local recurrence after caudal spleno-pancreatectomy of a G1 pNET for a patient with high serotonin levels and negative (inconclusive) CT exam. Somatostatin receptor scintigraphy (SRS): SPECT-CT acquisition at 2 hours after injection of radiolabeled somatostatin analogue (^{99m}Tc-Tekrotyd) (courtesy of Dr. Mirela Gherghe).

Therapy

The treatment strategy should be based on the patient's characteristics and the evaluation of tumor features. The options can vary from conservative management for small, non-functional and non-aggressive tumors to a more complex approach including surgical resection in a multimodal strategy for metastatic disease. The conservative approach implies active surveillance and endoscopic resection for lesions like gastric NETs type 1 over 10 mm. Surgical resection with lymphadenectomy can be the first option in localized disease for curative care, to relieve the symptoms in functional tumors and for any impending obstruction. But surgery should be considered for advanced disease as well.Several studies indicating an overall survival of 8-10 years, if the primary tumor and regional metastases are resected [7].

Other techniques that can be used supplementary to surgery include percutaneous or intraoperative radiofrequency ablation, bland, chemo- or radioembolization and selective internal radiation therapy (SIRT) with 90Y-microspheres as newer treatment options for liver metastases. Moreover, for advanced GEP NETs, several chemotherapy regimens and a broad spectrum of medical therapies can represent an option to prevent progression and increase survival.

For long-term systemic treatment, somatostatin analogs have been used for symptom control for decades but recent studies show that octreotide and lanreotide can also control neuroendocrine tumor growth [1 - 5]. Currently, combinations of somatostatin analogs and other agents such as α-interferon, bevacizumab, *etc*. are under clinical investigation. Novel therapies such as tryptophan hydroxylase inhibitor (telotristat ethyl) and peptide receptor radionuclide therapy (PRRT), were recently approved by FDA in 2017 and 2018 respectively. Telotristat ethyl can be used for symptom relief in patients with carcinoid syndrome, significantly reducing daily bowel movement frequency [8]. ^{177}Lutetium-DOTA-TATE (Lutathera), the first radio-labeled drug for GEP NETs, is an option for patients left with no other options and can improve patients' quality of life as well as slowing the disease progression. Moreover, other molecular targeted therapies have been proposed as a second-line alternative after first-line treatment with a somatostatin analog [9]. Thus, sunitinib, a tyrosine kinase inhibitor and everolimus, an mTOR (mammalian target of rapamycin) inhibitor have been proposed and approved for well-differentiated advanced/metastatic pNETs, with similar clinical benefits (significant improvement in progression-free survival) [10, 11].

Follow-up

Follow-up of GEP NETs is often shared among a healthcare team such as a family doctor, oncologist, gastroenterologist, endocrinologist and surgeon. The long-term care includes clinical symptom monitoring assessing carcinoid syndrome, tumor markers and imaging procedures such as abdominal ultrasound, endoscopy, EUS, CT, MRI, PET-CT with ^{68}Ga-DOTATOC or SPECT-CT with ^{99m}Tc-Tekrotyd (Fig. **7**). Follow-up should be lifelong, as maximum duration is not defined, with staging intervals at 3-6 months after curative treatment and then every 6-12 months for at least 5-7 years [5].

Perspectives

EUS imaging is useful not only for detection and characterization of small pNETs, but also for precise localization during laparoscopic/robotic surgery by performing EUS-guided tattooing preoperatively, using either sterile India ink or indocyanine green [12]. Although surgery is the mainstay for pNETs, other locally ablative therapies like EUS-guided radiofrequency ablation (EUS-RFA) have been described in a limited number of cases (N=61 patients with 73 tumors with the mean size of 16 mm, one third insulinomas) [13]. The overall effectiveness of 96%, for functional and non-functional pNETs, as well as the mild adverse events (13.7%) indicates the method might be reasonable for small pNETs, especially for patients unfit for surgery.

Targeting somatostatin receptors with similar agents for both imaging and therapy paved the way to theranostic applications and molecular targeting of these exotic tumors. Other studies targeted the C-X-C motif chemokine receptor 4 (CXCR4), because it is overexpressed in high-grade GEP-NETs and can be used as a therapeutic target, even in somatostatin receptor negative NETs. Other receptors are also targeted: glucagon-like peptide-1 (GLP-1) receptor for insulinoma, cholecystokinin 2 receptor (CCK2R) for gastric and pancreatic NETs, *etc*. [14].

Various other targeted radionuclides are currently assessed for both diagnosis and therapy (^{11}Carbon, ^{55}Cobalt, ^{64}Copper, ^{212}Bismuth, ^{166}Holmium, ^{212}Lead, ^{177}Lutetium, ^{90}Ytrium, *etc*.), using innovative approaches: targeting with antagonists instead of agonists, alpha instead of beta particles, intra-arterial *versus* intravenous administration, combination therapy, *etc*. [14].

gastro-entero-pancreatic neuroendocrine tumors, not being very frequent, are quite difficult lesions, but new developments in the diagnosis and management of these diseases give hope for a better therapeutic approach.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author confirms that this chapter contents have no conflict of interest.

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CHAPTER 6

Intestinal Microbiota and its Implications in Pathology

Paul J. Porr[1,*]

[1] *Polisano Med Life Clinics Sibiu, Romania*

Abstract: The intestinal microbiota develops as a results of various genetic, nutritional and environmental factors, becoming very specific for each individual. It totalizes more than 100 trillions of bacteria with a piece of genetic information more than 100x greater than the human genome. The functions of the microbiota can be grouped into metabolic, protective and structural. The microbiota-derived metabolites signal to distant organs of the host, which enable the microbiota to connect to the brain, the immune and endocrine system, metabolism and other functions of the host. These microbiota-host communications are essential to maintain the vital functions and health of our organism. So, microbiota, in eubiosis and especially in dysbiosis, has multiple effects on the human organism. The therapeutic possibilities for this are the administration of nonabsorbable antibiotics, pre-, pro, syn- or symbiotics, as well as FMT, which is in principle a complex human probiotic.

The most important digestive effects of microbiota are in Clostridium difficile-determined pseudomembranous colitis, in IBS, IBD, diverticulitis, functional dyspepsia, and in different digestive cancers: gastric, colorectal, liver and pancreatic cancer. Alcoholic liver disease is also influenced by microbiota.

The extra-digestive effects of microbiota are very complex. In some metabolic diseases, like obesity, NAFLD, atherosclerosis, dyslipidemias and T2D, special types of dysbiosis have important pathophysiologic implications. Microbiota has also implications in Alzheimer's disease, osteoporosis, CKD, different psychiatric disorders and some extra-digestive cancers.

In conclusion, it may be stated that the intestinal microbiota has multiple effects, even in diseases that apparently have no relation with the intestinal flora.

Keywords: Autoimmune diseases, Cancer, Digestive diseases, Intestinal microbiota, Metabolic diseases, Microbiome.

[*] **Corresponding author Paul J. Porr:** Polisano Med Life Clinics Sibiu, 1A Izvorului Str., Sibiu, Romania; Tel: 0040722689333; E-mails: porrpj@gmail.com or paul.porr@polisano.ro

Ioan Sporea and Alina Popescu (Eds.)

INTRODUCTION

The intestinal microbial flora, called ***microbiota***, is formed beginning with the birth, the fetal intestine being sterile [1]. Depending on the natural or caesarian birth, namely the first contact with the vaginal flora or with the flora of the mother's tegument, the microbiota will develop differently [2]. The further development of the microbiota is related to genetic factors, nutrition (an important moment will be the diversification), as well as other environmental factors. Thus, the microbiota becomes very specific for each individual, called even "the second finger-print". But, the microbiota undergoes extensive changes across the lifespan, and age-related processes may influence the microbiota and its related metabolic alterations [3].

The microbial density rises from jejunum to colon, along with the microbial diversity (approximate 5000 species), totalizing approximately 100 trillion bacteria, which means 10 x more than the total number of cells in the human organism. The genetic information of the microbiota, called the microbiome, is 100 x greater than the human genome. It is necessary to clarify these two notions: the ***microbiota*** is the total of intestinal microorganisms (bacteria, viruses, protozoa *etc*.), the ***microbiome*** includes, without the microbiota, the totality of the microbial genes, as well as the totality of the microbial ecosystems [1]. Despite these differences, even gastroenterologists used the two notions as synonyms. In normal conditions, the microbiota is in perfect symbiosis with the human organism, being even a vital partnership. The microbiota was even called "the last discovered organ of the human body". The microbiota was studied very intensively in the last years. In 2007 PubMed included approx. 500 citations about the microbiome, ten years after, there were more than 8,000 [4].

The functions of the microbiota can be grouped into metabolic, protective and structural. The metabolic functions consist of the fermentation of indigestible glucides with the production of energy, synthesis of amino acids, short chain fatty acids (SCFA) and vitamin B & K, interaction with bile acids metabolism and absorption of water and salts. These microbiota-derived metabolites signal to distant organs of the host, which enables the microbiota to connect to the brain, the immune and endocrine system, as well as to the metabolism and other functions of the host. These microbiota-host communications are essential to maintain the vital functions and health of our organism [5]. The protective functions consist of the prevention of pathological colonization (existing a direct competition between microorganisms, as well as a synthesis of antimicrobial peptides), regulation of inflammatory cytokines and development and activation of the immune system (B cells, regulator and helper T cells). The structural function consists of the modulation of the mucus layer [6].

Normally an equilibrium exists between the different components of microbiota, called eubiosis. This equilibrium could be disturbed frequently, by the appearance of different kinds of dysbiosis. The causes of dysbioses could be viral, bacterial or fungal infections of the intestine, sudden environmental or dietetic modifications, immunodeficiency, drugs, especially antibiotics, or different diseases. Microbiota, in eubiosis and especially in dysbiosis, has multiple effects on the human organism. These effects could be digestive (acute and chronic intestinal infections, inflammatory bowel disease, irritable bowel syndrome, digestive cancers) or extra-digestive (metabolic diseases, allergies, autoimmune, neurological and psychiatric diseases *et al*.). In all these cases it is important to reestablish the eubiosis. There are a number of therapeutic possibilities such as: nonabsorbable antibiotics (Rifaximin, Neomycin), probiotics (Bifidobacterium, Lactobacillus, Lactococcus, Bacillus, Bacteroides, Enterococcus, Escherichia, Faecalibacterium, Propionibacterium, Saccharomyces), prebiotics (nondigerable glucides like inulin, lactulose, fructo- and oligosaccharides), synbiotics (combinations of pro- and prebiotics), symbiotics (combination of probiotics) and fecal microbiota transplantation (FMT), consisting in general of a colonoscopic infusion of a fecal suspension from a healthy donator, which is in principle a complex human probiotic [7, 8]. Maternal FMT in caesarian-born infants after birth has also been proved [9]. A very important role in reestablishing eubiosis has also been the diet [10, 11].

Digestive Effects of Microbiota

One of the most important acute intestinal infections in the last years all over the world is **pseudomembranous colitis**, caused by Clostridium difficile, mostly of an iatrogenic cause, unfortunately. This disease becomes a real public health problem. For instance in the EU in only one year, the costs are over 3 billion of euros and, unfortunately, the frequency is rising all over the world [12].

Through the microbiome - bowel - brain axis, called before bowel – brain axis, complex interactions take place between microbiome, central nervous system, neuro-endocrine system, neuro-immune system, autonomous nervous system and enteric nervous system. Thus are possible interventions of the microbiome in the reactions to stress, anxiety, memory, behavior or intestinal function [13]. Also the **irritable bowel syndrome** (IBS) could be influenced by the microbiota even through this axis [14]. IBS is a functional disorder, influenced by genetic predisposition, psycho-social factors and is characterized by motor disturbances and hypersensitivity. Studies on feces samples show that the microbiome is different in healthy and IBS patients (Lactobacillus, Veillonella, Clostridia, Ruminococcus, Proteobacteria, Firmicutes and others).

The characteristic dysbiosis determines a hyperproduction of SCFA with production of gas, which determines meteorism, consecutive pain with anxiety, negative emotions and diminished quality of life. Studies on intestinal mucosa show that the microbiome forms a biofilm on the mucosa, which is different in healthy individuals, in IBS, and IBD. Both categories of studies demonstrated the significant differences between microbiota of IBS and normal individuals [15].

Modifications of microbiota appear also in other functional gastrointestinal disorders, like **functional dyspepsia** [16].

Inflammatory bowel disease (IBD) is also characterized by a specific kind of dysbiosis, with altered composition as high quantities of Enterobacteriaceae and low quantities of Firmicutes, as well as decreased diversity and stability, with more proinflammatory than immunoregulating properties. Crohn's disease is also characterized by diminished Faecalibacterium prausnitzii, existing an inverse correlation with disease activity. For ulcerative colitis diminished Clostridium coccoides and Clostridium leptum is characteristic [17]. Also a loss of immunological tolerance of the microbiota is a crucial component in the pathophysiology of IBD. Normalizing and maintaining regulatory immune cell function by correcting dysbiosis provides a promising approach to treat IBD [18].

It is suggested that the microbiota may be implied in several aspects of **diverticulosis**, ranging from risk factors, to progression into symptomatic disease, and also to treatment [19].

The microbiome can contribute to digestive carcinogenesis. It can generate nitrosamines, especially in the hypo - or anacid stomach, and has also indirect carcinogenic effect, as a consequence of nutritional excesses or deficits, favoring the appearance of **gastric cancer.** An uncontested role in the pathogenesis of gastric cancer has Helicobacter pylori [20]. By a decrease of SCFA secondary bile acids with 7-α-dehydroxylation activity, microbiota is involved in the pathogenesis of **colorectal carcinoma** [21]. There is also evidence for an implication in the pathogenesis of **liver cancer**, *via* enterohepatic circulation, and in **pancreatic cancer** [22, 23].

Alcoholic liver disease is also influenced by microbiota. Alcohol causes bacterial overgrowth and gut bacterial products like endotoxin may mediate inflammation and increased intestinal permeability, allowing translocation of bacteria and bacterial products to the liver. The degree of bacterial overgrowth correlates with the severity of cirrhosis [24]. A promising therapeutic possibility is FMT [25].

Extra-digestive Effects of Microbiota

Obesity is one of the metabolic diseases correlated with alterations of the microbiota. As a consequence of the western diet with predominant fast food, bacterial population ("western style-microbiota") diminish, with high quantities of Firmicutes and low quantities of Bacteroides [26]. Interesting is the fact, that after weight loss, the ratio between them normalizes. The microbiome inhibits the activation of proteinkinase, activated by AMP, and the expression of protein 4 angiopoietin-like – both being factors of adiposities induced by hunger. But also inversely, obesity can influence the microbiome. Obesity is characterized also by the little degree of inflammation, with rising levels of TNFα, IL-18, IL-1β, to which contributes also to the microbiome, maybe even as an initiator [27, 28]. Also, malnutrition is associated with changes in the microbiome (immaturity) [29]. Very interesting results were obtained in animal experiments: fecal transplantation from mice with obesity to normal mice determined the onset of obesity in the latter. Inversely, fecal transplantation from normal mice to mice with obesity determined the weight loss [30]. A beneficial effect of bariatric surgery was also described, namely an increased richness of microbiota and Bacteroidetes/Firmicutes ratio six months after intervention [31].

Nonalcoholic fatty liver disease, the hepatic manifestation of the metabolic syndrome, is characterized by an intestinal bacterial overgrowth, especially of Enterobacteriaceae, with the production of endotoxins and alcohol, as well as an increased intestinal permeability [26, 32]. Important are also volatile organic compounds as by-products of the microbiotic metabolism with hepatotoxic effects [33].

Atherosclerosis is another metabolic disease that correlates with high levels of Collinsella and low levels of Eubacterium and Roseburia in the microbiota. It is proven that the metabolic transformation of choline from the diet into betaine and trimethylamine-N-oxide by the microbiome, correlates directly with cardio-vascular events [26, 34]. Some studies show the implication of microbiota also in the pathogenesis of **dyslipidemias** [35].

Recent evidence shows that **hypertension** is associated with altered gut function, changes in microbiota and altered gut-nervous system connectivity [36].

Type 2 diabetes (T2D) is also characterized by a modified microbiome with low Faecalobacterium prausnitzii and Firmicutes after a high-fat and high-gluten diet. An indirect correlation between insulin resistance and butyrate producing microbiome has also been observed, especially in pregnancy diabetes. Pre- and

probiotics can be considered as potential therapeutic tools to improve gut integrity in T2D, and probiotics as Lactobacillus acidophilus could even have a prophylactic effect on T2D [26, 37].

Allergies are also correlated with the microbiome. Nowadays the hygiene theory was dislocated by the microbiota theory. It has also been observed that there is an epidemiologic correlation between high risk of allergy and antibiotic use, as well as the presence of dysbiosis in allergic babies. The explanation is high levels of proinflammatory cytokines. A very convincing study that demonstrate the microbiome involvement in allergies is the so-called MIPS study: prebiotics administrated to newborns reduced atopic dermatitis by 50% [38].

It is known that gut dysbiosis can determine gut inflammation with increased intestinal permeability, also, for different antigens and breakdown for immune homeostasis. A systemic immune activation and an imbalance between T helper and T regulatory cells occur, as well as different kinds of autoantibodies [39]. One of these autoimmune diseases is **rheumatoid arthritis** (RA), in whose pathogenesis the bacteria Porphyromonas gingivalis has an important role. Also characteristic are an increase of Prevotella and a decrease of Bacteroides. Administration of a probiotic (Lactobacillus rhamnosus GG) in moderate clinical form of RA reduced RA activity in 70%, compared to 30% of patients without associated probiotic therapy [39].

Intestinal microbiota may also be involved in the pathogenesis of **ankylosing spondylitis**, these patients are characterized by a distinct fecal microbiota [40].

Autoimmune liver diseases (primary sclerosing cholangitis, primary biliary cirrhosis, autoimmune hepatitis) are characterized also by distinct microbiota, which is the trigger for an abnormal or inadequate immune response [24, 41].

Also in the pathogenesis of **type 1 diabetes,** the intestinal microbiota has an important role, especially a low diversity and an increased Bacteroidetes/ Firmicutes ratio [39, 42]. Even therapeutic possibilities exist by FMT [43].

Moreover, in **autoimmune neurological diseases** such as multiple sclerosis (MS), amyotrophic lateral sclerosis or optical neuromyelitis the microbiota is involved. Characteristic for these diseases are inflammatory lesions of the central nervous system by Th17 cells, with consecutive severe disabilities. These Th17 cells are inhibited by regulator T cells from intestinal lymphatic tissue and these regulator T cells decrease in some types of dysbiosis. In active MS a much less diversified microbiota was described than the MS in remission or even in healthy subjects. There exist **e**ven some therapeutic trials with PO. administration of antibiotics or probiotics with a fiber-enriched diet [39, 44].

In **Alzheimer's disease** dysbiosis is certainly present, which determines a release of great quantities of amyloid and lipopolysaccharides with a role in the synthesis of some proinflammatory cytokines, with a pathogenetic role in Alzheimer's disease, especially in the deposition of β-amyloid fibrils. There were also associations with insulin resistance described, with all its consequences like T2D or metabolic syndrome. By special diet and administration of pre - and probiotics an amelioration of dysbiosis with decreased production of amyloid was obtained [45].

In various **psychiatric disorders** such as anxiety, depression or eating disorders (anorexia nervosa), a dysbiosis was also evidenced, which acts by the microbiome- gut - brain axis. Lower bacterial diversity was observed in greater depression and anxiety. Even schizophrenia or autism are associated with some special dysbiosis [46 - 49].

It has been shown that the intestinal bacterial overgrowth is correlated with a significant lower bone mineral density - an important risk factor of **osteoporosis.** The beneficial effect of probiotics in this disease has also been described [50].

Not only the bone mass is influenced by microbiota, but also the muscle mass, with the onset of **sarcopenia**. Thus a gut-muscle axis was proposed [51].

Also, **chronic kidney disease** (CKD) seems to be associated with dysbiosis [52]. Probiotics administration reduces uremic retention solutes and also the progression of CKD. Dysbiosis is also implicated in idiopathic nephrotic syndrome in children [53]. Lactobacilliales have been linked with less severe graft-versus-host disease and better transplant survival [54].

The implication of microbiota in **extradigestive cancers** is proved, also its implication in chemotherapy and immunotherapy. Of specific interest is the capacity of some commensal bacteria to modulate the tumor microenvironment and anticancer therapy. There are discussed strategies to manipulate the microbiome to enhance immunotherapeutic responses. Inversely, the toxic effects of chemotherapy on the gastrointestinal tract can be diminished by the administration of probiotics [55].

The microbiota is involved through its metabolites in hematopoiesis and also in some hematologic disorders: even in some **anemias**, like aplastic anemia or anemia in chronic inflammation, in some **lymphomas,** as well as in **some platelet disorders,** like thrombocytopenia or reactive thrombocytosis [56].

The gut microbiota undergoes extensive changes across the lifespan, and age-related processes may influence it, as well as its related metabolic alterations. But

also **aging** and longevity may be characterized by increased flexibility and stability of the intestinal microbiota. Moreover, a particular hallmark of successful aging may be a balance amongst core microbiota as well as a balance between pro- and anti-inflammatory activities [3].

Final considerations: it may be stated that the microbiome has multiple effects, even in diseases that apparently have no relation to the intestinal flora. Hippocrates may have been right, by saying more than 2000 years ago: "all diseases have beginning in the intestine". These pathological effects appear because of dysbiosis, often of iatrogenic origin. Restoration of eubiosis in these cases is essential. This can be obtained by at least adjuvant pre-, pro- or synbiotic therapy. A newer therapeutic chance is FMT. Today it seems to be science-fiction, but maybe some years later, there will be a possibility to bank frozen processed fecal material, specific for different diseases, *e.g*. T2D, obesity *etc*. There is also a question about the efficacy of using alternatives to donor stools, such as synthetic stool formulations or live bacterial products generated by *in vitro* fermentation, for administration to patients as treatment or prevention of specific diseases. It is obvious that the implications of microbiota in human pathology represent a new frontier of medicine [24, 57, 58]. We can expect that microbiome research will continue to grow at a rapid pace as methods become easier to apply and less expensive. Because we still do not know, what the majority of the microbiome genes are capable of doing, the tendency from the analysis of the genes or transcripts of the microbiome *i.e*. metagenomics and metatranscriptomics is now to study the proteins and metabolites of microbiome and the host *via* metaproteomics and metabolomics approaches. Also this information is relative, so that new analytical techniques, including artificial intelligence, are necessary, especially about the functional interrelations between microbiota and host [59].

Intestinal microbiota and its implications in pathology is a fascinating field in medicine, with a lot of development in the last years. Evidence based medicine will help us for the therapeutic use in clinical practice.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 7

Videocapsule Endoscopy

Ciprian Brisc[1,2,*] and **Timothy Kurniawan**[2]

[1] *University of Oradea, Faculty of Medicine and Pharmacy, Romania*

[2] *Clinical County Emergency Hospital Oradea, Gastroenterology Clinic, Oradea, Romania*

Abstract: Videocapsule endoscopy is a non-invasive and important innovation in diagnostic endoscopy. This technology was first launched in 2000 and it has been widely used by gastroenterologists worldwide. This method requires the patient to swallow a miniature high-resolution camera which will pass through the digestive tract, while transmitting images to the recorder in order to be evaluated. It has its main advantages which are the non-invasiveness, and the possibility to yield a diagnosis in severely ill patients who cannot support invasive endoscopy procedures, but it also has disadvantages which include the impossibility to perform a biopsy or other therapeutic procedures. Over the years, this method has been revolutionized by not only approaching the small bowel, but also the esophagus and the colon. This chapter will also discuss the application of the esophagus capsule as well as the colon capsule. There are multiple indications for which patients can be referred to videocapsule endoscopy. The most frequent cause of referral to capsule endoscopy is the obscure GI bleeding, but it may be used in detecting small intestine polyps or tumors, searching for the cause of iron deficiency anemia or reviewing the extension of Crohn's disease. The main risk of this method is represented by retention which is also minimal.

Keywords: Anemia, Crohn's disease, Non-invasive, Obscure bleeding, Small intestine, Technology, Tumors, Videocapsule endoscopy.

INTRODUCTION

Video capsule endoscopy (VCE) represents a non-invasive method utilized to visualize the digestive tract, by sending images from a one-time use capsule which has been swallowed, to a receiver device attached to the patient's body. At the beginning, it was not a frequently used method as a first line approach, but usually employed after gastroscopy or colonoscopy if the diagnosis remains uncertain. Over time, this method has been improved to provide images with better pixels, prolonged battery life and also capability to visualize other segments the digestive tube (esophagus, stomach, colon) [1].

[*] **Corresponding author Brisc Ciprian:** University of Oradea, Faculty of Medicine and Pharmacy, Romania and Clinical County Emergency Hospital Oradea, Gastroenterology Clinic, Oradea, Romania; Tel: +40722591956; Fax: +40259211014; E-mail: brisciprian@gmail.com

Ioan Sporea and Alina Popescu (Eds.)

HISTORY AND DEVELOPMENT

The first small bowel video capsule (M2A capsule) was produced by Given Imaging, Yokneam, Israel and was approved by FDA in August 2000. It was afterward remarketed as PillCam SB, which provides a 140-degree field of view. After years of application, VCE technology was improved hence newer versions of video capsules were launched. PillCam SB2 with better resolution images, and 156-degree field of view was released in 2007. In the same year, the FDA approved the Endocapsule launched by Olympus Medical Systems. Since then different manufacturers have launched their own version of video capsule endoscopy (OMOM pill-Jinshan Science and Technology, CapsoCam SV1-CapsoVision, MiroCam- IntroMedic,) [2]. The latest PillCam SB3 offers similar quality as the previous PillCam SB2 but offers a higher framerate, up to 6 frames per second. As for other parts of the digestive tract, different types of capsules were released. PillCam ESO (released in 2004) and the dual camera PillCam COLON (released in 2006) were released for viewing the esophagus and respectively, the colon.

TECHNICAL SPECIFICATIONS

The wireless video capsule system consists of three important elements: the camera-containing capsule, the image receiver which is attached to the patient's body, a personal PC workstation, with a proprietary program for image reviewing and interpretation. All capsules have the same elements: an external disposable casing, a power source, LED array sources, optical lens, CMOS image sensor or high-resolution charge-coupled device (CCD) image capture system, radiofrequency transmitter and antenna. The manufacturers have developed software that can reduce the time required to analyze the images, as well as minimizing the possibility of missing some lesions [3]. All the programs in the market are able to detect red pixels to help the examiner detect bleeding lesions. Although it reduces the reading time, it is not recommended without a complete capsule evaluation, due to the high miss rate (12%) [4]. Other additional features include quick reference image atlas, the stage of capsule passing through the GI tract, virtual chromoendoscopy, three-dimensional reconstruction software as well as the use of artificial intelligence for better diagnostic yield [5, 6].

INDICATIONS FOR SMALL BOWEL VCE

Obscure Gastrointestinal Bleeding

Obscure Gastrointestinal bleeding (OGIB) is a gastrointestinal bleeding, whose cause was not identified after bidirectional endoscopy (gastroscopy and colonoscopy). OGIB is the most common indication for VCE, and is responsible

for 5% of all GI bleeding, the small intestine being the most frequent site of bleeding [7]. Localization of the bleeding site is usually difficult. Patients with OGIB frequently need hospitalization, blood transfusions and at the same time other diagnostic investigations. Due to its safety and easiness, VCE is considered to be the first line examination for the small intestine.

Numerous diseases are accountable for OGIB. Angiodysplasia is the most common of OGIB in the elderly (30-40%), whereas tumors are the most frequent cause in patients around 30-50 years of age [8]. The excessive use of NSAIDs may cause ulcers, erosions also leading to OGIB. Other differential diagnoses are listed in Fig. (**1**) [9]. Studies have shown that VCE has a higher diagnostic yield rate in OGIB than small bowel radiography or push enteroscopy. VCE was reported to have a specificity and sensitivity of 95% and 88.9% respectively. The most significant accuracy rate was observed in those patients with obscure and active bleeding (44.2% and 92.3%, respectively), while those with recent overt bleeding has the lowest yield rate (12.9%). As to detecting the bleeding source, a recent study showed that VCE managed to detect the source in a higher percentage compared to mesenteric angiography and CT angiography (72% *vs* 56% and 24% respectively) [10].

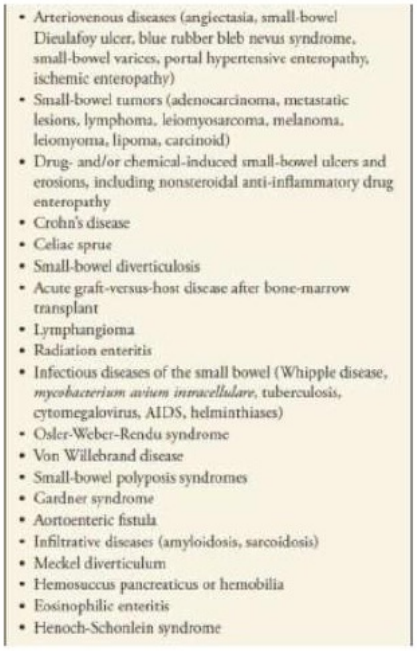

Fig. (1). Differential diagnoses in OGIB [9].

Iron Deficiency Anemia

Around 15% of the population of the globe are diagnosed with iron deficiency anemia (IDA). In the developed countries, IDA is estimated to affect 2-5% of male patients and women after climax and 5-10% of women of fertile age. Generally, this deficiency is responsible for 13% of gastroenterology complaints. A significant number of patients (30-50%) are still undiagnosed in spite of the increased application of bi-directional endoscopy. VCE is to be performed after a negative bi-directional endoscopy and the persistence of anemia in spite of adequate iron replacement therapy. The most frequent cause of IDA in young women is menstrual bleeding. Studies have shown that younger patients frequently bleed because of Crohn's disease, polyps, Dieulafoy lesions, small bowel cancers, Meckel diverticulum, while the elderly people develop anemia due to angiodysplasia or post NSAIDs ulcer.

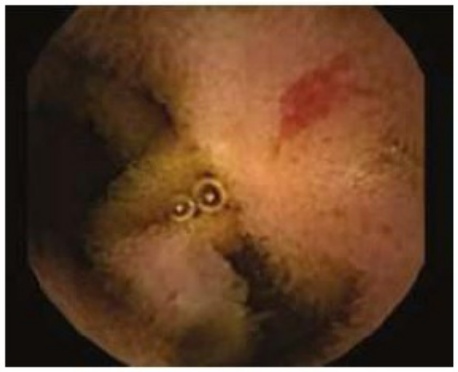

Fig. (2). Angiodysplasia.

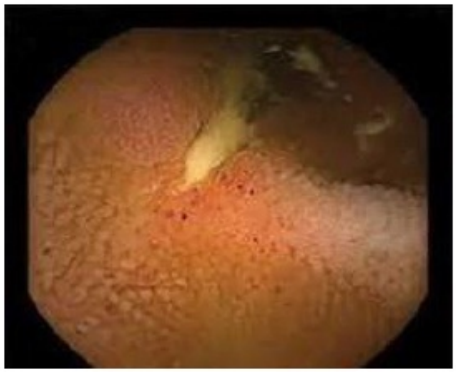

Fig. (3). Small bowel ulcer.

Fig. (4). Different capsule manufacturers.

Fig. (5). Belt sensor and recorder.

Small Intestine Polyps and Tumors

Even though the small intestine provides 90% of the absorption surface of the digestive tube and almost three quarters of its total length and, a small bowel tumor is a scarce diagnosis in daily practice. Only a small percentage of 6% of GI neoplasia appear in the small bowel and around 40 different histological types of small bowel neoplasia were diagnosed (Fig. **2-5**). The most commonly found are hematological neoplasia (lymphomas 15%-20%), carcinoid tumors (25%-30%) and adenocarcinomas (30%-50%). Small bowel metastases are more common

than primary tumors. Primary colonic, gastric, and gynecological neoplasms can spread to the small intestine by intraperitoneal route or local invasion, while primary skin, mammary and pulmonary neoplasm spread through the blood stream. In people with inherited polyposis syndromes such as Familial Adenomatous Polyposis or Peutz-Jeghers syndrome, or in those who developed Lynch syndrome, the risk of small bowel adenocarcinoma is higher. Also, the risk of small intestine T cell lymphoma is increased in patients with celiac disease.

The improvement and clinical application of video capsule endoscopy have shown an increased frequency of the small intestine tumor diagnosis in many studies [11]. Several studies have come to conclusion that routine application of VCE in the algorithm for OGIB, IDA or abdominal pain may hurry the diagnosis and treatment of small intestine tumors, hence improving the patients' prognosis regarding small bowel malignancies. VCE has shown comparable diagnostic yield with Device Assisted Enteroscopy (DAE), however VCE has certain limitations. There is a risk of false negative results, especially for small tumors situated in the proximal jejunum or duodenum, because of the rapid passing of the capsule. Submucosal growths may be missed due to intact overlying mucosa. Other disadvantages may include the impossibility of biopsy or polypectomy of the more proximally located polyps, in order to prevent the possibility of small bowel obstruction.

Crohn's Disease (CD)

Studies have shown that more than 50% of CD patients in the western countries and up to 87% of patients in Asia develop small intestine involvement at the moment of discovery. Thus, the investigation of the small bowel is important in this pathology, in order to determine its extension in the digestive tract and to establish a treatment strategy. Other indications for VCE are to assess the treatment response, and also to evaluate post-surgery CD relapse [12].

Crohn's disease is mostly diagnosed by ileocolonoscopy, but frequently lesions in the terminal ileum are visible during the colonoscopy. Due to the discontinuity of the lesions in CD, the involvement of the proximal part of the ileum is also common and often affects the patient's clinical manifestation. Several studies have shown that VCE can lead to precise diagnosis of CD, change the conduct of treatment and the patients' prognosis. VCE has also shown to have a high negative predictive value, therefore it is a valuable method to exclude the small bowel Crohn's disease [13].

VCE is valuable in reviewing patient's results to therapy as well as disease activity. There are two VCE- based scoring systems suggested in CD. The first one is the Capsule Endoscopy Crohn's Disease Activity (CECDAI) which uses

three characteristics (inflammation, extent of disease and stricture) found in the proximal and distal segments of the small bowel. The final score is the amount of both distal and proximal segment numbers obtained, and it may vary from 0 (normal) to 36 (severe). The second system is the Lewis score which also uses three endoscopic characteristics (villous edema, ulceration and stenosis) after dividing the small bowel in 3 segments. Every element is scored by taking into account its severity and extension. Lewis score is interpreted as normal (<135), mild to moderate (135-790), and severe disease activity (>790). Few studies suggested that VCE is better tolerated than ileocolonoscopy in the assessment of relapse after surgery, due to its non-invasiveness.

Celiac Disease

VCE is a valuable method in showing the endoscopic feature of intestinal mucosa in this disease such as scalloping, mosaicism, micro-nodularity of the small bowel lining and reduction of intestinal folds. VCE has a high positive predictive value and negative predictive value: 96.5%-100% and 71.4-88.9%, respectively. This shows that when VCE detects typical villous atrophy, patients have a higher possibility to develop celiac disease, but on the other hand, a lower negative predictive value predicts that a normal mucosal pattern cannot exclude celiac disease. Therefore, this diagnostic still depends on a biopsy taken from the duodenum revealing villous atrophy. The European Society of Gastrointestinal Endoscopy (ESGE) and American Gastroenterology Association (AGA) recommend VCE only in those patients which cannot or refuse to refer to a conventional gastroscopy and is also recommended to monitor patients undergoing celiac disease treatment, detect its complications (ulcerative jejunoileitis, T-cell lymphoma,) [14].

ADMINISTRATION

The performing physician will recommend the patient to fast at least 12 hours prior to capsule administration. At present, polyethylene glycol (PEG) based regimens are the primary recommendation for bowel preparation. The use of Simethicone is also recommended in many studies. On the day of capsule ingestion, the sensors connected to a receiver device join the patient's body. The capsule is then swallowed with water. The patient should pay attention to the blinking lights on the receiver device to be assured that the images are being transmitted. After capsule ingestion, the patient is allowed to drink clear liquid after 2 hours and is allowed to eat after 4 hours. The capsule then traverses the whole digestive tract for about 12 up to 48 hours when it is eliminated together with the feces. During this procedure, the patient is not allowed to stay within the range of certain magnetic field emitting devices such as mobile phones,

institutions with CT/MRI scans, *etc.* Once the capsule finishes the image capture (approximately nine-ten hours), the receiver device can be detached from the image source. The receiver is then linked to a personal workstation PC in order to move the obtained information. For the interpretation of the images obtained, the doctor must be competent in endoscopy, and have undergone formal capsule training [15].

ESOPHAGEAL CAPSULE ENDOSCOPY

Esophagus VCE is an option that offers a diagnosis of esophageal diseases, to patients who cannot tolerate a conventional esophagogastroduodenoscopy (EGD). The PillCam ESO (approved in 2004), and the PillCam ESO2 (used since 2007) have been in use to evaluate the esophagus. The PillCam ESO3 with even better specifications has also been introduced. The size, shape, and weight are comparable to the capsules used in the small intestine PillCam, but several improvements have been implemented in order to enhance the esophagus viewing, such as the shortened battery duration (20 minutes), the placement of cameras on both ends with a wider 174º viewing angle each, and also image acquisition up to 35 frames per second [16].

Procedure

The intake of food or water for the person who decided to undergo VCE is forbidden at least two hours prior to the procedure. The standing patient will drink 100 mL of water and then he will swallow the capsule in the supine position. Through a straw, extra sips of water can be swallowed. The patient will stay for two minutes in the supine and 30º inclined position, after which he will stay one minute at 60º inclined position. Afterward, the patient will stay for 15 minutes in the upright position. During this procedure, the images which are obtained will be transferred through the thoracic sensors attached to the receiver device.

Clinical Application

Esophagus CE has been applied to evaluate Barret's esophagus. However, multiple studies and meta-analyses have shown a low sensibility of 79% and 60% in the diagnosis of Barret's esophagus and esophageal metaplasia, respectively, therefore, authors have concluded that VCE is not able to correctly diagnose Barret's esophagus and that EGD remains the standard diagnostic modality [17].

A large meta-analysis and systematic review evaluated esophageal VCE in diagnosis and evaluating esophageal varices` grade (EV) in preexistent portal hypertension`s patients. The diagnostic accuracy of esophageal VCE in the diagnosis of EV was 90%, with the diagnostic pooled sensitivity of 83% and a

specificity of 85%. Diagnostic accuracy was 92% for the EV grading and the pooled sensitivity was 72% while the specificity was 91%. Regarding the results, authors have concluded that esophageal VCE was not considered capable to replace EGD to diagnose and determine the grade of EV, but is useful in patients who refuse EGD or in those with contraindication for EGD [18].

Limitations

Esophageal VCE has its limitations due to its impossibility to perform a biopsy or other necessary procedures in Barret's esophagus. Moreover, for an accurate diagnose of EV and for a correct grading of EV, air insufflation is also not possible by using VCE instead of endoscopic maneuvers.

COLON CAPSULE ENDOSCOPY

Colon Capsule Endoscopy (CCE) is defined as a new promising modality for colon evaluation. Its advantage relates to the noninvasiveness of the procedure, compared to conventional colonoscopy, which some patients may regard as an unpleasant experience. Since the first M2A small bowel capsule was launched in 2000, great interests have led to the development of the first capsule for the colon. The PillCam COLON is available since 2006, followed by its successor the PillCam COLON2 in 2009. This CCE contains 2 cameras on both sides, with viewing angles wider (up to 172º), creating the possibility of an approximate 360º image of the colon wall. During active movement, it can capture up to 35 frames per second [19].

Indications

Indications for CCE may include incomplete colonoscopy evaluation, in patients who refuse colonoscopy, the screening for colorectal cancer, monitoring and diagnosis of IBD. Meta-analyses have shown that CCE has a higher diagnostic yield rate in cancer of the colon and rectum than computed tomographic colonography (CTC), however with less sensitivity and specificity than conventional colonoscopy. Studies with CCE-2 have shown an 82% sensitivity rate and an 86% specificity rate for detection of the polyps larger than 6mm or any polyp detection; the sensibility was between 84% and 89% and specificity rate was between 64% and 88% [20, 21].

ESGE (European Society for Gastroenterological Endoscopy) guidelines recommend that CCE can be used to diagnose and review mucosal aspect for those who were diagnosed with ulcerative colitis, however in Crohn's disease, there is no sufficient data regarding the use of CCE. Therefore, due to these results, alongside its limitations regarding the impossibility to introduce air into

the colon, or performing therapeutic procedures, CCE has not been able to replace conventional colonoscopy as a standard modality in the evaluation of the colon [22].

CONTRAINDICATIONS OF CAPSULE ENDOSCOPY

VCE like any other procedure- has its contraindications. The absolute ones are represented by the obstruction of the digestive tract, pregnancy and fistula. The relative contraindications are pacemakers, cardiac defibrillators, esophageal stricture with dysphagia, and possible stricture in other parts of the intestine.

There are two delivery systems in case of esophageal stricture, dysphagia or other deglutition problems. The PillCam Express by Given Imaging Israel and The AdvanCE capsule endoscopy delivery service by US, Endoscopy are video capsule delivery services used on patients with deglutition problems or delayed emptying of the stomach.

In case of suspicion of stricture, the *patency capsule* may be used. It is the same size capsule as the normal VCE, with the particularity being its biodegradability and dissolvability. The patency capsule contains lactose and barium 10% to enable fluoroscopy imaging and a Radio Frequency Identification (RFID) tag to be able to locate the capsule. If this device does not pass through an obstruction, it will be destroyed inside the GI tract. Studies have shown VCE`s efficacy if the capsule transit through the GI tract under 30 hours. There are on-going studies over whether patency capsules should be administered in those with possible strictures before administering VCE in order to avoid capsule retention [23].

LIMITATIONS AND COMPLICATIONS

The main risk of VCE is capsule retention. VCE is riskier to perform in patients with Crohn's disease, post-radiation enteritis, Zenker's diverticulum or duodenal diverticulum. The overall retention rate is as low as 1.4%. For removing the retained capsule, we can use double balloon enteroscopy or, in the worst cases, we can call on surgery.

As to its limitations, VCE is still not able to take biopsies, or perform therapeutic procedures. Poor visualization and incomplete examination are also considered its limitation. Battery life is limited up to 9-10 hours, therefore in those patients who have a slow time of transit, the assessment of the small intestine wall may be incomplete.

Like **future development** of VCE, researchers are trying to implement ultrasonographic and autofluorescence imaging to VCE, improved localization

with virtual biopsy, drug delivering CE, computer-aided diagnosis and remote manipulation.

VCE is a non-invasive, comparable method that is also safe to apply in patients with higher risks to conventional endoscopy. The developments of VCE have allowed gastroenterologists to visualize and evaluate the whole digestive tract, however it has its own limitations for which researches are still taking place.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author confirms that this chapter contents have no conflict of interest.

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CHAPTER 8

Precision Medicine in Inflammatory Bowel Disease: Current Challenges

Eugen Dumitru[1,2,3] and **Cristina Tocia**[1,2,*]

[1] *Ovidius University of Constanta, Romania*

[2] *Gastroenterology Clinic, Clinic Emergency Hospital of Constanta, Romania*

[3] *Center for Research And Development of the Morphological and Genetic Studies of Malignant Pathology (CEDMOG), Romania*

Abstract: Inflammatory bowel diseases are chronic relapsing diseases with an increasing incidence worldwide, with variable and unpredictable evolution, as well as predisposition to complications throughout the disease. Despite the efforts of the academic world of research, their etiology remains incompletely elucidated, but intense research over the last decade showed that they are based on intricate complex pathophysiological mechanisms that occur in the genome, epigenome, microbiome, or immunome. Precision medicine is a new concept and its application in inflammatory bowel disease consists of adapting medical treatment to each patient who is viewed from an individual perspective, encompassing a multitude of evidence-based approaches in the literature, thus facilitating accurate medical decisions.

Significant progress has been made by studying genomic data such as genome, transcriptome, proteome, metabolome, and microbiome. With a wide range of treatments available, the demand for precision medicine in inflammatory bowel disease is of paramount importance. The goal of precision medicine is to provide individualized care so that the patient's voyage from diagnosis to treatment is based on the individual biological characteristics. Precision medicine, in order to adapt one specific therapy to a specific patient at one specific time based on the patient's biological characteristics, is an important aspiration in the medical world. Although much progress has been made in this area, some challenges remain unclear. In the future, precision medicine has the capacity to provide personalized care to patients with inflammatory bowel disease.

Keywords: Epigenome, Genetics, Genome, Immunity, Inflammatory bowel disease, Microbiome, Precision medicine, Proteomics.

* **Corresponding author Cristina Tocia:** Ovidius University of Constanta, Romania and Gastroenterology Clinic, Clinic Emergency Hospital of Constanta, Romania; Tel: 0740508441; E-mail: cristina.tocia@yahoo.com

Ioan Sporea and Alina Popescu (Eds.)

INTRODUCTION

The Role of Precision Medicine in Inflammatory Bowel Disease

Inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis, are chronic diseases with an increasing incidence worldwide [1], with variable and unpredictable evolution, as well as predisposition to complications throughout the disease. Despite the efforts of the academic world of research, their etiology remains incompletely elucidated, but intense research over the last decade showed that they are based on intricate complex pathophysiological mechanisms that occur in the genome, epigenome, immunome [2]. Understanding the etiopathogenesis of these diseases gives the advantage of being able to apply many therapeutic molecules, but this strategy may not be cost-effective; also, patients may often fail to respond to treatment or will never respond to some therapies. Therefore, there is a growing need to apply a personalized, targeted treatment based on the molecular characteristics of each patient, and thus to change the paradigm of approaching inflammatory bowel disease from the "reactive" approach driven by the complications of the disease to the "proactive" approach to prevent complications [3]. Precision medicine is a new concept and its application in IBD consists of adapting medical treatment to each patient who is viewed from a unique, individual perspective, encompassing a multitude of evidence-based approaches in the literature, thus facilitating accurate medical decisions.

Precision medicine has as objective a patient-centred medicine and adaptation of treatment according to personal genetic, epigenetic, biological characteristics and clinical features of each patient [2]. Although it is similar to the concept of personalized medicine, precision medicine also includes a complex approach based on the objective data to facilitate clinical decisions and to better identify the molecular processes of the disease, related to the molecular characteristics of each patient. In 2015, the national initiative "Precision Medicine" [4], was initiated, which aims to bring together multi-omic data from over 1 million subjects to deepen the comprehension of the pathogenesis of inflammatory bowel disease and the application of treatments. The classical strategy "step-up" risks to treat ineffective patients who could develop complications; also, the strategy "top-up" risks to overtreat patients who could have remained stable and uncomplicated over time with only standard, cheaper therapies and no major side effects. Therefore, several parameters have been identified as risk factors associated with the severity of the disease: the location and the phenotype of the disease, the age, serological markers, and the need for early introduction of corticosteroid therapy or lifestyle. However, none is enough to guide early therapy.

Precision medicine, in order to adapt one specific therapy to a specific patient at one specific time based on the patient's biological characteristics [5], is an important aspiration in the medical world. Although much progress has been made in this area (which will be summarized below), some challenges remain unclear. In the future, precision medicine has the capacity to provide personalized care to patients with inflammatory bowel disease.

Etiopathogenesis of Inflammatory Bowel Disease: Current Challenges

Genetics

Patients with IBD have a genetic predisposition, and the risk of acquiring the disease is higher in subjects who have families with IBD. Genome-wide association studies (GWAS) have enabled the detection of many risk genes. Genetic polymorphisms also play a role in the control of the intestinal barrier. Even significant progress has been made in this area, only 25% of the heredity of IBD can be proven today [6]. In the last decades, scientific advances in genomics and the availability of genetic data from large studies have considerably contributed to a greater understanding of the relationship between certain genes implicated in the pathogenesis of IBD. GWAS has found more than 300 genetic forms that affect several host functions, such as: local homeostasis, intestinal barrier, microbiota structure, autophagy, production and secretion of antimicrobial substances, or regulation of acquired immunity [7]. Although Crohn's disease and ulcerative colitis are known to be two distinct diseases (at least clinically), 30% of genetic changes are common, suggesting the existence of common genetic pathways responsible primarily for the immune response, cytokine release, and lymphocyte response. These findings emphasize the importance of genetic predisposition in the pathogenesis of IBD. However, there are gaps in the full understanding of the pathogenesis as there are patients who do not have a genetic susceptibility and can still have the disease, suggesting that an isolated study of genomics is not enough to complete the “puzzle” of the pathogenesis of IBD.

Microbiome

Research into the microbiome of healthy and sick patients based on the genetic sequencing of 165 RNA genes using state-of-the-art technology made possible the analysis of the composition and functions of the microbiome, and also facilitated the understanding of the effects of various external factors [8]. Among the many roles it plays in the human body, the microbiota also has an essential role in preserving the integrity of the intestinal barrier, synthesis of molecules, digestion, and the development of immune cells. The environment of the gastrointestinal tract based on microbial diversity maintains a state of symbiosis. Intestinal dysbiosis is characterized as a reduction in microbial diversity that leads to a

disparity between "good" and harmful pathogenic bacteria, resulting in excessive intestinal inflammation. The persistence of this intestinal inflammation can lead to a chronic, uncontrolled inflammation, observed in various diseases, including IBD.

Specific microbial signatures in inflammatory bowel disease: have we reached this point of knowledge?

The large number of studies in the literature that report the presence of dysbiosis in patients with IBD, especially in those with Crohn's disease, continue to increase and suggest the use of a microbial mark as a diagnostic instrument for subtypes of inflammatory bowel disease. However, there are inconsistent results, with great intra- and inter-individual variability between studies, emphasizing the need for further studies and for a better knowledge of the microbial pattern specific to these diseases. For example, Pascal and colleagues [8] collected microbiological samples at three-month intervals and found eight species that were present differently in patients with IBD. Similarly, in recent years, state-of-the-art technology has been used to analyse the intestinal, but inconsistent results caused by high microbial diversity failed to identify a universal microbial biomarker for predicting these diseases [9]. This discrepancy could be explained by the complex interaction between the microbiota and the host during the evolution of the diseases, the adjacent factors (such as diet, lifestyle, genetics), which have a different effect on the microbiota. To address these concerns about the interaction between the microbiota and the host (intrinsic or extrinsic host-related factors), Lloyd and colleagues [9] lately provided the most comprehensive analysis of the interaction between the microbiome and the host reaction in patients with IBD. However, due to great inter-individual disparities, the researchers were unable to identify a single microbial biomarker, but provided important information in this area and provided a dynamic view of the phenomenon which occurs at the microbiome level in the case of active disease, emphasizing that it is not just a simple interaction with the host, but a complex interaction involving several areas, including metabolomics, proteomics and transcriptomics.

Immunome

Immuno-proteomics

Latest published studies [10] have shown an intricated interaction between host genetics and environmental factors, which is the cause of the dysregulated intestinal barrier function; microbial antigens are translocated into the intestinal wall, resulting in an aberrant mucosal immune response. Thus, the excessive production of cytokines at this level, in addition to the induction of intestinal inflammation and the installation of clinical symptoms, also induces the systemic

effects associated with IBD. Various cytokine profiles have been observed: patients with Crohn's disease have an increased Th response and generate higher levels of IL-2 and Interferon-gamma than patients with ulcerative colitis. Numerous interleukins are targeted as treatment options, but with conflicting effectiveness, emphasizing the value of determining the immunological and proteomic profile in patients with inflammatory bowel disease.

Immuno-transcriptomics - specific immunological signatures of inflammatory bowel disease: have we reached this point of knowledge?

Immunological and transcriptomic markers that can distinguish between healthy subjects and patients with IBD or between Crohn’s disease and ulcerative colitis can be reliable diagnostic or prognostic biomarkers [10]. Unfortunately, such biomarkers are not yet well defined. The great number of genes involved in the pathogenesis, as well as the complexity of signaling pathways that regulate immune responses in these patients, highlight the fact that identifying a single biomarker is difficult, but several target genes have been identified and research is progressing. The network of genes involved in immunology consists of hundreds of cells and subpopulations, so finding an immunological and genetic pattern is extremely complex. The incorporation of genetic, immunologic, transcriptomic information and proteomic profiles in patients with IBD, especially on how they change during the phases of the disease or in response to various treatments, has huge potential to discover new specific molecular pathways and potential biomarkers [11].

Metabolomics and Lipidomics

Prior studies have shown variations between the metabolomics and lipidomics of the patients with IBD, compared to those of healthy patients or between Crohn's disease and ulcerative colitis [9]. A study that compared the lipid profile of patients with such diseases with that of healthy patients, identified 33 lipidomic specific signatures for Crohn's disease and 5 specifics for ulcerative colitis [8]. Also, other studies have identified the elevated levels of diacylglycerol and N-acyl phosphate diethanolamines and decreased levels of phosphatidylcholine, urobilinogen, and ceramide in patients with IBD. Recent analyses in this field have also demonstrated the possibility of differential diagnosis between Crohn's disease and ulcerative colitis. These studies emphasize the importance of studying lipidomics and metabolomics to find reliable biomarkers, but research is in its infancy and requires validation in large cohorts [12].

According to the concept of precision medicine, IBD patients should be classified into distinct genetic genotype and clinico-molecular phenotypes (Fig. **1**).

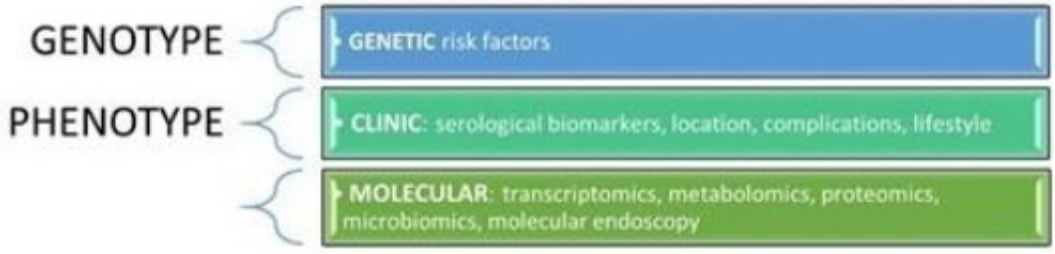

Fig. (1). The genotype, clinical and molecular phenotype of the patients with inflammatory bowel disease according to Precision Medicine.

Prediction of Disease Susceptibility and Clinical Phenotype

GWAS has provided numerous information on the etiology and evolution of IBD [13]. To date, more than 240 genetic loci specific to these diseases have been identified. Some findings from GWAS have been associated with various disease phenotypes. For example, NOD2/CARD15 can identify the signal triggered by microbial stimuli and is associated with the ileal disease with a stenotic phenotype. NOD2/CARD15 is also correlated with a high risk of surgery and complications. ATG16L1, involved in autophagy dysfunction (an important mechanism in the development of Crohn's disease), is related to ileal disease, and IGRM (another autophagy gene) is correlated with penetrating disease. IL23R, which has turned out to be an effective therapeutic target in Crohn's disease, has also been previously associated with an ileal disease [14]. Recent research in the genetics of inflammatory bowel disease has shown clinically distinct phenotypes [15]. A UK genetic study of 29,838 patients redefined (genetically) the subtypes of inflammatory bowel disease in ileal Crohn's disease, colonic Crohn's disease and ulcerative colitis [16].

Similarly, the microbiome can provide important information in identifying patients at high risk. A study of patients with the early-onset disease by Gevers and colleagues [17] investigated the ileal microbiome and found features that can support the diagnosis of Crohn's disease, even in the absence of obvious inflammation. It appears that these microbial signatures vary depending on the phenotype of the disease, the associated environmental factors (such as smoking), and the types of treatments administered. Longitudinal studies in this area have shown the ability of the microbiome to predict the phenotypes of inflammatory bowel disease and to diagnose these diseases. In the future, following thorough research, the possibility of implementing risk scores depending on the composition of the microbiome is expected.

Researchers have begun to explore new diagnostic biomarkers in metabolomics, proteomics, epigenetics, and their findings are encouraging. Multicenter

associations such as IBDCharacter, IBD-BIOM, and Biocycle are trying to discover new biomarkers, that can be used in clinical practice [18]. IBDCharacter and IBD-BIOM have focused on the development of multi-omic biomarkers, useful for diagnosis and prognosis and Biocycle is exploring innovative therapeutic regimens for moderate-severe Crohn's disease. The results of these studies have not yet been transposed into the clinic, but provide important information on the pathogenesis of inflammatory bowel disease.

Prediction of Disease Course

Advanced molecular research has found molecules that are associated with illness progression. The research of Lee and colleagues, in which CD8 + T cells were explored in patients with newly diagnosed IBD, was able to identify distinctive RNA sequences that were correlated with the need to escalate therapy and/or surgery over time in both Crohn's disease and ulcerative colitis. Another study identified four prognostic loci: FOX03, XACT, a region upstream of IGFBP1, and the MHC region. The molecular composition was further divided into proteome, methylome, glycome and sublevels, with findings showing that individuals with severe IBD have a distinct molecular architecture with unique methylome and proteome signatures [19]. Glycomeric biomarkers have recently been associated with the property of predicting the need for escalating therapy. Until now, the escalation of therapy was based on simple clinical and paraclinical criteria (based on calprotectin at most), but the identification of unique, specific biomarkers that can objectively argue the need to approach a patient from the beginning of the disease with a standard therapy “Top-down” and avoiding overtreatment in “risk-free” patients who would not need immunosuppressive therapies, is a real necessity. The RISK study [20] found specific multi-omic profiles that were associated with the evolution of ileal location of Crohn's disease. Therefore, the addition of information from transcriptomic profiles at the ileal level in the composition of a clinical and serological score can improve the prognostic accuracy, providing essential data in predicting the evolution of Crohn's disease. However, it remains to be seen whether the implementation of this strategy and the therapeutic adaptation according to this molecular characterization can change the evolution of the disease over time.

The microbiota may also have an essential role in predicting the evolution of inflammatory bowel disease. A study that investigated postoperative recurrence in Crohn's disease showed that the low population of Faecalibacterium prausnitzii discovered in resected ileal pieces, was associated with a high frequency of postoperative recurrence [8]. In a study of pediatric patients with Crohn's disease, certain distinct microbial profiles made it possible to predict 6 months of remission without corticosteroids. All of these studies provide promising

information that can help the clinicians identify patients at risk for aggressive development and complications and guide them in choosing the appropriate therapy.

The possibility of revealing the progress of IBD since diagnosis, using numerous specific multi-omic criteria in addition to the clinical and serological ones, will revolutionize the approach of these patients, getting closer to precision medicine.

Prediction of Drug Response

The range of therapeutic options in inflammatory bowel disease has been diversified considerably in last few years and many other new molecules are under approval. After all, precision medicine aims to choose a certain therapy based on the patient's biological characteristics, in optimal doses adapted to the patient that can ensure disease control, maintaining therapeutic effects and minimizing side effects. Prediction of response to treatment is important to facilitate early change of therapy if necessary.

Accurate prediction of treatment response before initiating therapy would allow a more appropriate and personalized selection of treatments for patients. However, at present, there is no biomarker available for this role, although numerous studies have been conducted. West and colleagues [16] have shown that elevated cytokine oncostatin M is associated with a lack of anti-TNF response. Verstock and colleagues [21] have shown that low serum TREM1 level measured immediately before initiation of therapy is a specific biomarker associated with an adequate response to future anti-TNF therapy. Telesco and colleagues have identified and validated a genetic profile consisting of 13 genes that can predict mucosal healing at 6 weeks with golimumab therapy in PURSUIT and Project studies. Morilla and colleagues identified a panel of 9 microRNAs in colonic biopsies from severe acute colitis and adequately classified the patients who respond or not to corticosteroids, infliximab, and cyclosporine.

Prognostication of the response to biologics with the help of data delivered by microbial profiles was also investigated [22]. In one study, the microbiota of pediatric patients who responded to anti-TNF therapy was similar to that of controls and different from that of patients who did not respond to treatment. Differences in the microbiota were also identified in the ileum in patients who responded to anti-integrin therapy; the authors identified abundant species of Roseburia inulinivorans and Burkholderiales in the ileum of those who acquired remission. Moreover, an algorithm based on neural networks was able to predict the response to treatments. For ustekinumab, Doherty and co-workers, using RNA sequencing, were able to divide patients at week 6 of treatment into remission patients and patients with activity based on microbiome characterization [8].

Although the results are promising, these tests need to be widely validated before they can be put into practice.

Researchers have also turned their attention to the role of glycosylation. This post-translational process in proteins alters their function and may be associated with inflammation. Pereira and colleagues showed that low N-glycan levels in colon biopsies of 131 patients with ulcerative colitis are correlated with an absence of response to conventional therapy (aminosalicylates, corticosteroids or immunomodulators).

Further modern studies [11] applied new gene amplification tools to improve the knowledge about molecular mechanisms of the treatment response. In active ulcerative colitis, there is a decrease in epithelial mitochondrial genes in the biogenesis of PPARGC1A mitochondria (PGC1a) and mitochondrial membrane potential (MMP). PGC1a reduction seems to play an important role in epithelial barrier dysfunction in ulcerative colitis. This study also identified a genetic profile that is related to a lack of response to anti-TNF or anti-integrins. In case of Crohn's disease, recent research has shown that cells in the inflamed ileum (Ig G-type cells, mononuclear phagocytes, activated T cells, and GIMATS-type stromal cells) are associated with a lack of response to anti-TNF therapy.

Current Technologies and New Molecular Technologies for the Practice of Precision Medicine

Nowadays, there are several technologies that could participate to this multi-omic characterization of patients with IBD [23]. These technologies are represented by the evaluation of transcriptomics and proteomics in the blood and tissue. Supplementary to the measurement of RNA and protein expression, important disturbances in the periphery and mucosal cells may further highlight differences between patients with IBD [24]. DNA regulation is a crucial factor of RNA and protein expression, and both analyzes of DNA sequencing variants by genotyping, as well as epigenetic analysis by DNA methylation, along with histone modification and ATAC-seq studies can offer an in-depth look at the mechanisms that regulate DNA [25]. Also, the possibility to make organoid cultures brought an extraordinary benefit. CRISPR-Cas9 and other target genes have permitted genetic modulation in these organoid culture systems, as well as in a range of other cells relevant to the pathogenesis of IBD. In conclusion, current microbiota technologies in both intestinal biopsies and faeces based on 16s-RNA sequencing, metagenomics, metabolomics and culture systems have allowed the description of the microbiome and its roles in IBD patients, with adjuvant information on disease phenotype, genetic susceptibility and types of treatment [12].

Studies based on RNA sequencing of cells in intestinal tissues are now underway. Characterization of the spectrum of cell differences by protein expression is also ongoing, for example using the CyTOF method, or in tissue sections using mass spectrometry or using multiplexed ion beam imaging (MIBI) technologies. An additional section of expansion was the sequencing of T-cell receptors and the description of the antibody epitope with important advances in providing further insight into the pathogenesis of IBD [23].

Also essential in advances in IBD is the application of artificial intelligence (AI) based on convolution algorithms for extracting subpopulations of cells from whole tissues and the implementation of "Big Data in IBD" [26]. The implementation of new systems for querying microbiome and novel algorithms that use learning models to distinguish findings in radiology, endoscopy, and pathology are also important for patients with inflammatory bowel disease. The evolution of precision medicine in IBD is summarized below (Fig. **2**).

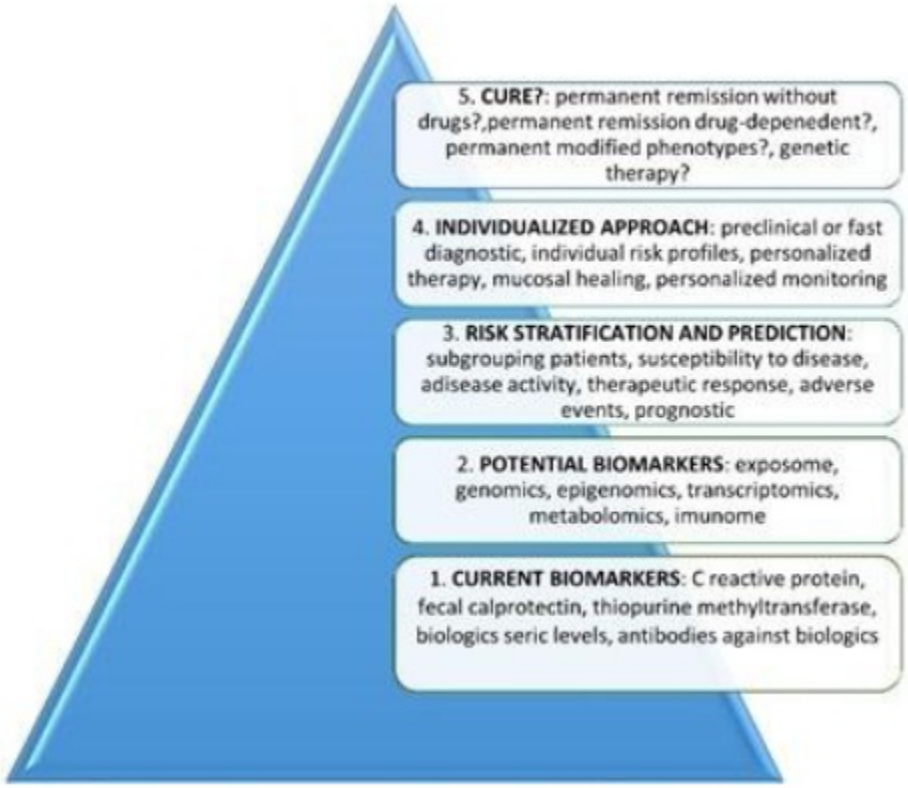

Fig. (2). The evolution of precision medicine in inflammatory bowel disease.

Significant improvement has been made by studying genomic data such as genome, transcriptome, proteome, metabolome, microbiome, *etc.* With a wide range of molecules available, the demand for precision medicine in IBD is high. The goal of precision medicine is to provide individualized care so that the patient's voyage from diagnosis to treatment is based on the individual biological characteristics.

The recent advance in the direction of precision medicine depends on the intricated links between multi-omic data in IBD. This concept of precision medicine will increase the ability to assess individually the patient's inflammation based on his or her genetic and biological characteristics, thus allowing the application of an individualized treatment and monitoring algorithm. With the help of precision medicine, the general goal will probably be to achieve complete healing, not just mucosal or histological healing.

However, many challenges persist in the application of precision medicine in IBD, requiring the integration of all multi-omic data obtained from numerous researches. This involves infrastructure modifications in the exchange of data between investigational groups, integrating clinical and research data into large databases. Another challenge is the way of analysing the data obtained. Currently, there is a software that facilitates the integration and analysis of multi-omic data at several levels, such as iCluster148 which classifies patients based on the entered multi-omic data. Following the efforts of several investigational associations to produce multi-omic data sets, it is time to outline the "interactome" of inflammatory bowel diseases [27], the "network" of the disease where disturbances in one of the "omics" produce intestinal inflammation which is produced by altered molecular pathways. Future innovations in treatment based on network interactions in IBD will revolutionize the therapeutic field in these diseases.

CONCLUSION

Precision medicine has a huge potential to impact the results of research and practice in IBD. Together we play an important role in providing superior care for inflammatory bowel disease patients, and in implementing "Big Data", so that in the future we can provide truly individualized and precise care to these patients.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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CHAPTER 9

Fibrosis in Crohn's Disease - From Evolution to Treatment

Adrian Goldiş[1,*]

[1] *Department of Gastroenterology and Hepatology, "Victor Babeş" University of Medicine and Pharmacy Timişoara, Romania*

Abstract: One of the major complications of Crohn's disease is the development of fibrosis, this causes the intestine to lose its mobility. The most frequent intestinal "damage" occurrences are considered fibrosis, fistula, abscess, resected bowel. The Lemann index has been developed to describe the entire gut damage score in CD. It is summarizes the clinical, imaging, endoscopic, and surgical findings from all the segments of the digestive tract into one global score and provides a superior quantification of the severity of bowel, destruction. Chronic inflammation, hypertrophy of MP (muscularis propria) and smooth muscle hyperplasia of SM (submucosa) were the most valid histopathological features characterizing the intestinal stricture. Imaging methods such as MRI, CT or IUS can detect penetrating disease and intra-abdominal abscesses in different accuracy grades. Although the current imaging techniques were not able to determine the degree of fibrosis, MRI was preferred in the US for pelvic fistulae, abscesses or deep-seated fistulae. By decreasing MRTF and p38 MAPK activation and increasing autophagy in fibroblasts, local ROCK inhibition prevents and reverses intestinal fibrosis. Fibrosis is certainly reversible in animal models. The duration of treatment and toxicity are challenging for the time being.

Keywords: Crohn Disease, Fibrosis, IL36A, Inflammatory bowel disease, Lemann index, MRFT, Penetrating disease, p38 MAPK, ROCK inhibition, Smooth muscle hyperplasia, Stricture.

INTRODUCTION

In Crohn's Disease there is a chronic inflammation which can develop and cause tissue damage, represented by thickening and hardening in the bowel wall, this process is called fibrosis. This may cause the intestine to lose mobility, causing a stricture (narrowing) of the bowel, which can then lead to blockage.

[*] **Corresponding author Adrian Goldiş:** Department of Gastroenterology and Hepatology, "Victor Babeş" University of Medicine and Pharmacy Timişoara, 2 Eftimie Murgu Square, 300041 Timişoara, Romania; Tel: 0256/490626; E-mail: goldisadi@yahoo.com

Ioan Sporea and Alina Popescu (Eds.)

Different proteins, such as collagens, which are normally involved in the tissue healing process, end up in a state of overproduction, consequently leading to fibrosis.

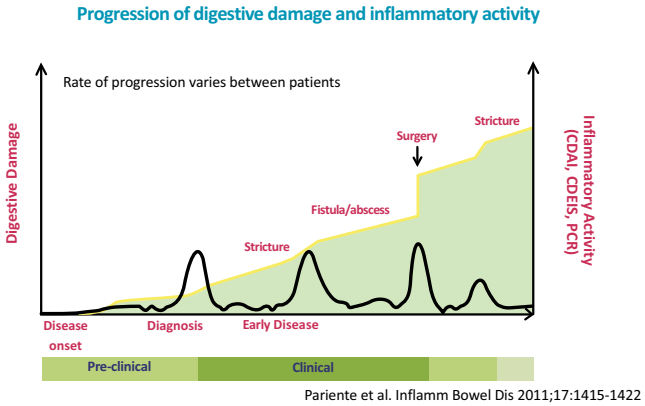

Fig. (1). Progression of digestive damage and inflammatory activity.

The Lemann index was recently created with the aim of determining the total gut damage score in CD. Medical, surgical, endoscopic, and imaging results from all parts of the digestive tract are combined into a single total score [1]. The Lémann score could be a clearer indicator of the magnitude of structural bowel injury and it should be used to monitor bowel damage development over time.

The slope of the digestive damage curve could be used to make decisions, regardless of the magnitude of the damage. As in the rheumatoid arthritis model, the slope of the curve may enable patients with rapid damage progression to be identified in order to propose accelerated therapy or, in other cases, to use less aggressive care. It will also be possible to assess the impact of medical treatments or interventions on disease progression. Such a score should allow better identification of patients with severe damage and those with rapid progression of damage [2]. During the follow-up period, the disease location and disease behavior has changed. Only biologic therapy was shown to be related to a shift in location. Changes in behavior or disease location in Crohn's disease patients have been seen to raise the risk of resection [3].

Regardless of early anti-TNF exposure, survival curve study of this matched cohort revealed comparable progression of stricturing behavior in patients. The transition in penetrating behavior was three times lower among those patients who received early anti-TNF, in contrast to patients who did not undergo early anti-TNF,, but this decrease did not achieve significance in the unadjusted study. The early anti-TNF response was described as achieving corticosteroid-free remission

6 months after diagnosis, and this outcome was noted in 124 (71%) of the 175 participants with available data. After 6 months, there was no discrepancy in the prevalence of B2 or B3 complications in anti-TNF responders and non-responders, despite the limited sample size of these subgroups [4].

Patients with strictures had genes regulating extracellular matrix aggregation induced at diagnosis, whereas those with penetrating disease had genes regulating the acute inflammatory response to microbes induced. In patients who experienced penetrating (B3) and stricturing (B2) complications, the balance between antimicrobial acute inflammatory and extracellular matrix aggregation pathways was investigated. In patients who experienced stricturing complications, the extracellular matrix of the structural constituent molecular function was mediated to a greater extent than those who remained complication-free (B1) and those who progressed to penetrating disease (B2) [4].

Internal penetrating disease and intra-abdominal abscesses can be identified with different degrees of accuracy using cross-sectional imaging such as MRI, CT or IUS [EL1]. For deep-seated fistulae, pelvic fistulae, or abscesses, MRI was preferred over ultrasound [EL4] [5]. The medical utility of MRI for diagnosing intraabdominal fistulas was calculated in five trials by van Gemert-Horsthuis, who looked at 51 lesions in a number of 210 patients. The plurality of lesions corresponded to enteroenteric fistulas, as in previous US and CT studies. As a comparison standard, four trials used a mixture of medical procedures, physical assessment (enterocutaneous fistulas), and surgery. In one analysis, there was no reference standard [6]. In a study from Panes, it was found that MRI had a sensitivity of 76 percent (95 percent CI 71–82 percent) and a precision of 96 percent (95 percent CI 92–98 percent) for the diagnosis of fistulas in a sample with appropriate comparison level [7]. The occurrence of intraabdominal abscesses was identified in four studies using MRI, with ten lesions found in 109 cases.

For the diagnosis of extraenteric lesions, one study did not use an acceptable reference level [8]; Lesions were confirmed in the majority of cases (8/10) in the remaining trials. The findings of the studies with an appropriate comparison level indicate that MRI has a sensitivity of 86 percent (95 percent CI 79–91 percent) and a precision of 93 percent (95 percent CI 88–97 percent) in detecting abscesses.

Small bowel strictures can be detected using cross-sectional imaging [EL2]. Since CT exposes patients to radiation, MRI and/or intestinal ultrasound [IUS] are the recommended approaches. In fact, none of the imaging methods will assess successfully the degree of fibrosis [EL3] [5]. A number of 239 patients,

distributed over eight studies found the importance of MRI for detecting stenosis in CD, finding 89 lesions, the most of which were in the small bowel. The sensitivity of MRI for detecting stenosis ranged from 75% to 100%, with specificity ranging from 91% to 100%. The sensitivity of MRI for diagnosis of stenosis was 89 percent (95 percent CI 84–92 percent) and specificity was 94 percent (95 percent CI 90–96 percent) when the findings of the seven studies were combined with an appropriate reference standard.

In three trials, using surgery as a reference norm, ultrasonography was shown to have a valuable predictive precision for detecting small bowel stenosis, finding 78 stenotic lesions in 156 patients. The sensitivity of ultrasound for stenosis diagnosis ranged from 74% to 100%, with specificity ranging from 89 percent to 93 percent. The sensitivity was 79 percent (95 percent CI 71–84 percent) and the specificity was 92 percent (95 percent CI 87–96 percent) when the findings from the 3 trials were combined [9].

Active inflammation and fibrosis usually co-exist, starting from the inflammatory wall thickening and advancing to the fibrotic thick wall which in time leads to fixed strictures. The two processes are commonly overlapping [10].

A comparison of intestinal segments with absent, moderate, and significant inflammation was made in another article by Rimola J [11], and it revealed a progressive and important increase in certain MR parameters, such as relative contrast enhancement, post-contrast wall signal intensity, lymph node enlargement, wall thickness and the presence of edema, pseudopolyps or ulcers.. At MR, relative contrast enhancement, wall thickness, the prevalence of ulcers, and the presence of edema were all independent predictors of CDEIS (Crohn's Disease Endoscopic Index of Severity) in a region. According to the logistic regression analysis coefficients, there was a strong association between the segment's CDEIS and the MR index. The MR index was highly accurate in detecting disease activity as well as ulcerative lesions in the colon and terminal ileum [11].

The use of MRI for the diagnosis of Crohn's Disease is becoming more widespread. The aim of this analysis was to identify and validate MRI predictors for an active CD or extreme CD, as well as a relatable Magnetic Resonance Index of Activity (MaRIA). The MaRIA Score is defined as following:1.5*wall thickness + 0.02*RCE + 5*edema + 10*ulceration. (RCE= relative contrast enhancement).

In another article from Rimola J [12], using CDEIS as a reference, it was observed that the following were independent predictors of disease severity: wall thickness, relative contrast enhancement (RCE), presence of edema, and ulcers on

MRI. For each of the segments, an estimation of activity was made using this regression model, or another one with simplified coefficients (MaRIA) correlated with data. Both of the used indexes had an elevated and equal accuracy in the diagnosis of active disease in the validation cohort.

In the article of Ordas I *et al*. [13], is shown the MaRIA score is an accurate measure of inflammation in CD, the correlation between overall CDEIS and overall MaRIA, including both baseline and week 12 was highly significant.

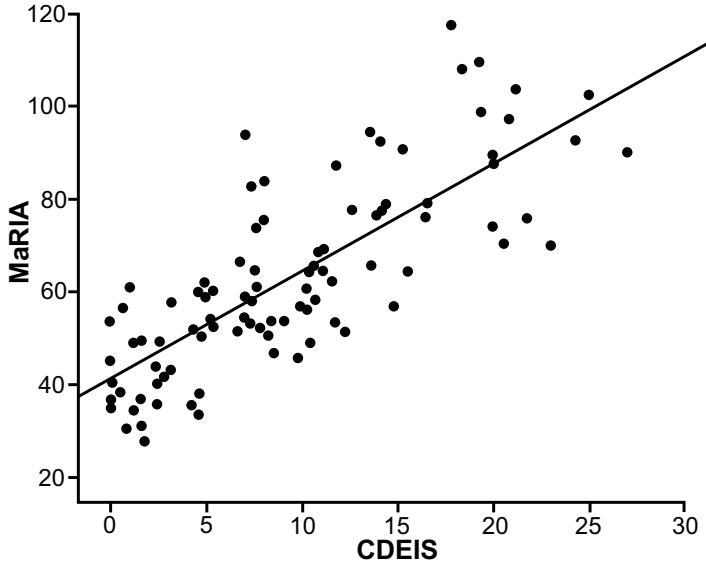

Fig (2). Correlation between CDEIS and MaRIA (per patient).

In the article by Punwani S *et al*. [14], the acute inflammatory score (AIS) (based on edema, mucosal ulceration, quantity and depth of neutrophilic infiltration) and the degree of fibro-stenosis were performed at each site and the findings were correlated with MRI features. On T2-weighted fat-saturated images, AIS was conclusively associated with mural thickness and mural/CSF signal strength ratio, but no association was identified with mural enhancement at 30 and 70 seconds. In layered mural enhancement, AIS was higher, a trend that is commonly correlated with coexisting fibro-stenosis (75 percent).

Identification of Damage Components

Regarding the Lémann Index, in order to create and properly analyse the index, the digestive tract had to be divided in separate organs, specifically into 4 categories as follows: upper digestive tract, small bowel, colon/rectum and anus. Each of these organs was then further divided into separate smaller segments:

upper digestive tract- 3 segments: esophagus, stomach and duodenum, for the colon/rectum – 6 segments: cecum, ascending colon, transverse colon, descending colon, sigmoid colon and rectum) and for the anus, one segment. As for the small bowel intestine, each of the discovered lesions within a length of 20 cm was considered to represent one segment. The total number of segments for the small bowel was capped at 20. In the protocol, surgical procedures were defined by the grade of severity for each of the mentioned organs. On a scale ranging from 0 to 3, where 0 is representing none and 3 is representing resection, stricturing and penetrating lesions were defined and illustrated similarly, also by the grade of severity, with a range starting from 0 to 3, where 0 is representing none and 3 is representing maximal, per investigational method. For each of the segments, a damage evaluation range was established. The damage evaluation was represented by a scale ranging from 0.0 to 10.0 where 0 is representing no damage while 10.0 represents maximal damage or complete resection. Also, in this evaluation it was taken into account the presence and length of stricturing lesions and/or the presence of penetrating lesions. Furthermore, for each organ, a cumulative damage evaluation was calculated.

The four calculated organ damage evaluations that resulted were then standardized to a scale ranging from 0.0 to 10.0, according to the total number of segments per organ [1]. For the validation of Lemman Score, 12 centers, between them and also our center, each including at least 10 CD patients and validated by entero-MRI and endoscopy the score-coordinator Pariente. As for the CROCCO study, that will follow starting from this year, a 3 year prospective study with the role of measuring the evolution of fibrosis using the Lemman score.

Patterns of Fibrosis Development A Histopathological Approach

Smooth muscle hyperplasia of the SM (submucosa), hypertrophy of the MP (muscularis propria), and chronic inflammation were the most notable histopathological characteristics of the stricturing intestines. Muscle modification was also found to be widespread in all layers. Chronic inflammation was shown to be strongly associated with total muscular hyperplasia or hypertrophy. In comparison, fibrosis was adversely associated with total muscular hyperplasia or hypertrophy. Muscular hyperplasia in the SM was also linked to aggressive inflammation within MU.

To summarise, the smooth muscle hyperplasia/hypertrophy contributed the most to the stricturing phenotype. Fibrosis was shown to be less significant in CD-associated 'fibro-stenosis'. Regarding the pathogenesis of Crohn's strictures, we might say that the 'inflammation-smooth muscle hyperplasia axis' can be the most important [14].

Pathophysiology of Intestinal Damage in CD: A Source of New Therapeutic Targets and Strategies

In the pathogenesis of stenosis and fistulizing lesions, several different pathways can be involved. Lesions in the transmural space, especially fibrostenosing strictures, are the product of increased tissue remodeling. Uncontrolled extracellular matrix (ECM) formation may result in the obstructive lesions as a result of tissue remodeling. Around 95% of intra-abdominal fistulas seem to emerge throughout or around the proximal end of a stricture. Mechanical variables such as intraluminal pressure appear to take a role in the formation of fistulae, as shown by the fact that intra-abdominal fistulae appear to pass through the muscular layer along penetrating vessels.

The production of chemokines, growth factors, and profibrotic cytokines by the innate and adaptive immune systems results in the activation of mesenchymal cells. This activation occurs during chronic inflammation in CD, when the epithelial and endothelial defenses are significantly compromised. Elevated ECM deposition and architectural distortion appear in the lack of ongoing inflammation due to an increase of profibrotic factor activity as a result of mesenchymal cell activation [15].

Which are the Current Therapeutic Approaches Able to Reduce the Fibrogenic Process and Possibly Induce Fibrosis Regression?

In a study from Van Assche, between the years 1995 and 2006, 237 dilatations were performed in 138 patients (with a mean age of 50.6613.4 and a 56% female) for a clinically obstructive stricture (defined as <5cm, 84% anastomotic).

A first dilatation was found to be effective in 97 percent of cases, with a 5% severe complication rate. Recurrent obstructive conditions necessitated a new dilatation in 46 percent of patients and surgery in 24 percent after a total follow-up of 5.8 years. A need for new intervention was not indicated by elevated C-reactive protein levels or endoscopic disease activity. None of the concurrent treatments had an impact on the result. This study shows that the long-term effectiveness of endoscopic dilatation for Crohn's disease surpasses the chance of complications. Repetitive dilatation or surgery is not expected by active illness at the point of dilatation or surgical therapy subsequently.

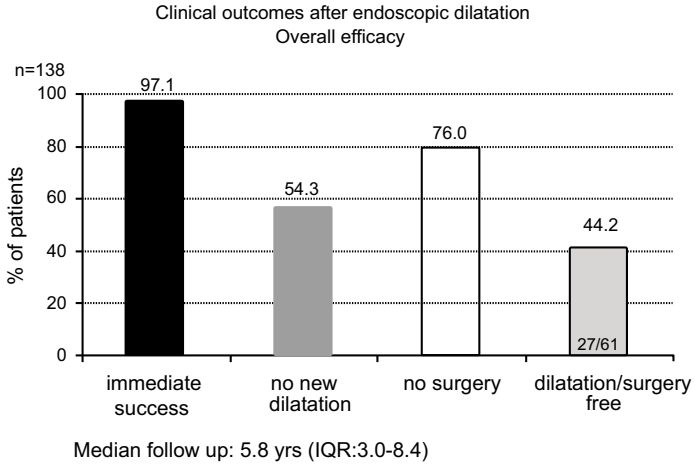

Fig. (3). Clinical outcomes after endoscopic dilatation.

At the conclusion of the study, 71.4 percent of patients treated with 5-aminosalicylic acid (5-ASA), 44.2 percent of patients treated with azathioprine, 47.8 percent of patients treated with anti-TNF treatment, 50 percent of patients treated with budesonide, and 47.4 percent of patients who were not treated required a further dilatation and/or surgery. Patients treated with 5-ASA have a prolonged follow-up than the other patient categories [16].

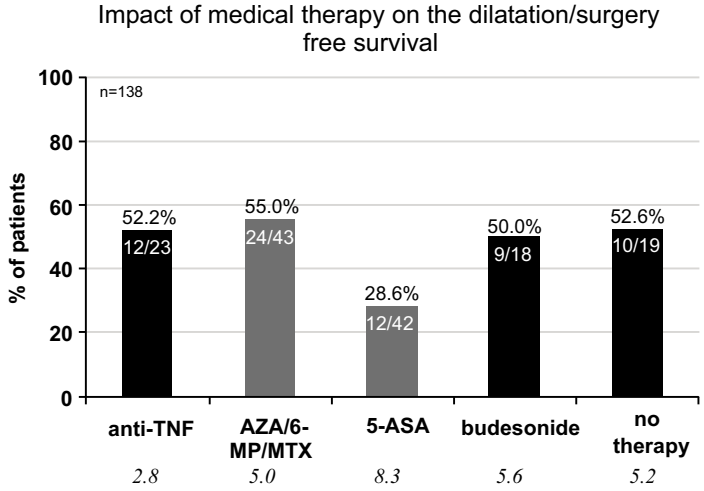

Fig. (4). Impact of medical therapy on the dilatation/surgery free survival.

In the study by Scheibe K *et al*., it was reported that the concentrations of IL36A in fibrotic intestinal tissues of patients with CD were higher than in control individuals. In fibroblasts, IL36 increased the expression of genes that control

fibrogenesis. Suppression or deletion of the IL36R gene results in a decrease in chronic colitis and intestinal fibrosis in mice. To conclude, agents that inhibit IL36R signaling can be introduced for the prevention and treatment of intestinal fibrosis in IBD patients [17]. Local ROCK inhibition prevents and reverses intestinal fibrosis by decreasing MRTF and p38 MAPK activation and increasing autophagy in fibroblasts, according to the article Holvoet *et al*. Overall, the findings suggested that local ROCK inhibition as a CD incorporate therapy may be effective in preventing fibrosis [18].

CONCLUSIONS

Early diagnosis of fibrosis is of great importance. Fibrosis is certainly reversible in animal models. Instruments that can be used in the clinical trials are in development. The duration of treatment and toxicity are challenging for the time being. The future looks promising, but there is a need for improvement in methodologies for target discovery and pre-clinical drug efficacy testing.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author confirms that this chapter contents have no conflict of interest.

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Declared none.

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CHAPTER 10

The Quality of Life of Patients with Inflammatory Bowel Disease: A Continuous Challenge

Mircea Diculescu[1] and **Tudor Stroie**[1,*]

[1] *Fundeni Clinical Institute Bucharest, University of Medicine and Pharmacy "Carol Davila" Bucharest, Romania*

Abstract: Inflammatory bowel diseases (IBDs) are chronic conditions of the gastro-intestinal tract with a remitting and relapsing course and an unpredictable evolution.

Patients affected by these diseases often have to deal with severe abdominal pain, diarrhea and loss of bowel control, fatigue, multiple surgeries and a wide range of extra-intestinal manifestations. Given these facts, the majority of them have a severely impaired health-related quality of life (HR QoL) and they are more prone to developing anxiety and depression.

Even though early clinical trials didn't show much interest in it, assessing the patients' QoL has become, over time, one of the main endpoints of the clinical trials, thus more and more articles involving the patients' QoL being published every year. Patients with active disease have a significantly lower HR QoL compared to those with inactive disease. Regarding the disease phenotype, especially when in remission, patients with Crohn's disease tend to have lower QoL than those with ulcerative colitis.

Anxiety and depression have a significant impact on the patients' HR QoL. Another concern regarding the patients with IBD is the high rates of fatigue. Fatigue is a common symptom in many other inflammatory conditions like rheumatoid arthritis or multiple sclerosis, and leads to a significant impairment of the QoL and lowers work productivity. In spite of this, it is frequently underdiagnosed or overseen by physicians, and many times remains unexplored and untreated.

Keywords: Anxiety, Depression, Fatigue, Inflammatory bowel disease, Quality of life.

INTRODUCTION

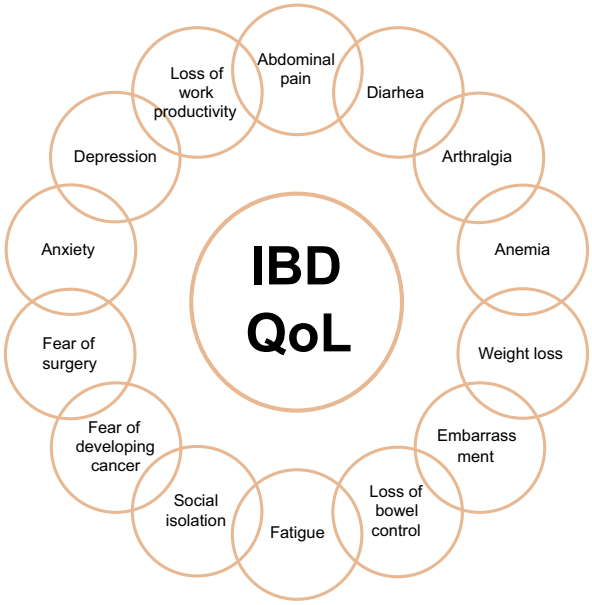
Inflammatory bowel diseases (IBDs) are chronic conditions of the gastrointestinal tract with a remitting and relapsing course. Crohn's disease (CD) and ulcerative

* **Corresponding author Tudor Stroie:** Fundeni Clinical Institute Bucharest, University of Medicine and Pharmacy "Carol Davila" Bucharest, Romania; Tel: +40744902086, Fax: +40213180447; E-mail: stroie.tudor@gmail.com

Ioan Sporea and Alina Popescu (Eds.)

colitis (UC) represent the 2 subtypes of IBDs. Their etiology is not completely elucidated, but there is strong evidence on its multifactorial nature, involving environmental factor, genetic predisposition, intestinal microbiome and the altered immune response [1, 2]. The prevalence of IBDs is increasing, in Europe 322/100.000 inhabitants being diagnosed with CD and 505/100.000 inhabitants with UC [3].

Being a chronic disease with an unpredictable course, with sometimes embarrassing symptoms like chronic diarrhea, urgency, abdominal pain, arthralgia, undesired weight loss, anemia and the possibility of perianal involvement or the need for an ostomy, they have a significant impact on the patients' quality of life (QoL).



Patients with IBD are prone to develop anxiety and depression. The rates of anxiety are up to 19.1% in patients with IBD (*vs* 9.6% in healthy controls) and those of depression up to 21.2% (*vs* 13.4% in healthy controls) as showed by a clinical study [4]. Patients show significant concerns about the course of their disease, the possibility of developing cancer or the need for surgery [1].

Even though early clinical trials didn't show much interest in it, assessing the patients' QoL has become, over time, one of the main endpoints of the clinical trials, thus more and more articles involving the patients' QoL being published every year (currently, more than 400 new articles every year) [1].

Health-related Quality of Life (HR QoL)

The HR QoL can be explored using either generic measures that allow us to compare groups of patients with different pathologies or disease specific measures. Patients with IBD have a significantly poorer QoL compared to the healthy or general population, involving both mental and physical functions [1].

A French study conducted on 1185 patients with IBD shows that half of the patients have an impaired HR QoL (SIBDQ<45: 53.3%), suffer from extreme fatigability and exhaustion (FACIT-F<30: 47.4%) or have depressive symptoms (HADS-D>7: 49.4%). One third reported symptoms of anxiety (HADS-A>7: 30.3%), 22.4% had a moderate and 11.9% severe disability according to IBD-DI score [5].

According to a clinical study conducted by Casellas *et al*., it seems that symptomatic activity of the disease and socio-demographic variables (gender, level of education), along with the need for hospitalization and recurrence/year index are the most important predictive factors for an impaired HR QoL in patients with IBD [6].

However, when comparing patients with IBD and those suffering from other chronic conditions, such as irritable bowel syndrome (IBS), rheumatoid arthritis, chronic hepatitis or multiple sclerosis, the HR QoL seems to be similar, many studies presenting divergent results, especially those comparing the HR QoL of patients with IBD and IBS [1].

Regarding the activity of the disease, it has been shown that patients with active disease have a significantly lower HR QoL compared to those with inactive disease, in both mental and physical scores, but more pronounced for the mental function [7].

Overall, patients with CD tend to have a lower QoL compared to those with UC, but the differences were borderlines significant in the recent meta-analyses. When in remission, patients with CD have a significantly lower HR QoL compared to patients with UC. However, patients with the active disease tend to have a similar HR QoL, regardless of the IBD subtype [7].

The HR QoL of patients may improve over time. Many clinical studies report that patients with longer disease duration have a better HR QoL, which may be due to an adjustment to the chronic condition and an improvement of the self-management strategies [7].

The HR QoL is also influenced by the unpredictable remitting-relapsing course of the disease. One study shows that the level of certainty is strongly associated with the HR QoL in patients with CD. Self-epistemic authority (the amount of confidence one has in his own personal expertise and knowledge in a domain in order to make a certain judgement) correlates with the level of certainty, while the information gathered by patients from the Internet was associated with decreased certainty [8].

Anxiety and Depression

Anxiety and depression represent the clinical condition that significantly impacts the patients' HR QoL. Even though there is strong evidence that patients with IBD have higher rates of anxiety and depression, only a minority of them receive the appropriate medical care for them [9, 10]. Major depression can increase symptom burden in chronically ill patients and determine additive functional impairment, increase the costs associated with medical care and lead to a lower self-care and adherence to medical recommendations [11].

The prevalence of depressive symptoms in IBD patients is up to 21.6%, with a higher prevalence in CD patients (25.3%) compared to UC patients (16.7%). Also, patients with active disease have higher rates of depression (40.7%), compared to those in remission (16.5%). The depressive disorder is reported to have a prevalence of 15.2% in patients with IBD, which is significantly higher compared to the general population (estimated to 5.9%) [10]. The prevalence of depression in patients with IBD seems to be similar to the one reported for other chronic conditions, such as diabetes, cancer, multiple sclerosis, rheumatoid arthritis and Parkinson's disease [10]. Coexisting depression is associated with a worse course of the disease and with a lower response to treatment [12].

The prevalence of anxiety symptoms in the IBD population is reported to be 35.1%, with no significant differences between IBD subtypes. Patients with active disease have significantly higher rates of anxiety symptoms (75.6%), compared to patients in remission (31.4%). The prevalence of generalized anxiety in a disorder is 20.7%, which is 2-5 times higher than in the worldwide general population (estimated to be 7.3%) [10].

The estimates for anxiety are similar to those reported for other chronic diseases such as diabetes and COPD, but higher compared to those reported for patients with cancer or multiple sclerosis. Considering only patients with active disease, the rates of anxiety are higher than those for patients with diabetes and COPD [10].

Patients suffering from anxiety and depression report more frequently digestive symptoms, even in the absence of inflammation. Moreover, anxiety and depression may increase the risk of disease flares and complications and may reduce the efficiency of the therapy [13].

Depression is increasingly considered a multi-system disease, inflicting changes in the endocrine, cardiovascular and immune systems. It can alter the immune function, inducing immunosuppression or inflammation. It has been shown that patients diagnosed with generalized anxiety disorder have dysregulated T-cell function [14].

Whether anxiety and depression contribute to the development of IBD or their onset is after the diagnosis of IBD is still a matter of debate. It has been demonstrated that stress can alter intestinal permeability and immune response, which plays a pivotal role in the etiology of IBD [15]. Pharmaceutical interventions, such as corticosteroids, may contribute to the onset of psychiatric symptoms [12]. Being a disease with an unpredictable course, the fear of the prognosis, the necessity of sometimes multiple surgical interventions and the possibility of cancer development can all lead to symptoms of anxiety [15].

There is also evidence that psychological comorbidities can augment the risk of IBD relapse. Studies on animal models of colitis show that psychological stress can induce inflammation at the level of the digestive tract. On the other hand, the presence of the mediators of inflammation can itself be the cause of mood disorders. The control of inflammation can lead in many cases to the improvement of the psychological disorder [13].

One study shows that patients dealing with anxiety and depression are more likely to develop extra-intestinal manifestations and they have a significantly higher symptom burden. They present more frequently to the emergency room, use more testing and have a higher number of hospitalizations, leading to higher costs associated medical care [13]. They also tend to have multiple abdominal imaging evaluations [16].

Other study reports that patients with CD previously diagnosed with anxiety or depression had a modestly higher rate of surgical interventions, and an increased use of corticosteroids, immunosuppressive therapies and anti-TNF agents [16].

Finally, it is important that clinicians screen the patients for these disorders at the time of the diagnosis and during each flare, and refer them for further medical care if necessary. Appropriate treatment can lead to a significant improvement in the patients' HR QoL and a better functioning [12].

Fatigue

Fatigue is a commonly encountered symptom in patients with IBD. It is defined as a lack of energy associated with a limitation of daily activities, and it is not relieved by rest [17].

Fatigue it is a common symptom in many other inflammatory conditions like rheumatoid arthritis or multiple sclerosis, and leads to a significant impairment of the QoL. In spite of this, it is frequently underdiagnosed or overseen by physicians, and many times remains unexplored and untreated.

Fatigue has multiple etiologies in patients with IBD, such as: inflammation, anemia (caused by chronic bleeding, deficiencies in iron, vitamin B12 or folate or anemia of chronic disease), micronutrients deficiencies (caused by malabsorption, accelerated intestinal transit and diarrhea, self-imposed dietary restrictions, adverse effects of medications, intestinal dysbiosis and dysregulations of the gut-brain axis) [17].

A clinical study reveals that approximately half of the newly diagnosed patients with IBD are also affected by fatigue: 42% - 47% of patients with UC and 48% - 72% of patients with CD [18].

At least twice as many patients with IBD suffer from chronic fatigue compared to healthy controls and it seems that it is more frequently encountered in patients with CD. The presence of IBD symptoms, the hemoglobin level and altered sleeping habits are among the predictive factors for fatigue in IBD [19]. Other factors associated with fatigue are the lack of education beyond primary school, part-time work schedule and other comorbid conditions [20].

Moreover, the lack of energy seems to be one of the most burdensome symptoms in patients' view, even more than gastrointestinal symptoms such as diarrhea [21]. In a Norwegian study encompassing 440 patients with IBD, those with active disease had significantly higher scores for fatigue compared to those with the disease in remission. Self-perceived disease activity, poor sleep quality, anxiety and depression were all associated with fatigue [22].

This could be explained by the fact that inflammation is associated with an accelerated catabolic state, patients with CD and active disease having a significantly increased resting energy expenditure per body weight compared to patients with inactive disease (28.8 ± 5.4 *vs* 25.9 ± 4.3 kcal/kg) [23].

Patients with the active disease also have to deal with sleep disturbances. A clinical study assessing the sleep quality of patients with CD through the

Pittsburgh Sleep Quality Index (PSQI) reveals that patients with active disease have a significantly impaired quality of sleep compared to patients in remission. Active disease was associated with significantly lower sleep duration, more sleep disturbances, lower efficiency of habitual sleep, higher use of sleep medication and daytime dysfunction [24]. Another study comparing patients with the inactive disease with healthy controls using polysomnography shows that even when the disease is in remission, patients still have more sleep disturbances compared to the control group. They had significantly less rapid eye movement (REM) sleep, light sleep percentage was higher and REM latency being longer. Furthermore, desaturation <90% was more frequently encountered in patients with IBD [25].

Because of its significant repercussions on the patient's HR QoL and on the disease course, screening for fatigue is strongly advised. There are several scales that can be used to identify symptoms of fatigue, but in clinical practice it can be done by simply asking the patients if they feel or have recently had symptoms of fatigue or exhaustion. Identifying and correcting the underlying cause of anemia could lead to significant clinical improvement [17].

Quality of Life of Patients with Inflammatory Bowel Disease is modified. Searching for QoL is then mandatory and the corrections of producing factors will improve this parameter.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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CHAPTER 11

Advances in Colorectal Cancer Screening

Eftimie Miuțescu[1] and **Bogdan Miuțescu**[2,*]

[1] *Department of Gastroenterology, Faculty of Medicine, "Vasile Goldiș" Western University of Arad, Romania*

[2] *Department of Gastroenterology and Hepatology, "Victor Babeș" University of Medicine and Pharmacy Timișoara, Romania*

Abstract: Colorectal cancer (CRC) is the second most commonly diagnosed cancer in women and the third most commonly diagnosed cancer in men. There is a 5% lifetime risk of developing CRC in many regions and despite treatment, 45% of persons diagnosed with CRC die as a result of the disease. The development of molecular biology techniques and methods has allowed a thorough knowledge of the carcinogenicity process in the CCR. Currently, multiple guidelines are available that provide guidance to clinicians who refer patients to screening. Although colonoscopy is the preferred tool for detecting and diagnosing CCR, non-invasive stool-based tests are widely used. In this section we reviewed the most important studies that have been published regarding molecular biomarkers to identify new approaches, as well as metabolomics for identifying new biomarkers for colorectal cancer. Death occurring from colorectal cancer can be prevented by detecting cancer and precancerous lesions at an early stage. For achieving this goal, new screening tools are mandatory and research for better screening tests is needed.

Keywords: Colonoscopy, Colorectal cancer, Faecal test, Metabolomics, Screening.

INTRODUCTION

Digestive cancers are highly ranked all over the world in the matter of incidence and mortality. Colorectal cancer (CRC) is the second most commonly diagnosed cancer in women and the third most commonly diagnosed cancer in men [1, 2]. In 2018, the International Agency for Research on Cancer reported a worldwide incidence of 23.6/100,000 in men and an incidence of 16.3 in women and mortality of 8.9/100,000 in both men and women. In Europe, the highest incidence is found in Northern Europe (32.1/100,000), but the highest mortality in

* **Corresponding author Bogdan Miuțescu:** Department of Gastroenterology and Hepatology, "Victor Babeș" University of Medicine and Pharmacy Timișoara, Blvd. Liviu Rebreanu 156, Timișoara 300723 Romania; Tel: 004047222739 Fax: 0040256488003; E-mail:bmiutescu@gmail.com

Ioan Sporea and Alina Popescu (Eds.)

Central and Eastern Europe (15.2/100,000) [3]. Although there is an increase in colorectal cancer incidence, a decrease in mortality is observed especially in developed countries. The survival rate of patients is higher, the earlier the disease is detected. Screening programs are already implemented in western countries due to their high incidence rate, but the rising mortality rates in the Eastern part of Europe can imply a limited access to healthcare and suboptimal treatment for CRC [4].

There is a 5% lifetime risk of developing CRC in many regions and despite treatment, 45% of persons diagnosed with CRC die as a result of the disease. The adenoma to cancer sequence is known to be a process that develops over years, making it an ideal target for early detection through screening. Having the opportunity to detect the lesions in an early stage, advances in the molecular pathogenesis of CRC led to new insights and this may have an impact over the years in the strategy of detecting the precancerous and cancerous lesions of the colon [5].

COLORECTAL CANCER PATHOGENESIS

The development of molecular biology techniques and methods has allowed a thorough knowledge of the carcinogenicity process in the CCR. Understanding the pathogenetic mechanism has enabled new treatments to be introduced and accurate diagnosis and prognosis to be established (Fig. **1**) [6].

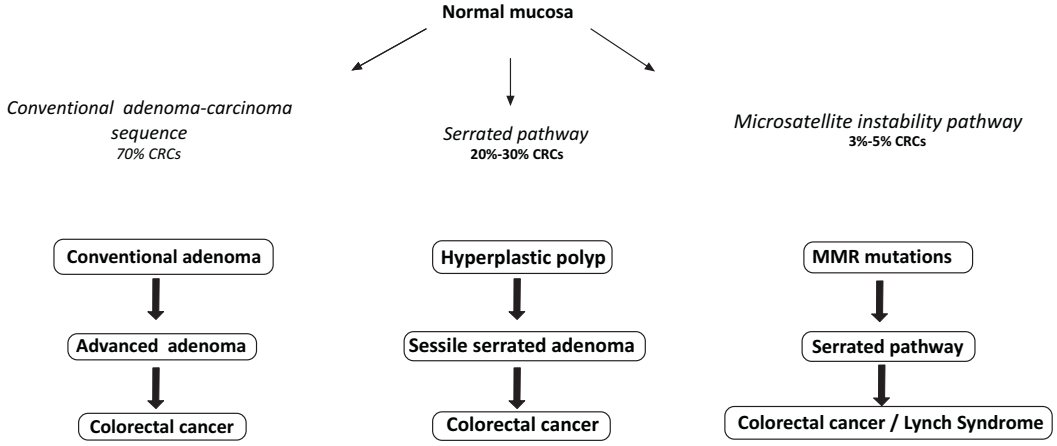

Fig. (1). Molecular pathways in CCR pathogensis. CCR: colorectal cancer. MMR: mismatch repair.

ADENOMA-CARCINOMA SEQUENCE

All the research published proves that CRC develops from precancerous lesions. Pathogenesis starts from an early dysplastic lesion to adenomatous polyps and invasive malignancy develops in the last stage. On the molecular setting, Vogelstein *et al*. published a genetic model for CRC, the adenoma to carcinoma sequence, stating that tumorigenesis usually begins with APC gene mutation and is followed by K-RAS and TP53 mutations.

SERRATED POLYP PATHWAY

This pathway is an alternative to CRC evolution from hyperplastic polyps or sessile serrated adenomas where BRAF mutations are the initial event. Dysbiosis in the intestinal microbiome has been implicated in the progression of the serrated polyp to adenocarcinoma, especially when excessive growth of Fusobacterium nucleatum is detected. The prognosis is difficult to be assessed, but a combination of different mutations such as high CIMP (CIMP-H), microsatellite stability (MSS) and BRAF mutation, can have the worst outcomes [7].

CHROMOSOMAL INSTABILITY PATHWAY/APC PATHWAY

Chromosomal instability occurs in 70% of patients with CCR. It is demonstrated by the loss of chromosomal material that causes the tumor suppressor genes to inactivate: APC gene at the level of the 5q chromosome arm, TP53 at the 17p arm. The CCR of phenotype LOH+ (loss of heterozygosity) is caused by genetic alterations such as aneuploidy, chromosomal instability, mutations of Kras (Kirsten Ras) and TP53. Colon cancers developing from CIN have worse outcomes than those with microsatellite instability [8].

MISMATCH REPAIR (MMR)

The instability of microsatellites is a genetic instability involved in colorectal carcinogenicity and is caused by the alteration of the genes involved in the mismatch repair genes. They are found in 80% of the cases of hereditary non-polypoid colon cancers (HNPCC) and in 15% of sporadic cancers. The HNPCC-characteristic MSI+ phenotype is the result of genetic instability. The genes involved are anti-mutation, stability (hMSH2, hMSH3, hMSH6, hMLH1, hPMS1, hPMS2) that repair defects that occur in DNA. The genome of these genes, called RER+ (positive replication error), precedes the mutations in the APC gene.

DNA errors are frequent in cells with mismatch repair. A deficient mismatch protein system leads to the expansion or contraction of these microsatellites, thus called microsatellite instability [9].

GUIDELINES RECOMMENDATIONS FOR CCR SCREENING

When referring to colorectal cancer (CRC), multiple guidelines are available that provide guidance to clinicians who counsel and refer patients to screening and the vast majority of them use colonoscopy or faecal tests as their first recommendation as a screening tool in average risk adults between 50 and 75 years. Guidelines have variable recommendations regarding the use of colonoscopy, optimal test to be first recommended, age interval for screening and screening interval [10]. There are several guidelines that have been published or updated in the past few years. A summary is presented in Table **1**.

Table 1. Colorectal cancer screening guidelines.

Location	Professional Society	Publication	Age Interval	Screening Option
Norh America	ACS	2018	≥ 45	Faecal tests: • FIT - annually • gFOBT - annually • DNA stool – every 3 yrs • Endoscopic examinations: • Colonoscopy (10 yrs) • CT Colonography (5 yrs) • FS (5 yrs)
	USPSTF	2017	50-75 76-85	First-tier: • Annual FIT • colonoscopy (every 10 yr) Second-tier: • CTColonography (5 yrs) • FIT/DNA stool test (3 yrs) • FS (5-10 y) Third-tier: Capsule colonoscopy (every 5 yrs) – if no screening history
Europe	Germany: GGPO	2014	≥ 50	Preferred test: Colonoscopy (10 yrs) Alternatives: - FS (5 yr) with annual FOBT - FOBT annually
	European Guidelines	2013	50-74	Recommended test: gFOBT or FIT (every 1-2 yrs) Alternatives: colonoscopy (10-20 yrs) \ FS (10-20 yrs)
Asia	Asia Pacific	2015	50-75	Preferred test: FIT Alternatives: FS/colonoscopy

In North America, in May 2018, the American Cancer Society (ACS) released updated colorectal cancer screening guidelines [11]. One of the most important changes to the previously published guideline is the age at which screening should start. The recently published ACS guideline recommends that screening should start at the age of 45 years in average risk adults and regular screening should be performed, according to patients' preference, with high sensitivity stool based or visual examinations [11]. Previously, the ACS recommended that this population start CRC screening at the age 50 years [12]. Patients that use for screening other tests than a colonoscopy and have an abnormal test result, should be scheduled for a colonoscopy. There is an evidence that patients will have a preference for one type of screening test over the others if provided sufficient information regarding these test attributes [13].

The American College of Gastroenterology (ACG) recommends that colorectal cancer screening should start at the age of 50 and colonoscopy should be performed at 10 years interval [14].

The US Preventative Services Task Force (USPSTF) recommends screening in average-risk individuals aged 50 to 75 years, with a decreased benefit after the age of 75, especially in adults with screening history. Nevertheless, a healthy person aged 76 to 85 without previous screening is very likely to benefit from screening. It is also recommended that patients aged 76 through 85 years to continue their screening if their overall health status indicates so. These recommendations are currently in the process of being reviewed and may be updated [15].

In 2012, The European Colorectal Cancer Screening Guidelines Working Group recommends screening individuals between ages 50 and 74. Authors conclude that current evidence is in favor of a 10 year surveillance period when colonoscopy is being used as a screening tool. Both faecal stool tests, gFOBT and FIT (fecal immunochemical test) are considered to be effective, but FIT is recommended to be superior in terms of specificity and sensitivity. The European Colorectal Cancer Screening Guidelines Working Group does not recommend FOBT with flexible sigmoidoscopy, virtual colonoscopy, faecal DNA testing or capsule endoscopy [16, 17].

In 2014, The German Guideline Program in Oncology (GGPO) recommended starting screening in average-risk atadtuhlets age of 50 years without establishing an upper age screening limit. The authors concluded that this is due to lack of studies concerning the benefit of screening for CRC older individuals.

They say that colonoscopy is recommended as "gold standard" (every 10 years) and CT and MR-colonography should be used in patients with incomplete colonoscopy, especially if they are requesting a complete colonic examination [18].

Like many other guidelines, the updated Asia Pacific Consensus Recomm endations on CRC screening, published in 2015, mentions that screening should be offered to average-risk adults between 50 and 75 years old. Colonoscopy is considered to be the gold standard and should be the preferred screening method among endoscopic examinations, but flexible sigmoidoscopy is also appropriate for screening. Stool based tests are recommended, but quantitative FIT should be preferred over gFOBT [19].

CURRENT STATE OF SCREENING METHODS FOR CRC

CRC is a global health problem and, still, the optimal screening test has not been established yet. There are several options and physicians should weigh before recommending a screening method. Colonoscopy and non-invasive stool tests, such as the faecal immunochemical test (FIT), are the most commonly used worldwide. Understanding the advantages and adverse events that are associated with each tool can guide health practitioners in recommending the optimal test for their patients [20].

Guidelines recommend stool-based tests and structural examinations as options for colorectal cancer screening [21]. The pros and cons of CRC tests currently in use are described in Table **2**.

RECENT UPDATES IN CRC SCREENING METHODS

Within the final decade, noteworthy research has been made concerning the adequacy of distinctive strategies accessible for screening of CRC. Therefore, our main focus will be on the different screening strategies for asymptomatic average-risk adults. In the United States of America and worldwide, a myriad of professional associations' guidelines recommends screening for CRC in average-risk adults in the interval age of 50 and 75 years, although the recently published guideline by the American Cancer Society (ACS) mentions starting CRC screening earlier, at 45 years [21].

As of now, the focus is centered on finding CRC particular tumor markers for the improvement of an unused and non-invasive screening method. Colorectal cancer has a heterogeneous nature, therefore, in order to understand the tumor genesis, different approaches are required and needed to be studied. In this section we reviewed the most important studies that have been published regarding molecular

biomarkers to identify new approaches, as well as metabolomics for identifying new biomarkers for colorectal cancer.

Table 2. Colorectal cancer screening tests currently in use.

Screening test	Interval	Efficacy	Comments
Endoscopic methods			
Colonoscopy	Every 10 years	Mortality Reduction in a prospective study	Considered "gold standard". Majority of cases performed with sedation. Requires complete bowel preparation.
Flexible Sigmoidoscopy	Every 5 years	Proved mortality reduction in RCTs	Does not examine the entire colon. Can detect precancerous lesions. Does not require a full bowel preparation.
Stool-based tests			
gFOBT	Every year	Mortality reduction in RCTs	Higher compliance compared to endoscopic methods. A positive result needs colonoscopic follow up.
FIT	Every year	Compared to gFOBt has a higher sensitivity and specificity, but RCTs are needed.	A positive result needs colonoscopic follow up.
FIT-DNA	Every 1-3 years	Higher sensitivity but lower specificity than FIT. Unknown reduction in mortality.	Higher costs compared to gFOBT and FIT. Limited sensitivity in detecting precancerous lesions. A positive result needs colonoscopic follow up.
Radiologic method			
CT colonography	Every 5 years	Unknown reduction in mortality.	Needs complete bowel preparation. Less sensitive than colonoscopy. A positive result needs colonoscopic follow up.
Biomarker			
Septin9 DNA	Unknown	Unknown reduction in mortality.	FDA approved for CRC screening. Lower sensitivity and specificity than colonoscopy.

MOLECULAR BIOMARKERS

CRC is a complex illness decided by distinctive hereditary adjustments in oncogenes, MMR genes, tumor silencer genes, and cell cycle directing genes of the colonic surface cells. They are thought to be possible CRC biomarkers, because these molecular changes offer valuable information for high quality diagnosis, prognosis, and information on treatment response. Microsatellite instability (MSI), chromosomal instability (CIN), and the CpG island methylator phenotype are the main molecular pathways accountable for carcinogenesis. Currently, a variety of new molecular detection tools is being assessed. However, most of these methods have not yet been validated in larger cohorts using randomized controlled studies [22, 23].

METABOLOMICS

Another choice as a screening instrument is metabolomics that could have a great impact in developing a new tumor marker for the detection of colorectal malignancy. It is imperative to have good knowledge of CRC metabolites and to precisely understand its pathways in order to develop new preventive and curative options. The characteristics, pros and cons of colorectal cancer screening tools that have been recently studied are presented in Table **3** [24 - 26].

Table 3. Characteristics of colorectal cancer screening of sample types (blood, urine, stool).

Sample types	Evidence of efficacy	Advantage	Disadvantage
Blood-based biomarkers • serum • plasma, • dried blood spot	A combination of 8 metabolites (99.3% sensitivity, 93.8% specificity)	Easily accessible Less affected by diet than urine Less diurnal variation	Affected by smoking status More invasive than urine and stool Analysis can be more complex than urine
Urine	Cross-validated panel of seven metabolites (97.5% sensitivity, 100% specificity) 10 different metabolites (100% sensitivity, 80% specificity) but a rather small sample size	Easily accessible Less invasive than blood	More affected by diet than serum samples More diurnal variation More inter- and intra- subject variability than serum A full day storing at room temperature or on cool packs altered metabolite concentration
Stool	A three metabolite panel (AUC 1.0 but very small sample size) A metabolomics panel (AUC 0.94)	Easily accessible Less invasive than blood	Inconvenient to collect stool samples Low compliance

COLORECTAL CANCER SCREENING: ORGANIZED SCREENING *VERSUS* OPPORTUNISTIC SCREENING

Organised screening programmes have proven to rapidly and significantly decrease the incidence and mortality of CRC, still, few countries worldwide have implemented such programmes for colorectal cancer. Opportunistic screening (patients' own will, physicians' indication) can be an alternative to detect cancer and remove precancerous lesions in the lower gastrointestinal tract, even though it is considered to be less cost-efficient. However, physicians adopting opportunistic screening for CRC need to be aware of its limitations (overscreening, poor follow-up, quality assurance). In the United States with a generous reimbursement policy, reports show that almost 60% of the population have been screened for colorectal cancer [27].

Organized screening programs are the ideal modality to screen a population for crucial healthcare diseases, but not all countries have one implemented. In this regions, an opportunistic program can be a good option to prevent increasing the incidence and mortality through colorectal cancer [28].

CONCLUSION

Screening tools that are currently in use have their limitations and research for new screening methods is further needed. Research on metabolomics is advancing and this could lead to new non-invasive screening methods that have high compliance, high sensitivity and specificity and are cost-effective.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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CHAPTER 12

New Guidelines on Post-polypectomy Colonoscopy Surveillance

Simona Băţagă[1,*]

[1] *GE Palade University of Medicine, Pharmacy, Science and Technology Târgu-Mureş, Emergency Hospital Târgu-Mureş, Romania*

Abstract: Colorectal cancer (CRC) remains a frequent tumor, in spite of the screening programs developed in most of the countries. It is well known that CRC is developing from polyps and that the polypectomy prevents the CRC and ultimately the death of the patient. One important debate is about the post polypectomy surveillance of the patients, in regard to the timing of the second colonoscopy after the baseline one. Appropriate intervals spare the patient from an unwanted colonoscopy, however, in the case of advanced lesions ensures no recurrence of the lesion. Last year, important guidelines were elaborated and revised by different societies. This chapter is summarizing the recent European, American and British guidelines which are mostly similar, with small exceptions. The updated guidelines are reducing the number of colonoscopies in patients with small adenoma and serrated polyps without dysplasia. The villous proportion of a polyp is not considered a risk factor. In the piece-meal resection is indicated a shorter period to reevaluate the patient to reduce the risk of incomplete resection. The present guidelines are decreasing the unnecessary colonoscopies in patients that are considered with no risk, reducing the costs and ensuring a better psychical comfort for the patients.

Keywords: Colorectal cancer prevention, Guidelines, Polypectomy, Surveillance, Timing of the second colonoscopy.

INTRODUCTION

Colorectal cancer (CRC) is still one of the deadliest cancers, being in the second place as a cause of death worldwide, and ranks in the third place in incidence. These facts are in spite of the actual CRC screening programs. The disease begins as polyps, and some of these untreated polyps develop into cancer and ultimately causing death.

[*] **Corresponding author Simona Băţagă:** GE Palade University of Medicine, Pharmacy, Science and Technology Târgu-Mureş, Emergency Hospital Târgu-Mureş, Romania; Tel: 0040744573787, Fax 0040265216681;, E-mail simonabataga@yahoo.com

Ioan Sporea and Alina Popescu (Eds.)

In the screening programs the detection and removal of colorectal polyps is the most effective method of preventing CRC and related deaths. Polypectomy is considered a very efficient method and the post-polypectomy surveillance is important. The timing of the second colonoscopy after polypectomy has to be precise, so the patient should have no risk of recurrence. In patients with low-risk polyps is important to reduce the number of unnecessary investigations, for the best psychical comfort of the patients.

It is a real fact that each polypectomy may save lives, and to understand the importance of colonoscopy and screening for the patients involved and further on for their relatives.

There are very recent important studies in the literature that are evaluating the best surveillance interval after polypectomies to avoid the development of colon cancer. All the studies are taking into equation the polyp characteristics, as a number, histology, size, the quality of colonoscopy in view of the current guidelines and also the clinical condition of the patient. However, the guidelines about following-up the patients with polyps, after polypectomy, are continuously being updated, as new pieces of evidence are discovered.

In 2020 there were several new guidelines elaborated by different societies most important being the European, American and British recommendations.

The 2020 ESGE, the European Society of Gastrointestinal Endoscopy developed new guidelines [1] that, as the older ones from 2013 [2], are based on some definition as:

Table 1. Definition used on ESGE guidelines [1].

Term	Definition
High quality colonoscopy	Complete colonoscopy with a meticulous inspection of adequately cleaned colorectal mucosa. Neoplastic lesions have also been completely removed and retrieved for histological examination
Index colonoscopy	First high-quality colonoscopy on which surveillance strategy is based
Metachronous lesion	Any lesion that is detected at surveillance colonoscopies

In 2020 ESGE guidelines the terms high/low risk polyps or population have been replaced with new categories as patients that after polypectomy do not require surveillance and patients that after polypectomy do require surveillance.

1. The first category: without surveillance after Polypectomy includes

• the removal of small adenomas, less than 10mm, one or maximum 4 adenomas

even with dysplasia if it is low grade dysplasia, **the** villous components are not taken into account, **or**

• any small **serrated polyp less than 10mm, with no dysplasia detected,**

• these patients should be returned to screening **as they do not need surveillance**

• or they should undergo another colonoscopy in 10 years

Multiple studies revealed that the patients with non-advanced adenomas have a very low incidence of CRC and also death, compared to or even lower than the patients with a clean index colonoscopy [3, 4]. In a recent study, a high number of patients were followed 14 years. The low-risk adenoma group of 10978 patients did not present, in the follow-up period, a significant increase in the risk of CRC (HR 1.29; 95% CI 0.89–1.88) and also did not present a significant increase in death. (HR 0.65; 95% CI 0.19–2.18) [5].

In the group of patients not requiring follow-up have been included additionally the patients with villous components if the polyps are <10 mm and also those with serrated polyps <10mm.

Patients Requiring Surveillance following Polypectomy

The recommendation of the ESGE guidelines is:

- In patients with complete removal of a large adenoma **more than 10mm or with high grade dysplasia**,
- Or in case of more than 5 adenomas, **or**
- Any large **serrated polyp more than 10mm or in case it has dysplasia.**
- **Colonoscopy is recommended after 3 years**

Multiple studies revealed that only advanced adenomas have a high risk for the development of CRC [3, 6, 7]. For example data from the Polish study demonstrated that only the adenomas ≥ 20mm had a higher risk of colon cancer incidence and death (age-adjusted HR 7.45, 95%CI 3.62 – 15.33; P < 0.001) compared to individuals with no adenomas [8].

Serrated polyp (SP) ≥ 10mm, traditional serrated adenoma (TSA), and serrated polyp with dysplasia require surveillance at 3 years [9]. A recent retrospective study evaluating 122 899 patients with 10 years of follow-up showed an increase in metachronous CRC (3.35, 95%CI 1.37 – 8.15) compared to negative

colonoscopy [10].

New from the last guidelines is the fact that the patients with polyps having villous histology are not considered high risk anymore. Recent studies are supporting this statement, and showed that the polyps with villous histology have the same risk as to the ones without it to develop neoplasia [11].

Usually in small adenoma villous features are very rare.

The important feature is for the patients with multiple adenomas, more than 10 to be adviced to make genetic tests. In the presence of more than 20 adenomas APC and MUTHY tests should be done [1].

Timing of the Second Colonoscopy

ESGE recommends that if no polyps requiring surveillance are detected at the first surveillance colonoscopy, a second colonoscopy should be scheduled after 5 years. This conclusion is similar to that from 2013, so there is no difference in the new guideline.

Continuing the idea, the patients can be included back into the screening programme, if at the second examination also no polyps requiring surveillance are detected.

In the presence of polyps requiring surveillance at first or subsequent surveillance examinations, the next colonoscopy is indicated to be scheduled at 3 years.

Most of the studies revealed that in the risk group there is a high incidence of CRC. In one study [6], the overall incidence of CRC in the high-risk group after 10 years of follow-up was nearly double than that of in the general population (SIR 1.91, 95%CI 1.39 – 2.56).

There are studies examining the interval between first and second surveillance [12]. One study showed an increased risk of advanced neoplasia per year increase (OR 1.11, 95% CI 1 – 1.24). This study showed that a 2-year interval was not statistically significant, but larger intervals, like 3 years, 4 or 6.5 years showed significance.

Another study compared the interval ≥3 with < 3 years for the timing of a second colonoscopy and found no risk [13].

Piecemeal Resection

An important issue is the follow-up of the patients after piecemeal resection, when

the polyps are very large or above 20mm. The ESGE recommendation is to repeat the colonoscopy after 3 – 6-month from the piecemeal resection, depends also on the experience of the endoscopist [1].

After the repeat colonoscopy the patients are scheduled for the next colonoscopy after 12 months to ensure that the excision was complete. Advanced imaging techniques are recommended to examine the site.

Compared to the guidelines from 2013, the repeat colonoscopy is shorter, after 3-6 months, due to the high risk of piecemeal resection. The rate of recurrence is usually high (12%– 24%) after a piecemeal resection, so a shorter interval is much safer to ensure a complete resection [14].

Family History

The ESGE guidelines recommend the same intervals of colonoscopy after polypectomy in patients with a family history of CRC.

When to Stop the Post-polypectomy Surveillance

The screening is generally recommended until 74 years of age, and it depends on every country and region guidelines [15].

Taking into account the overall life expectancy, ESGE recommends stopping post-polypectomy surveillance after 80 years. Considering colonoscopy as an invasive method, after 80 years, the indication is in the absence of cardiac or pulmonary comorbidities.

The U.S. Multi-Society Task Force (MSTF) Guidelines

The recommendations were published in 2020 for Follow-Up of the patients after colonoscopy and polypectomy in colorectal cancer by **MSTF**. These recommendations there are more stratified regarding the number of polyps and also the histology of adenomas and sessile polyps [16]. The high-risk adenomas (HRA) and the low-risk adenomas (LRA) are defined in detail.

- HRA is defined as an adenoma or serrated polyp larger than 10 mm, or with villous histology or high-grade dysplasia and the recommendations for colonoscopy are more frequent.
- LRA is defined as 1 to 2 tubular adenomas or serrated polyps smaller than 10 mm, and may not have a higher CRC risk than the patients with no adenomas. This is why they recommend extending the surveillance interval from 7 to 10 years for the LRA.

Table 2. MSTF recommendation on Follow-up patients with normal colonoscopy or adenoma [16].

Colonoscopy at Baseline	The Interval for Surveillance Colonoscopy Recommended
Normal	10 years
Small 1–2 tubular adenomas less than 10 mm	7-10 years
Small 3–4 tubular adenomas less than 10 mm	3- 5 years
Small 5–10 tubular adenomas less than 10 mm	3 years
Large Adenoma more than 10 mm	3 years
Adenoma with tubulovillous or villous histology	3 years
Adenoma with high-grade dysplasia	3 years
More than10 adenomas	1 years
Piecemeal resection of adenoma more than 20 mm	6 months

Regarding the serrated polyps the MSTF guidelines are also slightly different from the ESGE ones. They are including the hyperplastic polyps (HP), sessile serrated polyps (SSP) and traditional serrated adenoma (TSA), so all the known serrated polyps.

Table 3. MSTF recommendation on Follow-up patients with serrated polyps.

Colonoscopy at Baseline	The Interval for Surveillance Colonoscopy in Years
Less than 20 HPs in the rectum or sigmoid colon, small less than 10 mm	10
Less than 20 HPs proximal to sigmoid colon less than 10 mm	10
1–2 SSPs small, less than 10 mm	5-10
3–4 SSPs, small less than 10 mm	3-5
5–10 SSPs small, less than 10 mm	3
SSP more than 10 mm	3
SSP dysplasia present	3
HP more than 10 mm	3-5
TSA	3
Piecemeal resection of large SSP more than 20 mm	6 months

The British Society of Gastroenterology, and Association of Coloproctology of Great Britain and Ireland/Public Health England (BSG/PHE/ACPGBI) Guidelines

Recently, the BSG/PHE/ACPGBI also published new guidelines for post-polypectomy surveillance, mostly similar to the MSTF regarding the HRA [17].

They recommend 3-year surveillance for:

- Two or more premalignant polyps and at least one advanced colorectal polyp.
- The advanced colorectal polyp is defined as one adenoma larger than 10 mm with high-grade dysplasia, or serrated polyp of at least 10 mm in size or including any grade of dysplasia, or
- 5 or more adenomas.
- Villous histology is not included in their HRA risk stratification

These are considered the high-risk and for these patients' colonoscopy should be performed after 3 years [17].

The inclusion of tubulovillous/villous histology in the guidelines was considered t unjustified, compared with the additional colonoscopies that would be generated. In a recent study by Atkin *et al*, the patients undergoing surveillance for adenomas, the tubulovillous histology was not a risk factor for CRC incidence.

The BSG position on serrated polyps recommends colonoscopy at 3 years for patients with an advanced serrated polyp. The definition of the advanced serrated polyp is a sessile serrated lesion (SSL) more than 10 mm, or including dysplasia and also the traditional serrated adenomas [18].

In an analysis of the Norwegian flexible sigmoidoscopic screening study (NORCCAP), large serrated polyps, more than 10mm were associated with the same risk as advanced adenoma for colon cancer (HR 4.2 *vs* 3.3, respectively) at follow-up [10].

A recent study with 122 899 patients had the same conclusions, that the advanced adenoma and the large serrated polyps are high risk lesions for colon cancer [9].

The UK guidelines are also addressing the age of a patient. If a patient is >10 years younger than the lower screening age and has premalignant polyps but no high-risk findings, the endoscopist may schedule the surveillance colonoscopy at 5 or 10 years.

DISCUSSION

Polypectomy is the most important method in decreasing the incidence of CRC. Most guidelines are similar and all try to reduce the burden of unnecessary colonoscopies, twhich are expensive and also, usually unwanted by the patient [19, 20]. Surveillance colonoscopy, after polyp removal should be targeted at the patients who are really benefitting from it and the trend is to reduce the frequency in patients who are not at risk.

In all the guidelines, there are important factors that matter as:

1. Cleansing of the bowel

If the bowel is not well prepared, that, ESGE recommends a second colonoscopy in a year, so the patient has actually to repeat the colonoscopy. ESGE defines adequate bowel preparation as: Boston Bowel Preparation Scale ≥ 6, Ottawa Scale

≤ 7, or Aronchick Scale excellent, good, or fair [21, 22].

2. Quality of the baseline colonoscopy

In a well-prepared bowel, the polyps should all be detected and all the polyps are completely removed.

3. Adenoma detection rate (ADR)

The higher adenoma-detecting endoscopists have lower post-colonoscopy (interval) CRC incidence and mortality rates [23]. ADR is acceptable 30% in men and 20% in women.

4. Cecal intubation rate.

Cecal intubation rate should be above 90%, preferably >95%, this is a very important feature for a good colonoscopist.

Between the guidelines there are not many differences, as it could be seen in this table:

Table 4. Differences between the three guidelines.

Findings at Baseline Colonoscopy	ESGE	MSTF	BSG/PHE/ACPGBI
Small 1–2 tubular adenomas less than 10 mm	Return to routine screening	7-10 years	Return to routine screening
Small 3–4 tubular adenomas less than 10 mm	Return to routine screening	3-5 years	Return to routine screening
Small 5–10 tubular adenomas less than 10 mm	3 years	3 years	3 years
Large Adenoma more than 10 mm	3 years	3 years	3 years
Adenoma with villous histology or tubulovillous	3 years Only if >10mm	3 years	3 years Only if >10mm
Adenoma including high-grade dysplasia	3 years	3 years	3 years
Large adenoma more than 20mm Piecemeal resection	3-6 months	6 months	2-6 months

(Table 4) cont.....

Findings at Baseline Colonoscopy	ESGE	MSTF	BSG/PHE/ACPGBI
Less than 20 HPs in the rectum or sigmoid colon less than 10 mm	Return to routine screening	10 years	Return to routine screening
Less than 20 HPs proximal to sigmoid colon less than 10 mm	Return to routine screening	10 years	Return to routine screening
Small 1–2 SSPs less than 10 mm	Return to routine screening	5-10 years	Return to routine screening
Small 3–4 SSPs less than 10 mm	Return to routine screening	3-5 years	Return to routine screening
SSP more than 10 mm	3 years	3 years	3 years
SSP including dysplasia	3 years	3 years	3 years
Traditional serrated adenoma	3 years	3 years	3 years

Compared to the oldest guidelines, the small adenoma and also the small serrated polyps without dysplasia are considered with no risk, so most guidelines recommend returning the patient to the usual screening guidelines.

Another new feature is that in the ESGE guidelines and BSG/PHE/ACPGBI, the villous component is not considered as a risk factor in small polyps.

CONCLUSION

In the last years the number of colonoscopies has increased due to colon screening programs. An important question is how to follow-up the patients after polyps removal. The classification of patients who have to be followed-up and patients who do not,, and may be directed to routine screening programs is very important, very clear and is imperative to be applied.

In this way the number of colonoscopies is decreasing, together with the cost given for unnecessary investigations and the patients are less stressed.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 13

Artificial Intelligence in Gastrointestinal Endoscopy

Radu Bogdan Mateescu[1,2] and **Theodor Alexandru Voiosu**[1,2,*]

[1] *University of Medicine and Pharmacy Carol Davila, Faculty of Medicine, Bucharest, Romania*

[2] *Gastroenterology Department, Colentina Clinical Hospital, Bucharest, Romania*

Abstract: Artificial intelligence (AI) in endoscopy refers to the capacity of computer algorithms using "machine learning" to aid in the detection and characterization of lesions in the digestive tract. The field of AI in endoscopy is expanding at a very rapid pace and, while the potential for development is enormous, the only validated applications currently available in everyday practice are computer-assisted detection and characterization of colonic polyps. The main advantage of machine learning is the capability of analyzing vast quantities of data to detect patterns that are not readily available to the endoscopist, thus theoretically increasing the accuracy of detection and diagnosis of the predefined lesion. However, the current technology is still heavily reliant on adequate image databases which have to be appraised by expert endoscopists before the algorithms can be trained on these datasets. Furthermore, each individual algorithm is trained to answer very specific questions, usually in a binary fashion (*i.e.* – is the polyp neoplastic or hyperplastic?).

Endoscopists need to be aware of the developments in the field, because in the near future such applications as detection and characterization of early esophageal and gastric cancer might also be included in their diagnostic armamentarium. Finally, several ethical and practical questions regarding the implementation of AI-based diagnosis and treatment in everyday practice need to be addressed by the academic and medical community before the large-scale adoption of AI in endoscopy becomes a reality.

Keywords: Algorithms, Artificial intelligence, Cancer, Colonoscopy, Computer-assisted detection, Computer-assisted diagnosis, Deep learning, Endoscopy.

[*] **Corresponding author Theodor Alexandru Voiosu:** University of Medicine and Pharmacy Carol Davila, Faculty of Medicine, Bucharest, Romania and Gastroenterology Department, Colentina Clinical Hospital, 19-21 Stefan cel Mare Bvd, Bucharest, Romania; Tel: +40726732764; E-mail: theodor.voiosu@gmail.com

Ioan Sporea and Alina Popescu (Eds.)

INTRODUCTION

Artificial Intelligence: Basic Notions for the Endoscopist

Artificial intelligence (AI) is a very broad term which refers to the capacity of machines to mimic cognitive tasks such as "learning" or "problem-solving" [1]. The colloquial use for the term covers a wide range of functions performed by machines from autonomously operating cars to engaging in strategic games or understanding human speech. With respect to endoscopy, the main application of AI currently under development is *machine learning,* which refers to the iterative use of complex mathematical models and algorithms to capture structure in data [2]. This is currently achieved by using a form of machine learning called *deep learning*, which involves a neural network with several layers of algorithms, triggered in a cascade fashion, and exploiting the hierarchical relations in the data being analyzed.

While the mathematical and conceptual details behind machine learning are beyond the scope of this discussion, the basic construct of AI models used in endoscopy can be broken down in 3 main processes: training, validation and testing of the model [3]. Broadly speaking, the first phase requires feeding a large amount of labelled data (*i.e.* still images of videos of various lesions – polyps, tumors, *etc.*) into the algorithm, which can break down the data according to the salient features it recognizes as useful discriminators (size, shape, color, mucosal pattern *etc.*). This is followed by a second step in the process, wherein a new, unlabeled set of data is used to assess the performance of the AI algorithm and perform a sort of "fine-tuning" which ensures that the model is not over-fitted to the training data set, meaning that it can be applied successfully to previously unseen data. Finally, the algorithm is assessed by using a third, independent set of data to check its performance in real life.

In many ways, deep learning medical applications have been likened to a "black box" – data goes in the machine and, through an inscrutable, opaque process, a decision, or "label" is returned by the algorithm [4]. The decision process, because of its complexity, cannot be fully explained to the clinician and, indeed, to some extent, it remains impenetrable to the programmer or algorithm constructor. However, it is important that the clinician understand the main limitations of current AI applications, in order to ensure adequate use in real life practice.

Firstly, current models are developed using "supervised training" – which means that training data has previously been analyzed and labeled by a human operator, which in this case is an expert endoscopist. As a result, it is important that the dataset be as large as possible, and as representative as possible of real life

conditions, otherwise systemic errors or biases will be inherently built into the algorithm. Early experience with AI development showed that still images used for training were usually high quality images, carefully selected and labeled by expert endoscopists, which would not be usually found in real life conditions, leading to the selection bias and, ultimately, to overfitting, which means that the algorithm performed very well on the training dataset, but poorly in the real life [5]. Current algorithms are developed with an aim to minimize the risk of selection bias and rely on videos rather than still images, preferably from different operators, using different examination protocols, to ensure a wide variety of captured data and, ultimately, a robust AI algorithm [6].

Another significant factor that needs to be considered is the fact that AI algorithms are designed to perform very straightforward tasks (*i.e.* detecting polyps in the video feed from the colonoscope or classifying a lesion as neoplastic or non-neoplastic). The end product of the algorithm is, in fact, a probability calculated by the AI model, based on which the program returns a "label" that is usually binary in nature (*i.e.* labeling a polyp as neoplastic or hyperplastic). What this means is that AI applications currently available in endoscopy can only be used for very specific tasks and can only perform within very clear confines. A simple example of these limitations was showcased in a recent study which showed a non-negligible rate of false positive lesions classified by the AI as colonic polyps which turned out to be feces, submucosal tumors, cysts or normal mucosal folds [7].

CURRENT APPLICATIONS OF ARTIFICIAL INTELLIGENCE

AI Applications in the Lower Gastrointestinal Tract

Screening colonoscopy represents an ever-increasing burden on medical systems worldwide. However, current data suggests that up to 27% of post-colonoscopy colorectal cancers (interval cancers) are related to the missed lesions at the index colonoscopy, highlighting the need for better detection of neoplastic lesions during colonoscopy [8]. Almost two decades have now passed since the first reports of computer-aided detection of polyps using a computer program that analyzed white light images obtained at colonoscopy [9], which were then followed by the attempt to use computer-assisted diagnosis for characterization of narrow-band imaging (NBI) of colonic polyps [10]. However, more than a decade passed before computing power and the development of image-recognition software based on deep learning algorithms could allow real-time implementation of AI software, demonstrating high diagnostic accuracy for polyp detection [11] - computer-aided detection (CADe) and for computer-aided diagnosis (CADx), which usually referred to discriminating neoplastic from non-neoplastic lesions.

Currently, all three major endoscope manufacturers offer AI platforms for computer-aided detection of colonic polyps: Endo-AID/Olympus®, CAD EYE/ Fujifilm® and Discovery/Pentax®, with Fujifilm's CAD EYE also offering an integrated CADx solution, which is based on virtual chromo-endoscopy modality and which characterizes the polyps as either neoplastic or hyperplastic, based on their appearance in blue-light imaging (BLI) (Fig. **1** and **2**).

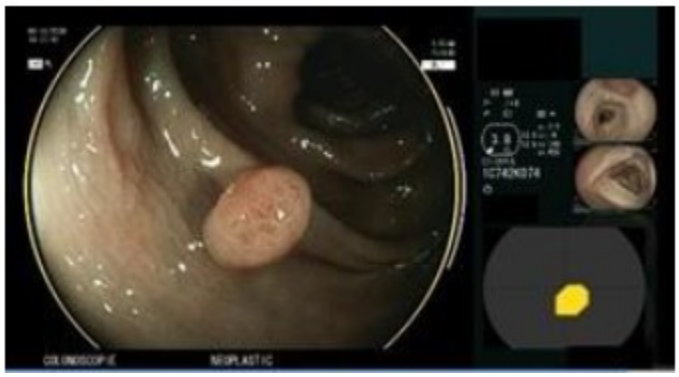

Fig. (1). Adenomatous polyp characterized as neoplastic using BLI and CAD EYE.

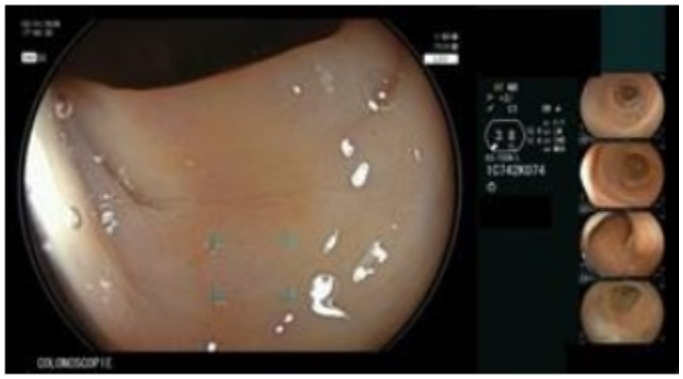

Fig. (2). Small hyperplastic polyp detected using BLI and CAD EYE.

There is limited data evaluating the utility of these CAD systems in real life, with the first randomized trial (RCT) showing significant improved adenoma (ADR) and polyp (PDR) detection rates [12] in the CAD group compared to the control group. Interestingly, these results were confirmed in a subsequent double-blinded RCT conducted by the same study group [13], with both studies demonstrating an increase in the number of small polyps and adenomas (<5mm), with no significant difference in the detection of larger lesions between the two study groups. Of note, both studies were conducted using an independent AI algorithm (EndoScreener; Wision AI, Shanghai, China) which was custom designed and

linked to the live endoscope feed and not one of the commercially available platforms. The first meta-analysis of all available data from RCTs of CAD-assisted colonoscopies suggests an advantage of CAD-assisted colonoscopies over conventional examinations in terms of ADR and PDR, irrespective of polyp size and location, with AI systems outperforming the control group on all these categories [14].

While research into CAD is more advanced than simple polyp detection, aiming at predicting the histology, two recent trials have also shown high diagnostic accuracy in classifying diminutive polyps into hyperplastic and adenomatous [15, 16], with both studies meeting the prespecified PIVI (Preservation and Incorporation of Valuable endoscopic Innovations of the American Society of Gastrointestinal Endoscopy) criteria required for implementing a "diagnose-a--leave" policy.

Further applications in colonoscopy have also been evaluated in limited studies so far, including AI algorithms for endocytoscopy, magnification chromoendoscopy, autofluorescence endoscopy and confocal endomicroscopy [17], with promising preliminary results for detecting and characterizing neoplastic features.

AI Applications in the Upper and Middle Gastrointestinal Tract

Early neoplastic lesions arising in the upper gastrointestinal endoscopy are considered to be more challenging for the endoscopist, both in terms of detection and characterization, than lesions in the colon. Consequently, detection of neoplastic lesions developing in Barrett's esophagus (BE) patients and early gastric cancer have been proposed as potential applications for the development of AI models, but currently available data is limited, usually stemming from small, single-center retrospective series [18].

For early neoplasia in BE, a study comparing an AI algorithm performance with that of expert endoscopists analyzing images from 44 patients with BE demonstrated reasonable sensitivity (86%) and specificity (87%) for the AI algorithm, with an overall diagnostic accuracy which was lower than that of expert endoscopists. The same group later reported a very good diagnostic accuracy of another AI algorithm, trained to analyze images obtained by volumetric laser endomicroscopy, a refinement of the optical coherence tomography technique, with sensitivity and specificity of >90% when compared to histology for differentiating non-dysplastic from dysplastic BE [19]. Recently, another study comparing detection and characterization of lesions suspicious for squamous cell carcinoma showed that AI outperformed a panel of experts in correctly detecting suspicious lesions and further classifying them into cancerous and noncancerous lesions [20].

Diagnosing Helicobacter pylori (HP) infection has also been the focus of several studies which developed AI algorithms that outperformed endoscopists with regard to diagnosing HP infection based on white light or virtual chromoendoscopy images [21, 22]. Detection of early gastric cancer lesions [23] and prediction of deep submucosal invasion in cases of gastric cancer [24] have also undergone preliminary studies which proved that AI algorithms could be successfully applied in these clinical settings. While the number of studies and proposed applications for AI in the upper GI tract continues to grow at an enthusiastic pace, it is important to understand that most of the studies represent proof-of-concept or *ex-vivo* studies, with little evidence from real-life settings to back up these initial results. Furthermore, none of the endoscope manufacturers currently provides any authorized AI platform for clinical use in the upper GI tract.

Small bowel endoscopy was one of the first fields to benefit from the use of AI in endoscopy, mainly for the detection of bleeding sources. While the upper and the lower digestive tract could be easily investigated by conventional endoscopy, the arrival of the wireless capsule endoscopy (WCE) marked a big step ahead in the investigation and management of small-bowel pathology (*e.g.* the obscure gastrointestinal bleeding). Combining the WCE excellent tolerability with machine learning algorithms using color-based feature extraction (hue, saturation, intensity or texture) allowed the automated detection of bleeding, celiac disease or intestinal parasitic infestation with high accuracies [25].

THE ROAD AHEAD FOR AI IN ENDOSCOPY

There is currently sound evidence supporting the potential utility of AI in various clinical applications in diagnosing diseases of the gastrointestinal tract. With the possible exception of colonoscopy, where CAD is now commercially available, further studies are required before AI platforms can be implemented elsewhere in the GI tract or even in the field of endoscopic ultrasound, as some form of very early data suggest [26]. Clinical trials, especially randomized trials with hard endpoints, relevant to the daily practice of medicine should be used to validate all the candidate AI systems prior to their adoption in real life.

Unlocking the full potential of deep learning and computer-assisted diagnosis in endoscopy depends on several critical factors. First, the availability of very large databases of videos and images of relevant lesions correctly annotated by expert endoscopists is a prerequisite for training algorithms that can be applied successfully in a real life setting. Secondly, while currently available solutions are usually designed for a very limited, usually, binary question, AI platforms of the future will have to be versatile and the same machine should be able to multitask

in real-time – *i.e.* detect and characterize various types of lesions in various locations throughout the GI tract. Another important point is the fact that endoscopists should be adequately trained and prepared to interact with AI in a way that enhances their diagnostic and therapeutic capabilities. Several authors have already underscored some of the caveats of over-relying on AI systems [27, 28] while encouraging an increase in the competency of the human operator as well. Finally, physicians and patients alike should be adequately prepared to address the financial, ethical and legal issues which might arise from the implementation of AI in real life [29, 30]. As medicine is seen as a fundamental human activity, the answer to the question that who will be accountable for the consequences of AI error or malfunction, is still open to debate and needs careful consideration before we move forward with the mass implementation of AI algorithms.

CONCLUSIONS

Based on the current state of the field, it is reasonable to assume that AI will be part of our future and some form of computer-assisted diagnosis will be routinely available in most endoscopy suites over the following decade and that, by correctly integrating AI systems, we will be significantly enhancing the standard of care for our patients. However, performing the endoscopic procedure itself, making complex clinical judgements and performing therapeutic maneuvers will make human operators indispensable in the endoscopy room, at least for the near future.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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Declared none.

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CHAPTER 14

Gallbladder Tumors

Ioan Tiberiu Tofolean[1,2,*] and **Mihaela Țanco**[1,2]

[1] *Ovidius University Constanța - Faculty of Medicine, Romania*

[2] *Gastroenterology Clinic, Constanța County Clinical Emergency Hospital "Sf. Apostol Andrei", Constanța, Romania*

Abstract: Conventional ultrasound (US) is the most important and fundamental imaging method for gallbladder diseases.

Biliary disorders are still very common nowadays, especially the ones affecting the gallbladder. Either benign (in most cases), or malignant, their diagnosis still relies on the abdominal ultrasound. Gallstones and their complications represent a major public health issue in Europe and other developed countries, and affect > 20% of the population.

According to GLOBOCAN 2020 data, gallbladder cancer is the 23rd most incident, but the 20th most deadly cancer worldwide, which could be explained by the late discovery of gallbladder cancer. Worldwide, gallbladder cancers represented 0.6% of the total cancer cases in 2020, with a mortality of 0.85% among all cancers.

US becomes more appropriate than computed tomography (CT) and magnetic resonance imaging (MRI) for the detection of gallbladder diseases, having the advantages of safety (without radiation), real-time imaging, considerable cost effectiveness and high spatial resolution.

Regardless of the previously mentioned advantages, the accuracy and sensitivity of US are not satisfactory, particularly when gallstones or other gallbladder lesions occupy the entire gallbladder lumen. Contrast-enhanced ultrasound (CEUS) is considered to increase the diagnostic precision of US.

Keywords: CEUS, Elastography, Gallbladder, Tumors, Ultrasound.

INTRODUCTION

Conventional ultrasound (US) is the most important and fundamental imaging method for gallbladder diseases. The biliary disorders are still very common now-

* **Corresponding author Ioan Tiberiu Tofolean**: Ovidius University Constanța - Faculty of Medicine, Romania and Gastroenterology Clinic, Constanța County Clinical Emergency Hospital "Sf. Apostol Andrei", Constanța, Romania; Tel: +40722294863; E-mail: tofoleanioan@yahoo.com

Ioan Sporea and Alina Popescu (Eds.)

adays, especially the ones affecting the gallbladder. Either benign (in most cases), or malignant, their diagnosis still relies on the abdominal ultrasound. Gallstones and its complications represent a major public health issues in Europe and other developed countries, and affect > 20% of the population. According to GLOBOCAN 2020 data, gallbladder cancer is the 23rd most incident, but the 20th most deadly cancer worldwide, which could be explained by the late discovery of the gallbladder cancer. Worldwide, the gallbladder cancers represented 0.6% of the total cancer cases in 2020, with a mortality of 0.85% among all cancers. The incidence and mortality of gallbladder carcinoma were the highest in Asia, followed by Europe and Latin America and Caribbean Region [1]. For Romania, the incidence is lower than the world average, and slightly higher in males than in females. Gallbladder cancer is the 30th most incident cancer, representing 0.23% of the total cancer cases in 2020. At the same time is the 25th most deadly cancer, accounting for 0.35% of the total number of cancer deaths [2].

US becomes more appropriate than computed tomography (CT) and magnetic resonance imaging (MRI) for the detection of gallbladder diseases, having the advantages of safety (without radiation), real-time imaging,considerable cost effectiveness and high spatial resolution [3]. Regardless of the previously mentioned advantages, the accuracy and sensitivity of US are not satisfactory, particularly when gallstones or other gallbladder lesions occupy the entire gallbladder lumen [4, 5]. Contrast-enhanced ultrasound (CEUS) is considered to increase the diagnostic precision of US. A meta-analysis of sixteen studies that was completed in 2016 has found that the specificity and sensitivity of CEUS in defining gallbladder carcinoma with diameterless than 1 cm was 92 and 91%, respectively, having an AUROC of 97% (IC 95% 0.94–0.98). Nonetheless, the authors suggested that additional studies need to be performed in order to clarify the utility of CEUS, because the methodological quality was only moderate [6]. Regarding bigger tumors, Zhuang *et al.* observed that branched intralesional vessels, hypo-enhancement in the late phase, and the irregular shapewere characteristics indicating malignancy in gallbladder disease. By combining any two of these three characteristics, the diagnostic sensitivity was 90%, specificity was 92.4%, and AUROC 0.91 [7].

THE CLASSIFICATION OF GALLBLADDER TUMORS

Intraluminal Polypoid Mimickers

Tumefactive Sludge/Pseudotumoral Gallbladder Sediment

Conventional 2D-US and Doppler US present difficulties in differentiating the immobile gallbladder sediment from gallbladder carcinoma (Fig. **1** - **3**).

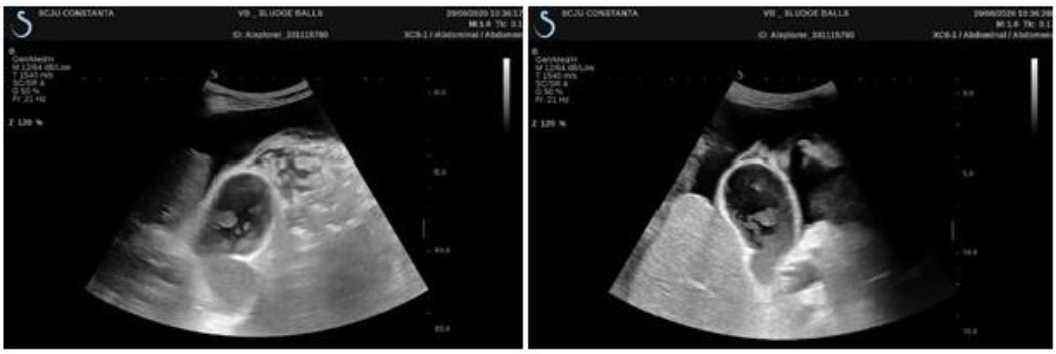

Fig. (1). Gallbladder sediment occupying > 50% of gallbladder lumen in conventional 2D-US.

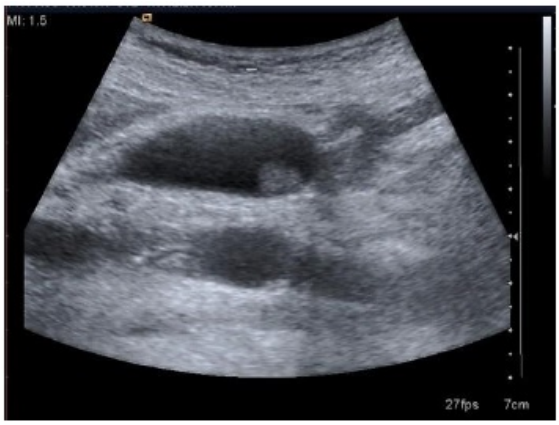

Fig. (2). Conventional 2D-US of gallbladder showing Tumefactive sludge presenting with polypoid aspect.

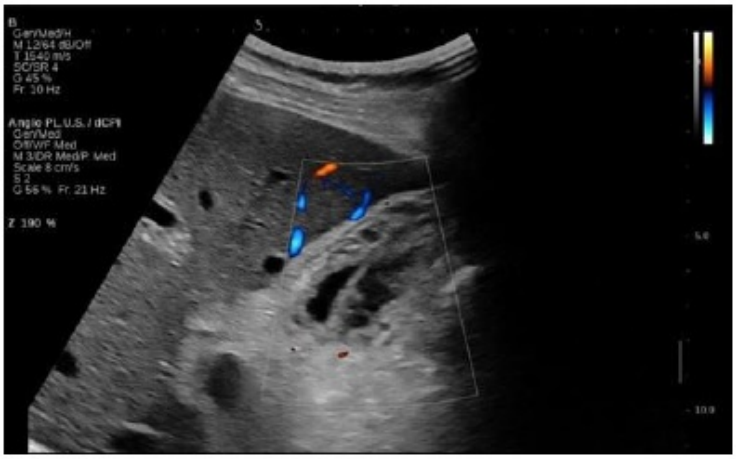

Fig. (3). Doppler US shows no vascularization present in the intraluminal hyperechoic structure in gallbladder.

CEUS aspect: without enhancement during both arterial and late phases, due to the absence of vascularization in the sediment (Fig. **4**).

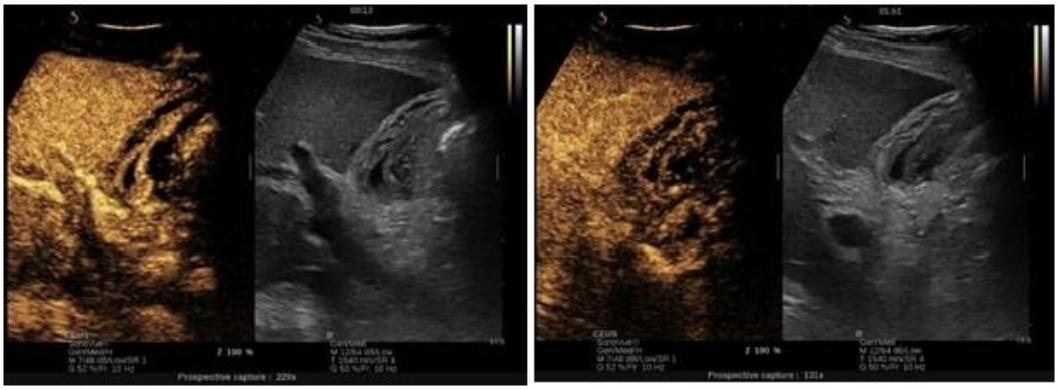

Fig. (4). CEUS examination of an inhomogeneous, hyperechoic/isoechoic gallbladder mass showing no enhancement.

Gallstones

On conventional 2D-US the gallstones may resemble polypoid lesions because of the posterior acoustic shadowing beingcovered by the prominent fatty tissue or the superimposed bowel gas. The presence of gallbladder stones does not exclude the possibility of malignancy (Fig. **5** and **6**).

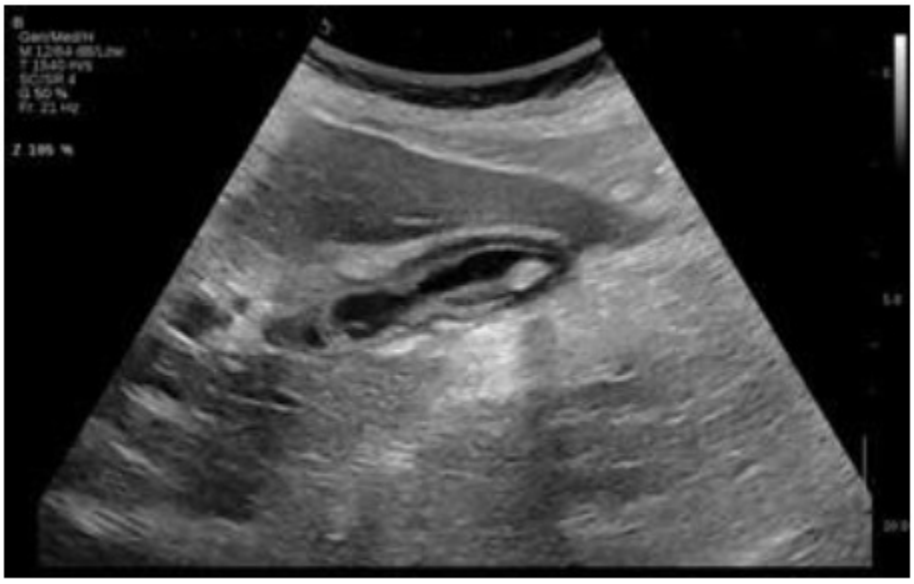

Fig. (5). Hyperechoic mass with posterior acoustic shadowing and thick gallbladder wall in acute cholecystitis.

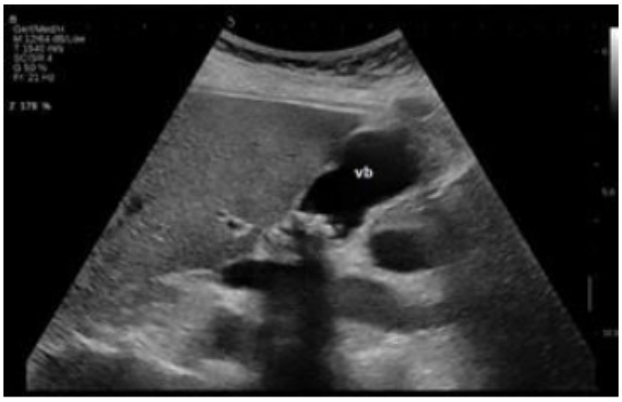

Fig. (6). Hyperechoic conglomerate with posterior acoustic shadowing.

Doppler US shows the lack of vascularization. CEUS – no enhancement during arterial or late phases, due to the absence of vascularization inside the gallstones.

Pseudotumors

Cholesterol Polyps

They develop from the encompassment of triglyceride by phagocytes and usually gather on gallbladder wall, leading to an aspect of "balls-on-the-wall sign" (Fig. **7**).

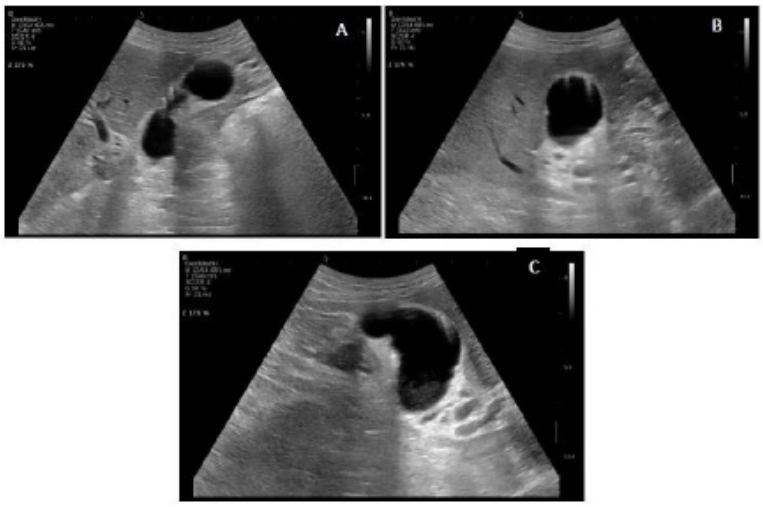

Fig. (7). (A, B, C) show multiple echoic/hyperechoic structures attached to the gallbladder wall with "balls-on-the-wall" appearance.

Doppler US – less frequent, cholesterol polyps may appear as a solitary polypoid lesion with slight vascularity.CEUS shows:

- heterogeneously enhanced lesions
- Invasion of the adjacent liver parenchyma and incomplete gallbladder wall suggest malignancy.

Inflammatory Polyps

These are formed by fibrous and granulation tissue proliferation inside the gallbladder.

Conventional 2D-US aspect of the polyps (Fig. **8**):

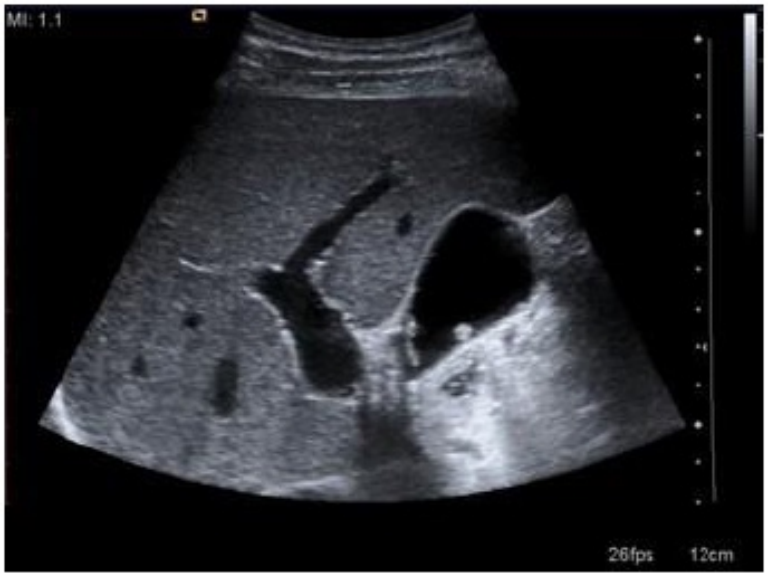

Fig. (8). Conventional 2D-US showing hyperechoic small mass on the gallbladder wall – polyp.

- small, between 5-10 mm
- sessile or pedicled

Polyps larger than 10 mm may be sometimes confused with malignant ones [8].

Doppler US shows vascularization inside the polyp (Fig. **9**).

CEUS reveals:

- focal arterial hyper-enhancement
- no significant washout in the venous phase.

Elastography does not provide additional diagnostic information in this disease.

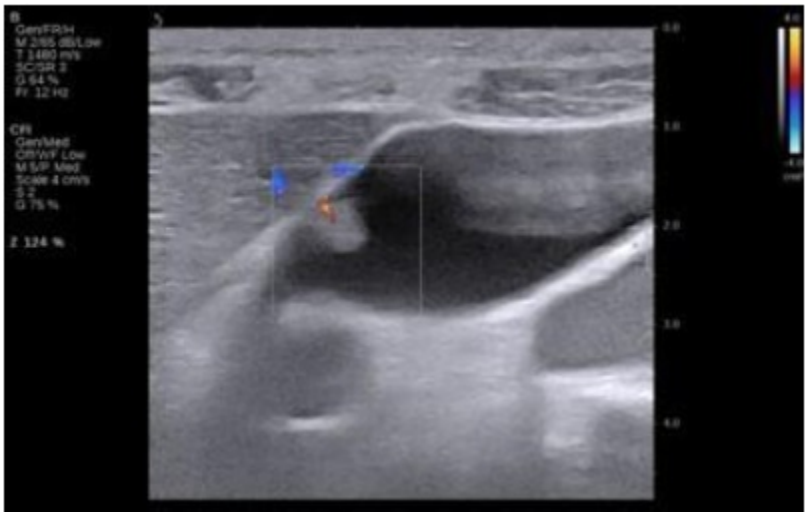

Fig. (9). Polyp with vascular pedicle present in Doppler US examination.

Adenomyomatosis

Represented by bile acid crystals that are precipitating in the intramural diverticula (also named Rokitansky-Aschoff sinuses) of the gallbladder and often coincides with thickening of the gallbladder wall. The typical "comet tail" artifact is produced by the multiple interfaces formed due to crystals. Distinct morphological changes may display three types of adenomyomatosis: diffuse, segmental, and focal types. Ootani *et al.* suggested, after examining over 3000 gallbladder specimens, that segmental adenomyomatosis has a strong connection with gallbladder cancer. The development of inflammatory polyps appearsbecause of chronicinflammation and chronic cholesterol precipitates, which stimulate the mucosal wall and lead to the formation of granulation and fibrosis. Inflammatory polyps may progress to mucosal dysplasia [9].

Conventional 2D-US may detect:

- echoic foci due to cholesterol deposits
- comet-tail artifacts – a highly specific sign [10, 11]
- gallbladder wall thickening – diffuse of localized (when differentiation from a gallbladder carcinoma is required) (Fig. **10**).

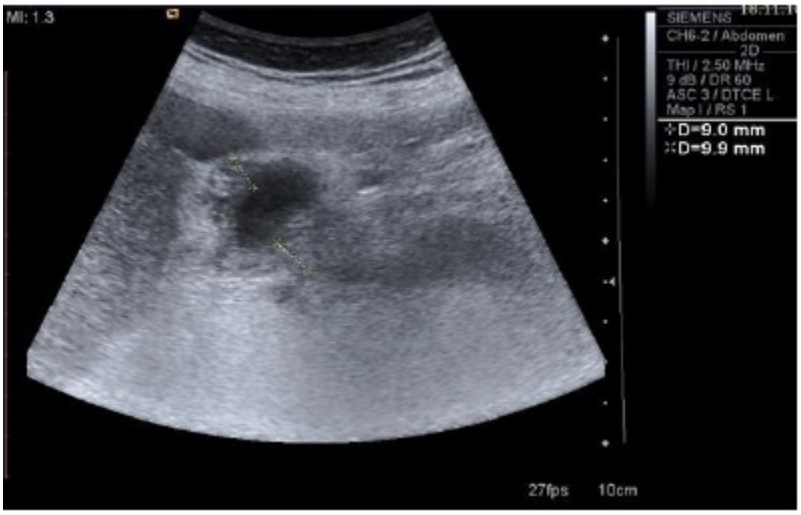

Fig. (10). Diffuse gallbladder wall thickening.

Doppler US does not provide additional information for the diagnosis. The crystals in the intramural diverticula may present with tinkle artifacts.CEUS reveals:

- uneven, focal enhancement of the gallbladder wall
- no washout in the venous phase. Elastography is not relevant for the diagnosis.

Benign Polypoid Tumors

Adenomas

Adenomas are mainly observed in individuals with primary sclerosing cholangitis and intestinal polyposis syndrome and it is very hard, even almost impossible to discriminate adenoma from adenocarcinoma on conventional and Doppler US since both are correlated with internal vascularity. In clinical practice it is widely accepted the use of 1 cm diameter as an indicator of greater risk of malignancy and it is applicable at screening ultrasonography. The management of lesions with a diameter less than 1 cm in a patient having no other risk factors depends on the clinical setting, keeping in mind that malignant transformation of the smaller polypoid lesions can occur [12, 13].

There are usually subtle differences between adenoma and adenocarcinoma on ultrasonography.

Conventional 2D-US shows (Fig. **11** and **12**):

- echoic, pedicled structure
- narrow implantation basis
- the absence of mobility at the patient's movement and absence of the shadow cone differentiates it from calculi.

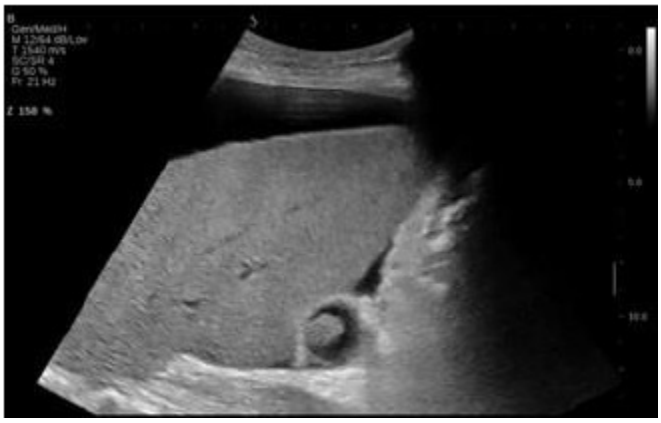

Fig. (11). Echoic pedicled structure, without mobility and shadow cone.

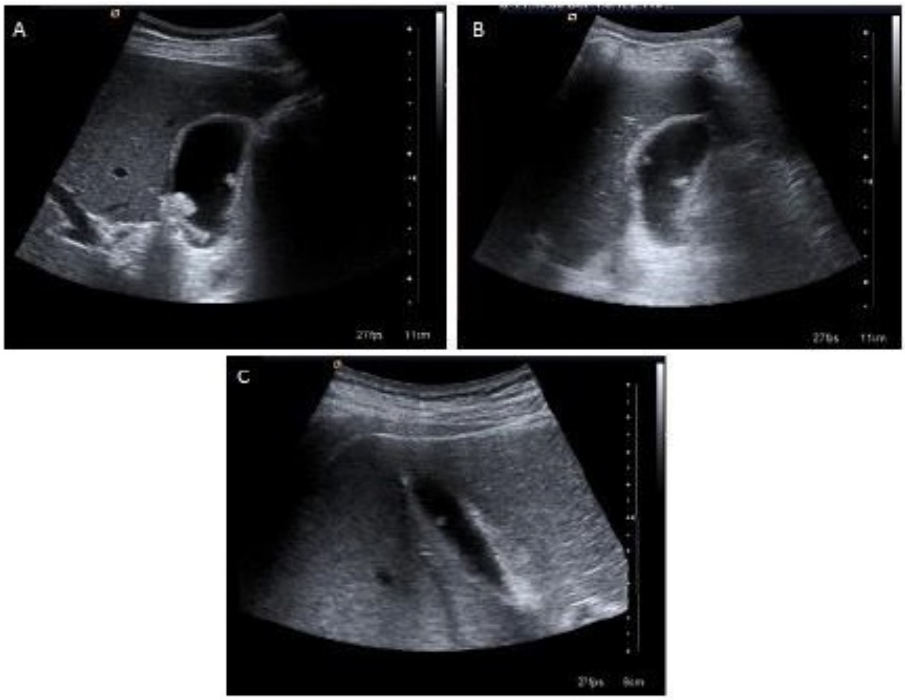

Fig. (12). (A, B, C) Echoic pedicled structures, with narrow implantation basis, without mobility and shadow cone.

The Doppler US can differentiate malignant from benign lesions based on:

- the presence of the color flow
- vascularization patters
- flow velocity
- low sensitivity for small tumors due to the low flow velocities [14 - 16].

CEUS (Fig. **13**) shows:

- eccentric enhancement in the arterial phase (78%), or iso-enhancement (28%)
- intact gallbladder wall underneath the lesion
- in the late phase they show lower (44%) or the same enhancement (56%) as the liver parenchyma.

Elastography does not bring diagnostic benefits.

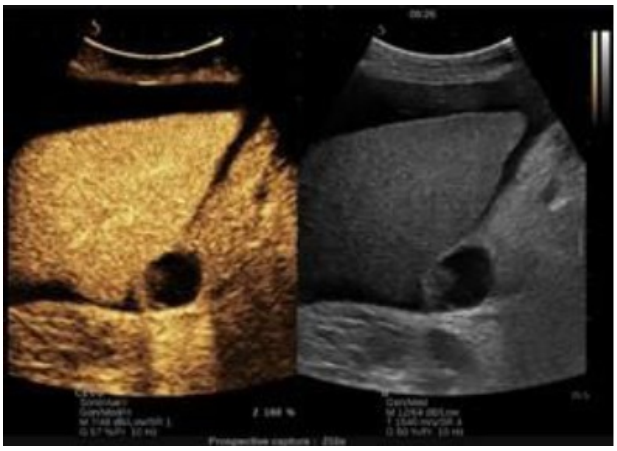

Fig. (13). Isoenhanced lesion.

Papillomas

These lesions are relatively rare, commonly seen in elderly males, and have malignant potential to producepapillocarcinomas [17, 18].

Malignant Polypoid Tumors

The early-stage malignant tumors of the gallbladder do not present with particular clinical symptoms and exhibit advanced or extracholecystic invasion when they are diagnosed. A part of the imaging characteristics of malignancy are:

- size over 10 mm
- focal thickening of the gallbladder wall (>3 mm)
- Incomplete gallbladder wall beneath the lesion [19, 20].

Adenocarcinomas

The most common malignant tumors of the gallbladder, having risk factors that vary from older age to chronic cholecystitis, gallstones, porcelain gallbladder, choledochal cysts and primary sclerosing cholangitis. Unifocal polypoid lesions have a higher probability to be malignant.

The imaging features of typical adenocarcinomas of the gallbladder include:

- relatively bigger size
- incomplete gallbladder wall
- wide base
- co-existence of chronic cholecystitis.

As blood flow can be noticed in both adenomas and adenocarcinomas, the presenceof vascularity is not specific. Therefore, more important than the presence of color Doppler signal may be theultrasound lesion'ssizes and morphological changes. The invaded liver parenchyma is usually hypoechoic and associated with an unclear border with the gallbladder.

Conventional 2D-US shows different macroscopic models, according to the tumor stage.

a) In the initial polypoid stage – no characteristic aspect.

- malignancy criteria: wide implantation base, usually 10 mm, and the exacerbated erratic arterial Doppler signal
- Infiltration of the gallbladder wall layers (high suspicion of malignancy) (Fig. **14**).

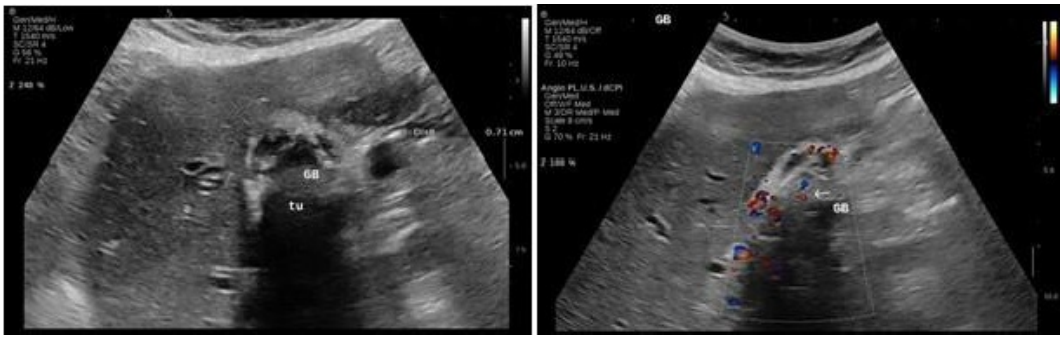

Fig. (14). Conventional and Doppler US showing infiltration of gallbladder walls.

In advanced stages (Fig. **15**):

- parenchymatous mass involving the gallbladder bed
- often centered by a gallstone image
- intrahepatic ducts dilation is frequently associated, due to hilum invasion.

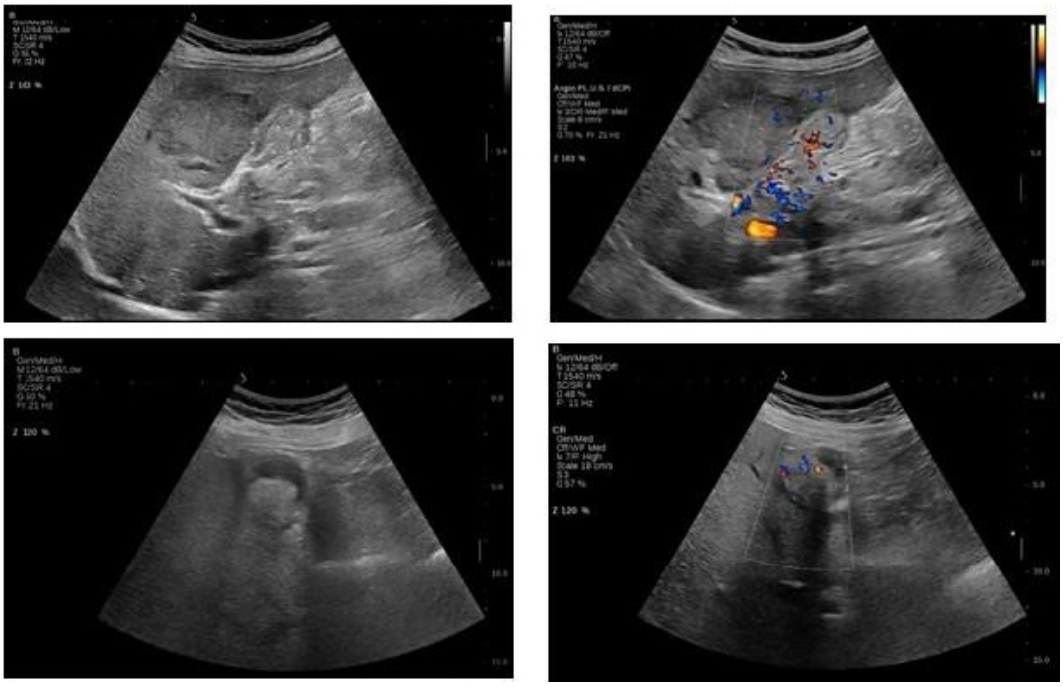

Fig. (15). Advanced stages of gallbladder carcinoma showing mass involving the gallbladder lumen and bed.

The Doppler US has a controversial diagnostic value (Fig. **16**).

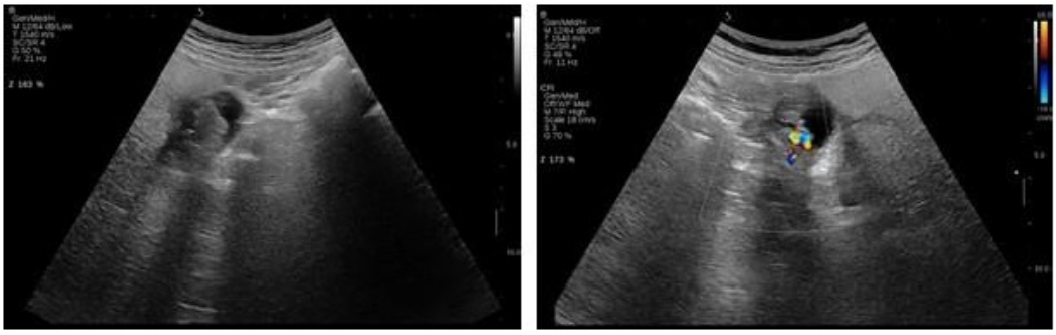

Fig. (16). Doppler US shows rich vascularization of the gallbladder mass.

CEUS shows:

- hyperenhancement in the arterial phase (Fig. **17**).
- hypoenhancement in the late phase (Fig. **18** and **19**)
- the differential between benign and malignant lesions can be made using the arterial phase and the venous phase, because malignant tumors have a faster washout time (41.4 seconds) than benign ones (58.2 seconds).
- destruction of gallbladder wall and liver infiltration is highly suggestive of malignancy.

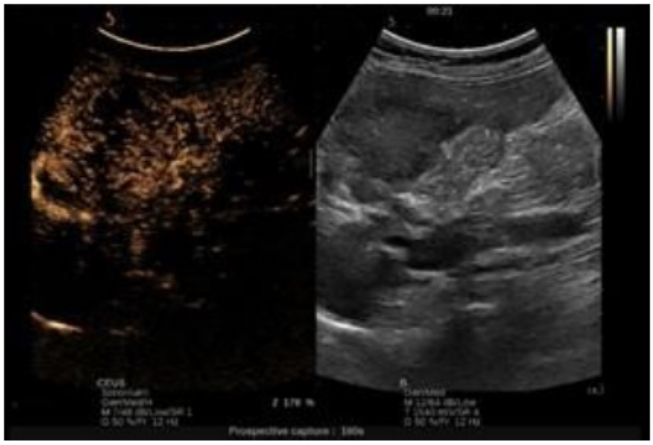

Fig. (17). Arterial phase–hyperenhancing.

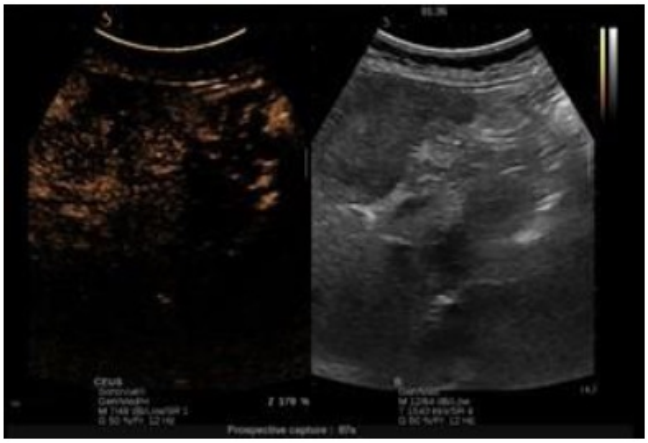

Fig. (18). Late phase–hypoenhancing.

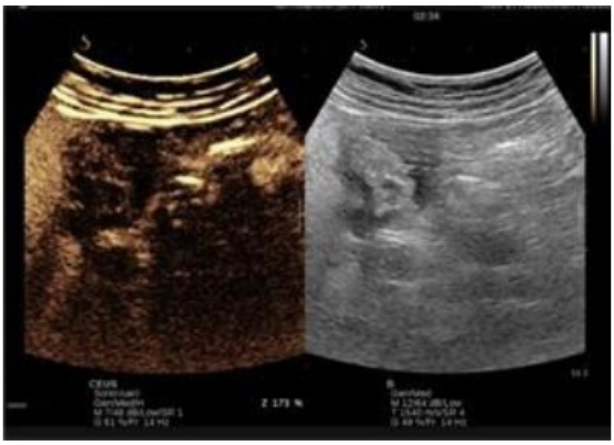

Fig. (19). Late phase – hypoenhancing.

Elastography:

- useful only when the tumor is large and superficially located
- shows increased stiffness, with an uneven distribution.

Metastatic Tumors in the Gallbladder are Exceptional

Metastasis can originate from the stomach (most common among Asian patients), skin (melanoma), kidneys or lung cancer. Direct invasion of cholangiocarcinoma's and hepatocellular carcinomas are not uncommon and may resemble the imaging aspect of direct invasion of liver parenchyma by gallbladder cancer.

Conventional 2D-US:

- wall thickening
- calcifications
- parenchymatous masses adhering to the wall and protruding into the lumen and/or infiltrating the liver.

  Doppler examination is not relevant for the diagnosis of gallbladder metastases.

  CEUS:
- gaps situated in the gallbladder lumen or wall
- marked enhancement in the arterial phase
- washout in the venous phase, distinct from that of the gallbladder wall.

  Elastography detects stiffness and is relevant within large tumors.

Lymphomas

Primary gallbladder lymphomas are less commonlydiscovered than secondary ones and may derive from chronic cholecystitis. More frequent are the secondary lymphomas with adjacent lymphadenopathy. Ultrasonographic findings are not unequivocal, and intra-abdominal lymphadenopathy may be noticed.

For gallbladder tumors, an appropriate selection of other imaging procedures is also very important for the final diagnosis. We present the approach algorithm in the ultrasonography diagnosis of gallbladder intraluminal lesions (Fig. **20**):

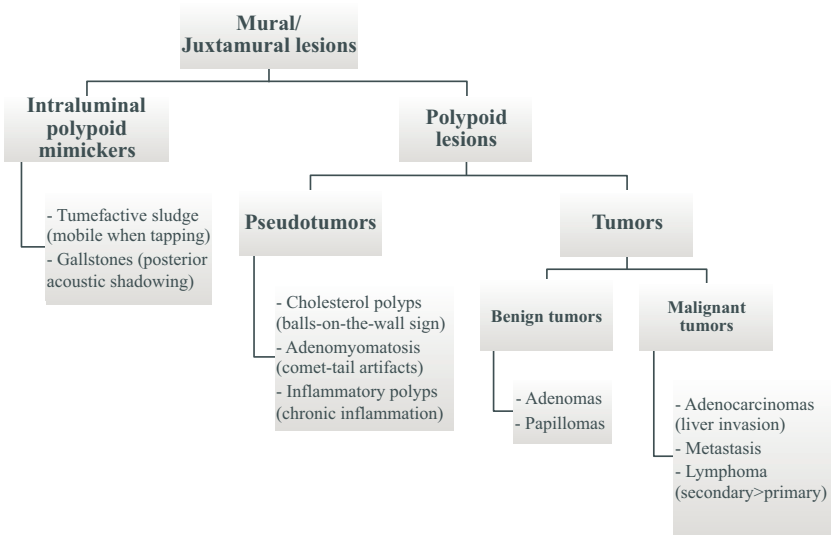

Fig. (20). Algorithm for ultrasound diagnosis of gallbladder intraluminal lesions (adapted from Wu, Chia-Hung & Luo, Yukun & Fei, Xiang & Chou, Yi-Hong & Chiou, Hong-Jen & Wang, Hsin-Kai & Lai, Yi-Chen & Lin, Yung-Hui & Tiu, Cm & Wang, Jane. (2018). Algorithmic approaches to the diagnosis of gallbladder intraluminal lesions on ultrasonography. Journal of the Chinese Medical Association. 81. 10.1016/j.jcma.2018.01.002).

CONCLUSIONS

Ultrasound examination of the gallbladder remains the first-choice method for all gallbladder disorders. Current techniques include various methods of examination, some of them being focused on morphology (2D-US), some on circulation (Doppler US, CEUS), and others assessing rigidity (elastography). Depending on the underlying condition, any combination of these techniques leads to the establishment of an accurate diagnosis in most inflammatory or tumoral gallbladder diseases, but the patient's clinical picture remains an essential criterion.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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Declared none.

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CHAPTER 15

Management of Severe Acute Pancreatitis

Mircea Manuc[1,*] and **Doina Istratescu**[1]

[1] *Department of Gastroenterology and Hepatology, Fundeni Clinical Institute Bucharest, Romania*

Abstract: One of the most important gastroenterological emergencies is acute pancreatitis. It is classified into mild, moderately severe, and severe pancreatitis depending on occurring complications. Establishing etiology and assessing disease severity is the first step of the management.

Severe pancreatitis is encountered in 25% of patients and carries the highest mortality. The therapy in these cases is structured on 4 interventions: fluid resuscitation, nutritional support, pain management, specific measures addressed to etiology or complications.

Fluid resuscitation for prevention of necrotizing pancreatitis is the foundation of early management. Quality of life in these patients relies on prompt pain management. Early enteral nutrition might reduce mortality, multiple organ failure and infection rate when compared to late enteral nutrition and parenteral nutrition.

Pseudocysts and infected necrosis can complicate severe pancreatitis. These symptomatic patients will need appropriate interventional maneuvers depending on imaging and disease extension. Antibiotics should only be given when infection is highly suspected, particularly when necrotizing pancreatitis is involved. Percutaneous drainage is recommended when the collected necrosis has less than 1 month from constitution. In walled-off pancreatic necrosis, endoscopic drainage and subsequent necrosectomy is preferred to percutaneous drainage.

Surgery has to be taken into account after failure of endoscopical/percutaneous procedures, intra-abdominal compartment syndrome, or acute on-going bleeding.

Keywords: Management, Pancreatic necrosis, Severe acute pancreatitis.

INTRODUCTION

Acute pancreatitis can frequently involve adjacent organs or other systems, representing one of the common gastroenterological diagnostic emergencies. The majority of cases can be self-limiting due to the mild edema, but severe pancreatic

[*] **Corresponding author Manuc Mircea:** Department of Gastroenterology and Hepatology, Fundeni Clinical Institute Bucharest, Sos. Fundeni 258, Bucharest, Romania; E-mail:m_manuc@yahoo.com

Ioan Sporea and Alina Popescu (Eds.)

inflammation triggering necrosis, organ failure and death are also possible. The distinguishing feature of severe acute pancreatitis is the occurrence of persistent organ failure (48 h or longer) [1].

Epidemiology

Worldwide incidence of acute pancreatitis is 5 to 30 per 100 000 population, with an increasingly higher incidence since the late 2000s in UK and USA. Other factors like male gender, age and low economic status were also tied to an elevated incidence of acute pancreatitis [2].

Mortality rate ranges from 1%–7% and can rise to 15- 20% in patients with pancreatic necrosis. Persistent organ failure is generally associated with the highest mortality reaching 60% in some series [3].

Etiology

The main etiologies of acute pancreatitis are gallstones and alcohol, with the latter being the most common cause reported. Alcoholic intoxication is more frequent in men, while biliary lithiasis is more frequent in the female gender. Establishing the etiology is an important step because it will influence disease management. Other causes of acute pancreatitis are rare (listed below in Table **1**). The pancreatitis can be classified as idiopathic if we exclude all other causes. However, the most probable potential causes of "idiopathic pancreatitis" are believed to be microlithiasis and Oddi dysfunction [4]. In terms of risk factors, obesity has been proven to be frequently associated with severe acute pancreatitis [5].

Table 1. Causes of acute pancreatitis [4].

Toxic	**- Alcohol** **- Smoking**
Obstructive	- Gallstones - Pancreatic cancer - Pancreatic cystic tumor - Sphincter Oddi dysfunction
Iatrogenic	- ERCP - Drugs [thiazides, azathioprine]
Metabolic	- Hypertriglyceridemia, hypercalcemia, hyperuricemia
Autoimmune	- IgG4 pancreatitis
Genetic	- Mutations in PRSS1, SPINK1, CFTR genes
Infection	- HIV, Coxsackie, Mycoplasma, Legionella, Leptospira, Toxoplasma, *etc.*
Unknown	- Idiopathic

(Table 1) cont.....

Toxic	**- Alcohol** **- Smoking**
Endocrine	- Hyperparathyroidism
Other	- Abdominal trauma, hypovolemic shock, hypo/hyperthermia

Diagnosis

The hallmark of acute pancreatitis is abdominal pain which has an acute onset, is persistently severe, with typical epigastric localization, often radiating to the back. All other manifestations are usually related to complications [6].

The diagnosis of acute pancreatitis must be considered in all instances where acute abdominal pain is present. History and examination can be indicative of acute pancreatitis, however, for a definite diagnosis two out of the following three criteria should be met:

- Characteristic abdominal pain
- Elevated serum amylase or lipase (>3 x normal upper limit)
- Imaging [Computed tomography (CT), magnetic resonance imaging (MRI) or ultrasound] consistent with acute pancreatitis

Despite frequent clinical practice, routine CT is not recommended for diagnostic purposes if there are typical presenting symptoms. Laboratory testing includes complete blood count, plasmatic lipase, C-reactive protein, electrolytes and glycemia, hepatic enzymes. In addition, an abdominal ultrasound can identify gallstones, gallbladder complications and bile duct dilation, all indicative of a calculous etiology. Initial abdominal CT-scan should be preferred in cases of diagnostic uncertainty, signs of perforation or suspected abdominal bleeding [6, 7].

Severity Grading

In mild acute pancreatitis, patients have no complications (local or systemic). When transitory organ dysfunction [lungs, kidneys or cardiovascular system] is present, patients have moderately severe pancreatitis. In the presence of persistent organ failure (beyond 48 hours) the pancreatitis is classified as severe, imposing surveillance in an intensive care unit when accessible [6].

Local complications are frequently present in moderately severe and severe pancreatitis. Up to 25% of cases develop severe pancreatitis, which carries the worst prognosis in terms of mortality [8]. Infected necrosis in pancreatitis has a worse prognosis compared to sterile necrosis, with an average in-hospital

mortality of 30%. If organ failure is present in addition to infection of necrosis, mortality rises to 40% [9].

There are many scores that can be used to classify the severity of acute pancreatitis (*e.g.* Ranson, Balthazar, SOFA, APACHE II, Marshall), but there is no gold-standard. The Harmless Acute Pancreatitis Score (HAPS) can accurately predict a mild course of disease at admission. If the patient has no sign of peritonitis, normal serum creatinine and normal hematocrit there is a chance of 98% of a non-severe disease course [10].

For severe acute pancreatitis assessment we can utilize the Bedside Index for Severity in Acute Pancreatitis (BISAP) (Table **2**). A BISAP score ≥3 predicts a severe disease - 83% sensitivity [11].

Table 2. Bedside Index for Severity in Acute Pancreatitis (BISAP) score [11].

B	**BUN [Blood Urea Nitrogen] Level >25 mg/dL**
I	Impaired mental status [Glasgow Coma Scale score <15]
S	Development of systemic inflammatory response syndrome [SIRS]
A	Age >60 years
P	Presence of pleural effusion

SIRS is characterized by two or more of the following criteria: Body temperature below 36 °C or above 38°C; Heart rate greater than 90 beats/min; Respiratory rate greater than 20 breaths/min or partial pressure of carbon dioxide (pCO_2) less than 32mmHg; White blood cell count greater than 12 000mm, less than 4000mm, or greater than 10% immature (band) forms.

Recently, a more complex Pancreatitis Activity Scoring System (PASS) was elaborated. In addition to organ failure and SIRS parameters, it takes into account pain and opioid doses, as well as digestive tolerance [12].

Higher levels of C-reactive protein (>150 mg/L) at the 72h timepoint have prognostic significance and often imply a severe disease. Increased hematocrit (>44%) is an independent risk factor for pancreatic necrosis. A shorter time-lapse (<18 h) between pain onset and arrival at the hospital is also associated with severe disease [12, 13].

Prediction scores are helpful but can also have limitations due to shifting clinical patterns. An initially mild clinical disease can rapidly become severe and quite possibly life-threatening. A dynamic measurement system for immediate changes

in disease management according to patient responsiveness could be a useful tool in clinical practice and clinical trials [1].

Anticipating severity in acute pancreatitis is a major research key point. Star candidates for accurate forecasting include angiopoietin-2, resistin, and interleukin-6. Probably future studies will explore whether cytokines can improve significantly current clinical practice [14 - 16].

Imaging

In moderate to severe pancreatitis it is recommended to perform either a contrast-CT scan or MRI, preferably 72-96 h after pain onset. Patients with unknown etiology should be evaluated *via* magnetic resonance cholangiopancreatography or endoscopic ultrasound, the preferred imaging techniques for occult bile duct lithiasis [17].

The CT severity index (CTSI) is generally used for acute pancreatitis grading. It evaluates the degree of inflammation, fluid collections and the extent of necrosis. Higher scores imply higher morbidity and mortality.

In severe cases (CTSI>3) a follow-up CT scan has to be done within 7-10 days after initial evaluation. If invasive interventions are needed or clinical status deteriorates, the patient might benefit from repeated imaging studies [18].

Practical Management Algorithm in Severe Pancreatitis

The comprehensive management of these cases is presented in the Fig. (**1**) [3]

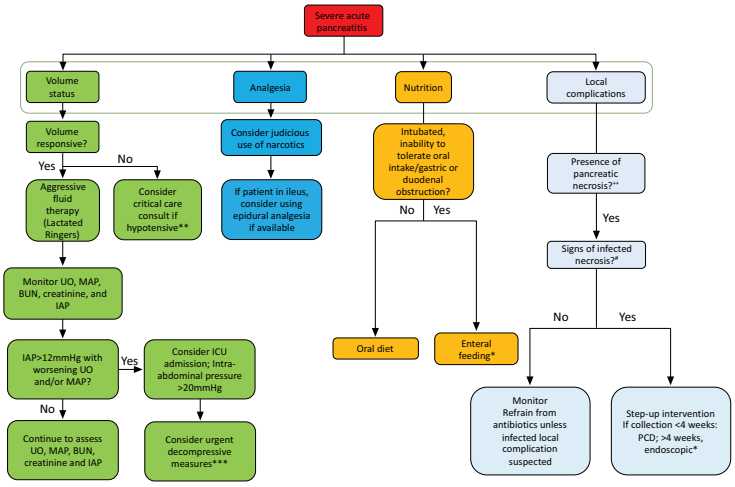

Fig. (1). Practical management algorithm in severe pancreatitis.

UO- urine output; MAP- mean arterial pressure; BUN- blood urea nitrogen; IAP- intra-abdominal pressure; ICU-intensive care unit; PCD- percutaneous drainage.

+Nasojejunal administration route is preferred; ++ contrast enhanced CT for evaluation; # clinical evaluation - organ failures, CT evaluation - air within a necrotic collection

- transmural drainage, followed by endoscopic necrosectomy if lack of benefit within 72 h;
- * risk of volume overload; *** decompressive laparotomy or percutaneous drain placement.

Fluid Resuscitation

Early aggressive IV fluid therapy is the groundwork for all patients with acute pancreatitis. However, the type of IV perfusion, the volume and extent of fluid administration are controversial. Oliguria, lower cardiac output and declining blood pressure are prevalent during severe acute pancreatitis and need appropriate monitoring. In subjects with anticipated severe acute pancreatitis, prolonged high-volume administration can become deleterious, particularly in cases of systemic vascular leak syndrome. Excessive IV perfusions may expand third-space fluids and elevate intra-abdominal pressure (>12 mmHg), which in turn can precipitate abdominal compartment syndrome and respiratory insufficiency. Diuresis, mean arterial pressure, oxygen saturation/ respiratory rate and intra-abdominal pressure are parameters to be dynamically monitored starting from admission [13, 19].

IV fluid protocols should be goal-oriented in line with clinical response, time-lapse (marginal benefits of massive fluid administration after 24 h), and patient's susceptiveness for fluid accumulation (and risk of abdominal compartment syndrome).

Important elements to be considering when developing an IV protocol:

1. Aggressively perfuse with a volume between 150 and 250mL/h in the first 24–48 h; postponing or administering less than the recommended volume can increase in mortality.

2. Higher volume and debit (1,000 mL/h) should be avoided since it may worsen the prognosis.

3. Ringer's lactate is the best fluid. The infusion rate should be adapted based on diuresis (target: 0.5–1 mL/kg/h) and vitals. During the first 24 h the patient should be under continuous surveillance.

Central venous pressure is influenced by intra-abdominal pressure and therefore is not accurate in determining volume response [20 - 22].

Enteral Nutrition

Enteral nutrition can limit complications and improve patient outcome in severe acute pancreatitis while maintaining intestinal functionality and integrity. It can be started as early as 48 hours within admission. Early enteral nutrition has benefits in terms of lowering mortality, multiorgan failure and infectious complications when compared to late enteral nutrition and parenteral nutrition. Parenteral nutrition can favor intestinal atrophy and promote damage of the mucosal barrier even though it reduces pancreatic enzyme secretion [23, 24]. A parenteral nutrition can be recommended in select cases presenting oral intolerance or if the patient has contraindications for enteral nutrition.

However, whether early nasogastric nutrition is better than "on-demand" oral feeding is controversial. For now, it is recommended to start an oral diet [low fat, soft oral diet] which can be enriched if tolerated. In intubated patients, a basic nasogastric feeding tube is indicated. If there is evidence of duodenal obstruction from peri/pancreatic edema or fluid, nutritional support can be ensured by a nasojejunal feeding tube (placed endoscopically) [25].

A basic nutritional evaluation should rely on the patient's weight and severity of disease. If energetic requirements are not covered by the enteral route, supplementing with parenteral nutrition is possible. A good nutritional intervention will help improve plasmatic parameters including prealbumin and albumin [26].

Antibiotics

Empiric antibiotic treatment is not supported in non-infected pancreatitis. Infected necrosis (pancreatic or adjacent) will be suspected in patients who regress (clinical instability or sepsis physiology, elevation of leukocytes, fever) or fail to ameliorate 7 to 10 days after admission.

A few studies have shown a modest benefit of antibiotic prescription in cases of severe necrotizing pancreatitis; therefore, antibiotic use is limited to cases highly suspected of infection. Furthermore, injudicious antibiotic administration in walled-off necrosis may promote multidrug-resistant organisms when the infection does develop [27, 28].

The diagnosis of infected pancreatitis is difficult; however, its timing culminates in the second to fourth week. Procalcitonin is a highly sensitive parameter for pancreatic infection and lower values (<0.5 ng/ml) could be used as a negative predictor for infection. There is no established procalcitonin threshold for identification of sepsis in acute pancreatitis [29]. The CT-guided fine-needle aspiration is no longer routinely used for diagnosis of pancreatic infection. Gas in the retroperitoneal cavity indicates an infected pancreatitis, but it is rarely observed [17].

Empiric antibiotherapy should include molecules that can penetrate pancreatic necrosis (*e.g*, a carbapenem alone; or a quinolone, ceftazidime, or cefepime combined with metronidazole) [30].

Pain Management

Pain is the most bothering symptom for patients, lowering the quality of life. Therefore, it is imperative to be managed appropriately within 24 hours. Treatment can include fentanyl, meperidine, non-steroid anti-inflammatory drugs (NSAIDs). Pain management is based on WHO analgesic ladder:

1. NSAIDs

2. low potent opioid ± NSAID ± adjuvant drugs

3. high potent opioid ± NSAID ± adjuvant drugs

4. interventional treatment ± high potent opioid ± NSAID ± adjuvant drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) have shown promising results preventing severe acute pancreatitis. However, renal dysfunction frequently associated with severe acute pancreatitis can significantly limit their usage. Opioids are currently the most potent short-term analgesic agent in severe acute pancreatitis. Nonetheless, cautious dispensation is required, especially when ileus is present [31, 32].

Specific Measures Addressed to Etiology ERCP and Surgery in Biliary Pancreatitis

Patients with predicted severe acute pancreatitis secondary to biliary lithiasis commonly have elevation of transaminases and jaundice, since pancreatic head edema or concomitant biliary lithiasis can lead to obstruction of the intrapancreatic bile duct. For these select patients, biliary drainage with sphincterotomy had been shown to lower morbidity and mortality. As such, in clinical practice is recommended to reserve ERCP for acute biliary pancreatitis

complicated with detectable obstructive choledocholithiasis or clinical suspicion of cholangitis. Biliary drainage should be achieved within 24 to 48 h [33].

Rapid cholecystectomy should be performed in mild biliary pancreatitis (same admission) due to the 60-80% incidence of recurrent pancreatitis in the ensuing months. After a severe episode of biliary pancreatitis cholecystectomy should be considered when subsequent local complications have subsided [1].

Specific Treatment of Local Complications

Acute Pancreatic Fluid Collection

The term applies to an early pancreatic collection without evidence of pancreatic necrosis, developing within the first month after the episode. These collections rarely pose infectious risks and usually don't require treatment [6].

Pseudocysts

Pseudocysts are encapsulated fluid collections well defined by an inflammatory wall. These usually occur after 4 weeks and may resolve spontaneously. The size of the pseudocyst will not necessarily establish the therapeutic approach, although some studies suggest that masses over 5 cm will probably need intervention. A symptomatic pseudocyst refers to the presence of pain, signs of gastroduodenal obstruction, weight loss or biliary obstruction. Seldom, acute complications can arise. These include rupture causing pancreatic ascites, intracystic hemorrhage, infection, or erosion into adjacent blood vessels. Patients with symptomatic pseudocysts will need drainage interventions. Pseudocysts can be drained percutaneously, surgically or endoscopically [34, 35].

Pancreatic Necrosis

Pancreatic necrosis can occur in two conditions: acute necrotic collection and walled-off necrosis. Acute necrotic collections appear immediately and contain both fluid and necrosis of the pancreas and adjacent tissues. These collections are distinct from walled-off necrosis, which involve the formation of an inflammatory wall. Walled-off collections develop later, one month or more from presentation, the time necessary for a wall to form [1, 6, 36].

Management of patients with symptomatic sterile necrotic collections is quite challenging since data regarding the optimal strategy is scarce [7, 37, 38]. Acute necrotic collections or walled-off pancreatic necrosis benefit from drainage. Drainage in infected walled-off necrosis should be advised when the patient medical status stagnates in spite of maximum medical therapy. A "step-up" to

minimally invasive necrosectomy should be pondered over for patients who fail to improve within 72 h.

Percutaneous drainage is recommended if the necrotic collection has less than 1 month from constitution, whereas, in walled-off pancreatic necrosis, transmural endoscopic drainage followed by necrosectomy seems to be superior, based on a lower rate of pancreatico-cutaneous fistula formation [17].

Surgical Indications in Complications of Pancreatitis

A multidisciplinary team should evaluate the potential surgical patients in order to establish the indication, timing and type of intervention.

Firstly, surgery has to be taken into account in the step-up approach after endoscopical/percutaneous procedures. Timing of surgery is case-tailored. However, postponing the procedure for more than 4 weeks [if possible] allows delimitation of necrosis from other tissues, resulting in less bleeding and therefore less mortality.

Another indication is the intra-abdominal compartment syndrome (>20 mmHg). For this particular complication, we should consider an emergency decompressive laparotomy for prevention of advanced end-organ failure. One alternative decompressive measure is the percutaneous drainage, to be performed when feasible. The timing as well as the type of decompressive techniques would benefit from further studies regarding their impact on morbidity and mortality [39, 40].

Acute bleeding is frequently caused by erosion in the splenic or gastroduodenal artery or other vessels near the pancreas. Tipically IT can present with hypovolemia and a lowering hematocrit. CTangiography will establish the diagnosis and may spot the vessel involved. Patients with severe acute pancreatitis should have an endovascular first attempt to stop bleeding. Emergency surgery is needed when radiological procedures fail [41].

Rare other surgical indications include bowel ischemia or acute necrotizing cholecystitis or bowel fistula extending into a peripancreatic collection.

Specific Measures in Systemic Complications

The systemic complications of acute pancreatitis include worsening of preexisting illnesses such as heart or chronic lung disease, renal. The treatment is supportive and includes oxygen with assisted ventilation for patients with lung failure, renal support therapy (dialysis) in patients with renal failure, and inotropic support in patients with circulatory shock [6, 42].

In the management of severe acute pancreatitis some progress was made and new protocols are proposed by guidelines.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 16

Endoscopic Treatment in Chronic Pancreatitis

Alina Ioana Tanțău[1,*]

[1] *Department of Gastroenterology, 4th Medical Clinic, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania*

Abstract: Chronic pancreatitis is a debilitating disease. A common symptom is a pancreatic pain, sometimes with an impact on the patient's life quality. The goal of the endoscopic approach of chronic pancreatitis with pain resisting standard drugs is the drainage of Wirsung duct and reducing the severity of pancreatic pain. Furthermore, biliary obstruction and pseudocysts are locoregional complications that may endoscopically be resolved. The long term safety and efficacy of the endoscopic approach is under investigation.

Keywords: Chronic pancreatitis, Endoscopic treatment, Pancreatic pseudocysts, Pancreatic stones, Pancreatic strictures.

INTRODUCTION

During the last decade, endoscopic treatment in patients with chronic pancreatitis has become an important therapeutic tool, due to the development of non-invasive imaging techniques [1]. Guidelines recommend endoscopic management in patients in whom the standard medical treatment fails [1].

The chronic pain is developed due to obstruction of pancreatic duct by stones and strictures with secondary ischemia [2], therefore the endoscopic duct drainage seems to be a rational approach [3, 4] and it may be successfully repeated if the episode of pain is relapsing. In patients unfit for surgery or those who are refusing surgery, endoscopic drainage can be chosen as a first-line treatment. Moreover, the endoscopic approach can be practice before surgery as a rescue therapy [4]. As few studies mentioned the quality of life can be improved [3]. The surgery remains the optional approach in the absence of success of endoscopic therapy [5].

* **Corresponding author Tanțău Alina:** Department of Gastroenterology, 4th Medical Clinic, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, 18, Republicii street, Cluj Napoca, Romania; Tel: +40751110150, Fax: +40264598278; E-mail: alitantau@gmail.com

Ioan Sporea and Alina Popescu (Eds.)

The endoscopic therapy of pain in chronic pancreatitis is performed by several procedures, to improve drainage of the Wirsung duct. These include pancreatic sphincterotomy, removal of pancreatic stones, stenting of pancreatic and biliary ducts, and the drainage of pseudocysts with standard endoscopy or with endoscopic ultrasound (EUS) [6 - 8]. Endoscopic procedures may be combined with extracorporeal shock wave lithotripsy (ESWL) for removal of pancreatic stones. In some cases the ESWL alone could be sufficient [6 - 8]. EUS provides important information regarding pancreatic stones and stenosis (Fig. **1**).

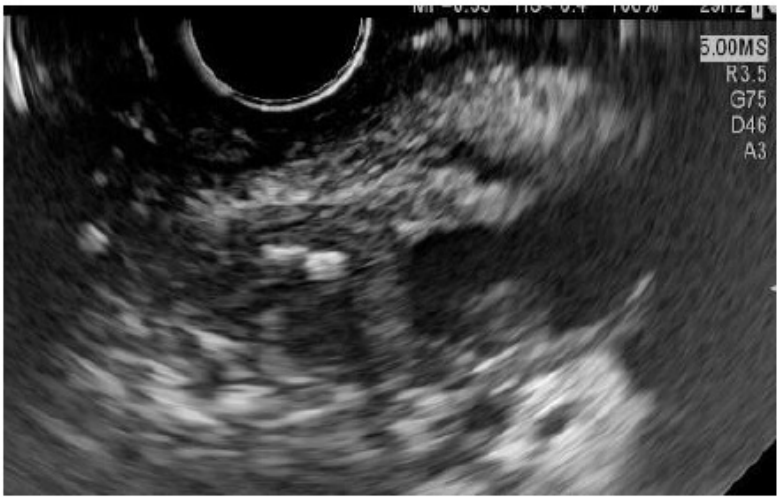

Fig. (1). EUS. Chronic pancreatitis. Pancreatic stones and dilated MPD (main pancreatic duct).

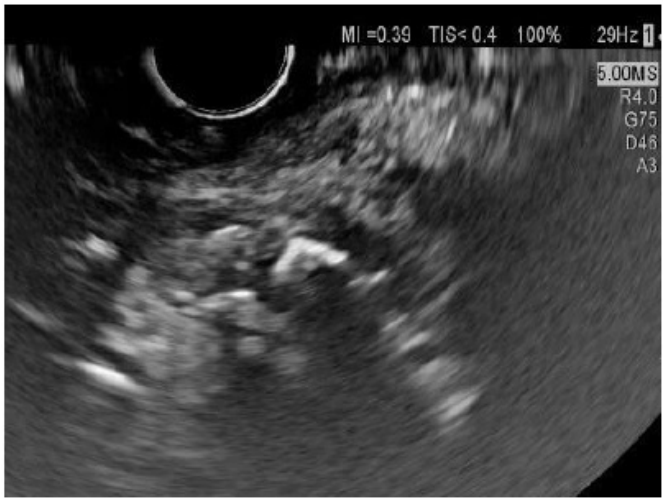

Fig. (2). EUS. Chronic pancreatitis. Intraductal pancreatic stones and head MPD stricture.

Several studies have highlighted the short-term and/or long-term efficiency of endoscopic treatment *versus* surgery in chronic pancreatitis [6]. Regarding the long-term efficiency in patients with pancreatic stones, strictures and dilated pancreatic duct surgery showed better results [6].

Pancreatic Strictures

In chronic pancreatitis, the pancreatic strictures are the consequences of chronic inflammation, fibrosis and pancreatic stones [7]. There may occur single/multiple and dominant/nondominant strictures of the main pancreatic duct (MPD) [8]. Technically successful treatment of dominant MPD strictures is obtained by stent insertion across the stricture. Clinical success is defined as the absence of pain at 1 year after the removal of pancreatic stent removal [8]. The pancreatic brushing and the endoscopic ultrasound with fine needle aspiration (EUS-FNA) rule out a pancreatic malignancy in cases with increased risk [9, 10] (Fig. **3**).

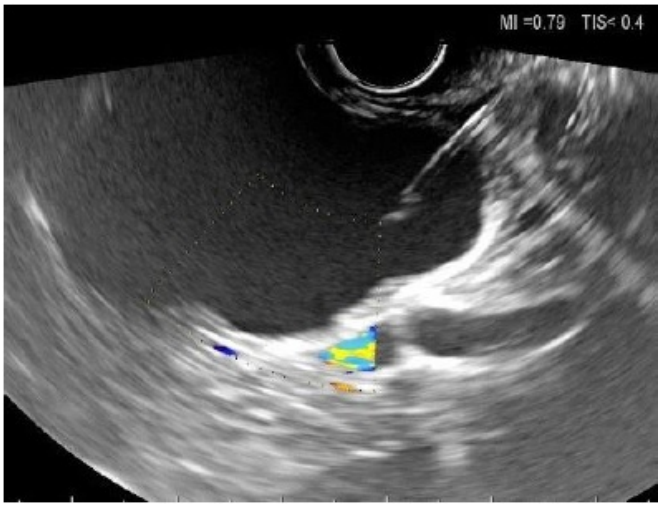

Fig. (3). EUS-FNA. Cyst of the tail of the pancreas.

Dilatation and stenting are endoscopic techniques for benign pancreatic strictures management [7]. Prior to MPD stenting, pancreatic sphincterotomy (Fig. **4**) is preferred in all studies [8, 11 - 13].

The difficult cannulation of MPD, jaundice with cholangitis, cholestasis or dilated CBP are the situations when the biliary and pancreatic sphincterotomy are performed [7, 8]. The pancreatic stenting is recommended in symptomatic cases with only one cephalic stricture of the MPD [7, 8]. In up to 90% of cases the pain is relieved immediately and in up to 50% of cases the pain start decreasing during the follow-up [11 - 13]. For dilatation, wire guided balloons and bougies are

preferred. The widely used stents are the polyethylene pancreatic stent (diameter 10 Fr). The plastic stents will be exchanged every three months or in case of occlusion development [7, 8]. Data showed that in cases where 10 Fr plastic stent was used, the hospitalization rate decrease more than in cases where the pancreatic stents ≤ 8.5 Fr were used [14]. Another study showed that in more than half of patients with ductal strictures, there was a successful placement of plastic pancreatic stents, and it maintained the response after definitive stent extraction. Multiple simultaneous stents should be inseted in patients with Wirsung duct strictures persisting after one year of single plastic stenting. Some authors showed that in majority of the cases with multiple pancreatic stents, a median of three stents is an effective approach. The stents were placed for a median time of 7 months. In this case, the pain was decreased after more than 3 years of follow-up [15]. More than two-thirds of patients (86%) with pancreatic strictures and temporary placement of fully covered SEMSs for 2 or 3 months, remain asymptomatic at least 5 months after the stent extraction [27 - 29].

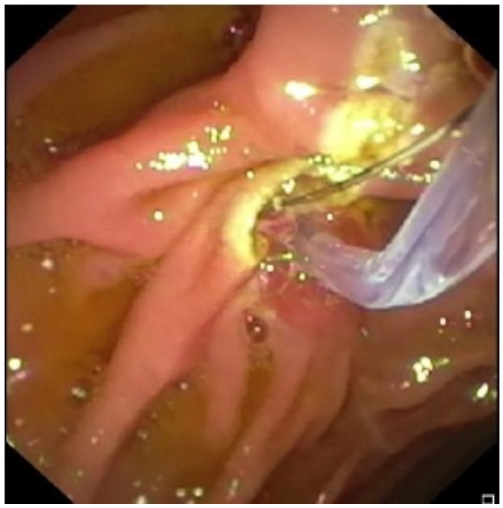

Fig. (4). Endoscopic view of the ampulla and pancreatic sphincterotomy.

Table 1. Selected series of plastic stenting of MPD strictures in chronic pancreatitis [17 - 18].

Study	No. of Patients	Follow-up Months	Early Pain Relief %	Long-term Pain Relief %	Surgery %
Morgan *et al.* [19]	25	NA	65	NA	NA
Ishiara *et al.* [11]	20	21	95	90	NA
Weber *et al.* [12]	17	24	89	83	NA

NA not available.

Pancreatic Stones

The pancreatic sphincterotomy with stones extraction, ESWL with stones fragmentation or association of these two procedures is proposed for the complete removal of pancreatic stones. The endotherapy is efficient in two third of the cases and in more than a half of the cases (68%) pain improvement was achieved [20]. A large prospective study (1006 patients) showed a very high (93%) success rate for ESWL technique [21]. The endoscopic removal of MDP stones is facilitated by ESWL. Pain relief by using ESWL approach has been demonstrated in a large meta-analysis (17 studies) [22]. The success of MPD clearance after ESWL was obtained in cases of a single cephalic stone [23]. ESWL alone was compared with ESWL followed by ERCP in 55 patients [23]. The authors noted a longer hospitalization rate with a higher cost in patients with ESWL followed by ERCP [23]. In cases with pancreatic stones and pancreatic strictures, ESWL following by endoscopic treatment showed good short and long-term results [21].

The success rate of intraductal laser and electrohydraulic lithotripsy after ESWL failure varies (47-83%) in a small case series [24]. In Table **2** are presented two studies regarding the efficacy of endoscopic therapy in short and long-term.

Table 2. Results of pain treatment in chronic pancreatitis after endoscopic extraction and ESWL of pancreatic stones.

Study	No. of Patients	Follow-up Months	Early Pain Relief %	Long-term Relief %	Surgery %
Inui *et al.* [25]	504	44	97	78	4
Tadenuma *et al.* [26]	70	36	97	70	0

Pancreatic Pseudocysts (PPC)

Intraductal hypertension and the rupture of a pancreatic duct may cause the development of pancreatic pseudocyst in twenty to forty percent of patients with chronic pancreatitis [27]. The symptoms, the PPC infection or the PPC enlarging with secondary obstruction [27] are the indications for endoscopic treatment (Figs. **5** - **7**).

In patients with a direct communication between PPC and the MPD (40-66% of cases), the insertion of one or few stents from the digestive lumen into PPC or through papilla or a combination of these methods represent the therapeutic options. The ability to insert at least one stent from PPC to digestive lumen or resolution of PPC define the technical success [8]. The complete improvement of the symptoms and the reduction of PPC about 30-50% in one month after

treatment define the short-term clinical success [60]. Transmural drainage has a technical success rate in the majority of cases, the recurrence rate is present in 15% of cases and the complication rate is present in up to 30% of patients [28 - 31]. Transmural PPC drainage under EUS guidance should be performed in cases with collateral circulation and in cases without luminal bulging [27]. The cystoduodenostomy approach is preferred instead of the cystogastrostomy technique [27]. The morbidity rate is the same (10%) between these two techniques, but the long-term success rate is higher in cystoduodenostomy (83,1% *vs.* 64%). Studies showed that the insertion of more than a double-pigtail plastic stent is required for at least 2 months. In case of stents removal before resolution, the cyst may occur [32, 33]. The transmural drainage under EUS guidance in case of portal hypertension decreases the risk of bleeding [7, 8, 27]. In the case of an arterial pseudoaneurysm the arterial embolization should be performed anterior to pseudocysts drainage [34].

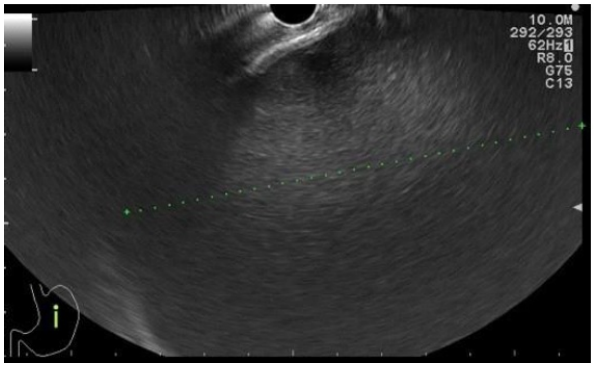

Fig. (5). EUS. Large pseudocyst producing bulging of the stomach and symptoms.

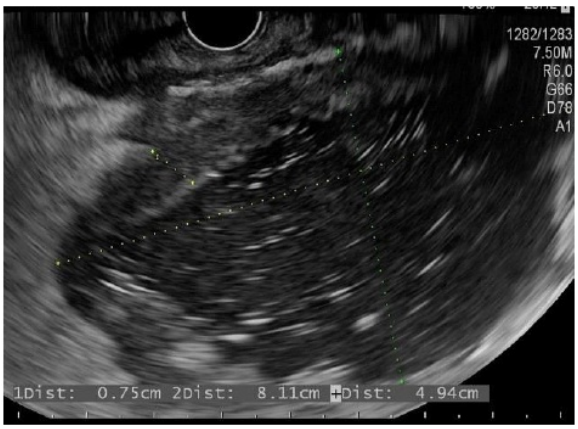

Fig. (6). EUS. Pseudocyst with thick walls and debrides.

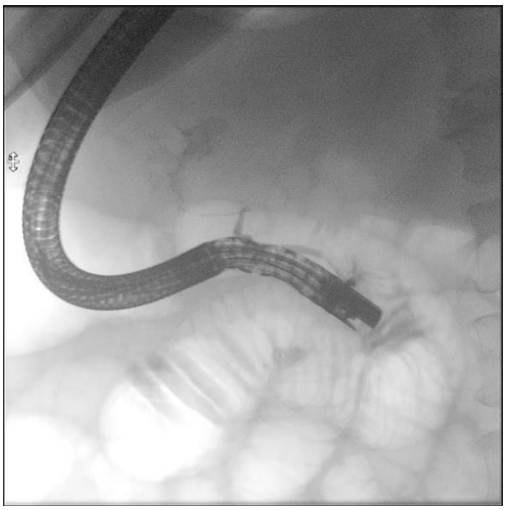

Fig. (7). Radiologic view. Ductal pancreatic rupture at the level of the head of the pancreas. MPD communicates with a pseudocyst.

Biliary Duct Strictures

Up to a quarter of patients with chronic pancreatitis, biliary duct strictures is developed by fibrosis, pseudocysts and cancer [35]. The endoscopic treatment of strictures is challenging, especially in cases with calcifications in the head of the pancreas. The recurrence rate may occurred in 1/3 of cases after temporary multiple plastic stents placement (Fig. **8**) simultaneously or covered SEMSs insertion and in 2/3 of cases after using a single plastic stent [7, 8].

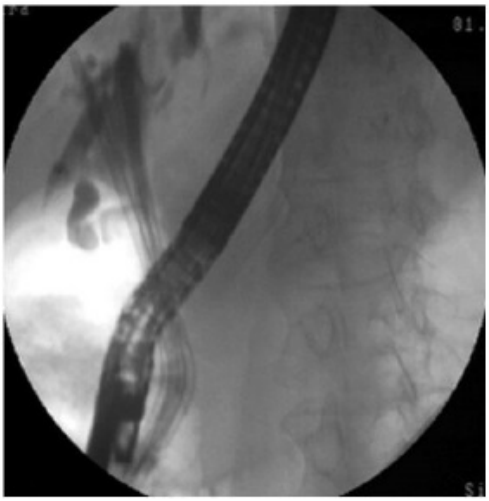

Fig. (8). Multiple Biliary Plastic stenting for chronic pancreatitis related stricture.

The digestive symptoms, biliary stones, secondary biliary cirrhosis, progression of biliary stricture, increased levels of serum alkaline phosphatase and serum bilirubin for more than 30 days [27] are the indicators for endoscopic treatment. A suspect malignancy should be ruled out before the endoscopic stenting using IMR and/or EUS [7]. Clinical success rate by using temporary simultaneous plastic stents is 94%, 80% in the placement of uncovered SEMS and 60% in a single plastic stent according to the studies. The uncovered SEMSs have a higher complication rate (40%) compared with single plastic stents (36%) and multiple plastic stents (20%) [36]. Since 12 months after uncovered biliary SEMSs insertion the patency decreases [37]. Initially, Costamagna *et al.* obtained good long-term results for pain improving and biliary strictures resolution with multiple stent placement in cases of postoperative stricture [38]. Several studies showed also a median rate of 10-33% strictures resolution [36, 38, 39]. The possibility of occurrence of epithelial hyperplasia, occlusion of the stent, chronic inflammation, cholangiocarcinoma development and the impossibility of stent retraction are the major limitations of the uncovered self-expanding biliary metal prosthesis (USEMS) in chronic pancreatitis [7, 8]. The long term success rate of the partial covered self-expanding prosthesis (PCMS) and the fully-covered self-expandable metal prosthesis (FCSEMS) is present in more than half of cases with a low recurrence rate of 14%. The removal rate of these stents from patients with biliary stricture related to chronic pancreatitis is 75% [36]. The patients in whom plastic stent therapy failed, these stents are useful for a temporary placement due to their easy removal [40]. The patency of PCMS decreases if these are left in place over time and additional endoscopic interventions are needed [41].

EUS-Guided Celiac Plexus block

Considering the success rates close up to 100% and the small complication rate, EUS-celiac plexus block (CPB) is recommended in patients with painful chronic pancreatitis over CT-guided celiac plexus block. Unfortunately the long-term success rate is low. Almost half of the patients had a short-term pain improvement, but only 10% of them for 6 months only [42].

Our Experience

We analyzed 129 patients with painful chronic pancreatitis (106 males and only 23 females). These patients were endoscopically treated using various endoscopic procedures (pancreatic or biliary stenting, dilatation of structures, stones removal, and cysts drainage) [43]. The follow up period was around 15 months. Technical success of pancreatic, biliary and pseudocysts drainage was obtained in the majority of the cases (81.39%, 74.29% and 96.43% respectively). In half of the

patients the pain was completely disappeared and in the other half of the patients the pain was improved [43].

In patients younger than 40 years, the technical success was higher than in patients older than 40 years. The ERCP and procedures rate were more efficient in cases with pancreatic stones and strictures, compared to the cases with either strictures alone or stone alone [43].

Smoking and alcohol drinking were two factors affecting the efficacy of endoscopic treatment. Therefore, in our experience, the clinical success rate was higher in non-smokers patients. There was a high rate of admission in cases with alcohol drinkers and smokers. Better prognosis was achieved in non-smokers and patients with non-alcohol consumption [43].

CONCLUSIONS

The endoscopic approach has an important role in the treatment of chronic pancreatitis. The chronic pain management remains a therapeutic challenge and an interdisciplinary collaborative team formed by radiologist, gastroenterologist and surgeon is mandatory to face this challenge. In patients with painful chronic pancreatitis and without an optimal answer to the medical therapy, a minimal endoscopic treatment should be the first approach.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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Declared none.

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CHAPTER 17

EUS Drainage of Peripancreatic Fluid Collections

Gabriel Constantinescu[1,2,*] and **Mădălina Ilie**[1,2]

[1] *Department 5, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*

[2] *Department of Gastroenterology, Clinical Emergency Hospital of Bucharest, Romania*

Abstract: Endoscopic ultrasound (EUS) has revolutionized the management of peripancreatic fluid collections (PFCs). In the last decades, new treatment strategies have been widely approached and recommended, by shifting from surgical interventions to minimally invasive modalities such as EUS-guided drainage. PFCs complicate the evolution of acute or chronic pancreatitis, traumas or surgical interventions. It is generally accepted, among scientific community, that PFCs may be managed conservatory in the first 4-6 weeks and that delayed intervention is currently preferred over early intervention in order to decrease morbidity and mortality. PFCs may be drained using different endoscopic approaches: transpapillary/transductal, transmural or in selected cases by a combination between both. Nowadays, transmural drainage by stents insertions under EUS-guidance represents the mainstay technique used in the management of pseudocysts or WONs. There are two types of stents: plastic stents and metal stents. Double-pigtail plastic stents are generally used to drain pseudocysts with mostly fluid content. Innovative stents, namely lumen-apposing covered self-expanding metal stents (LAMS) have been developed to simplify the procedure from a technical point of view. In addition, LAMS are preferred in drainage of WONs because of their large diameter which allows direct endoscopic necrosectomy by passing the endoscope through the stent lumen. In conclusion, EUS-drainage by placement of stents is currently the best option for the management of PFCs in terms of safety and efficacy.

Keywords: Drainage, Endoscopic ultrasound, Metal stents, Plastic stents, Pseudocyst, Walled-off necroses.

INTRODUCTION

Traditionally, surgical treatment was considered the procedure of choice for peripancreatic fluid collections (PFCs), unfortunately carrying the risks of recurrence (5-20%), morbidity (10-30%) and mortality (1-5%) [1].

* **Corresponding author Gabriel Constantinescu:** Department 5, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania and Department of Gastroenterology, Clinical Emergency Hospital of Bucharest, Calea Floreasca 8, 014461 Bucharest, Romania; E-mail: gabrielconstantinescu63@gmail.com

Ioan Sporea and Alina Popescu (Eds.)

Recently, the management of PFCs faced a paradigm shift towards minimally invasive techniques which encompasses percutaneous and endoscopic drainage. Endoscopic methods have several advantages over percutaneous drainage: safer access to collections, lack of an external catheter, lower risks of complications (pancreatic-cutaneous fistula in particular), higher success rates, lower recurrence rates [2, 3].

Over the last two decades, EUS (endoscopic ultrasound)-guided drainage of PFCs has gained popularity in terms of safety and efficacy, being generally preferred over surgical and percutaneous drainage, as the standard procedure in many centres [4 - 6]. Data concerning technical and clinical success show high rates for EUS-guided transmural drainage (>90%) [7].

DEFINITION AND CLASSIFICATION OF PFCS

Peripancreatic fluid collections (PFCs) represent accumulation of fluid inflammatory contents and/or necrotic tissues, complicating the course of pancreatitis (acute or chronic), traumatic injuries or surgical interventions [8]. Based on duration of disease and their content, PFCs are subdivided, according to the revised Atlanta Classification stated in 2012, into four categories: ***acute peripancreatic fluid collections, acute necrotic collections, pseudocysts and walled-off necroses*** (Table **1**) [9].

Table 1. Revised Atlanta classification pancreatic/peripancreatic fluid collections [9].

Duration from the Onset of Acute Pancreatitis	Type of Collection	Features	Evolution
≤ 4 weeks	acute peripancreatic fluid collection	Homogeneous, no well-defined walls, no solid material, single/multiple	resolve spontaneously/ progress to pseudocyst
	acute necrotic collections	Heterogeneous, no well-defined walls, necrotic material, single/multiple	resolve spontaneously/ progress to pseudocyst
> 4 weeks	pseudocyst	Encapsulated, homogeneous, fluid content	resolve spontaneously asymptomatic→ symptomatic sterile → infected
	walled-off necroses	Encapsulated, heterogeneous, solid content	resolve spontaneously asymptomatic→ symptomatic sterile → infected

Acute peripancreatic fluid collections appear in the early phase of interstitial oedematous pancreatitis, have no well-defined walls, being confined to the

retroperitoneum and adjacent organs. Most of them resolve spontaneously, while 5% to 15% of cases evolve into pancreatic pseudocyst, after 4 weeks [10]. Pseudocysts are encapsulated, having no solid material inside and result from leakage due to the disruption of the main pancreatic duct or its branches [9].

Necrotizing pancreatitis accounts for 10% of all cases of acute pancreatitis. 20-40% of them may be complicated by *acute necrotic collections* [11, 12]. These distinguish from acute fluid collections because of their inhomogeneous necrotic content involving pancreatic parenchyma and/or peripancreatic tissues. They are seen within the first 4 weeks from the onset of necrotizing pancreatitis and may be single or multiple and multiloculated [8]. In their course, most of them progressively resolve, while in 1-9% persist as walled-off necroses (WONs). WONs is the term used to define encapsulated necrotic collections, after 4 weeks from the acute episode; 50% of them remain asymptomatic, while the other half become symptomatic [12].

Both pseudocysts and WONs are sterile in the beginning and might get infected by bacterial translocation or following iatrogenic maneuvers. If a necrotic collection is infected, the mortality rate is up to 30% [13]. Pseudocysts are more commonly seen in chronic pancreatitis associated with obstruction of the pancreatic duct due to strictures and stone formation, while WONs develop more frequently after acute pancreatitis. Regarding the etiology, the most frequent causes are alcohol consumption, biliary tract stones, iatrogenic procedures (endoscopic/surgical) [14].

INDICATIONS FOR DRAINAGE OF PFCS

Data concerning management of PFCs are vast and controversial among studies. However, it is generally considered that acute collections do not require specific therapy.

Pancreatic pseudocysts should be managed conservatory between 4 to 6 weeks, as studies have shown that almost one third of them regress spontaneously [15]. The main indications for interventional procedures of pseudocyst drainage are the presence of symptoms, progressive increase in size and persistence [1]. While collections smaller than 3 cm are not amenable to drainage, those larger than 5 or 6 cm are predisposed to complications, especially when the size does not decrease in six weeks. The larger is the size, the higher are the risks of complications and mortality. Rupture into nearby viscera (stomach, duodenum, colon) and peritoneum may cause melena, hematemesis, hematochezia, pancreatic ascitis, peritonitis or even hemorrhagic shock [16, 17]. Therefore, early drainage is mandatory for large pseudocysts, especially over 10 cm [1]. Symptomatic pseudocysts are also responsible for abdominal distension, nausea, vomiting, pain,

but may manifest signs and symptoms attributable to their effects upon adjacent organs and vessels (Table **2**). Besides, infection or bleeding inside the pseudocyst may complicate the evolution and require interventional therapy [4].

Table 2. Signs and symptoms of pancreatic pseudocysts that represent indications for interventional therapy.

Adjacent Organs/Vessels Involvement	Signs and Symptoms
Biliary tract	Stenosis → Obstructive jaundice
Esopaghus	Compression → Dysphagia
Stomach	Gastric outlet obstruction, cystogastric fistula, intramural gastric mass
Duodenum	Duodenal outlet obstruction, fistula
Portal vein, splenic vein	Fistula, thrombosis, portal hypertension
Gastroduodenal artery, splenic artery	Erosion → haemorrhage Pseudoaneurysm
Pleura, mediastinum	Pancreatic-pleural fistula, pleural effusion, mediastinal extension
Skin	Subcutaneous fat necrosis
Genitourinary tract	Stricture, fistula, ureter obstruction

When referring to WONs, there is well-known that similar to pseudocysts, these may cause compressions and obstructions of the gastrointestinal tract, biliary duct and blood vessels or may fistulize to adjacent organs [17]. Besides, WONs bear higher risk of infection, with 44-70% of them getting infected during their evolution [18, 19]. Delayed intervention is currently preferred over early intervention because the last one is associated with increased morbidity and mortality. The most acceptable treatment for asymptomatic WONs is the "step-up" approach, starting with "watchful waiting" and conservative treatment with antibiotics and nutritional support [20]. The most common bacteria encountered inside WONs are *Escherichia coli, Klebsiella pneumonia, Enterococcus faecalis, Pseudomonas aeruginosa* which are also found in gut microbiota [19]. Antibiotics should be selected according to their potential to penetrate into the WONs. The most effective are 3rd-generation cephalosporins, carbapenems and metronidazole [20]. Interventional drainage is recommended when conservative management fails to treat WONs. There is general consensus among studies that endoscopic drainage is the treatment of choice for symptomatic or infected PFCs, at least 4 weeks after the acute episode, in order for surrounding walls to constitute [12, 17, 21 - 23].

Imagistic techniques such as MRI (magnetic resonance imaging), S-MRCP (secretin-enhanced magnetic resonance cholangiopancreatography) or EUS should be performed before drainage in order to confirm that the PFC does not represent a cystic neoplasm, pseudoaneurysm, duplication cyst or other noninflammatory fluid collection (*ASGE, 2016; ESGE, 2019*). In addition, pre-treatment management should also focus on discontinuation of anticoagulant and antiplatelet drugs [23, 24].

TECHNICAL ASPECTS REGARDING EUS DRAINAGE OF PFCS

Endoscopic drainage creates a connection between PFC cavity and the gastrointestinal lumen. PFCs may be drained using different endoscopic approaches: transpapillary/transductal, transmural or in selected cases by a combination between both. The approach depends on the anatomic relation of the collection with stomach and duodenum, the content, the size, whether or not it communicates with the pancreatic duct [23]. Transpapillary/transductal drainage is performed *via* endoscopic retrograde cholangiopancreatography (ERCP), mostly for small collections located in the head or body of the pancreas that communicate with the main pancreatic duct. Transmural route is recommended to drain large PFCs which do not communicate with the main pancreatic duct and are near the duodenal/gastric walls. Transmural approach may be performed either conventional, as a blind procedure or assisted by EUS [4, 5].

In contrast to conventional endoscopy, EUS does not rely on bulging to locate the collection, offering the possibility to accurately establish the localization of PFCs. In addition, EUS provides several other advantages: identify and avoid vascular interposing structures, characterize the material within the PFCs, assess the apposition with the gastric/duodenal wall and guide the entire procedure of drainage [17]. EUS can be used in two ways for the drainage of PFCs. Initially, a radial or linear echoendoscope may be used to assess the localization of the collection and afterwards withdrawn and replaced by a therapeutic duodenoscope to complete the procedure. This technique has been lately abandoned in favour of the one-step procedure that uses a single linear therapeutic echoendoscope [25].

The first successful EUS-guided drainage was performed in 1992 by Grimm *et al*. [26] in a patient with chronic pancreatitis complicated by a large pseudocyst, thus promoting a new and safer method for endoscopic pseudocysts drainage. The first drainage of WON was described in 1996 by Baron *et al*. [27] by endoscopic transmural approach and lavage through a nasocystic tube. In 2000, Seifert and his colleagues [28] introduced the concept of direct endoscopic necrosectomy (DEN), by advancing the endoscope into the necrotic cavity with subsequent removal of necrotic tissue using endoscopic accessories. In the following years,

the overall success rate for EUS-guided drainage of PFCs increased and the complication rate significantly decreased because of continuously improvements of techniques [17, 29].

Placement of stents are nowadays widely used in the management of PFCs. There are two types of stents described to drain these collections: plastic stents and metal stents. Double-pigtail plastic stents (DPPSs) (Fig. **1**) were initially used in the 90s [21]. Starting with 2009, multiple small case series described the use of biliary or esophageal fully-covered self-expanding metal stents (FCSEMS) for the drainage of PFCs [30 - 32]. The advantages of FCSEMS (Fig. **2**) consist of the larger lumen for drainage and the possibility to pass the endoscope for necrosectomy. Even so, the adverse reactions are high, ranging from 15% to 33%, due to the fact that after the collection collapses, the stent may be pushed outside and result in stent migration. Besides, conventional metallic stents may also erode the pancreatic bed and cause severe bleeding [30].

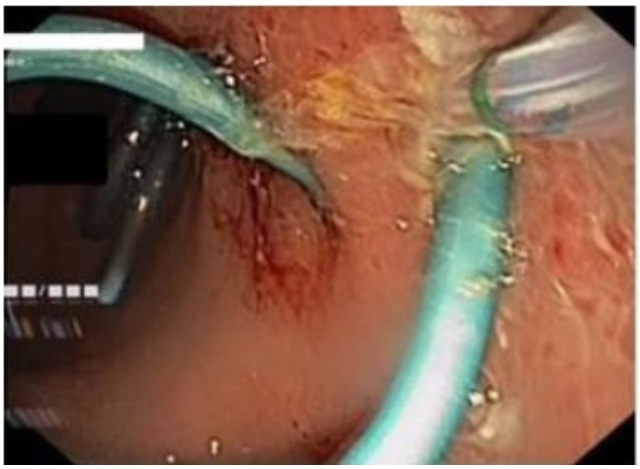


Fig. (1). Endoscopic view of DPPSs.

Transmural drainage with double-pigtail plastic stents (DPPSs) was the mainstay of endoscopic therapy for PFCs until the introduction of lumen-apposing covered self-expanding metal stents (LAMS) in 2011 [33]. LAMS are dedicated devices for EUS-guided drainage of PFCs with the latest version having a single-step deployment mechanism, thereby simplifying technical steps of the procedure. Due to their proximal and distal flanges they allow tissue apposition and decrease the risk of stent migration. LAMS are preferred in drainage of WONs because of their large diameter (10-20 mm) which allows DEN by passage of the endoscope through the stent lumen, improving efficacy and decreasing adverse events associated with these procedures [21, 34 - 36].

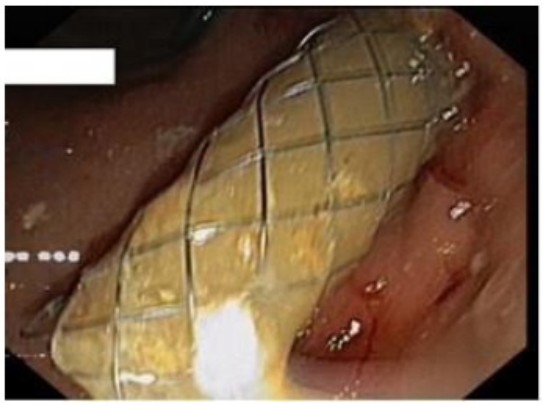
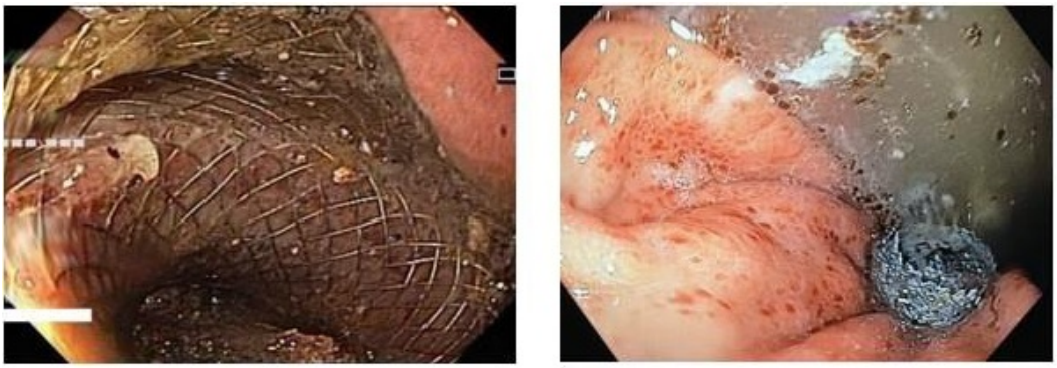


Fig. (2). Endoscopic view of FCSEMS.



a. **b.**

Fig. (3). (a) Endoscopic view of LAMS. **(b)** Endoscopic view of LAMS.

Recently, several studies [34 - 37] compared the efficacy of plastic stents with metal stents. Siddiqui *et al.* [34] demonstrated in a multicenter study that EUS-guided drainage of WONs using FCSEMS and LAMS is superior to DPPSs in terms of overall long-term clinical efficacy. The number of procedures and endoscopic reinterventions were lower in the LAMS group, while the complete resolution was significantly higher using FCSEMS and LAMS compared to DPPSs [34]. Bang *et al.* [35] failed to demonstrate improved outcomes of metal over plastic stents for the drainage of either pseudocysts or WONs. Lang and his colleagues [37] reported equal rates of clinical success regarding DPPSs and LAMS, but significantly more adverse events with LAMS compared to DPPSs. DPPSs recorded a lower clinical success rate (93.6%) with a single stent and a higher success rate (97.4%) with multiple stents [37].

Technically, the EUS-guided drainage consists of several steps as follows: excluding interposing vessels, measuring the distance between gastric/duodenal

wall and collection (less than 1 cm is recommended) and establishing the appropriate site to puncture. Afterwards, a 19 Gauge needle is inserted into the collection through which a 0.035-inch guidewire is advanced under fluoroscopic guidance. Then, a 10 Fr or 6 Fr cystotome creates a fistula between the collection and the gastric/duodenal cavity, which is further dilated using a 6-15 mm balloon. The final step involves the deployment of one or multiple 7 Fr or 10 Fr double-pigtail plastic stents using the double wire technique [3]. The European Society of Gastrointestinal Endoscopy (ESGE) [24] recommends the insertion of at least two double-pigtail plastic stents. Moreover, transmural stents should not be retrieved before evaluating the resolution of the pseudocyst by cross-sectional imaging or at least 6 weeks after its regression. In patients with disconnected pancreatic duct syndrome, transmural double-pigtail stents should be kept indefinitely [24].

The use of LAMS has become further simplified by the development of an electrocautery-enhanced delivery system using the electrocautery catheter tip without need for needle puncture and wire-guided entry [21]. PFCs tend to resolve faster with LAMS; therefore, it is recommended to assess treatment response by cross-sectional imaging in 3 weeks post-insertion. LAMS should be removed within 4 weeks if the collection has resolved or is less than 3 cm in size, in order to prevent the complications like bleeding or "buried stent" (mucosal overgrowth) [36]. Multiple debridement sessions are recommended and should be performed every 48 to 72 hours [23]. A controversial aspect is represented by the use of PPI in patients with LAMS for WONs. It seems that stent occlusion and the need for DEN are higher in patients treated with PPI [17].

EUS-guided drainage is efficacious for both simple and infected pseudocysts. However, infected pseudocysts are more difficult to drain and associated with a higher complication rates [10]. Varadarajulu *et al.* [38] noted a higher success rate for pseudocysts compared to necrosis (93.5% *vs.* 63.2%) and a lower complication rate for pseudocysts compared to necrotic collections (5.2% *vs.* 15.8%). The authors used plastic stents with additional nasocystic catheter placed to facilitate periodic flushing and aspiration of necrotic tissue [38]. For drainage of pseudocysts, LAMS have an estimated technical success of 95-99% and a clinical success of 96-100% [17].

Regarding EUS-guided drainage of WONs, the transmural route is generally preferred to allow evacuation of solid tissue and endoscopic debridement [23]. These techniques require skilled endoscopists and the post-procedure management after initial drainage is more extensive than the one for uncomplicated pseudocysts. Traditionally, multiple transmural plastic pigtailstents were placed along with nasocystic drainage for lavage *via* one guidewire [23]. An advanced, new method named multiple transluminal gateway technique (MTGT),

based on creating 2-3 unique transmural tracts with multiple guidewires for multiple stent insertion and 1 for nasocystic lavage, is considered significantly more efficient than conventional drainage with single tract for 2 plastic stents and a nasocystic drain [39]. Irrigation of the WONs with normal saline solution with a flushing volume of 50-500 ml, three to six times per day was described in several studies [40, 41]. In addition, hydrogen peroxide or antibiotic irrigation may also be used during necrosectomy sessions [42]. However, according to ESGE (European Society of Gastrointestinal Endoscopy) [43] no prospective randomized trials have assessed the duration, type and volume of irrigation. Therefore, ESGE suggests "restraint regarding the use of high-flow water-jet systems, hydrogen peroxide, or vacuum-assisted closure systems to facilitate debridement of necrosis in walled-off necrosis" [43]. Furthermore, no significant difference was found regarding clinical success between with or without nasocystic tube placement [44].

COMPLICATIONS OF EUS DRAINAGE

Developments in EUS-guided drainage techniques and LAMS have significantly reduced the complication rates which have been varying widely from 5% to 35%, bleeding and perforation being the most common and significant complications [45].

Adverse events may by divided into 2 types: complications that occur during the procedure or immediately after procedure and delayed complications. Immediate or early complications encompasses technical failure, improper stent deployment, bleeding at the site of puncture, during dilatation of the tract or within the cavity, perforation, air embolism (is rare, <1% but fatal), shearing of the guidewire by needle. Delayed complications refer to bleeding due to formation of a pseudoaneurysm caused by an indwelling transmural stent. Other delayed complications are buried stent, especially encountered in LAMS left *in situ* for long time (>6 weeks), secondary infection caused occlusion of stents with either food or solid debris [45].

CONCLUSIONS

Over the last 3 decades the evolution of endoscopic treatment of PFCs went from simple aspiration, fistulotomy, nasocyastic catheter drainage, EUS-guided puncture to necrosectomy, insertion of metal stents, multiple gateway access techniques and development of EUS specific stent systems. The most important discoveries are probably the development of novel lumen-apposing metal stents and linear echoendoscopes which have brought tremendous advancements in terms of accuracy, safety and efficacy in the management of PFCs.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 18

Update in the Management of Pancreatic Cysts

Mariana Jinga[1] and **Daniel Vasile Balaban**[1,*]

[1] *"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest Romania*

Abstract: Pancreatic cystic lesions (PCL) comprise a wide spectrum of pathological entities, from benign lesions such as retention cysts and pseudocysts to potentially malignant ones such as mucinous cystic neoplasms and intraductal papillary mucinous neoplasms. Due to the widespread use of cross-sectional imaging for various indications, PCLs are being increasingly identified in clinical practice and they can pose diagnostic challenges sometimes. Among the broad differential diagnosis of a PCL, the stake is to accurately detect lesions with a malignant potential. Along with the medical history of the patient and the imaging features of the PCL, endoscopic ultrasound (EUS) plays an important role in the management of these lesions, by providing detailed morphologic assessment including vascular pattern and detection of solid component, cyst fluid analysis and tissue diagnosis. We herein summarize the currently available evidence with regard to diagnostic updates in PCLs, focusing on recent advances in tissue acquisition and diagnosis – the micro-biopsy forceps, confocal laser endomicroscopy and cyst fluid markers. Although in an early phase, artificial intelligence applications in PCLs are briefly discussed. In summary, there has been significant progress in PCL diagnosis over the last few years and there is growing evidence that accuracy will be further improved by routine use of molecular markers in cyst fluid.

Keywords: Confocal laser endomicroscopy, Cyst fluid, Endoscopic ultrasound, Mucinous, Micro-biopsy forceps, Neoplastic, Pancreatic cyst.

INTRODUCTION

With the growing use of cross-sectional imaging for various indications, often pancreas-unrelated, pancreatic cystic lesions (PCLs) are being increasingly encountered in routine practice. The prevalence is 8% in asymptomatic individuals and increases with age [1]. This has led some authors to consider pancreatic cysts a "disease of technology". Although the vast majority of them are benign, detection of a PCL can generate significant anxiety for patients and pose diagnostic challenges for clinicians. In front of a patient with a PCL, the stake is

* **Corresponding author Balaban Daniel Vasile:** "Carol Davila" University of Medicine and Pharmacy Bucharest, 37 Dionisie Lupu, 030167; Tel: +4074251793; E-mail: vbalaban@yahoo.com

Ioan Sporea and Alina Popescu (Eds.)

to differentiate if the cyst is neoplastic or non-neoplastic and to assess its risk of progression to malignancy. This translates into a decision to either follow-up the cyst, when it has a low-risk of malignant transformation, or to sent it for surgery, when the risk is high. Misdiagnosing a cystic lesion can thus bear a risk of either missing an early cancer and the opportunity for curative resection or sending a patient for unnecessary, high morbidity and mortality surgery. Over the time there have been several guidelines published on PCLs, with some recommendations of low-quality evidence. Some of these guidelines are favoring a surgical approach, while others are more balanced towards a conservative, follow-up approach [2 - 7] (Fig. **1**). Several issues have been revealed when analyzing management decisions based on these guidelines [8, 9].

Fig. (1). – Evolution of guidelines on PCLs over the time.

Although currently available diagnostic techniques allow accurate characterization of PCLs and even subtyping of cysts, sometimes a definite diagnosis can be difficult. While diagnosis has improved considerably from the mere characterization of PCLs on imaging to cyst fluid analysis, *in-vivo* histology and sampling of cyst wall, management of PCLs is still far from being satisfactory. However, even if some PCLs may harbor cancer, we should keep in mind that the vast majority of lesions will not progress to malignancy. Surveillance on the other hand can be costly for health care systems and generates uncertainty for patients.

In this chapter, we aim to discuss the technological advances and the most recent evidence regarding the improvements in diagnosis and management of PCLs.

Approach to the Patient with PCL

In front of a patient with a PCL, the clinician should make use of all features and tools that could provide an insight for the diagnosis of the lesion. Age and gender are important to note, as some lesions are found mostly in young females (solid pseudopapillary neoplasm – SPN), others in females in their 30-40s (mucinous cystic neoplasm – MCN) and others in their 60-70s (serous cystic neoplasm – SCN). A thorough medical history is also warranted, particularly checking for episodes of acute pancreatitis and risk factors for pancreatic tumors. With regard to the characterization of the cysts, computed tomography (CT) is known to accurately demonstrate calcifications, while magnetic resonance imaging (MRI) better depicts the cystic structure of a hypodense lesion on CT scan and also

provides details about the communication with the main pancreatic duct. Besides morphological assessment, endoscopic ultrasound (EUS) is undoubtedly essential by providing cyst fluid and tissue sampling by fine needle aspiration (FNA) – (Figs. **2** - **5**).

Fig. (2). – Role of endoscopic ultrasound in the characterization of PCLs.

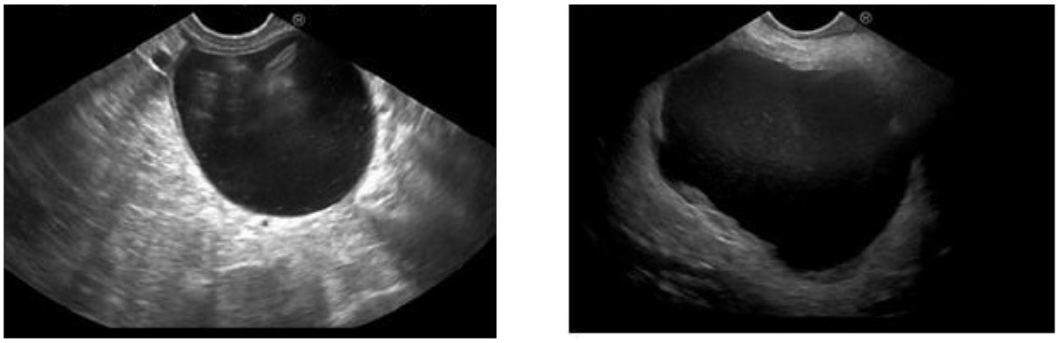

Fig. (3). – EUS images showing peripancreatic fluid collections in the setting of acute pancreatitis.

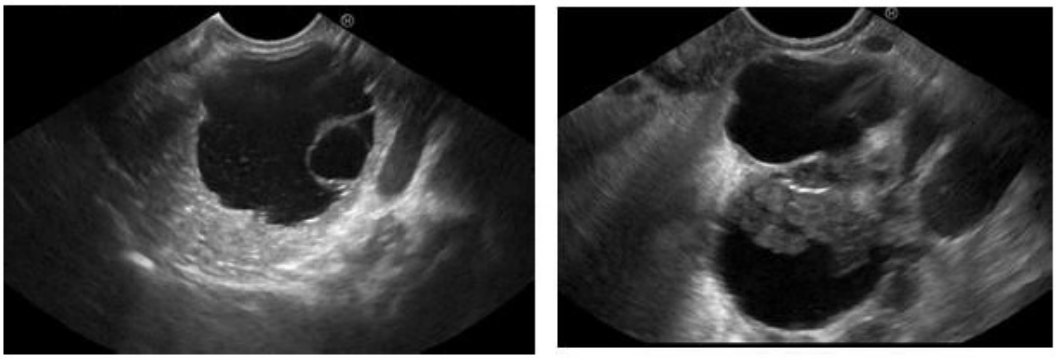

Fig. (4). – EUS images of mucinous cystic neoplasms – A: PCL with "cyst in cyst" appearance, B. Multiloculated PCL with solid component.

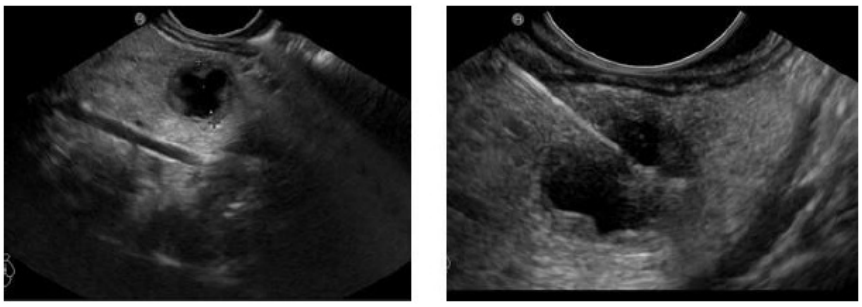

Fig. (5). – EUS image revealing PCL with thick, irregular internal wall and tissue sampling with microforceps biopsy – cystic neuroendocrine tumor.

Micro-biopsy Forceps

The major limit of EUS-FNA in PCLs is represented by scant cellularity which is insufficient for diagnosis. With standard techniques, up to 30% of PCLs are misdiagnosed as mucinous lesions, which results in unnecessary surgery [10]. To overcome this, the microforceps biopsy (MFB) has emerged as a novel tissue acquisition tool that provides the opportunity to sample the cyst lining and thus set a definite diagnosis. The MFB is mounted through a 19G needle used to puncture the cystic lesion and provide histology specimens from the cyst wall – (Fig. **5**). Also, the MFB can target mural nodules or septations in PCLs. Currently available data have shown high technical success in using this method, with possible difficulties for transduodenal punctures, when the EUS probe is in the fully flexed position [11]. The technique has been reported to have high diagnostic accuracy (82.76%) in a systematic review and meta-analysis of 11 studies comprising 518 patients [12], with a low rate of serious adverse events (1.08%). However, some authors have reported high rates of adverse events – up to 22.9% [13], with intracystic bleeding, usually self-limited, being the most frequently reported. Of note, a change in the management of PCLs was reported in up to 1 in 4 patients, which is quite significant considering that this would translate into reducing unwarranted surgery and optimizing follow-up decisions [11]. Also, compared to cytology alone, MFB can provide a definite diagnosis of the specific cyst histotype, for example intraductal papillary mucinous neoplasm (IPMN) subtype (gastric, intestinal, pancreatobiliary, oncocytic), which is known to be associated with risk of recurrence, invasive progression and overall prognosis [14]. Although IPMN subtyping seems feasible with MFB, there are several issues worth to note: first, some lesions may contain different coexisting epithelium subtypes and the sample provided by MFB could be nonrepresentative and miss a more aggressive subtype, and second, the interobserver agreement for IPMN subtyping is unsatisfactory even for the surgical specimens, probably lower for MFB samples [15].

Given the high technical success and tissue acquisition yield, improved diagnostic accuracy compared to the standard FNA and low adverse events rate, MFB is a promising tool for managing PCLs. Prospective data is however limited and the growing experience with the MFB is expected to provide more insight about indications, safety and cost-effectiveness of this technique.

Confocal Laser Endomicroscopy

Confocal laser endomicroscopy (CLE) is also a through-the-needle technique that allows real-time *in-vivo* microscopic assessment of the epithelial lining of cysts. Several patterns have been described for CLE images of PCLs, which have been translated into diagnostic criteria – superficial vascular network for serous cystadenoma, papillary projections for IPMN, epithelial border for mucinous cystadenoma, dark aggregates of cells surrounded by gray areas of fibrosis and vessels for neuroendocrine lesions [16]. A meta-analysis of available data has shown a high diagnostic accuracy of 88.6% for needle-based CLE (nCLE), clearly outperforming standard FNA [17]. As with MFB, the safety issue of nCLE evaluation of PCLs refers to the risk of acute pancreatitis, and also bears the potential side effects associated with fluorescein administration. Other limitations of nCLE are the difficult learning curve, the need to standardize and validate findings in PCLs and improve interobserver variability.

Compared to the standard approach, studies have shown a 28% change in PCL management using CLE [18] and 52.3% using the combination of MFB+CLE [19]. With regard to risk-stratification of IPMNs, papillary epithelial features seen with CLE have been shown to predict dysplasia in these lesions [20].

nCLE is undoubtedly a promising technology but warrants several improvements before widespread adoption among endosonographers.

Intracystic Glucose in Differentiating Mucinous and Non-mucinous Lesions

Several markers in the cyst fluid have been studied over time as predictors for malignancy in PCLs, with amylase and carcinoembryonic antigen (CEA) being mostly used to differentiate among different types of cysts – (Table **1**). A value of 192 ng/ml for CEA has been adopted in clinical practice for the differential diagnosis between mucinous and non-mucinous PCLs, this cut-off having demonstrated 75% sensitivity and 84% specificity in the study of Brugge *et al.* [21].

Table 1. Amylase and CEA values in different PCL [22].

-	**MCN and IPMN**	**SCN**	**Pseudocyst**
Amylase	Variable (IPMN>MCN)	Low	High
CEA	High	Low	Low

MCN – mucinous cystic neoplasm, SCN – serous cystic neoplasm, IPMN - intraductal papillary mucinous neoplasm, CEA – carcinoembryonic antigen.

Recent studies have looked however on a simple, cheap and readily available marker – cyst fluid glucose. Several authors have shown similar or even higher diagnostic accuracy for glucose compared to CEA in distinguishing between mucinous and non-mucinous cysts – (Table **2**). Some have even proposed on-site assessment of intracystic glucose using point-of-care tests (glucometers) or glucose reagent strips.

Table 2. Summary of studies looking at intracystic glucose for differentiating mucinous from non-mucinous lesions [23 - 27]

Author, year	***No of patients***	***Glucose threshold***	***Diagnostic accuracy***
Carr, 2018	153	≤ 50 mg/dL	Sn 92%, Sp 87% specific, Acc 90%
Zikos, 2015	65	<50 mg/dL	Sn 95%, Sp 57%
Simons-Linares, 2020	113	≤ 41 mg/dL	Sn 92%, Sp 92%, PPV 96%, NPV 86%, AUC 0.95
Faias, 2020	82	< 50 mg/dL	Sn 89%, Sp 86%, AUC 0.86
Ribaldone, 2020	56	< 50 mg/dL	Sn 93.6%, Sp 96%

Sn – sensitivity, Sp – specificity, Acc – accuracy, PPV - positive predictive value, NPV - negative predictive value, AUC – area under the curve

Given its wide availability, ease of measurement and good diagnostic accuracy, glucose should be routinely used for cyst fluid analysis, along with other validated markers, to better discriminate between mucinous and non-mucinous lesions.

Antibiotic Prophylaxis for FNA of Cystic Lesions

While guidelines suggest antibiotic prophylaxis for EUS-sampling of PCLs, the recommendation is acknowledged as being low quality evidence, based on old data [28 - 30]. Early studies provided conflicting results with regard to the protective effect of prophylactic antibiotic administration against infections after EUS-FNA of PCLs, and most of the data were retrospective [31 - 33]. Moreover, a meta-analysis looking at the risks of EUS-FNA of PCLs revealed a 2.77% incidence of adverse events associated with prophylactic periprocedural antibiotic use, suggesting that prophylaxis could be in fact harmful and not beneficial [34].

More recent data, including one multicenter, randomized clinical trial, have demonstrated that prophylactic antibiotics do not reduce the risk of infection and suggest that their routine use could be abandoned [35, 36]. As current practice will be difficult to change, some authors have suggested transitioning to a single dose and shorter course of antibiotics [37].

Artificial Intelligence

As with other digestive diseases, artificial intelligence has gained significant interest in the management of PCLs. Using machine learning techniques, researchers have developed algorithms based on cyst characteristics and demonstrated that AI is more accurate than conventional management and can cut unnecessary surgeries. The CompCyst test developed by Springer *et al.* [38] outperformed the standard of care in accurately classifying PCLs into surgical, surveillance and discharge groups (69% *versus* 56%, p=0.000073). Such AI-based interventions have a huge potential to improve the management of PCL and reduce costs and morbidity related to the misdiagnosis of these lesions.

CONCLUSIONS

Driven by an unmet need for early and accurate detection of precancerous cysts, significant progress has been made on techniques, tools and accessories to accurately characterize the heterogeneous group of PCLs. Despite several guidelines being published in the last few years, decision-making can sometimes be difficult with regard to impactful decisions such as sending a patient to surgery or abandoning surveillance. Optimized imaging protocols and novel EUS-based techniques have been demonstrated to enhance the diagnosis of PCLs. Molecular markers are on the horizon and their routine use will enhance patient outcomes even more.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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Declared none.

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CHAPTER 19

Emerging Techniques for Assessment of Chronic Liver Diseases: The "Omics" Cascade

Dana Crisan[1,*] and **Mircea Grigorescu**[1]

[1] *University of Medicine and Pharmacy Cluj-Napoca, 11 Tabacarilor Str., 400139 Cluj-Napoca, Romania*

Abstract: Chronic liver diseases are carrying an important social and economic burden, as they are having a high prevalence and are accompanied by many comorbidities. Furthermore, their progression ends frequently in a cirrhotic stage with its complications, the most fearful of these being the hepatocellular carcinoma. Therefore, diagnosing the disease at an early stage, then classifying the severity of the disease properly is mandatory. In addition, identifying the forms of liver diseases that are prone to progression towards severe fibrosis and cirrhosis is also very important. The invasive methods of diagnosis are almost completely replaced by noninvasive techniques, some of them failing to prove a high diagnostic accuracy, others being very expensive or not applicable or reliable. Consequently, the researchers are diving lately into a new domain of noninvasive diagnosis, namely OMICS cascade, which is very complex and through its multiple faces, addresses the different pathogenetic pathways of liver disease, increasing the probability of diagnosis, staging and prognosis to a higher level. The aim of this review is to present the data we have gathered until now from the field of genomics, proteomics, transcriptomics and metabolomics in the assessment of liver diseases.

Keywords: Genomics, Liver diseases, Metabolomics, Proteomics, Transcriptomics.

INTRODUCTION

The noninvasive approach of liver diseases is gaining weight in the last few years especially regarding the staging of the disease when we speak about fibrosis or when it comes to the severity of the disease. On the other hand, a noninvasive assessment is also important for the accurate diagnosis of nonalcoholic fatty liver disease, as the progression towards nonalcoholic steatohepatitis is still completely in the hand of liver biopsy. As the noninvasive approach, lacking the risks of liver

[*] **Corresponding author Dana Crisan:** University of Medicine and Pharmacy Cluj-Napoca, 11 Tabacarilor Str., 400139 Cluj-Napoca, Romania; Fax: 004 0735 406 258; E-mail: crisan.dc@gmail.com

Ioan Sporea and Alina Popescu (Eds.)

biopsy, is equally awaitedby the hepatologists and patients also, we aimed to make a literature review with the latest data regarding the newest molecular assessment of liver disease, meaning the "omics cascade". It includes genomics, proteomics, transcriptomics and metabolomics (together with lipidomics and glycomics), with the highest tribute being given to metabolomics which is the most extensively studied lately.

GENOMICS

Over the past two decades, extraordinary advances have been made in the field of genetics, generating a vast amount of information regarding different maladies, including liver diseases. Genomics centers on identifying genetic variants linked to the disease, treatment response, or prognosis. This has been possible through the rapid development of new genomic techniques, including tests for single nucleotide variants (SNV), whole-genome sequencing or exome sequencing, which provide the possibility for the identification of a large number of genes that create an individual's predisposition to multifaceted, erratic or mutual traits [1].

The first major breakthrough in the field of hepatology was in 1993, through the cloning of the ATP7B gene, involved in Wilson's disease [2]. This paved the way for the next step, which was differentiating between monogenic diseases, where a single mutation in one gene is responsible for the disease, and polygenic diseases, which are the result of the collective breakdown of a number of traits and are associated with an abundant number of gene variants [3]. The first genome-wide association study (GWAS) in hepato-biliary diseases, identified the cholesterol transporter ABCG5/G8 as the main predisposition factor for the appearance of gallstones [4]. Since then, numerous studies have involved different genes in the development of hepatic diseases, leading to progress in the field of precision medicine.

When talking about etiologies of chronic liver disease, the main contributors seem to be chronic hepatitis B, chronic hepatitis C, alcohol-related liver disease and non-alcoholic fatty liver disease (NAFLD). Hepatitis B and C have a decreasing incidence due to advancements in treatment, but NAFLD is on an ascending path, mainly owing to the global epidemic of obesity. NAFLD's incidence has risen at an alarming rate, and non-alcoholic steatohepatitis (NASH) is now considered to be the second most common indication for liver transplantation in the USA [5]. NAFLD also leads to extrahepatic morbidity, through its association with cardiovascular disease, cancer and diabetes. Given its trajectory, there is a clear need also to understand this disease's genetic foundation, hence, contributing to the development of a specific treatment.

Data derived from different studies points to an existing heritable component to NAFLD [6]. Currently, there are at least five variants of genes that have been strongly correlated with the predisposition to and progress of NAFLD, explicitly: amino acid substitution p.I148M of the lipid droplet-associated triglyceride lipase PNPLA3, transmembrane 6 superfamily member 2 (TM6SF2), glucokinase regulator (GCKR), membrane bound O-acyltransferase domain-containing 7 (MBOAT7) and hydroxysteroid 17β- dehydrogenase (HSD17B13) [7].

GWAS studies have confirmed different variants of PNPLA3 and TM6SF2 as risk loci for alcoholic cirrhosis as well; these variants may also potentially play a role in hepatic steatosis in both hepatitis B and C [8]. In addition, variants of PNPLA3 and TM6SF2 have also been correlated to cardiovascular risk [9]. All this data highlights the importance of gene polymorphism, which is able to increase the intricacy of the clinical phenotype of the disease.

GWAS studies were also able to identify variants in the IL28b gene encoding interferon (IFN)-k3 which are related to the response to IFN therapy in patients with chronic hepatitis C virus infection [10], involving the significance of gene variants in treatment response. IL28b allele was also discovered to be a risk factor for the development of hepatocellular carcinoma (HCC) in patients with HCV infection, regardless of the sustained virologic response [11].

Another genome study for drug-induced liver injury (DILI) caused by amoxicillin-clavulanate exposure, found two human leukocyte antigen (HLA) genotypes that are related to the development of DILI; in addition, other studies found stirring evidence that relates HLA genotype to DILI susceptibility [12]. On one hand, drug toxicity related to the liver is one of the most common reasons for withdrawal of a drug and, on the other hand, there are many drugs being frequently used that can cause DILI, therefore, there is a logic in studying the potential mechanism that could diminish the development of DILI in predisposed patients or even the manufacture of hepatotoxic drugs.

With regards to autoimmune and immune-mediate liver diseases, genome-wide association studies have connected HLA variants (SH3B2, CARD10) with autoimmune hepatitis type 1 (AIH type 1); these variants overlap with the ones found in primary biliary cholangitis (PBC), and sclerosing cholangitis (SC). The variants of SH3B2 are also associated with hypothyroidism, type 1 diabetes or celiac disease. Even though in the study regarding AIH neither of these associations reached the acknowledged level of significance mandatory to proclaim “genome-wide significance”, the implication is that part of the genetic susceptibility of this disease overlaps with other immune-mediated diseases [13]. The importance of the studies mentioned in regard to diagnosis or treatment is still

not clear, but they could pave the way to a better understanding of these diseases.

Despite all the advances in the field, genome studies are only the first step in the development of personalized medicine. There is an immense gap between the data collected and the impact on the management of these patients. Following the identification of different gene variants associated with disease risk, *in vitro* and *in vivo*, studies need to be developed in order to further explain the pathological foundation for the effects interceded by a risk allele.

TRANSCRIPTOMICS

Transcriptomics allows the study of gene expression, through the analysis of the complete set of ribonucleic acid (RNA), comprising of messenger RNAs (mRNAs), non-coding small RNAs -microRNAs (miRNAs), long noncoding RNAs (lncRNA), among others [14]. Transcriptomics has revolutionized our understanding of genome expression in different pathologies, including chronic liver diseases. Two of the main transcriptomics techniques are: microarray analysis, a method used to detect a specific predefined sequence and RNA-sequencing (RNA-seq) which allows the sequencing of the whole transcriptome.

RNA-seq comprises whole tissue analysis and single-cell RNA sequencing (scRNA-seq), an innovative method that provided novel insight into liver zonation, the characterization of endothelial and mesenchymal cells in liver fibrosis or the role of the immune system in hepatocellular carcinoma and cholangiocarcinoma [15].

Despite the progress in the diagnosis and the treatment, the global burden of chronic liver diseases and their end-result – cirrhosis, is the proof that there is a clear need for a better depiction of the pathological process behind the disease, in the hope of developing a superior antifibrotic therapy. ScRNA-seq is currently one of the techniques that can enhance our grasp on liver cell biology. Dobie *et al.* [15] used scRNA-seq to illustrate the heterogeneity of the hepatic mesenchyme, confirming that hepatic stellate cells (HSC) are the key contributors to liver fibrosis; the experiment also allowed depicting the zonation of function in HSC in a model CCl4-induced hepatic injury. Zonation is determined by the difference of gene expression along the lobule axis, which according to a study by Harpen *et al.* [16] is not monotonic, with some genes having the highest expression in the mid-lobule layers. This was assessed through the analysis of the transcriptomes of thousands of mouse livers, thus challenging the traditional periportal and pericentral classification. ScRNA-seq technique was also used for identification of progenitor cells in the context of liver regeneration [17], the study of the tumor microenvironment in hepatocellular carcinoma [18], or for the accurate description of non-parenchymal liver cells and their role in the pathogenesis of the

chronic liver disease [19]. This type of technique is expected to have significant applications in the characterization of the molecular mechanisms underlying liver diseases; still, it is imperative to integrate the data in a multi-omics approach.

Other techniques, like microarray profiling and real-time PCR can be used to detect microRNAs (miRNAs), which are small, non-coding RNA molecules that play a central role in the modulation of gene expression at a post-transcriptional level. They contribute to numerous physiological and pathological processes in the human body and could be used as potential biomarkers. Most of the hepatic metabolic processes are under the control of miRNAs that circulate in the blood in extracellular vesicles or bound to specific proteins; many studies have highlighted their potential role as liver disease biomarkers. miRNAs have specific attributes that could marque them as superior compared to the traditional biomarkers: they are tissue specific (miR-122 and miR-192 are specific to the liver tissue, and exceedingly abundant), sensitive (a study revealed that miRNA can be detected in acetaminophen induced drug injury model, even before changes in alanine aminotransferase levels), predictive (miR-122 and miR-1 were found to be the potential prognostic markers in hepatocellular carcinoma [20], miR-122 was correlated with disease severity in hepatitis C virus (HCV) infection [21] or as a prognosis marker in the liver cirrhosis [22]) and can be detected through non-invasive techniques.

Studies have also involved miRNAs in the progress and pathogenesis of liver diseases. In alcoholic liver disease (ALD), some miRNAs have proved to induce hepatocyte apoptosis (miR-34a and miR200a), while miR-122 was found to have a defensive role [23, 24]. It appears that even HSC is controlled by miRNAs, such as let-7, its down regulation being able to activate HSC in ALD [25].

Regarding NAFLD, miR-34a, miR-122, and miR-155 have been repeatedly connected with its pathogenesis, playing different roles, including the progress of hepatic fibrosis, deregulation of lipid metabolism [26] and even hepatocarcinoma development [27].

miRNAs are showing promising results in the field of hepatology, however, to present-day, there are no validated studies or standardized techniques that could implement them for clinical use. Also, there are no current clinical trials for targeted miRNA therapy, so there are still obstacles for their application.

Present-day medical care is based on the common characteristics of an average population, with a “one size fits all” tactic; scRNA-seq or miRNA detection expedites the progress to precision medicine, through revealing critical pathological deviations in certain diseases. Still, in spite of the existing data, correlating transcriptomic with genomic and proteomic information is

complicated by the restricted correlations between the fields due to biological differences. Further studies are needed to overcome these barriers.

PROTEOMICS

Proteomics encompasses all technologies used for the study of the proteome, which is the total count of proteins in a cell. Mass spectrometry (MS) is one of the main techniques used, among others; it is suitable for the determination of protein expression, protein interactions or post-translational modifications [28].

Substantial efforts have been made on this subject: one of the Human Proteome Organization initiatives includes the Human Liver Proteome Project (HLPP), a database encompassing 6788 proteins; it is an ongoing project, providing public access to cohesive information on the human liver proteome [29]. Also, an integrated transcriptomic and proteomic analysis of the liver tissue, using RNA-seq and antibody-based immunohistochemistry identified 477 protein-coding genes having the highest expression in the liver; the study identified protein localization, biological and metabolic function, showing the impact of integrated omics studies and contributing to further understanding of liver phenotype in different states [30]. Other studies have similarly added to the outlining of the proteomic repertoire of the liver [31].

Continuous improvements in proteomics studies have led to the discovery of potential new biomarkers for diagnosis, prognosis and therapy response. A study on HCV infected patients at different stages of fibrosis found 210 proteins individually associated with the stages – suggesting different protein expression depending on fibrosis evolution, due to mechanisms like oxidative phosphorylation and fatty acid oxidation; the study's conclusion steered to a better understanding of HCV infection pathogenesis [32]; another proteomic study on cirrhotic liver tissue samples from HCV infected patients (genotype 1) found human microfibril–associated protein 4 (MFAP-4) as a promising biomarker for liver cirrhosis [33]. Tissue samples from 76 patients with HCV cirrhosis were compared to ALD cirrhosis; they found a galectin-3-binding protein (G3BP) as a potential marker for HCV cirrhosis, and also found different protein expression related to the fibrosis stage [34]. Using proteomics technology, a study determined potential biomarkers for HCV treatment response within 24 hours from the initiation of treatment [35]. The same conclusions regarding protein expression depending on fibrosis stage were found in HBV infected patients; additionally, the study established peroxiredoxin-2 as a potential biomarker for early fibrosis [36].

Regarding ALD, it is a known fact that many changes occur at a protein level concerning their stability, structure or expression in pathological conditions such

as alcohol abuse – a proteomic study on ethanol-fed rats showed different levels of protein expression offering new biomarkers for ethanol-induced steatosis. Mass spectrometry was furthermore used to assess protein expression in 69 patients with different stages of NAFLD; the study is just one of many that tried to create a biomarker panel useful in diagnosis and prognosis of NAFLD, therefore limiting the use of liver biopsy [37].

These studies are just examples of the advances made in the field; since 2000, over 200 studies have been made only on plasma or serum proteome in liver diseases; the main limitations of the studies include limited cohorts, lack of validation methods and high costs. Still, cutting-edge technologies have the ability to better explain liver disease mechanism, the main focus being the identification of markers that are shed into circulation long before the symptomatology occurs, consequently, offering a window of opportunity for faster and personalized treatment.

METABOLOMICS

About twenty years before, Jeremy Nicholson *et al.* introduced the concept of metabolomics, aiming for the description of different metabolite profiles that represent a consequence of constitutional and genetic differences or can be derived from toxic exposures [38]. The initial protocols were based on high resolution proton nuclear magnetic resonance spectroscopy (HNMR) of biological fluids. Later on, other technologies were added, based on mass spectrometry.

Since the biochemical complexity was increasing, the old definition of metabolomics was abandoned and we are now considering that metabolomics studies metabolites with low molecular weight (<1.5kDa) found in plasma, serum, urine and cell cultures, using especially MS and NMR. There is also confusion regarding the eventual differences between metabolomics and metabonomics. Even if specialists in the field are pleading for a non-technical difference between the two terms, sustaining that in fact the terms are interchangeable, the research published on metabonomics is almost exclusively based on NMR.

Untargeted metabolomics focuses on the separation of biological analytes that are sharing similar physical and chemical features, using ultra-performance liquid chromatography (uPLC). In targeted metabolomics, some specific metabolites like amino acids or acylcarnitines are quantified, using stable standards of isotopes.

Another method used in metabolomics is gas spectrometry (GC-MS). This brings with it the benefit of a high specificity in metabolite identification, but for a lesser number of metabolites.

Lipidomics refers to the total number of lipid species that exist in the cells, tissues, organs, biofluids. Even if there is surely an overlap with the human metabolome, it is considered that the lipidome is even more complex, due to its high variability in the length of chains, the degrees of saturation together with the structural isomerism. Data gathered until now is showing that the metabolome has 200000 members, and that also includes the lipidome.

Regarding the role of metabolomics in liver diseases, many recent studies proved the importance of identification and quantification of metabolites originating in the liver. Most liver diseases targeted by metabolomics are ALD, NAFLD and cholestatic diseases; other studies entail the assessment of fibrosis stage in different viral or non-viral hepatic diseases.

The spectrum of diseases in *ALD* comprises different aspects like steatosis, steatohepatitis, alcoholic hepatitis, fibrosis, cirrhosis and hepatocarcinoma. The liver is the main organ involved in metabolizing alcohol and it is consequently the main organ affected by excessive alcohol consumption; its pathogenesis implies the conversion of alcohol into acetaldehyde by alcohol dehydrogenase. In patients having ethanolic steatosis, researchers identified increased levels of plasma triglycerides while in those with signs of cirrhosis, N-Lauroylglycine and decatrienoic acid were associated with the severity of the disease [39]. In patients with ethanolic hepatitis, several oxylipins that proved to be related to this type of hepatitis were identified in serum and 9 oxylipins were identified in feces [40].

In *cholestatic diseases*, metabolomics tried to answer the challenge of differentiating between the mechanisms of cholestasis. In animal models, studies showed that bile acids, valine, methyl malonate are probable markers of cholestasis, although nonspecific [41]. PBC and SC were some of the cholestatic diseases targeted by the metabolomic studies, especially with regards to bile acids. In PBC with cholestasis, cholic and chenodeoxycholic acids were 13 folds higher compared to patients without cholestasis, especially those conjugated with taurine. In PBC, some other metabolites were also identified in high concentrations beyond primary bile acids like phospholipids, oleic and linoleic acids [42]. In SC, primary bile acids were found in higher concentrations, while secondary bile acids and 6 alpha-hydroxylated bile acids were significantly lower. Regarding intrahepatic cholestasis during pregnancy, different predictive metabolites were found in urine, belonging to the primary biliary acids, like glycocholic and chenodeoxycholic acid 3-sulfate [43].

Probably most studies in this field were performed for the assessment of *fibrosis and cirrhosis* in different diffuse liver diseases, both on animals and humans. Many metabolites were identified from the class of amino acids, simple sugars

and substances belonging to the Krebs cycle. Even though these metabolites detected through H-NMR have different advantages like rapidity, simplicity, reproducibility, they also have a major drawback *i.e.* low sensitivity.

In NAFLD, the progression of fibrosis was correlated with lower levels of etiocholanolone sulfate and dehydroepiandrosterone sulfate with a concomitant increase of 16 alpha-hydroxy-dehydroepiandrosterone sulfate. Their ratio was able to separate different stages of fibrosis with sensitivities and specificities reaching values between 76 and 85% [44]. Moreover, metabolomics based on MS showed increases of bile acids in cirrhosis, accompanied by modifications of urinary corticosteroids [45]. Another frequent modification met in severe fibrosis and cirrhosis was the increase of phospholipids in the serum of these patients [46].

Overall, the metabolomic profile of patients with severe fibrosis is characterized by the decreased levels of phosphatidylcholines (PC) and an increase in bile acids [47] and this profile remains unchanged independent of the etiology of liver disease or the development of a hepatic tumor.

In hepatitis C with advanced fibrosis, the level of HDL-cholesterol and choline are decreased compared with the patients without fibrosis or with mild fibrosis [48].

In patients with liver cirrhosis and minimal hepatic encephalopathy, researchers identified low levels of glucose, lactate, methionine and glycerol on one hand. On the other hand, low levels of choline, amino acids with branched chains, alanine, glycine and lipid products have been identified in this group of patients [49].

In addition, in patients with chronic liver failure (CLF), the profile of metabolomic products is different depending on the disease stage assessed through the MELD score [50]. In patients with a higher MELD, a lower level of HDL cholesterol, choline and phosphatidylcholine (PC) have been described. Other products like lactic acid, butyrate, pyruvate and citrate are increased in CLF. The protein metabolism is also modified because of the catabolism of skeletal muscle. Some studies also proved the prognostic level of serum PC and lyso-PC in liver cirrhosis, as being correlated with survival [46]. Moreover, there were studies that highlighted the role of metabolomic products in the diagnosis of acute or chronic liver failure (ACLF), expressed by low levels of HDL-cholesterol and increased levels of lactic acid, pyruvate, and aromatic amino acids, but these markers seem to rather be associated with the severity of the disease [51].

An impressive number of studies addressed *NAFLD or NASH,* but the great desiderate of using metabolomics to reach the diagnosis of NASH or determine

the progression from NAFL to NASH was not yet accomplished. As a short conclusion from most of the studies, an increase of fatty acids and acylcarnitines, but also of bile acids and aromatic amino acids were seen in patients with NAFLD. In order to avoid biases related to metabolic diseases, non-diabetic steatosis patients were also studied. Increased levels of gama-glutamyltyrozine have been signalized in patients with fatty liver [52]. In addition, the analysis of urine and serum in non-diabetic NAFLD patients with or without alterations in liver function showed a variability of metabolites depending on the stage or severity of NAFLD: in patients with NASH, higher levels of indole-acetic acid, pyroglutamic acid and indole-lactic acid were described [53]. In the progression from simple steatosis to NASH, some other metabolites have been proving to be increased: leucine (with 127%), isoleucine (139%) and valine (147%), but also lauroyl-carnitine and hexanoyl-carnitine, suggesting that acyl-carnitines could be a marker of progression from steatosis to NASH; still, further studies are needed [54]. From the field of lipidomics and eicosanoid metabolites, we are having some data regarding the increase of 11-hydroxyeicosatetraenoic acid in patients with NASH compared to NAFL, this metabolite being a non-enzymatic product of arachidonic acid oxidation [55]. This observation highlights again the potential role of oxidative stress in the development of NASH.

CONCLUSION

The biomarkers derived from “omics” are still under an intense and elaborated evaluation. An iterative assessment and a sustained validation is mandatory before their acceptance and entry in the clinical practice as diagnostic markers or as prognostic markers for the identification of patients at risk for a progressive disease. Moreover, there is a chance that including omics in current practice will lead to the development of a personalized molecular signature useful for a personalized diagnosis and treatment.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 20

Where are we Now with Ultrasound-based Liver Elastography?

Ioan Sporea[1,*] and **Felix Bende**[1]

Regional Center of Research in Advanced Hepatology, Academy of Medical Sciences, Timişoara, Romania WFUMB Center of Education, Romanian Academy of Medical Science, Romania

Abstract: While the spectrum of liver diseases has changed in last few years and non-alcoholic fatty liver disease (NAFLD) becoming the main field of activity in hepatology, the evaluation of patients with chronic liver disease has shifted mainly from invasive methods (liver biopsy) to non-invasive methods. Liver ultrasound-based elastography, as a non-invasive method for predicting liver fibrosis, has been extensively studied and developed in the last fifteen years, demonstrating its good value for the evaluation of chronic liver diseases of different etiologies. Current elastography guidelines advise on how and when to use these elastographic methods in clinical practice and highlight their advantages and also their limitations too. Moreover, the rapid innovation of ultrasound systems has allowed the development of new software tools that allow, in addition to quantifying fibrosis, the quantification of steatosis and the viscoelastic properties of tissues, such as inflammation, thus turning the ultrasound systems into multiparametric methods (multiparametric ultrasound-MPUS). Also, besides liver stiffness, spleen stiffness is a good predictor for liver cirrhosis complications, such as portal hypertension and there are current recommendations and clear criteria for when to use elastography for evaluating portal hypertension.

Keywords: 2D-SWE, Liver elastography, Liver steatosis, pSWE, Shear-wave elastography, Spleen stiffness, Steatosis quantification, Transient elastography.

INTRODUCTION

The etiology spectrum of chronic liver diseases is wide, and nowadays the number of patients with such diseases is increasing. Many years ago, chronic viral hepatitis B or C were the main fields of activity for hepatologists, today this spectrum is changing. Nowadays, the fatty infiltration of the liver represents the main field of daily activity in hepatology. Alcoholic liver disease (ALD) is also a

[*] **Corresponding author Ioan Sporea:** Regional Center of Research in Advanced Hepatology, Academy of Medical Sciences, Timişoara, Romania 156 Liviu Rebreanu Str., 300736 Timişoara, Romania; Tel: +40256309455, Fax: +40256488003; E-mail: isporea@umft.ro

Ioan Sporea and Alina Popescu (Eds.)

problem worldwide, but the pathological condition with increasing prevalence is a non-alcoholic fatty liver disease (NAFLD). Why is NAFLD an emerging problem? Because the prevalence of overweight and obese population worldwide overpasses 2 billion, and one in eleven people in developed countries has type 2 diabetes mellitus (T2DM) patients [1] and the proportion of the dyslipidemic population has increased during the time. All these conditions are factors associated with the development of NAFLD. More recently, the term NAFLD was replaced with MAFLD (metabolic associated fatty liver disease) [2], this new terminology seems to be more appropriate in terms of etiopathogenic because the patients with fatty liver are mainly dysmetabolic.

Facing this very high number of subjects with liver diseases, with MAFLD and ALD, without losing sight of other liver diseases such as cholestatic or autoimmune, we must have simple solutions to evaluate these patients, especially for the decision of therapy, prognosis and follow-up. The main driver of the progression in chronic liver diseases seems to be liver fibrosis and this is why its assessment is important in clinical practice [3].

Evaluation of patients with chronic liver diseases can be performed invasively (by means of liver biopsy), or non-invasively, by using biologic or elastographic methods.

Liver biopsy (LB) was the traditional evaluation method of patients with liver diseases, percutaneous liver biopsy being used for more than 50 years. LB is usually performed echo-guided, allowing a precise evaluation of fibrosis, inflammation and steatosis [4]. LB is still considered the "gold standard" method of liver evaluation, but considering the very high number of liver associated pathological conditions, this method is not practicable in all patients. At the same time, LB has some limitations, mainly it is not well accepted by patients, it is rarely repetitive, can lead to complications (very rare mortality) and the liver specimen obtained is not always of the best quality. In a systematic review (that included more than 8,700 patients) on the quality of LB specimens [5], major and minor complications occurred in up to 6% of LB, 0.04 to 0.11% of them life-threatening. In this review, the LB specimens had an average length and number of portal tracts well below the recommended minimum sample size requirements in more than half of the cases (only 42% of LB with a large 17-gauge needle contained 10 or more portal tracts). Therefore, the size and quality of the liver specimen obtained by LB is a problem, when using this approach for evaluating liver diseases. In a meta-analysis performed between 2010-2020, that included 30 studies, reporting on complications following 67,552 percutaneous LB, the incidence of minor complications was 12.60% (mainly minor pain), major complications were reported in 2.44% (1/40 cases), with mortality of 0.01%

(1/10.000) cases, major bleeding in 0.48% (1/200) cases and hospitalization in 0.65% of cases [6].

An alternative for non-invasive evaluation of patients with liver disease, extensively developed during the last 15 years, is **liver elastography**. Starting from the physical properties of the tissue and an external excitation of the liver tissue, these elastographic methods can provide information regarding liver stiffness. They are quite simple and repetitive. Elastography can be divided into *ultrasound-based elastography* and *magnetic resonance elastography (MR-E)*. In this chapter, we will cover only the ultrasound-based methods, especially the development of these methods during the last years.

Many guidelines advise how and when to use these elastographic methods. The first guideline published was the EFSUMB guideline (European Federation of Societies for Ultrasound in Medicine and Biology) [7], which made the first classification of ultrasound-based elastographic methods. We can divide ultrasound-based elastographic methods into:

1. Shear Waves Elastography (SWE): a) Transient Elastography-TE (FibroScan); **b) Point Shear Wave Elastography - pSWE** [using Acoustic Radiation Force Impulse Quantification (ARFI): VTQ (Siemens), Elast PQ (Philips), Samsung, Hitachi, Mindray, Esaote, others] **c) Real-Time Shear Wave Elastography - 2D SWE** (Aixplorer, General Electric, Canon, Samsung, Philips, Siemens, others)

2. Strain Elastography (Hi RTE**)**

More recent guidelines [8, 9] describe exactly how and when to use these elastographic methods, the advantages and limitations of the methods.

Which are the ***advantages of SWE***? The probe produces the impulse that generates the shear waves inside the liver tissue, without any manual pressure. Thus, by pressing a button the result is immediately displayed, expressed either in kPa (such as in Transient Elastography -FibroScan) or in meters/second or both (available now on all ultrasound machines with elastography modules). The learning curve is not very long (at least 50 examinations) [10], however, for 2D SWE some ultrasound examination experience is necessary [10].

Considering all published papers, the EFSUMB and WFUMB (World Federation of Societies for Ultrasound in Medicine and Biology) liver elastography guidelines consider that strain elastography is not ready for clinical practice. Some Japanese studies showed quite good results for strain elastography [11], but these results can possibly be suited for a “slim” Asiatic population. Considering

the recommendations from the aforementioned guidelines, this chapter will cover only SWE techniques.

Ultrasound-based Elastography for Liver Fibrosis Staging

Transient Elastography (TE) is the oldest ultrasound-based elastographic method. It is implemented on a FibroScan (Echosens, Paris, France) device. TE can be easily performed by doctors or technicians, and operators can be trained quite rapidly (learning curve of at least 50 examinations). For TE evaluation, the patient is placed in a dorsal position with their right arm in maximum abduction and the examiner tries to find an intercostal space corresponding to segment V or VIII of the liver. Afterward, a semi-blind examination is performed and by pushing a button on the probe, the result is provided immediately on the screen. Ten valid measurements are necessary and the median of these values represents the result. IQR/M (interquartile range/median) is used as a quality parameter, and for a valid result, it is necessary to be below 30%. The first examinations were performed with the M probe (for adults) and the feasibility of the TE was between 70-85% [12], with difficulties in obtaining valid results in obese subjects. More recently, the XL probe for obese patients overpassed these limitations and the feasibility of TE, with both probes, increased to 93% [13]. The main impediments for TE in practice are severe obesity, narrow intercostal spaces, and the presence of ascites [14].

The first results of TE in clinical practice were published regarding HCV patients, followed by different meta-analyses [15 - 17], all showing a good accuracy of this method, which increases with the severity of fibrosis. For moderate fibrosis ($F\geq2$) the accuracy is higher than 80%, for advanced fibrosis ($F\geq3$) higher than 85%, and for cirrhosis (F4) approximately 90-95%. Cut-off values for staging different grades of liver fibrosis were proposed for TE and an important meta-analysis [15] recommended 7 kPa for $F\geq2$, 9.5 kPa for $F\geq3$ and 12 kPa for cirrhosis. Further studies evaluated patients with HBV chronic infection and again published papers showed good correlations with fibrosis severity. Papers on the value of TE in ALD, cholestatic liver diseases, or in autoimmune chronic hepatitis, also demonstrated the good value of TE in these categories of patients. Many studies were published regarding the value of TE in NAFLD patients and the recommended cut-off values were slightly different from those in other types of chronic hepatopathies, considering that all studies used liver biopsy as the gold standard. The latest EFSUMB and WFUMB Guidelines published in 2017 and 2018 describe the cut-off values for TE in each pathological condition [8, 9]. These cut-off values are slightly different for every liver disease and those who use them in their daily practice, must know them.

Considering the good results of TE as compared with liver biopsy and the validation of this method in more than 1500 published papers, in 2015, the EASL Guidelines recognized TE as a valuable assessment method for fibrosis severity and thus the need for treatment in HCV chronic infection [18]. Nowadays, the number of centers using TE as a non-invasive evaluation tool for liver fibrosis is very high (in Romania there are more than 50 centers). The duration of an evaluation is less than 5 minutes; the method is repetitive, without any discomfort for the patient and with good practical accuracy.

Some years ago, Echosens implemented in their system a module for liver steatosis quantification. The technique used is called CAP (Controlled Attenuation Parameter) and its principle is to quantify the attenuation of the ultrasound beam in the liver. Many papers were published regarding the clinical value of CAP, using liver biopsy as the gold standard. The accuracy of CAP for quantification of mild (S1), moderate (S2), and severe (S3) liver steatosis ranges between 82-87% [19, 20]. Some factors such as obesity and the presence of T2DM can influence CAP values and accuracy. Thus, a correction formula of CAP values etiological factors was proposed [20].

FibroScan with CAP module. For adults, the system is provided with two probes (M and XL probe – for normal weight and obese subjects), and besides the liver stiffness measurements expressed in kiloPascals (kPa), the system also displays values of steatosis expressed in dB/m.

Fig. (1). Liver fibrosis and steatosis evaluation using FibroScan and CAP.

From the practitioner's point of view, CAP implemented into the FibroScan device has a good value for the evaluation of NAFLD or MAFLD patients.

Patients with metabolic syndrome, those with T2DM, with obesity are at high risk of important liver steatosis and liver fibrosis. FibroScan has the advantage that, in the same session, in less than 10 minutes, a quantitative evaluation of liver steatosis can be obtained, and it can also detect if liver fibrosis is present. Concerning the cut-off values for different degrees of steatosis, the producer proposed 230, 275 and 300 dB/m. Later published papers, in which liver biopsy or MRI-PDFF (magnetic resonance imaging-derived proton density fat fraction) were used as a reference, and recommended higher values. In a paper published in 2018 by Chan [21], the cut-off values for ≥S1, ≥S2, and S3 were 248, 268, and 280 dB/m, respectively. The same study showed that the cut-off values for M and XL probes were similar. In a more recent paper, in which liver biopsy was also used as a reference in patients with NAFLD, the cut-off values for steatosis grades ≥ S1, ≥ S2, and S3 were 302, 331 and 337 (dB/m) [22] and these results seem to be more realistic for clinical practice in MAFLD subjects. A few studies showed that if IQR/M is below 30 or 40 dB/m, the correlation with histology is better [23].

Regarding the practical value of CAP, we can discuss the study performed in France on 5,323 examinations: CAP failure has occurred in 7.7% of cases [24]. By multivariate analysis, CAP values were influenced by the following parameters: metabolic syndrome, BMI, waist circumference, the presence of diabetes or hypertension. In a sub-cohort of 440 patients with liver biopsy, the AUROCs of CAP for the diagnosis of steatosis >10%, >33%, and >66%, were 0.79, 0.84, and 0.84, respectively.

TE is very useful for screening metabolic subjects. A prospective study that evaluated 776 T2DM patients [25] revealed that 60.3% of them had severe steatosis, while 19.4% had advanced fibrosis. Female gender, BMI, waist circumference, elevated levels of AST, total cholesterol, triglycerides, blood glucose, and high liver stiffness values were associated with severe steatosis (all p-value<0.05).

Which are the weak points of FibroScan? First, it is quite expensive, especially if we want both M and XL probes and/or the pediatric probe. Secondly, it is quite a blind method (and to overcome this problem, the last model has an ultrasound system near the FibroScan). Thirdly, these probes must be calibrated annually, and this is quite expensive.

Development of ultrasound-based elastography continued after TE with **point SWE.** Point SWE (pSWE) uses the ARFI technology (Acoustic Radiation Force Impulse) and it is implemented in an ultrasound system. The first pSWE technique available in the market, more than 10 years ago, was VTQ (Virtual

Technology Quantification) from Siemens. VTQ uses an acoustic impulse produced by the probe that generates shear waves into the liver, whose speed is used to quantify liver stiffness in a point (point SWE). In clinical practice, under a real-time B mode ultrasound image, a measuring box of 10/5 mm is placed at least 10 mm below the liver capsule and a button in the system is pressed. Immediately the measurement result is displayed, expressed either in m/s or in kPa. Ten valid measurements must be obtained and their median is calculated.

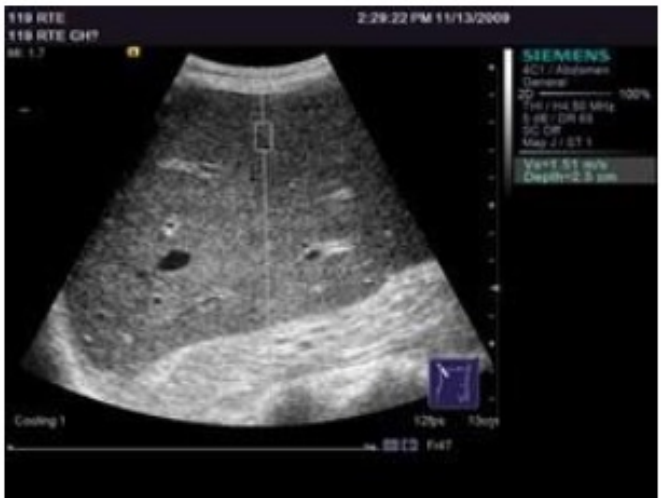

Fig. (2). Liver fibrosis evaluation using pSWE (VTQ).

The first published papers compared pSWE by VTQ with liver biopsy, mainly in HCV chronic infections, but later studies were performed also on HBV chronic infections and the other chronic liver diseases as well. In a meta-analysis published by Nierhoff [26], which included 36 studies with 3,951 patients, the mean diagnostic accuracies of VTQ expressed as AUROCs were: 0.84 for the diagnosis of significant fibrosis ($F \geq 2$), 0.89 for the diagnosis of severe fibrosis ($F \geq 3$) and 0.91 for the diagnosis of liver cirrhosis ($F = 4$). A meta-analysis published by Bota *et al.* [27] on 13 studies, with a total of 1163 patients, demonstrated that for predicting the significant fibrosis ($F \geq 2$), the summary Se of VTQ was 0.74 (95% CI: 0.66-0.80) and the summary Sp was 0.83 (95%CI: 0.75-0.89). In comparison, for TE, the summary Se was 0.78 (95%CI: 0.72-0.83), the summary Sp was 0.84 (95%CI: 0.75-0.90). The diagnostic odds ratios of VTQ and TE did not differ significantly [the mean difference in rDOR = 0.27 (95%CI -0.69 to 0.14)] [27].

After VTQ, other companies developed pSWE techniques, simple methods to use for liver fibrosis evaluation. The examination position is the same as for TE, the

patient must be in a fasting condition, the examiner must perform the measurements avoiding the capsule of the liver and the hepatic vessels and the patient must sustain breath-hold in a neutral position (not in deep inspiration) for a few seconds. Several pWE techniques are available on the market, such as the ones from Philips (Elast PQ), Hitachi, Esaote, Mindray, *etc.*

pSWE from Philips (ElastPQ) was evaluated in a multicentric study performed in five European centers that included 664 patients, 83.1% with viral hepatitis, and 7.5% with NAFLD [28]. The optimal cut-off values of ElastPQ for staging significant fibrosis (F≥2), severe fibrosis (F≥3), and cirrhosis (F=4) were, 7.04 kPa, 8.83 kPa, and 9.11 kPa, respectively. The diagnostic performance of ElastPQ for fibrosis staging increased if LSM values were obtained with IQR/M ≤ 30%. Furthermore, this study showed that the number of measurements (5 *vs.* 10) did not modify the results.

2D-SWE is an elastographic method integrated into a standard ultrasound machine that uses numeric and color-coded quantification of liver fibrosis. Published papers revealed its good results for liver fibrosis assessment. Super Sonic Imagine (Aixplorer) 2D-SWE SSI was the first used in practice [29], followed by 2D-SWE GE (General Electric Healthcare) and other methods (Toshiba/Canon, Philips, Samsung).

Fig. (3). Liver fibrosis assessment using 2D-SWE SSI (Aixplorer) (left) and Canon (right).

Some important papers were published with the Aixplorer system. In a meta-analysis [30] 2D-SWE was compared to liver biopsy in 1134 patients from 13 sites. Most patients had chronic hepatitis C (HCV, n = 379), hepatitis B (HBV, n = 400) or non-alcoholic fatty liver disease (NAFLD, n = 156). Regarding histology, 40.8% of the patients had minimal or no fibrosis, 19.3% had significant fibrosis, 14.0% had severe fibrosis and 26.0% had cirrhosis. The AUROCs of 2D-SWE in patients with HCV, HBV and NAFLD were 86.3%, 90.6% and 85.5% for

diagnosing significant fibrosis (F≥2) and 92.9%, 95.5% and 91.7% for diagnosing cirrhosis, respectively. The study concluded that 2D-SWE has good to excellent performance for the noninvasive staging of liver fibrosis. In another meta-analysis on twelve studies that included 1635 patients (mixed etiologies), the pooled sensitivity and specificity were 0.84 and 0.81 for F≥2, 0.89 and 0.84 for F≥3, and 0.88 and 0.86 for F=4, and the AUROCs were 0.85 for F≥2, 0.93 for F≥3, and 0.93 for F=4 [31].

Comparative studies between different elastographic methods have been published. The group of Cassinotto made a comparative study [32] in a cohort of 349 consecutive patients with chronic liver diseases who underwent liver biopsy. In each patient, liver fibrosis was assessed by 2D-SWE SSI (Aixplorer), pSWE (VTQ, Siemens), FibroScan (M and XL probes). 2D-SWE SSI, FibroScan, and VTQ correlated significantly with histological fibrosis score (r=0.79, p<.00001; r=0.70, p<.00001; r=0.64, p<.00001, respectively). The study concluded that no significant difference between the methods was observed for the diagnosis of mild fibrosis and cirrhosis. In a second study [33] that included a cohort of 291 NAFLD patients prospectively enrolled where liver biopsy was compared with 3 elastographic methods, the AUROCs for SSI, FibroScan, and VTQ were 0.86, 0.82, and 0.77 for the diagnosis of ≥F2; 0.89, 0.86, and 0.84 for ≥F3; and 0.88, 0.87, and 0.84 for F4, respectively.

For ultrasound-based elastography, some aspects must be remembered: different elastographic systems give different values for the same stages of liver fibrosis, so that the cut-off values must be known for the system with which we work. There are some confounding factors in liver elastography: fasting or not (liver elastography must be performed in fasting conditions), increased aminotransferases (confident values if ALT < 100 U/L), obstructive jaundice, right hearth failure (all of them increasing the elastographic values).

But what is new in ultrasound-based elastography? More and more ultrasound systems developed ***multiparametric methods*** (***MPUS***-multiparametric ultrasound). This new development is intended to provide in the same system not only a module for liver fibrosis evaluation, but also for steatosis quantification and the evaluation of viscoelastic properties (inflammation). Thus, in a few minutes, one can evaluate the severity of steatosis, quantify fibrosis, and can have information regarding inflammation.

Ultrasound-based Techniques for Liver Steatosis Quantification

Quantification of steatosis with ultrasound systems became possible in the last years, with published papers showing good and promising results.

Attenuation imaging (ATI) was developed by Canon and some papers were published evaluating its value for detecting and quantifying liver steatosis [34 - 36], showing promising results. In a study performed on 114 subjects potentially at risk of steatosis and 15 healthy controls, ATI results were compared to the ones obtained with CAP, using MRI-PDFF as the reference [34]. ATI showed a higher correlation with MRI-PDFF (r=0.81) as compared to CAP (r=0.65). In another study [35], using liver histology as a reference in a series of 108 subjects, it has been reported that the severity of steatosis was the only significant determinant factor for ATI results and that the AUROCs of ATI for predicting different degrees of steatosis ranged from 0.84 to 0.93.

Ultrasound-Guided Attenuation Parameter (UGAP) from General Electric Healthcare was recently developed and a study evaluated the diagnostic accuracy of UGAP for the detection of hepatic steatosis as compared with CAP, using histopathology as the reference. A cohort of 163 consecutive chronic liver disease patients who underwent UGAP, CAP and a liver biopsy on the same day were evaluated. The AUROC's of UGAP for identifying >S1, >S2 and S3 were 0.900, 0.953 and 0.959, respectively, which were significantly better than the results obtained with CAP [37].

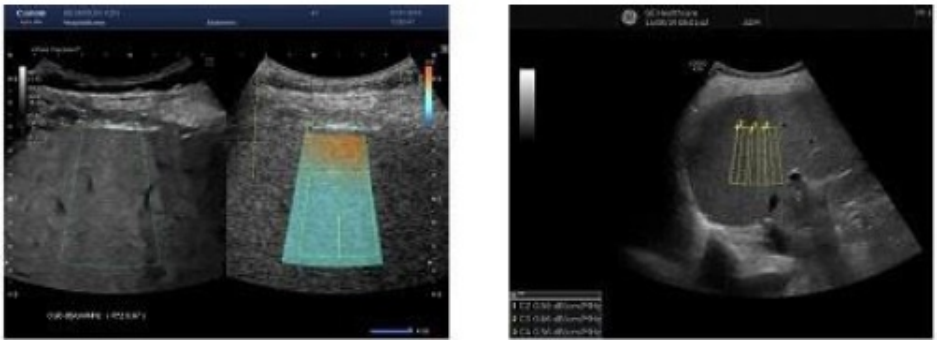

Fig. (4). Liver steatosis quantification using ATI (Canon) (left) and UGAP (GE Healthcare) (right).

Attenuation (ATT) from Hitachi has also shown good results. In a multicenter prospective study with 351 patients, the AUROC's corresponding to $S \geq 1$, $S \geq 2$, and $S \geq 3$ were 0.79, 0.87, and 0.96, respectively [38].

Speed of sound estimation (SSE) implemented on the Aixplorer US system (MACH 30) presume that an increase of fat in the liver causes a decrease in the speed of sound. In a pilot study with 100 patients [39], that compared SSE with MRI-PDFF, the SSE repeatability was excellent, with an intra-class correlation coefficient of 0.93. An SSE cut-off of ≤ 1.537 mm/μs had 80% sensitivity and 85.7% specificity in detecting steatosis (S1-S3).

What is more exciting in the last period is that the ultrasound companies try to find solutions for the non-invasive evaluation of inflammation, taking into consideration the viscoelastic properties of the liver tissue.

Combi-Elasto from Hitachi includes pSWE, Strain and Attenuation is a system that can quantify liver fibrosis, liver inflammation and liver steatosis. In a cohort of 388 patients with liver biopsy [40], the AUROC's for predicting different fibrosis stages were 0.87, 0.80, 0.83, and 0.80 (for F1, F2, F3 and F4, respectively), while the AUROCs for predicting different activity grades were 0.94, 0.74, and 0.76 (for A1, A2 and A3, respectively). This study concluded, "using strain and shear wave imaging (Combinational Elastography) might increase the positive diagnosis of liver fibrosis and inflammation".

Other systems, such as MACH 30 from Aixplorer can be used for inflammation assessment.

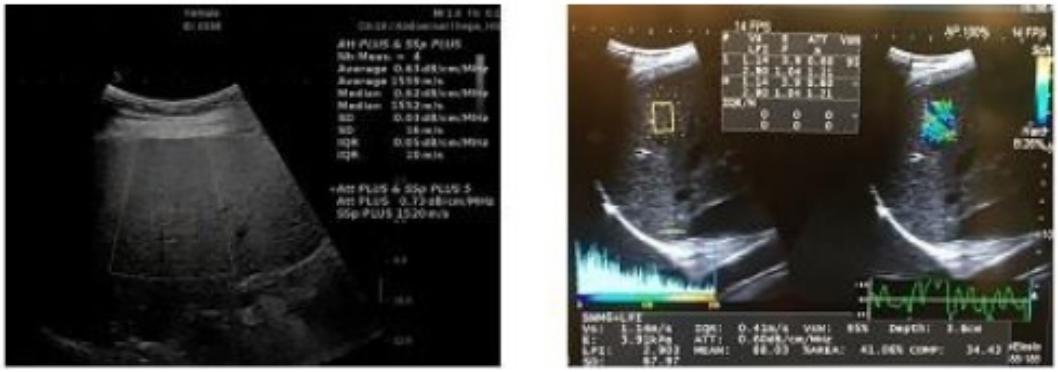

Fig. (5). Liver steatosis quantification using SSE (Aixplorer) (left) and Combi-Elasto (Hitachi) (right).

Ultrasound-based Elastography for the Evaluation of Portal Hypertension (PH)

Ultrasound-based elastography has earned its place as a non-invasive marker for the prediction of portal hypertension. The development of non-invasive markers has emerged from the need to assess patients with advanced liver disease in an accessible, easily reproducible, and most importantly, well accepted by the patients' manner. At the same time, these non-invasive markers do not aim to outperform the gold standard methods, such as hepatic venous pressure gradient (HVPG) and upper digestive endoscopy, but to apply them, only when this approach brings a definite benefit, such as to a new patient diagnosed with advanced liver disease, when it is preferable to screen with non-invasive markers to define the best time to perform endoscopy or other invasive techniques.

Liver stiffness (LS) is the most validated non-invasive marker as a predictor for portal hypertension and it was studied for more than 10 years. Initially, studies were performed using TE and demonstrated, a clear and reproducible correlation between LS values and the presence and severity of PH, in addition to the good feasibility of the method [41 - 43]. Later, the usefulness of LS as a predictor for PH was assessed using other elastography techniques, such as pSWE and 2D-SWE. In the last years, spleen stiffness evaluation, or a combination of liver and spleen stiffness seems to increase the accuracy of PH assessment.

Liver stiffness assessment by TE for predicting clinically significant portal hypertension (CSPH) has been evaluated in many studies, but a published meta-analysis [41] concluded that TE alone cannot replace endoscopy for esophageal varices (EV) screening. However, the Baveno VI Consensus [44] concluded that in patients with virus-related chronic liver disease, non-invasive methods are sufficient to rule-in CSPH, using a liver stiffness TE cut-off value ≥ 20-25 kPa. Moreover, combining a platelet count > 150000 and liver stiffness by TE < 20 kPa patients have a very low risk of having varices requiring treatment and can avoid screening endoscopy. Expanding the Baveno VI Consensus criteria, different thresholds of platelets and LS values have been tested for the identification of patients at very low risk (< 5percentage) of having varices needing treatment. The best new expanded classification rule of platelet count >110000 and LS <25 kPa could potentially spare 40% of endoscopies [45].

For the evaluation of PH, the most studied pSWE technique is VTQ. One of the first studies [46] that evaluated the performance of LS assessed with VTQ for predicting significant EV showed an AUROC of 0.59, similar to the one found in another study (AUROC 0.58) [47]. Better results were obtained later, studies showed that LS is a useful tool for predicting the presence of any grade EV with AUROCs ranging between 0.74-0.84 [48 - 50]. Regarding the evaluation of LS with the ElastPQ technique, the studies are more limited. A recent study showed that LS by ElastPQ significantly correlated with portal pressure ($R = 0.482$, $p < 0.001$) [51].

The performance of LS assessed using 2D-SWE for predicting PH was also studied. The majority of studies used 2D-SWE SSI technique and found good correlations between LS values and CSPH [52, 53]. Fewer studies are available regarding the performance of 2D-SWE GE for predicting PH. In a recent study, Stefanescu *et al*. [54] showed that LS by 2D-SWE GE was strongly correlated with HVPG ($r = 0.704$; $p < 0.0001$), especially if HVPG < 10 mmHg. LS values were significantly higher in patients with CSPH (15.52 *vs.* 8.14 kPa; $p < 0.0001$). For a cut-off value of 11.3 kPa, the AUROC of 2D-SWE GE-LS to detect CSPH was 0.91.

Spleen stiffness (SS) has been also studied for the evaluation of PH using different elastographic systems, with promising results. SS by TE showed a significant correlation with HVPG ($r = 0.433$. $p < 0.001$) [55] and high AUROC values (0.90 and 0.82) for predicting EV grade 2 and 3 [56, 57]. SS by TE of < 46 kPa in combination with Baveno VI criteria (LSM <20 kPa and platelets>150.000) was demonstrated to have 0% chance for missing high-risk varices [58]. SS has also been evaluated in combination with LS in a prospective multicenter study including 158 subjects, evaluated by 2D-SWE SSI in the liver and spleen and by HVPG measurement [59]. LS > 29.5 kPa and SS > 35.6 kPa were able to "rule-in" CSPH with high sensitivity (89.2%) and specificity (91.4%).

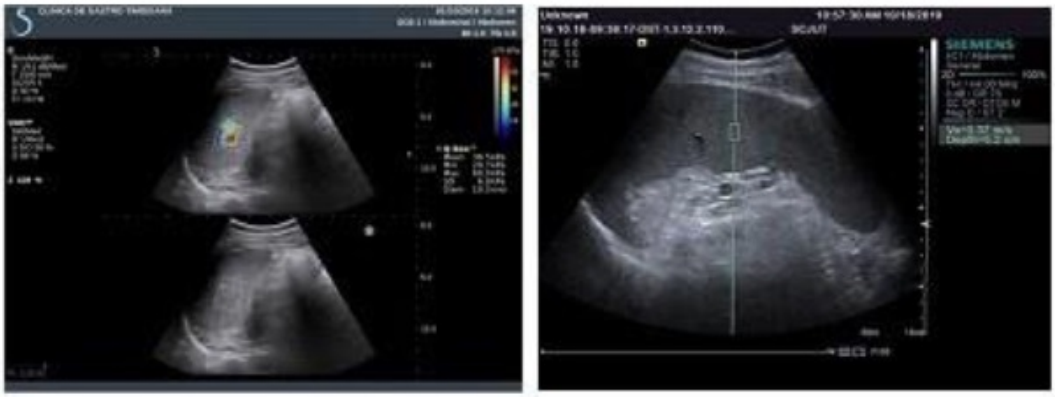

Fig. (6). Spleen stiffness evaluation with 2D SWE (Aixplorer system) (left) and pSWE (VTQ) (right).

CONCLUSIONS

Liver and spleen stiffness using ultrasound-based elastography are useful tools for the evaluation of patients with chronic liver disease. New developments in this field will make the non-invasive evaluation of the liver a very attractive clinical tool.

CONSENT FOR PUBLICATION

Not Applicable.

CONFLICT OF INTEREST

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CHAPTER 21

New Insights into NAFLD (Diagnosis, Risk Stratification, Treatment)

Carmen Braticevici Fierbinţeanu[1,2] and **Alexandru Moldoveanu**[1,2,*]

[1] *Internal Medicine II and Gastroenterology – University Hospital Bucharest, "Carol Davila" University of Medicine and Pharmacy Bucharest, Romania*

[2] *Department of Gastroenterology, University Hospital Bucharest, Romania*

Abstract: Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease, with a worldwide prevalence of 25%. Considering the ongoing obesity epidemic, the rise in diabetes, and other features of metabolic syndrome, the prevalence of NAFLD along with the proportion of those with advanced liver disease is expected to increase continuously.

NAFLD/NASH patients have a high comorbidity burden; those with advanced liver disease have significantly higher costs, especially for patients requiring hospitalization. Early identification and effective management is needed to minimize the disease progression and costs.

Experts reached a consensus that NAFLD does not reflect current knowledge, and metabolic (dysfunction) associated fatty liver disease "MAFLD" was suggested as a more appropriate overarching term.

Until now the biggest unmet need is a performant biomarker that can diagnose and stage NASH to replace the need for liver biopsy. Such a biomarker, will increase the ability to identify patients at risk, monitor disease progression, and response to the therapy.

Treatments need a multidisciplinary approach and include: drugs targeting intake and disposal energy, lipotoxic liver injury, inflammation and fibrogenesis that lead to cirrhosis.

Keywords: Biomarkers, Diabetes Mellitus, Disease Progression, Liver Biopsy, Liver Cirrhosis, Metabolic Associated Fatty Liver Disease, Metabolic Syndrome, Non-alcoholic Fatty Liver Disease, Non-alcoholic Steatohepatitis, Obesity.

[*] **Corresponding author Alexandru Moldoveanu:** Department of Gastroenterology, University Hospital Bucharest, Splaiul Independenței 169, sector 5, Bucharest, Romania; Tel: +40742224204; E-mail: alexandru@moldoveanu.tel

Ioan Sporea and Alina Popescu (Eds.)

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), a part of a multisystemic disease, is considered the hepatic manifestation of metabolic syndrome. The disease has risen in prevalence, involving a quarter of the population, with a major impact on the clinical and economic burden on the society [1]. NAFLD encompasses two sub-types of conditions with different prognoses: fatty liver, which, in general, follows a benign non progressive clinical course, and steatohepatitis or NASH, a more serious form of NAFLD, which may progress to cirrhosis and end stage liver disease.

Although the term nonalcoholic fatty liver disease, an acronym, was introduced by Ludwig and colleagues in 1980, to describe fatty liver disease arising in the absence of significant alcohol intake, until now the nomenclature and criteria for a diagnosis have not been revisited [2]. The heterogeneous pathogenesis of this disease represents an important impediment to the discovery of effective drug treatments. That is why, recently, a group of experts suggested a change in terminology, to better reflect the heterogeneity of individual pathogenesis of the disease and proposed a more appropriate term, instead of NAFLD, Metabolic (dysfunction) Associated Fatty Liver Disease "MAFLD". This update of nomenclature will be a step ahead in an accurate identification of particular disease subtypes, to better characterize the disease and detecting new therapeutically targets with major implications on clinical practice and public health policy [3].

Natural History of NAFLD

Although a substantial proportion of the population (25%) has NAFLD, only a minority progress to advanced liver disease (patients with NASH) and it is a challenge for a physician to identify them within the large NAFLD population. Fig. (**1**) presents the natural history of NASH based on 40 different studies.

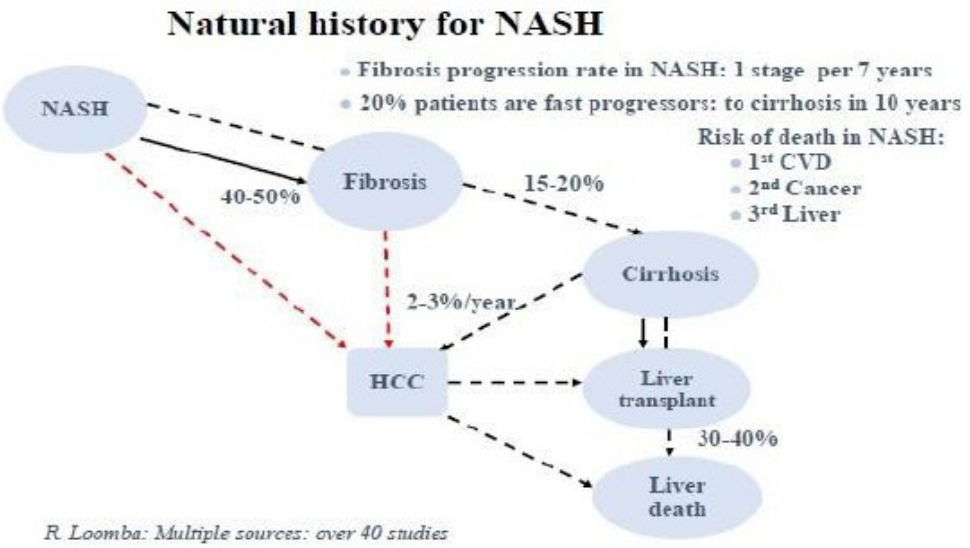

Fig. (1). Natural history of NASH.

From patients with NASH about 50% have fibrosis and 20% of them will develop cirrhosis in about 20-30 years. But 20% of patients are fast progressors and will develop cirrhosis in 10 years and a new challenge is to identify the patients with rapid progression to cirrhosis. There is an exponential manner of increased risk parallel to the increase in the fibrosis stages [4]. Once cirrhosis is developed the patients are at even higher risk for poor hepatic prognosis (hepatic decompensation, HCC, and liver-related mortality) [5]. In addition, those with NAFLD/NASH have a two times higher risk for death related to cardiovascular disease and non-liver cancers as compared to those without NAFLD [6, 7].

Liver-specific and overall mortality rates among NAFLD and NASH patients are 0.77 (range, 0.33–1.77) per 1000 and 11.77 (range, 7.10– 19.53) per 1000 person-years and 15.44 (range, 11.72– 20.34) per 1000 and 25.56 (range, 6.29–103.80) per 1000 person-years, respectively [8]. Factors identified to influence the NAFLD evolution with the established association are comorbidities (features of metabolic syndrome), genetic factors (PNPLA3, TM6SF, A1AT PIZ), microbiome products and nutritional factors (alcohol, cholesterol, fructose) [9].

The new data published from the largest prospective cohort of NASH patients revealed the dynamic nature of the disease evolution regarding the progression of NASH and the progression of fibrosis. The study showed that a large number of patients with NAFLD are likely to progress to NASH (46.9%) and fibrosis can be

modified (improve or progress) in 30% of patients during a mean period of 4.9 years [10].

Diagnosis of NAFLD and Risk Stratification

The diagnosis of NAFLD requires evidence of liver steatosis either by imaging or histology. NASH is a histological diagnosis and requires liver biopsy and is characterized by hepatocytes steatosis, lobular inflammation and the key diagnostic feature of NASH-liver cells ballooning (Fig. **2**).

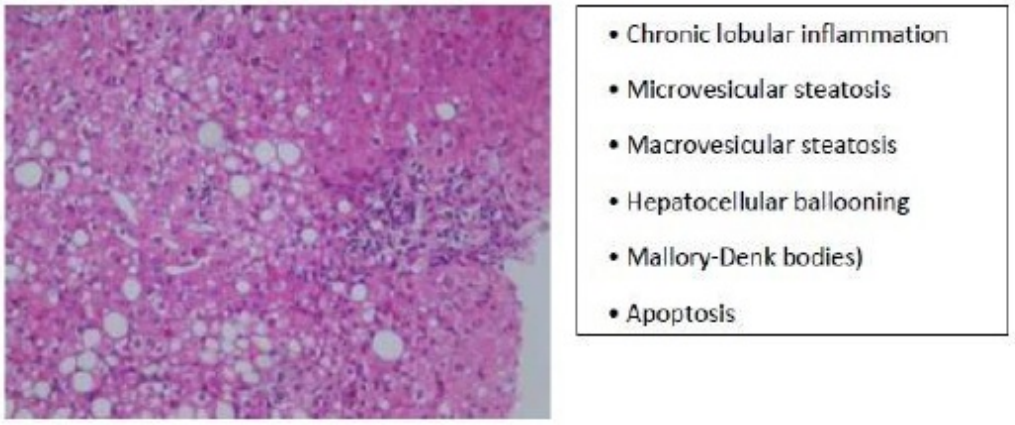

Fig. (2). Lesions and patterns of NASH - the liver tissue sample with macrovesicular steatosis with lobular inflammation and ballooning degeneration.

Until now we do not have an ideal biomarker instead of a liver biopsy. Liver biopsy is required to confirm the diagnosis, to stage the disease and to stratify the progression risk. But liver biopsy, to classify such a large population is impractical, is an invasive procedure with a low but a real risk of complications and has sampling and reading variability.

It is interesting to ask if we can identify the patients at risk of progression toward advanced liver disease without liver biopsy? For this purpose, it is important to look at the clinical profile of the patient, and the presence of features of metabolic syndrome. Metabolic syndrome is a strong predictor for NASH in patients with NAFLD. Even more, the presence of multiple components of metabolic syndrome is associated with advanced disease. That is why the European Association for the Study of the Liver (EASL) recommends routine screening for NAFLD in all patients with metabolic syndrome and in high risk patients (aged >50 years, diabetic patients), the assessment of liver fibrosis is advisable [11].

Due to the limits of liver biopsy, it was a significant interest in developing non-invasive biomarkers for risk stratification of patients with NAFLD. Fibrosis is the most important prognostic factor in NAFLD as in any chronic liver disease. In a longitudinal study of patients with NAFLD, only fibrosis and no other histological features (inflammation, ballooning), was associated with long term overall and hepatic mortality [5].

Clinical prediction rules (*e.g.*, Fatty liver disease Fibrosis Score [NFS], Fibrosis-4 [FIB-4] Index, Aspartate Aminotransferase to Platelet Ratio Index) and others, combine available demographic variables with laboratory markers providing prognostic information in the clinical practice at no cost [12 - 14]. Fig. (**3**) presents the most common scores to diagnose advanced fibrosis in NAFLD.

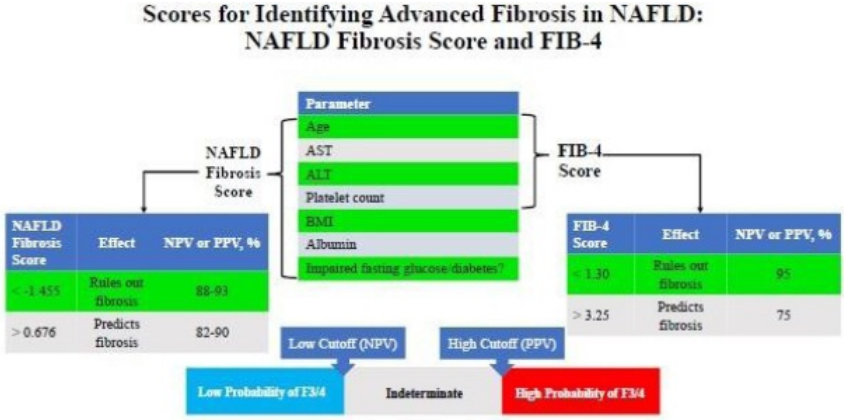

Fig. (3). Diagnostic scores for advanced fibrosis.

There are two different cut-offs. The low value is very sensitive, and it is used to exclude advanced fibrosis (the Negative Predictive Value - NPV is excellent). The high value predicts a high risk for advanced fibrosis but requires liver biopsy for confirmation. Between low and high values of cut-offs there is a middle zone (grey zone) where fibrosis is undetermined.

When we apply these two tests in clinical practice, both lack sufficient Positive Predictive Value (PPV) to be used alone to predict NASH and fibrosis.

What about imaging biomarker? Vibration-Controlled Transient Elastography (VCTE - Fibro-Scan) it is an ultrasound-based device that evaluates hepatic steatosis (using Controlled Attenuation Parameter - CAP) and liver stiffness (as a surrogate marker of liver fibrosis) [15]. VCTE is approved by the US Food and Drug Administration for use in adults and children with liver disease. Ultrasound -based acoustic force impulse elastography and supersonic shear-wave

elastography are additionally recognized modalities. Magnetic resonance elastography (MRE) is more sensitive but is limited by restricted availability, cost, presence of metal implant and history of claustrophobia [16]. In this context, VCTE is more accessible and easier to use. Regarding liver fibrosis, this technique is most reliable in ruling out advanced fibrosis (NPV stronger than PPV). Overestimation of fibrosis can occur in cases of acute inflammation, cholestasis, and liver congestion. Its use is also limited in patients with severe obesity and ascites. Higher values of stiffness can predict the risk of decompensation and correlate with portal pressure [17]. However, VCTE lacks sufficient PPV to be used alone to predict NASH and fibrosis.

To optimize the evaluation of fibrosis in NAFLD, it was proposed a stepwise approach with the sequential use of non-invasive tests to minimize the intermediate zone. This approach maintains the sensitivity and specificity enabling the classification of a larger proportion of patients.

To establish the performance of combination of noninvasive tests (simultaneous or sequential) for the diagnosis of NASH with advanced fibrosis, data was used from Stellar trials which enrolled patients with bridging fibrosis and compensated cirrhosis. The patients were staged according to the NASH Clinical Research Network classification. The study evaluated associations between fibrosis stage and noninvasive tests including: NFS, FIB4, Enhanced Liver Fibrosis Test (ELF) and liver stiffness by vibration-controlled transient elastography (VCTE). The performance of these noninvasive tests to discriminate advanced fibrosis, either alone or in combination, was assessed using areas under the receiver operating characteristic curve (AUROCs). By using a single test (NFS or FIB4), 50% percent of patients led to up in indeterminate aria. However, by adding the second line of noninvasive tests (FIB 4 than ELF or VCTE) only sequential and not simultaneous combinations of two noninvasive tests, the indeterminate aria was reduced by half [18].

Once the patients with NAFLD were identified there is an additional challenge in selecting the subset of patients with NASH and fibrosis. For this purpose, a fast modality was proposed by Koneman and co-authors which stratified the patients into three categories regarding the probability for NASH and fibrosis: low, intermediate, and high risks. This approach is based on clinical parameters, biological values, and results of non-invasive tests of fibrosis (Fig. **4**).

According to this approach, a patient included in high risk category is older than 50 years, has features of metabolic syndrome as comorbidities, values more than 8.5 kPa at FibroScan and high cut-offs for advanced fibrosis calculated for NFS and FIB [19].

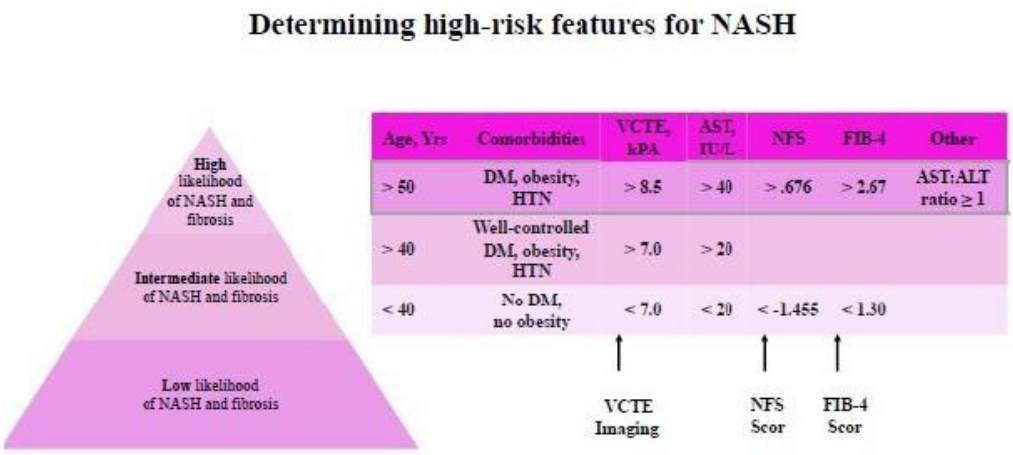

Fig. (4). Pre-screen criteria for NASH.

Due to the magnitude of the NAFLD epidemic, an algorithmic approach is welcomed in the initial evaluation of patients with NAFLD. Although, most patients have mild disease, patients with severe disease should be identified due to the dangerous complications, as hepatocellular carcinoma, and liver-related mortality.

Promising diagnostic algorithms were developed to identify patients at risk, with a certain level of NASH severity and fibrosis.

One of them, used for risk stratification of patients with NAFLD, divided patients into low or high risk for advanced disease based on their clinical profile Xs. (Fig. **5**) [20]. This algorithm, based on the clinical and biological profile of the patients and the results of the most frequent non-invasive tests, is used to screen patients with NAFLD for liver fibrosis, in a cost-effective fashion.

More recently, a new score was developed to identify the patients with NASH with significant activity, fibrosis, and FAST Score. This score is based on steatosis and fibrosis evaluated by using Fibro-Scan, and inflammation estimated with AST. The score was developed by analysis of a prospective multicenter study of patients undergoing liver biopsy for suspected NAFLD. The aim of the FAST score is to identify patients with active NASH (NAS $\geq$ 4) and significant fibrosis (F$\geq$2). FAST combined liver stiffness measurements, CAP by VCTE and biological marker (AST). This score showed a good performance in detecting active NASH with significant fibrosis with an aria under the receiver operating characteristic curve (AUROC) of 0.82-0.93) [21].

There are also many other non-invasive tests that are in development for selecting patients with active NASH and significant fibrosis, at higher risk for end-stage liver disease or liver-related mortality. Table **1** presents these non-invasive tests, which are blood-based biomarkers, correlated with the histological features provided by liver biopsy.

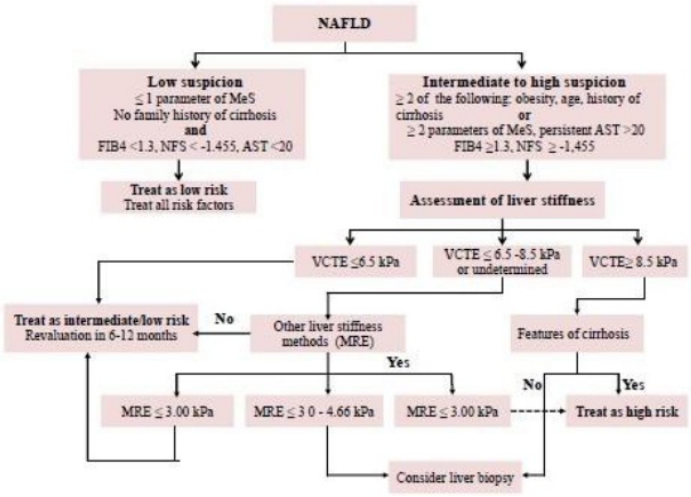

Fig. (5). Risk stratification of patients with NAFLD.

Table 1. Non-invasive tests for diagnosis of active NASH with advanced fibrosis [22 - 24].

Test	Requirements	Components
NIS4 [1]	Blood test	Alpha-2-macroglobulin, A1C, miR-34a, YKL - 40
FIBROSpect [2]	Blood test	Alpha-2-macroglobulin, hyaluronic acid, TIMP metallopeptidase inhibitor1
ADAPT/Pro-C3 [3]	Blood test	Age, diabetes, Pro-C3, platelets
A1C- glycated hemoglobin, miR-34a- MicroRNA, YKL-40 - Chitinase 3-like Protein 1(an inflammatory glycol-protein associated with endothelial dysfunction), Pro-C3- a marker of type III collagen formation.		

The mentioned components were incorporated into an algorithm and developed these new scores trained and validated for the identification of at-risk for NASH. These scores demonstrated a good performance in differentiating patients with NASH and mild fibrosis, from those with advanced fibrosis.

However, until now the biggest unmet need is a performant biomarker that can diagnose and stage NASH to replace the need for liver biopsy. Such a biomarker will increase the ability to identify patients at risk, monitor disease progression, and response to the therapy.

Treatment of NASH

As NASH is a metabolic disorder of nutrients, the most important recommendations for both the prevention and treatment of NASH are lifestyle modifications with a specific focus on healthy eating, regular exercise, consumption of excess energy substrates.

In overweight patients, a weight loss of 7 to 10% of their body mass is recommended, as it has been proven to improve histopathological features of the liver, including fibrosis [25]. A reduction of calories intake by 500 to 1000 kcal per day, either alone or in combination with physical exercise, is likely to provide a sustainable weight loss for most of the patients.

Discoordination between central and peripheral circadian rhythms is a key feature of nearly every genetic, dietary, or environmental model of metabolic syndrome and NAFLD. Time-restricted feeding can restore the coordination of circadian rhythms, which in turn can prevent or even treat metabolic syndrome and hepatic steatosis [26].

Physical exercise has been shown to have clear benefits on metabolism, independent of weight loss [27]. It is also recommended that patients with NAFLD should avoid excessive alcohol consumption, but there is insufficient data to warrant complete abstinence [25].

Interventional therapies, including bariatric surgery or endoscopically placed balloons improved weight loss by increasing satiety and reducing food uptake. A study has attempted to evaluate the usefulness of bariatric surgery in morbidly obese patients with NASH and has found that about 85% of patients had reduced histological features after 1 year [28]. Oral hydrogel is a non-invasive alternative, that has been shown to help overweight patients achieve greater sustained weight loss than through diet alone [29].

Pharmacological therapies are currently recommended to be limited to patients with biopsy proven NASH and fibrosis [25]. Leptin has been pursued as a potential satiety modulator, but animal studies have raised the concern of profibrotic and proinflammatory effects.

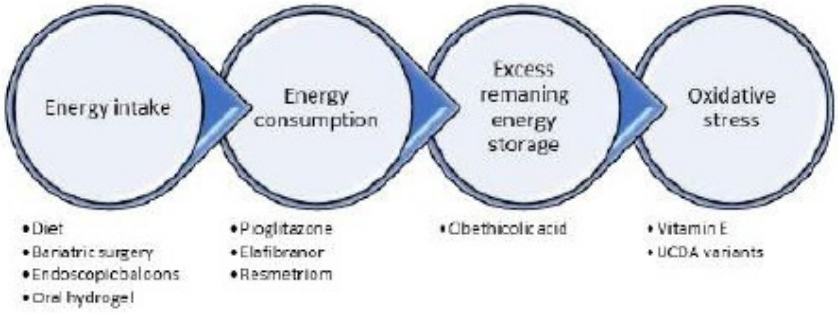

Fig. (6). Treatment options for NASH.

The storage of fatty acids triggers a local inflammatory response when the adipocyte storage capacity is exceeded. The PPAR receptor activation increases skeletal muscle, liver and adipose tissue metabolism and facilitates energy consumption, avoiding the excessive accumulation of lipids.

Drugs targeting the PPAR-γ, like pioglitazone – a drug used in increasing tissue sensitivity to insulin in type 2 diabetes patients, can reduce the local inflammatory response, and has been shown to improve the histological features in both diabetic and non-diabetic NASH patients. However, the side effects of pioglitazone include potential weight gain, which may have potential effects on different pathologies, as well as others including fluid retention as well as bladder cancer.

Elafibranor activates both the PPAR-α and PPAR-δ receptors and has been shown to improve liver histology features in patients with higher activity. Other drugs with similar mechanisms currently being studied include saroglitazar and lanifibranor.

The liver regulates the metabolism of excess metabolic energy, transforming excess glucose to fat. Farsenoid X receptor activating drugs have been shown to inhibit the production of lipids by the liver. Obeticholic acid was shown to significantly improve histological features in NASH, including fibrosis [30, 31]. Other molecules targeting similar pathways, like cilofexor, tropifexor are in early studies.

Thyroid hormone receptors in the liver have also been the target of experimental drugs, as stimulating the liver thyroid hormone receptor β has been shown to reduce both serum lipids and liver lipids by increasing hepatic fat metabolism.

Resmetriom was also shown to improve liver histology features in phase 2 trials and phase 3 trials are currently underway [27].

Oxidative stress is also an important component of NASH. Vitamin E in doses of 800 UI daily was used as an anti-oxidative treatment, but improvement in liver histology features was not clearly demonstrated in all patients. However, the vitamin E treatment has shown improvement in some patients and is currently recommended for non-diabetic non-cirrhotic adults with biopsy-proven NASH [25].

Oxidative stress in the endoplasmic reticulum specifically is also an important parameter and was investigated as a potential treatment target. Ursodeoxycolic acid (UDCA) was used as a treatment and showed promise in initial small-scale studies, however large studies show no significant improvement in histology features [32]. Variants of UDCA, including tauro-UDCA and nor-UDCA show more promising results, but have only been tested on small populations and with no histological evaluation of results [33].

It is worth mentioning that patients with NAFLD or NASH have increased cardiovascular risk and should receive treatment addressing this risk. Statins are commonly used to improve cardiovascular risk, but clinicians are often reluctant to administer them to patients with preexisting liver diseases. Large studies have shown that administering these drugs to patients with increased liver enzymes, including patients with NASH, have not shown increased liver toxicity risks [34, 35]. The current guideline recommends administering statins to all patients with cardiovascular risk with NAFLD or NASH, except for decompensated cirrhosis [25].

CONCLUSIONS

NAFLD is the most common liver disease, with a worldwide prevalence of 25%. Experts reached a consensus that NAFLD does not reflect current knowledge, and metabolic (dysfunction) associated fatty liver disease “MAFLD” was suggested as a more appropriate overarching term. Until now the biggest unmet need is a performant non-invasive biomarker that can diagnose and stage NASH to replace the need for liver biopsy. Treatments of MAFLD need a multidisciplinary approach and include drugs targeting intake and disposal energy, lipotoxic liver injury, inflammation and fibrogenesis that lead to cirrhosis.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this chapter contents have no conflict of interest.

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Declared none.

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CHAPTER 22

The Role of Cytokines and Inflammatory Mediators in Alcoholic Liver Disease

Ligia Bancu[1,*]

[1] *1st Department of Internal Medicine, UMFST " George Emil Palade" Târgu Mureş, Romania*

Abstract: Cytokines are low molecular weight substances, mediating intra and inter-cellular communications. They are produced by several cell types, including the liver with a special focus on Kupffer cells. In the liver, pathological stimuli induce cytokines release and are responsible for cell lesions, destruction, necrosis, apoptosis and regeneration. In alcoholic liver disease (ALD) inflammatory cytokines such as interleukin-8 (IL-8) tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6 (IL-6) as an acute phase-cytokine are involved in the liver injury. Another pro-inflammatory interleukin is interleukin-12 (IL-12), which seems to be related to chronic alcoholism. Transforming growth factor β (TGF-β) has the most important fibrogenic properties in the liver and it is also involved in regulating apoptosis along with tumor necrosis factor. Several types of cytokines are described to induce anti-inflammatory effects on the liver with chronic alcoholic exposure: Kupffer cells produce the hepatoprotective cytokine IL-6 and the anti-inflammatory cytokine interleukin- 10 (IL-10) during liver injury induced by alcohol. IL-6 acts in a protective manner *via* the activation of transcription 3 and induction of hepatoprotective genes in hepatocytes. IL-10 inhibits alcoholic liver damage in Kupffer cells/macrophages. Interleukin-22 (IL-22) is another important hepatoprotective cytokine against acute and chronic alcoholic liver injury. Adipocytokine adiponectin decreases hepatic insulin resistance and attenuates liver inflammation and fibrosis. Thus findings in the complex "puzzle" of ALD could launch the research for new therapeutic perspectives.

Keywords: Adiponectin, Alcoholic liver disease, Cytokines, Fibrosis, Inflammation, Interleukins.

INTRODUCTION

Alcoholic liver disease (ALD) is a syndrome consisting of a large spectrum of abnormalities, in which chronic ethanol intake induces progressive inflammatory liver injury.

[*] **Coresponding author Ligia Bancu:** 1st Department of Internal Medicine, UMFST " George Emil Palade", 50, Gh Mariescu st, 540138 Târgu Mureş, Romania; Tel +40722689490, Fax: +40265212886;, E-mail: ligiabancu@yahoo.com

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The liver injury evolves through different stages from simple fatty liver (steatosis) to fatty liver associated with inflammation (steatohepatitis), to destruction of the liver structure (fibrosis/cirrhosis) and higher risk of liver cancer (hepatocellular carcinoma) [1]. However, only 30-35% of chronic alcohol consumers develop clinically significant ALD, thus multiple co-factors may be involved.

The risk factors include, drinking habits, obesity, genetic, metabolic factors, cigarette smoking and sex [2]. A higher sensibility to alcohol-induced liver lesions is encountered in females. Obesity also facilitates alcohol induced liver damage, due to the activation of pro-inflammatory cells such as macrophages, thus determining the appearance of resistance to insulin and adiponectin [2 - 5]. Genetic factors including genetic polymorphism of patatin-like phospholipase domain-containing protein 3 (PNPLA3) have been recently described. Their expression induces the development of alcoholic cirrhosis in patients with ALD [2, 5 - 8].

Pathogenesis of this progressive destruction is evident multifactorial. ALD is the result of the innate and adaptative immune responses. The inflammatory pathways in ALD includeadaptive immune cell types, signaling receptors/pathways, together with anti- and pro-inflammatory responses.

Cytokines and their Role in ALD

In immune responses, cytokines are cell signaling molecules that help cell to cell communication and aids the movement of cells towards sites of inflammation, infection and trauma. They are polypeptide mediators of cellular communication that are produced and released by different cell types [9]. The production of cytokines is very low or even absent in most tissues, including the liver; nevertheless, in special situations, upon physiologic or pathologic stimuli, cytokine production is upregulated and these molecules induce the tissue response to the stimuli. Within the liver, cytokines as a response to pathological stimuli are involved in inflammation, cell necrosis and apoptosis. They are also responsible for fibrosis and regeneration following liver injury [10]. In the liver, several cytokines mediate hepatic inflammation, necrosis, and fibrosis but there are some hepato-protective and anti-inflammatory types, too.

In alcoholic hepatitis, there is a raising of tumor necrosis factor (TNF-α), a major trigger that leads to a succession of metabolic changes that harms the liver. Kupffer cells, activated *via* their toll-like receptors (TLR 4) by a high amount of lipopolysaccharides (LPS) – (intestinal derived gram-negative bacteria) [11], which secrete TNF-α and other pro-inflammatory cytokines determining free radical formation that are involved in the steatosis and fibrosis of the liver [12].

TNF- α

TNF- α is a pleiotropic cytokine that is produced by various types of cells in the organism. In the liver it is mainly produced by activated Kupffer cells and intervenes in the pathophysiology of various pathologies such as viral hepatitis, alcoholic liver disease, and nonalcoholic fatty liver disease (NAFLD) [13, 14]. TNF- α has a role in various physiological processes like cell proliferation, inflammation, and cell death (apoptosis).

More studies evidenced that ethanol intake may raise the liver's sensitivity to inflammatory cytokines, in two ways. Firstly, alcohol consumption leads to a stimulation of Kupffer cells to produce and then release TNF–α into the small vessels that allow blood flow in the liver. One indirect mechanism is represented by the augmentation of bacterial endotoxin concentration in the blood and its further explained mechanism [15]. The second mechanism is a higher response of hepatocytes to TNF–α in presence of alcohol [16]. This could lead to an increased production of small oxygen–containing molecules called reactive oxygen species (ROS) in the mitochondria. Unless they are rapidly eliminated or converted into harmless molecules by antioxidants, they can damage complex molecules in the cells (*e.g.*, proteins and DNA). ROS activates a protein called nuclear factor kappa B (NFκB), that influences the expression of numerous genes, including those encoding TNF–α, that promote apoptosis. Thus, hepatocytes could be activated a vicious cycle: TNF–α initiates ROS production, which activates NFκB, and NFκB and thus induces a larger production of additional TNF–α that promote apoptosis [16]. Within the liver, endotoxin levels are extremely low due to the intestinal barrier and Kupffer cell mediated detoxification role [17]. Ethanol consumption will increase endotoxin levels in the blood by damaging the permeability of the intestinal wall, as a consequence endotoxin cross that wall more easily. This "leaky gut", was demonstrated in animal studies [16]. By this mechanism gut-derived endotoxins invade the portal circulation activating Kupffer cells through the LPS/TLR-4 pathway.

IL-1/ IL-1β

IL-1β is a potent inductor of inflammation augmenting the expression of a large number of pro-inflammatory molecules. IL-1β is an endogenous pyrogen, apromoter of other proinflammatory mediators [18]. On hepatocytes induces steatosis by a direct effect. IL-1β also sensitizes hepatocytes to the killing effect of TNF-α, thereby causing a synergistic effect between pro-inflammatory cytokines regarding hepatocyte injury [19].

IL-6

IL-6 has a dual effect: it belongs to mediators involved in the acute inflammatory phase but it has anti-inflammatory properties, too. After alcohol consumption, in the liver, activated macrophages/Kupffer cells induce the release of IL-6 together with IL-10, TNF-α and other cytokines. IL-6 level is correlated with the stage of ALD [20]. Mice in presence of deficiency in IL-6 became vulnerable to alcohol induced steatosis [21].

IL—8

The increase of IL-8, in alcoholic hepatitis patients is linked to neutrophil infiltration [22].

IL -12

Interleukin -12 (IL-12) is a proinflammatory cytokine produced by antigen-presenting cells upon stimulation by different pathogens. This cytokine has direct effects on natural killer cells and T cells. Laso *et al.* [24] have demonstrated that serum IL-12 levels are increased in chronic alcoholism, in the presence or absence of alcoholic liver disease. Serum IL-12 levels return to within normal limits 1 year after cessation of alcohol consumption. The clinical significance of serum IL-12 in the diagnosis of alcoholism and ALD has to be demonstrated.

IL-17

The Th-17 cells are a recently discovered class of T helper lymphocytes, the main source of interleukin-17 (IL-17), that contributes to host immune response against microorganisms like in autoimmune diseases. The role of IL-17 in ALD in humans was not long ago suggested [25].

TGF –β

TGF-β represents the most important profibrogenic cytokine inducing fibrosis in some fibroproliferative conditions, especially in liver sufferance. Furthermore, TGF-β, generally considered an immunoregulatory cytokine is the second cytokine involved in regulating apoptosis in both normal and tumor cells. TGF–β along with TNF–α are responsible for apoptosis in hepatic cancer. It triggers hepatocytes that are very sensitive and responsive to this cytokine, to suffer apoptosis, generates space for hepatic stellate cells (HSC) proliferation and collagenous matrix formation. Anti TGF-β has been identified and with good results utilized for the treatment of experimental fibrogenesis [26]. Studies have demonstrated that TGF–β is produced in larger amounts in the liver of patients with alcoholic fibrotic transformation than in the liver of healthy people, it could

suggest that TGF–β is responsible for the appearance of alcohol–induced liver diseases [26].

Anti-Inflammatory Cytokines in ALD

Although IL-6, IL-10, and interleukin 22 (IL-22) stimulate (*via* STAT3) similar signaling pathways in the liver, they reach different types of liver cells. In hepatocytes, both IL-6 and IL-22 induce signal transducer and activator of transcription 3 (STAT3) and have important roles in hepatoprotection and liver regeneration. In Kupffer cells, IL-6 induces transient STAT3 activation and induces the pro-inflammatory response, as long as IL-10 induces prolonged STAT3 activation and induces the anti-inflammatory response. In sinusoidal endothelial cells, IL-6 promotes STAT3 activation and cell survival. In liver stellate cells, IL-6 starts STAT3 activation. The roles of STAT3 in stellate cell activation and fibrogenesis still remains obscure [27].

IL-6

IL-6 has a complex role in ALD. It possesses some protective effects on the liver. After alcoholic liver injury, IL-6 may diminish the apoptosis and intervenes in mitochondrial DNA repair. Serum and hepatic IL-6 levels are raised in patients with ALD [28] and in animal models with alcoholic liver injury [27]. IL-6 has been associated with the acute phase response, helping liver regeneration and offering protection against liver injury. IL-6 also has protective effects on the liver in steatohepatitis including ALD [29].

IL-10

Interleukin-10 (IL-10) is considered to be the powerful anti-inflammatory cytokine and was first identified in 1989 as a pleiotropicTh-2 cytokine that inhibits interferon-gamma (IFNγ) synthesis by T helper 1 (Th1) lymphocytes [10]. IL-10 inhibits cell-mediated immune responses while stimulating humoral immunity [30]. IL-10 is very important in limiting the lesions in inflammatory and autoimmune diseases. IL-10 lowers the production of pro-inflammatory cytokines from activated macrophages. IL-10 also protects from proliferation and fibrosis [71].

Adiponectin

Adiponectin is a 30-kDa adipokine: the adipocytes are the cells in which it is synthesized and secreted. The pathways through which adiponectin is activated in the liver are those involving activation of 5-AMP-activated protein kinase and peroxisome proliferator-activated receptor-α pathways and inhibition of toll-like

receptor-4 mediated signaling. There are many pieces of evidence that adiponectin could diminish hepatic and systematic insulin resistance, lowers liver inflammation and fibrosis. Adiponectin is immune active by adhering to the specific membrane-bound receptors (AdipoRs): adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) [31]. The above data about the role of cytokines in ALD are summarized in Table **1**.

Table 1. Cytokines effects in ALD.

Cytokine	Effects
TNF α	Proinflammatory -promotes ROS production,pro-apoptotic action,necrotic action,stimulates neutrophils and macrophages
IL-1/IL-1β	Inducer of proinflammatory molecules, pyrogen, sensitizes hepatocytes to TNF-α
IL-6	Has dual effect: involved in the acute inflammatory phase, promotes other proinflammatory cytokines production (*e.g* IL-17) **but** protects against hepatocyte apoptosis and participate in mitochondrial DNA repair
IL-8	Proinflammatory-acts through neutrophils attraction
IL-12	Proinflammatory-primes Th-1 response activates NK
IL-17	Proinflammatory-stimulates production of proinflammatory cytokines/chemokines
TGFβ	Proinflammatory-promotes collagen synthesis
IL-10	Antiinflammatory -decreases macrophages activity, production of cytokines
IL-22	Antiinflammatory-upregulates antiapoptotic, antioxidative genes
Adiponectin	Antiinflammatory effects, decreases insulin sensitivity

CONCLUSION

Through various types of action, cytokines regulate biochemical processes in hepatocytes as well as in other cells. In ALD, cytokine production with some known and some not very well identified effects leads to chronic inflammation hepatitis, fibrosis, and cirrhosis. Because of their various functions, cytokines help to accomplish the complex pathogenesis of ALD and could be important in the prevention or treatment of ALD.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author confirms that this chapter contents have no conflict of interest.

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CHAPTER 23

Noninvasive Assessment of Steatosis and Fibrosis in Alcoholic Liver Disease

Alina Popescu[1,*] and **Tudor Moga**[1]

[1] *Department of Gastroenterology and Hepatology, "Victor Babeş" University of Medicine and Pharmacy Timişoara, 156 Liviu Rebreanu Str., 300736 Timişoara, Romania*

Abstract: Alcohol-related liver disease (ALD) is the most frequent cause of severe chronic liver disease in Europe and worldwide. The diagnosis of ALD is usually suspected when there is the documentation of regular alcohol consumption of >20 g/day in females and >30 g/day in males and in the presence of clinical and/or biological abnormalities suggestive of liver injury. Non-invasive methods of evaluation in chronic liver diseases, including ALD, gain a lot of interest in the last years due to the large number of studies that have proven their usefulness and accuracy and due to the easy acceptability by patients, even that liver biopsy is still considered the gold standard method of evaluation. In ALD non-invasive techniques are available for the evaluation both of steatosis and fibrosis, including biological tests, ultrasound, attenuation imaging, elastography. Most noninvasive techniques allow a prediction of steatosis and advanced liver fibrosis with good accuracy, allowing also the dynamic follow up in these patients.

Keywords: Alcohol-related liver disease, Biological tests, Liver elastography, Liver fibrosis, Liver steatosis, Noninvasive assessment.

INTRODUCTION

Worldwide, harmful use of alcohol is associated with more than 3 milion deaths every year [1], with impact on over 200 diseases and types of injuries, the liver being one of the most important targets.

Alcohol-related liver disease (ALD) is the most frequent cause of severe chronic liver disease in Europe and worldwide [2]. The spectrum of alcohol-induced liver pathology is wide. ALD can progress from alcoholic fatty liver to alcoholic steatohepatitis, which is characterized by hepatic inflammation, to alcoholic liver

* **Corresponding author Alina Popescu:** Department of Gastroenterology and Hepatology, "Victor Babeş" University of Medicine and Pharmacy Timişoara, 156 Liviu Rebreanu Str., 300736 Timişoara, Romania, Tel: +4025 6488 003, Fax: +4025 6488 003, E-mail:alinamircea.popescu@gmail.com

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cirrhosis, with the risk of developing hepatocellular carcinoma as a complication. In addition, severe alcoholic hepatitis (with or without cirrhosis) is an acute liver injury associated with high risk of liver failure and mortality.

The diagnosis of ALD is usually suspected when there is the documentation of regular alcohol consumption of >20 g/day in females and >30 g/day in males and in the presence of clinical and/or biological abnormalities suggestive of liver injury [2].

Liver biopsy can distinguish between different stages of ALD based on the histopathological features, with macro- and microvesicular steatosis in alcoholic steatosis, hepatocellular injury with ballooning, necrosis and lobular inflammation in alcoholic steatohepatitis and the presence of severe fibrosis in alcoholic liver cirrhosis [3]. Even if liver biopsy has also the advantage of establishing the positive diagnosis of ALD or offering an alternate diagnosis when this is not the case, it is still an invasive procedure, with risk of complications and not so easily accepted by patients.

Non-invasive methods of evaluation in chronic liver diseases gain a lot of interest in the last years due to the large number of studies that have proven their usefulness and accuracy and due to the easy acceptability by patients. In ALD non-invasive techniques are available for the evaluation both of steatosis and fibrosis, the major role players in the prognosis of these patients.

Steatosis Evaluation

Liver steatosis is a central pathological element in ALD, identified as an independent prognostic factor for these patients [4]. It is estimated that up to 90% of heavy drinkers can have steatosis [5]. The non-invasive methods for steatosis evaluation in ALD include biological tests, ultrasonography, Controlled Attenuation Parameter (CAP) and Magnetic Resonance Imaging (MRI).

Biological tests for the diagnosis of *presence of steatosis* were mainly developed for evaluation of patients with non-alcoholic fatty liver disease. These tests include formulas based on simple parameters, such as Fatty Liver Index (FLI) or Hepatic Steatosis Index (HSI), but also patented formulas, such as SteatoTest [6], the later with the disadvantage of the cost. The advantages of these tests would be the large availability, acceptability by the patients, while the major disadvantage is that they lack large prospective studies on accuracy and effectiveness. Their utility may be more in rule in and rule out the presence of steatosis, and identify those patients that most need further investigations.

Ultrasound on the other hand proved its utility for screening and assessment of fatty liver [7]. It is also a non-invasive technique, widely available, well accepted by the patients and rather inexpensive that showed to have sensitivity ranging between 60–94% and specificity ranging between 88–95% in detecting steatosis [8 - 10], with better performance for severe steatosis as compared to mild forms. While ultrasound is a useful imaging technique for liver evaluation, that has also the advantage to demonstrate alternate abdominal disorders, the evaluation of steatosis is qualitative and subjective, based on the liver brightness, the gradient between the liver and the kidney parenchima echogenicity and the posterior attenuation of the ultrasound beams [11], the degree of attenuation allowing a subjective grading in mild, moderate and severe steatosis. It is also an operator dependent technique and can not give information related to the presence of fibrosis, with the exception of advanced liver cirrhosis.

Controlled Attenuation Parameter (CAP) is a new non-invasive tool that uses the same features as ultrasound for the assessment of liver steatosis, but has the advantage of being quantitative and objective. It is incorporated in the Fibroscan (Echosens, Paris, France) equipment, thus allowing in the same session the evaluation of steatosis by CAP and the evaluation of fibrosis by Transient Elastography. The technique proved to have good accuracy for diagnosis moderate and severe steatosis in studies and meta-analysis mainly in NAFLD and mixed cohorts [12, 13]. The technique was studied also in ALD [14], as compared to ultrasound and liver biopsy and showed for mild, moderate and severe steatosis AUROCs of 0.77, 0.78 and 0.82, respectively, and proved to be superior to ultrasound in diagnosing steatosis in ALD.

MRI using ***PDFF*** (proton density fat fraction), can also be used with good accuracy for liver fat quantification. Several studies have compared the accuracy of PDFF to CAP, all in favor of the MRI method [15, 16]. These studies showed an accuracy of approx. 90% for PDFF and 73% for CAP. A meta-analysis that included a total of 6 studies (n = 635) showed very good summary AUROC values of PDFF for differentiating steatosis grades 0 *vs.* 1-3, 0-1 *vs.* 2-3, and 0-2 *vs.* 3 (0.98, 0.91, and 0.90, respectively) [17]. However, the main disadvantages of this technique are the availability and the costs, and no specific studies in ALD patients are available until now.

Fibrosis Evaluation

Fibrosis evaluation is the landmark for assessment prognosis in chronic diffuse liver diseases. Liver biopsy is still considered the gold standard for fibrosis assessment, but it is continuously challenged by non-invasive tests, which are easier to use in clinical practice and are currently considered viable alternatives.

The use of biological tests and ultrasound based elastography were also studied in ALD patients.

Biological tests, as was mentioned previously, have the advantage that they are simple tests, using laboratory investigations, accessible to any doctor (especially those without a patent-protected formula), hence their wide applicability (>95%) [18], feasibility and good reproducibility [19]. They showed an acceptable diagnostic accuracy with AUROC> 0.8 (APRI: 0.82, BARD: 0.81, FIB-4: 0.80, NFS: 0.88, Fibrotest: 0.81-0.92) [20], but more importantly, have good negative predictive values for advanced fibrosis (APRI 95%, BARD 96%, FIB-4 90%, NFS 93%, Fibrotest 98%) [20]. Thus, their utility is not in discriminating between the different the degrees of fibrosis, but in excluding severe liver fibrosis.

In ALD patients a study performed by Thiele *et al*. [21] compared the accuracy of two patented biological tests, the Enhanced Liver Fibrosis Test (ELF), the FibroTest (FT), with liver stiffness measurements made by Transient Elastography (TE) and found that ELF and FT had comparable diagnostic accuracy for advanced fibrosis, with AUROCs of 0.92 and 0.90 respectively, higher as compared with simple non-patented tests like APRI or FIB4 (0.80 and 0.85 respectively), but lower as compare to TE (AUROC 0.97). However more importantly ELF values below 10.5 and FT values below 0.58 had very good negative predictive values for advanced liver fibrosis, of 98% and 94%, respectively, allowing to rule out severe disease. Other patented biomarkers, Fibrometer and Hepascore, show comparable accuracy with FT in patients with ALD [22, 23].

Ultrasound based elastography methods are by far the most studied non-invasive techniques for fibrosis assessment also in ALD patients.

These methods developed rapidly, due to applications in multiple liver pathologies, the fact that are fast methods, easily accepted by patients, which can be repeated, some of them implemented in ultrasound machines and also not very expensive [24, 25]. They are the most commonly used methods today for the noninvasive assessment of liver fibrosis. Compared to biological tests, ultrasound-based elastography techniques are more accurate in predicting liver fibrosis, allow discrimination between different degrees of fibrosis, but are more difficult to apply in obese patients and are influenced by higher values of transaminases, the presence of heart failure, biliary obstruction or possible post-feeding assessment [26].

Most of the studies performed in ALD patients used TE for liver fibrosis assessment (Table **1**), only few being performed with point share wave (pSWE) or 2D share wave (2D SWE) techniques. The number of patients included in these

studies were very small in some cases and the results showed a wide range of values for advanced fibrosis cut offs.

Table 1. Elastography in ALD patients.

-	Number of Patients	Elastography Method	Cut-off Values for Different Fibrosis Stage		
			F2	F3	F4
Anastasiou 2010 [27]	14 patients	TE	>7.15 kPa	-	>12.5 kPa
Bardou-Jacquet 2013 [28]	8 patients	TE	>7.15 kPa	-	>17 kPa
Boursier 2009 [29]	106 patients	TE	>7.15 kPa	>9.5 kPa	>12.5 kPa
Carl 2012 [30]	4 patients	TE	>7.15 kPa	-	>15.1 kPa
de Ledinghen 2012 [31]	34 patients	TE	>7.15 kPa	>9.5 kPa	>12.5 kPa
Dolman 2013 [32]	20 patients	TE	>7.15 kPa	>9.5 kPa	>12.5 kPa
Fernandez 2012 [33]	139 patients	TE	>7.15 kPa	>10.5 kPa	>15.7 kPa
Janssens 2010 [34]	49 patients	TE	>7.15 kPa	-	>19.6 kPa
Kim 2009 [35]	45 patients	TE	>7.15 kPa	>9.5 kPa	>12.5 kPa
Lannerstedt 2013 [36]	16 patients	TE	>7.15 kPa	>9.5 kPa	>12.5 kPa
Lemoine 2008 [37]	48 patients	TE	>7.15 kPa	-	>34.9 kPa
Mueller 2010 [38]	101 patients	TE	>7.15 kPa	>8 kPa	>12.5 kPa
Nahon 2008 [39]	147 patients	TE	>7.15 kPa	-	>22.7 kPa
Nguyen-Khac 2008 [40]	103 patients	TE	>7.15 kPa	>11 kPa	>19.5 kPa
Muller 2014 [41]	364 patients	TE	>6 kPa	>8 kPa	>12.5 kPa
Thiele 2016 [42]	199 patients	2D-SWE	>10.2 kPa	-	>16.4 kPa
Kiani 2016 [43]	69 patients	pSWE	>1.63 m/s	>1.84 m/s	>1.94m/s
Zhang 2015 [44]	112 patients	pSWE	>1.27m/s	>1.40 m/s	>1.65 m/s
Voican 2017 [45]	188 patients	TE	-	>13 kPa	>20.8 kPa
Cho Y 2020 [46]	251 patients	pSWE	>1.46 m/s	>1.47 m/s	>1.66 m/s

2D-SWE, 2D shear wave elastography (Aixplorer, Supersonic Imagine); pSWE, point-shear wave elastography; TE, transient elastography (FibroScan, Echosens); Mueller *et al*. [41] demonstrated that this was related to the presence of inflammation as assessed by aminotransferase levels, and liver stiffness decreases in these patients during alcohol withdrawal.

In a systematic review and meta-analysis by Pavlov *et al*. (Cochrane review) [47] that aimed the assessment of diagnostic utility of TE in ALD, 5 retrospective and 9 prospective cohort studies with 834 participants were reviewed. The authors

could not identify the optimal cut-off values for the fibrosis stages, TE may rule out cirrhosis with a negative likelihood ratio of 0.07 when liver stiffness is below 12.5 kPa.

A more recent individual patient data meta-analysis [48], ten liver biopsy proven studies with a pool of 1026 patients that used TE for liver fibrosis assessment were included. The authors showed good accuracy of TE especially for diagnosing advanced fibrosis, with the following liver stiffness cutoffs: F≥1: 7.0 kPa (AUROC 0.83 [SE 0.02; 95% CI 0.79-0.87]), F≥2: 9.0 kPa (0.86 [0.02; 0.82-0.90]), F≥3: 12.1 kPa (0.90 [0.02; 0.86-0.94]), and F=4: 18.6 kPa (0.91 [0.04; 0.83-0.99]). Higher levels of AST and bilirubin had a significant effect, increasing the liver stiffness values ($p<0{\cdot}0001$), with significantly higher cut-off values for diagnosis significant and severe fibrosis.

Even that there are still unknown issues regarding the place of ultrasound based elastography in ALD, Share Wave Elastography can be used in these patients to rule out advanced disease [24, 25].

Magnetic Resonance Elastography (MR-E), is a technique that interrogates the whole liver and is not limited to a defined target volume, is less dependent operator compared to ultrasound-based techniques, but more expensive and which had reported in meta-analyzes diagnostic accuracy of 93–98% for advanced liver fibrosis (F≥3), with sensitivities of 85–92% and specificities of 85–96% [49, 50], was much less investigated in ALD. Only one study [51] that included 90 alcoholic patients focused on MR-E and used TE as reference method, showing the following MRE cut-off values for different stages on fibrosis F1: 2.20 kPa, F2: 2.57 kPa, F3:3.31 kPa, and F4: 4 kPa.

CONCLUSION

Most noninvasive techniques allow a prediction of steatosis and advanced liver fibrosis with good accuracy in ALD patients, allowing the dynamic identification and evaluation of these patients, being much more easily accepted as methods of investigation as compared to liver biopsy.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author confirms that this chapter contents have no conflict of interest.

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CHAPTER 24

Can we Stop Nucleos(t)ide Analogs in HBV Chronic Hepatitis?

Roxana Şirli[1,*]

[1] *Department of Gastroenterology and Hepatology, "Victor Babeş" University of Medicine and Pharmacy, 156, Liviu Rebreanu Bv, 300723, Timişoara, Romania*

Abstract: Chronic infection with Hepatitis B Virus (HBV) is a public health problem, since more than 240 million people are infected worldwide. Not all of them require antiviral treatment, but only those with chronic hepatitis, either HBeAg positive or HBeAg negative.

Complete cure of HBV infection is impossible due to the persistence of covalently closed circular DNA (cccDNA) integrated into the hosts' liver cells. An ideal end-point is the functional cure: HBsAg loss with or without HBs seroconversion, which is also rather hard to achieve, especially after nucleos(t)ide analogs (NA) treatment. Thus, the main endpoint of all current treatment strategies is long-term suppression of HBV DNA levels. All NA therapies have a potent inhibition effect on HBV replication. The problem is that after NA cessation the viral replication restarts.

The only firm indication to stop NA therapy is HBsAg loss, preferably with seroconversion to anti-HBsAb. In HBeAg positive non-cirrhotic patients, NA therapy can be stopped if HBeAg seroconversion and HBV DNA undetectability are achieved, but only after 12 months of consolidation therapy. In HBeAg negative chronic hepatitis, life-long NA long-term treatment is recommended. However, published data showed that viral relapse following NA cessation in these patients can trigger an immune response that would lead to a durable remission. In HBeAg-negative patients, treatment discontinuation can be considered after more than 3 years of on-treatment undetectable HBV DNA and only if close monitoring is possible. NA treatment should be continued indefinitely in cirrhotic patients.

Keywords: HBeAg seroconversion, HBsAg loss, Hepatitis B virus, Nucleos(t)ide analogs, Stop treatment, Virologic response.

[*] **Corresponding author Roxana Şirli:** Department of Gastroenterology and Hepatology, "Victor Babeş" University of Medicine and Pharmacy, 156, Liviu Rebreanu Bv, 300723, Timişoara, Romania; Tel: +40256488003, Fax: +40256488003; E-mail: roxanasirli@gmail.com

Ioan Sporea and Alina Popescu (Eds.)

INTRODUCTION

Chronic infection with Hepatitis B Virus (HBV) is a public health problem considering that more than 240 million people around the world are currently infected with this virus [1]. However, the prevalence is not the same, varying from low (< 2%) in Western Europe for instance, to high endemic areas (>8%) such as in South Eastern Asia and Africa [2]. In the last few years, the prevalence in high endemic areas seems to decline due to vaccination and efficient treatment [3], while in well-developed countries, with historical low endemicity, a small increase was observed, mainly due to migration from high endemic areas [4].

Not all patients chronically infected with HBV (HBsAg persistent for more than 6 months) require antiviral treatment. According to the latest EASL Guidelines, chronic HBV infection can be divided into 5 phases according to the HBeAg status, HBV DNA level, cytolysis and presence of liver lesions, as shown in Table **1**. Those 5 phases are not necessarily successive and repetitive assessment of HBeAg, HBV DNA and ALT levels is needed in order to categorize patients and assess their need for treatment. The fifth phase, the "occult HBV infection" is characterized by the absence of HBsAg in the serum, with anti-HBcAb positive and anti-HBsAb positive or negative, most often with undetectable serum HBV DNA but with cccDNA detectable in the liver [1, 5].

Table 1. The new classification of Chronic HBV infection. Adapted from the EASL Guidelines on the management of hepatitis B infection [1].

Chronic HBV Infection	**HBeAg Positive**		**HBeAg Negative**		**-**
	Phase 1	**Phase 2**	**Phase 3**	**Phase 4**	**Phase 5**
	Chronic Infection	**Chronic Hepatitis**	**Chronic Infection**	**Chronic Hepatitis**	**Resolved HBV Infection**
HBsAg	High	High/Intermediate	Low	Intermediate	Negative
HBeAg	Positive	Positive	Negative	Negative	Negative
HBV DNA	$>10^7$ IU/ml	10^4 - 10^7 IU/ml	< 2000 IU/ml	> 2000 IU/ml	<10 IU/m
ALT	Normal	Elevated	Normal	Elevated	Normal
Liver disease	None/Minimal	Moderate/Severe	None	Moderate/Severe	None
Old terminology	Immune tolerant phase	Immune reactive HBeAg positive	Inactive carrier	HBeAg negative chronic hepatitis	HBsAg negative /anti-HBc positive

Treatment Goals and Who Should be Treated

Complete cure of HBV infection is impossible to achieve due to the persistence of covalently closed circular DNA (cccDNA) integrated into the hosts' liver cells. The treatment goal in chronic HBV hepatitis is to improve survival and quality of life by preventing disease progression and the development of hepatocellular carcinoma (HCC) [1]. To reach this goal, the ideal end-point is the functional cure: HBsAg loss with or without HBs seroconversion (apparition of anti-HBsAb), which has proven to further reduce the risk of HCC in patients with viral suppression under NA therapy [6].

HBsAg loss is unfortunately rather hard to achieve, especially after NA therapy [1, 7, 8]. Thus, the main endpoint of all current treatment strategies in chronic HBV hepatitis is long-term suppression of HBV DNA levels. On-treatment virologic response is defined for nucleos(t)ide analogs (NA) treatment as undetectable HBV DNA in the serum by a sensitive assay. For PegInterferon (PegIFN) treatment, the virologic response is defined as HBV DNA <2000 IU/ml 6 months following PegIFN discontinuation, while sustained virologic response as HBV DNA <2000 IU/ml for at least 12 months following discontinuation [1].

In HBeAg-positive chronic hepatitis, it is considered that HBeAg loss with or without seroconversion to anti-HBeAb achieves immune control and thus is considered a treatment end-point [1, 7, 8]. Normal ALT levels (biochemical response) are generally obtained in patients with efficient long-time suppression of HBV replication.

As mentioned before, not all patients chronically infected with HBV need treatment. Only those with chronic hepatitis, either HBeAg-positive or HBeAg-negative should be treated (phase 2 and 4 from the new EASL classification) [1]. Cirrhotic patients should be treated by NA regardless of the HBV DNA level if detectable.

Treatment Options

Regarding treatment options, two strategies are available:

Interferon Based Therapy

Treatment with Interferon alpha (IFN-α) became available in 1992, followed in 2005 by Pegylated Interferon (PegIFN). It is a difficult treatment, with parenteral administration, with numerous side effects and contraindications, and should be considered as a first line therapy only in patients with mild or moderate liver disease as well as in selected cases with compensated cirrhosis [1, 7, 8]. It is a

finite therapy, with rates of HBeAg loss of 20-30%, and viral suppression (HBV DNA < 2000 IU/ml) in approximately 23% of HBeAg-positive patients, and 20-44% in HBeAg-negative patients (less in genotype D and E patients) [1]. Regarding HBsAg loss, it occurs in 3-9% of patients 6 months after finishing treatment, but it increases progressively, reaching 30% in those with virologic response after 5 years follow-up, both in HBeAg-positive and negative patients [1, 5].

Nucleot(s)ide Analogs (NA)

All NA therapies have a potent inhibition effect on HBV replication. The problem is that after NA cessation the viral replication restarts in most cases. The first NA was Lamivudine, followed by Adefovir and Telbivudine, but they were abandoned due to the high rate of resistance developed over time. Currently, Entecavir (ETV), Tenofovir Disoproxil Fumarate (TDF) and Tenofovir Alafenamid (TAF), antivirals with a high barrier to resistance, are the recommended first line monotherapy in most chronic hepatitis HBV patients, cirrhotic or non-cirrhotic, including those with decompensated cirrhosis [1, 7, 8]. ETV, TDF and TAF are easy to use (one tablet/day in oral administration) with very few side effects: minimal renal function decline during long-term therapy with ETV and TDF, higher for TDF; bone density loss for TDF. However, at least in HBeAg-negative patients, the recommended treatment duration is indefinite [1, 7, 8].

Regarding efficiency, it was demonstrated that long term ETV or TDF therapy stopped the progression of fibrosis, resulting in significant improvement of necroinflammation and fibrosis, and decreased risk of HCC [1, 5, 9]. Furthermore, viral suppression in decompensated HBV cirrhosis leads to a marked improvement of liver function, leading to an important decrease in the need for transplantation [1].

In HBeAg-positive chronic hepatitis, after 5 years of ETV treatment, HBeAg loss was achieved in 53% of patients [10], while after 5 years of TDV treatment it was observed in 49% of cases [11]. The cumulative on-treatment virologic response (undetectable HBV DNA by a sensitive assay) was observed in 97 – 99% cases in HBe-positive patients [10, 11] and in 90 – 93% of HBe-negative ones [11, 12].

HBsAg loss following long-term NA therapy (5-8 years) occurs in 10-12% of patients with HBeAg-positive chronic hepatitis and is rare in HBeAg-negative patients (1–2%) [1, 13, 14].

When to Stop NA Treatment?

The only firm indication to stop NA therapy in chronic HBV hepatitis is HBsAg loss, preferably with seroconversion to anti-HBsAb. International guidelines recommend that treatment should be discontinued only after at least 6 months of consolidation treatment [1, 7, 8].

HBeAg-positive Chronic Hepatitis

Widely accepted stopping rule of NA treatment, applicable only in non-cirrhotic patients is if HBeAg seroconversion and HBV DNA un-detectability are achieved, but only after 12 months of consolidation therapy (EASL and AASLD guidelines) and after at least 12 months, preferably 36 months, according to the APASLD guidelines [1, 7, 8]. According to published data, HBeAg seroconversion will be maintained in most patients (~90%), while virologic remission (HBV DNA <2,000 – 20,000 IU/ml) will be maintained in ~ 50% of them, 3 years after NAs cessation [15]. However, the decision to stop treatment or to continue until HBsAg loss is left to the physician's discretion [1, 8]. Regarding the relationship between response, durability and duration of consolidation therapy, there is conflicting data. In a study published in 2013, in HBeAg-positive patients treated with Lamivudine, it was shown that the rates of maintained response were 26%, 39% and 71% in patients with consolidation therapy of <12 months, 12-18 months and >18 months, respectively [16]. In a more recent study, this time in patients treated with TDF, the relapse rates were similar in patients with 12 *vs*. 18 months consolidation therapy (56.5% *vs*. 52.2%) [17]. A predictor of maintained response was HBeAg decline > 25% after 6 months of treatment as compared to baseline [17]. Another predictor of response durability is the age at cessation of NA treatment. A study published in 2012 on patients treated with NA, showed that age younger than 40 years and consolidation therapy longer than 15 months were independently associated with lower relapse rates [18]. Thus, a lower relapse rate was observed in the group of patients younger than 40 with consolidation therapy longer than 15 months. Nevertheless, all guidelines agree that a consolidation therapy of at least 12 months is needed after HBeAg loss. Because relapse occurs most often in the first year after NA cessation, a close monitoring is recommended (recurrent viremia, ALT flares, seroreversion) every three months for at least 12 months [1, 7, 8].

HBeAg-negative Chronic Hepatitis

In HBeAg-negative chronic hepatitis, NA long-term treatment, (maybe life-long) is recommended, and there is no stopping rule, unless HBsAg seroconversion is achieved, due to the high relapse rate and the potential of a severe acute hepatitis flare after cessation [1, 7, 8]. However, published data showed that virologic

remission (HBV DNA < 2,000 – 20,000 IU/ml) was maintained in approximately 30% of HBeAg-negative patients treated with NA who had at least 2 years on-treatment undetectable HBV DNA, 3 years after NAs cessation [19]. These observations lead to the idea of trying to shorten the treatment, but not in patients with cirrhosis, in whom overt hepatitis flares and life-threatening episodes can occur, even if in a small number of cases [19 - 21].

Considering the high efficacy of NA treatment in HBeAg-negative chronic hepatitis, ***why stop it?*** First of all, it is a problem of compliance and costs. Considering that nowadays most patients are diagnosed at a young age, the high number of patients (more than 240 million patients chronically infected with HBV worldwide) [1] long-life treatment is difficult, implying high costs for the society. Secondly, even if modern NA have a very good safety profile, side effects still exist, especially regarding TDF. A slow decrease of glomerular filtration rate and bone density was observed after 96 weeks in TDF treated patients, significantly higher than in TAF treated patients [22]. This is why ETV or TAF should be preferred in patients with bone disease (chronic steroid use, osteoporosis, history of fragility fracture) as well as in those with or at risk for renal disease (diabetes, eGFR <60 ml/min/1.73 m^2, albuminuria >30 mg/24 h, hemodialysis) [1]. Thirdly, there are family planning issues in patients of reproductive age.

Last, but not least, probably the most important motive, is the possibility to induce immune clearance of HBV. Following NA cessation, an HBV DNA increase is almost universal, but its intensity varies from only a slight increase to a marked increase, the latter associated with ALT flare. Viral relapse is a prerequisite to induce an immune response that would lead to a durable remission of HBV infection, either as an undetectable HBV DNA, or ideally with HBsAg loss (functional cure) [23]. After a lag period of 1-12 months following NA cessation, the reactivation phase of HBV of approximately three months occurs, followed by a consolidation phase of approximately one year [23]. After the reactivation phase, several long-term outcomes are possible. The best outcome is the functional cure – HBsAg loss – which can occur in up to 20% of cases followed up for 2-3 years [23]. Another favorable outcome is durable undetectable HBV DNA, with or without HBsAg decline, which occurs in 20-30% cases [23]. Approximately 40% of patients experience a relapse of chronic hepatitis, with persistently increased ALT and HBV DNA, requiring retreatment, and finally 10-20% of cases do not fulfill the criteria to resume NA treatment [23].

After the review article of Papatheodoris [19] who pooled the results of small, individual studies, several prospective studies were performed trying to evaluate the benefits and risks of NA discontinuation in HBeAg-negative patients. The FINITE study included 42 non-cirrhotic HBeAg-negative patients, treated for

more than 4 years with TDF, with undetectable HBV DNA for more than 3.5 years, randomly assigned to either stop (n = 21) or continue (n = 21) TDF monotherapy [24]. At week 144 following TDF cessation, 62% of patients were still of treatment (HBV DNA < 2000 IU/mL and normal ALT), 19% [4] had HBsAg loss, 3 of them with seroconversion to anti-HBsAb.

The DARING-B study included 57 non-cirrhotic HBeAg-negative patients, treated for more than 4 years with ETV or TDF, with undetectable HBV DNA for more than 3.5 years who stopped treatment [25]. The rate of HBsAg loss at 6, 12 and 18 months after cessation of NA therapy were 5%, 16% and 25%, respectively. Virologic relapse (HBV DNA > 2000 IU/ml) occurred in 56%, 70% and 72% of patients at 3, 12 and 18 months following cessation. The probability of HBsAg loss was unaffected by patients' age, gender, fibrosis severity, nor by the pretreatment HBV DNA and ALT levels. However, probability of HBsAg loss was significantly higher in patients with low HBsAg levels at the end of treatment (HR per 100 IU/l - 1.35) and one month following cessation (HR per 100 IU/l - 1.28), as well as in patients who experienced high ALT levels one month following cessation (HR per 10 IU/l - 1.12) [25]. In another study, age younger than 55 and HBsAg < 150 IU/ml at NA cessation were predictors of HBsAg loss [26].

Which are the predictors of maintained response following NA treatment cessation in HBeAg-negative patients? As shown before, age, and most importantly, HBsAg levels at the end of treatment are the main predictors of maintained response. The question is which cut-off of HBsAg to use. In a Taiwanese study, HBsAg <100 IU/mL was the strongest predictor of response [21]. In the Hadziyannis study, all patients who had HBsAg <1000 IU/mL had a sustained virologic response and/or HBsAg loss [27]. In the FINITE study, an HBsAg cutoff of 25,000 IU/mL separated those with a better chance of HBsAg loss [24]. In a very recent study published at the 2020 AASLD Meeting, 1509 HBV patients (70.5% of them HBeAg-negative) from 12 centers in Europe, North America and Asia were included [28]. All of them were followed up for 4 years following NA discontinuation after at least three years of on-treatment undetectable HBV DNA. The proportion of patients with HBsAg loss increased from 3% at 1 year after NA cessation, to 8% after 2 years, 12% after 3 years, and 14% after 4 years. The rate of relapse raised from 30% one year after NA discontinuation to 43% at 2 years, 50% at 3 years, and 56% at 4 years. The rate of HBsAg loss was significantly higher in Caucasians *.vs* Asians (HR 5.8, 95% CI 3.6 to 9.5, P < 0.001). Also, the rate of need for the retreatment was significantly higher in patients over 50 years than in those younger than 50 (HR 1.6, 95% CI 1.3 to 1.9, P < 0.001) [28].

New Predictors of Sustained Response After NA Treatment Cessation in Chronic HBV Hepatitis

Hepatitis B core-related antigen (HBcrAg) is one of the many viral proteins synthesized following viral entry into the hepatocyte. It consists of three viral proteins, which have in common an identical 149 amino acid sequence, namely the HBV core antigen (HBcAg), the HBeAg and a truncated 22 kDa precore protein (p22Cr) [29]. HBcrAg levels have been proven to be associated with the risk of HCC, both in naïve and treated patients [30], as well as with reactivation risk after immunosuppressive therapy in occult HBV infection [31].

Regarding the risk of relapse following NA cessation in patients with HBeAg-negative chronic hepatitis, HBcrAg and HBsAg were independently associated with the risk of relapse. A score including HBcrAg, HBsAg, age and ALT had high accuracy in predicting relapse (0.85-0.90), 1-5 years following cessation of TDF [32]. In another study, an end-of-therapy HBcrAg > 3.7 log IU/mL led to a 3.7-fold higher risk of virologic relapse one year after NA cessation [33].

Pre Genomic HBV RNA

Earlier concepts did not acknowledge the presence of HBV RNA outside the hepatocytes. Recent studies demonstrated the presence of serum HBV RNA in HBV infected patients as full-length pregenomic RNA (pgRNA) encapsidated by HBc protein [29]. In a cohort of 33 patients treated for more than 3 years with NA with undetectable serum HBV DNA, in whom the treatment was stopped, 21 had detectable HBV RNA at end of treatment. The viral rebound occurred in all of them (100%), as compared to 25% of those with undetectable HBV RNA [34]. In a very recent study, it was demonstrated that only patients who had severe alanine transaminase flares had HBcrAg and HBV RNA present at the moment of NA treatment cessation and had a high risk of viral relapse [35].

Which are the Risks of NA Treatment Cessation?

As said before, al HBeAg-negative chronic hepatitis patients who stop NA treatment experience some level of HBV DNA increase, which, in some cases, can lead to the immune control of HBV infection. Even if small, there is always the risk of severe ALT flares (ALT > 10x upper limit of normal) and decompensation [19, 24, 27]. This is why NA treatment should not be discontinued in cirrhotic patients, the risk of death due to hepatic failure being significantly higher in this category of patients. In order to avoid these possible severe adverse effects, close monitoring is needed: every 2 weeks in the first 3 months, then every 4 weeks for the following 9 months, and subsequently every 12 weeks [24]. If a viral relapse is observed, treatment should be reinstated,

generally with no impact on regaining viral control, but with decreased chance to achieve HBsAg loss [21, 23].

Considering that a viral relapse can induce both immune control of chronic HBV hepatitis, but it can also lead to severe ALT flare and decompensation that require treatment reinstatement, which reduces the chance of HBsAg loss, the question is if and when to recommence NA treatment. In a presentation at the 12th Paris Hepatology Conference [available at https://www.aphc.info/wp-content/uploads/2019/01/pre49-mangia-alessandra.pdf], Alessandra Mangia recommended that NA therapy should be resumed immediately if direct bilirubin increases with more than 1 mg from baseline, or if total bilirubin is higher than 3 mg% in two consecutive assessments, or if INR >1.3, or if clinical decompensation occurs, regardless of HBV DNA and ALT levels. Another indication for retreatment is HBV DNA > 10.000 IU/ml associated with ALT > 1000 IU/l, suggestive of a severe flare. In patients with HBV DNA > 10.000 IU/ml associated with persistent ALT > 300 IU/l more than 4 weeks, or with persistent ALT > 150 IU/l more than 12 weeks, treatment should also be resumed.

CONCLUSIONS

NA treatment should be continued indefinitely in cirrhotic patients. NA treatment can be safely discontinued in patients with HBsAg loss, with or without seroconversion to anti-HBsAb, as well as in HBeAg-positive patients who achieved HBeAg loss, with or without seroconversion to anti-HBeAb, but only after at least 12 months of consolidation therapy. In HBeAg-negative patients treatment discontinuation can be considered only after more than 3 years of on-treatment undetectable HBV DNA and only if close monitoring is possible.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this chapter contents have no conflict of interest.

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CHAPTER 25

Hepatitis C Virus and Chronic Kidney Disease – What is New?

Cătălina Mihai[1,*] and **Cristina Cijevschi Prelipcean**[1]

[1] *"Grigore T. Popa" University of Medicine and Pharmacy, "Sf. Spiridon" Emergency Hospital, Institute of Gastroenterology and Hepatology, Iasi, Romania*

Abstract: It is a close, bidirectional relationship between hepatic C virus (HCV) infection and chronic kidney disease (CKD). On one hand, HVC patients have an increased risk of CKD, the most frequent form being cryoglobulin-immune-mediated glomerulonephritis. On the other hand, CKD patients, especially those in dialysis units, have an increased risk of HCV infection, with an increased cardiovascular and all-cause mortality. Direct acting antiviral agents (DAA) has revolutionized the treatment of HCV, including patients with CKD, dialysis, and kidney transplantation (KT). Patients with CKD stage 1-3b can be treated with any DAA approved regimen. In patients with CKD stages 4-5, including hemodialysis patients, there are three regimens approved: glecaprevir/pibrentasvir, elbasvir/grazoprevir and paritaprevir/ritonavir/ ombitasvir/dasabuvir. However, more recently, there are many pieces of evidence that, in spite of initial recommendations, Sofosvubir-based regimens can be safe and effective in patients with end-stage CKD. Many DAA regimens demonstrated very good results (sustained viral response – 98-100%) and very well tolerability in KT recipients, the main concern being drug-drug interaction between DAA and immunosuppressive therapy. One of the major challenges of the last years is the possibility to transplant an HCV- positive kidney in an HCV-negative recipient, with DAA treatment following transplantation, with the increase of the organ supply and the avoidance of long term dialysis complications. With preventive measures in dialysis units and DAA treatment in all categories of patients, the elimination of HCV infection in CKD patients can be a realistic goal.

Keywords: Chronic kidney disease, Direct acting antiviral agents, Hemodialysis, Hepatitis C virus, Kidney transplantation, Sustained viral response.

INTRODUCTION

Hepatitis C virus (HCV) infection is a major problem of public health. More than 71 million people (global prevalence 1%) are infected with HCV worldwide, with

[*] **Corresponding author Catalina Mihai:** "Grigore T. Popa" University of Medicine and Pharmacy, "Sf. Spiridon" Emergency Hospital, Institute of Gastroenterology and Hepatology, Iasi, Romania; Tel: +40745258797;, E-mail: catalinamihai@yahoo.com

Ioan Sporea and Alina Popescu (Eds.)

an increased risk of morbidity and mortality [1]. Liver cirrhosis and hepatocellular carcinoma are the main liver-related complications, but, at the same time, there are a lot of extra-hepatic manifestations linked to HCV infection: type 2 diabetes mellitus, lichen planus, porphyria cutanea tarda, non-Hodgkin's lymphoma, cardiovascular and renal diseases [2].

Chronic kidney disease (CKD) has an estimated worldwide prevalence between 8% and 16% [3]. It is classified by Kidney Disease: Improving Global Outcomes (KDIGO), based on cause, albuminuria (A1-3) and glomerular filtration rate (GFR): G1-G5. (Table **1**) [4].

Table 1. Current KDIGO classification of CKD based on GFR (mL/min/1.73 m^2) [4].

G1	**Normal or high**	**≥ 90**
G2	Mildly decreased	60-89
G3a	Mildly to moderately decreased	45-59
G3b	Moderately to severely decreased	30-44
G4	Severely decreased	15-29
G5	Kidney failure	< 15

Patients with end-stage kidney disease (ESKD) have a GFR < 15 mL/min/1.73 m^2, requiring hemodialysis or peritoneal dialysis.

It is well-known that between HCV infection and CKD it is a bidirectional, close relationship: it has an increased prevalence of HCV in CKD patients and, at the same time, increased proteinuria and CKD in HCV-infected patients. More than that in CKD – HCV positive patients, there is an increased risk for cardiovascular and all-cause mortality [5]. In the last years, direct acting antiviral agents (DAAs) have revolutionized the treatment of HCV, including those with CKD, dialysis, and kidney transplantation (KT).

Renal Impairment in HCV Patients

HCV infection is associated with microalbuminuria and the development of CKD. The main risk factors for renal involvement are: age < 50 years, male sex, diabetes, hypertension, hyperlipidemia, and liver cirrhosis [6]. The most frequent form of renal involvement is membranoproliferative glomerulonephritis, although tubulointerstitial injury also appears. There are two major mechanisms for HCV – related glomerulopathy: cryoglobulin immune-mediated tissue damage and the direct cytotoxic effect of the virus. The major glomerular diseases associated with HCV infection are: mixed cryoglobulinemia syndrome, membranous nephropathy, and polyarteritis nodosa. Mixed cryoglobulinemia is a small vessel vasculitis leading to immune complex deposition in many organs: skin, joints,

nerves, liver, and kidneys [6]. Renal involvement is reported in 20-35% of HCV-cryoglobulinemia patients with variable clinical presentations: proteinuria, hematuria, nephritic or nephrotic syndrome, acute or chronic renal failure [7]. Achievement of sustained viral response (SVR) is associated with remission of hematuria, proteinuria, decrease of cryoglobulin levels, and improvement of GFR [8]. As cryoglobulins persist even after successfully HCV eradication in patients with nephritic syndrome and progressive kidney failure, immune-suppressive therapy with rituximab with/without plasma exchange is recommended. Rituximab should be considered as first-line therapy before DAA in patients with rapidly progressive kidney failure, acute cryoglobulinemic flare, or nephrotic syndrome [9].

Implications of HCV Infection in CKD Patients

Prevalence of HCV in CKD

The prevalence of HCV infection in persons with CKD, particularly in dialysis units, is higher compared with the general population, with a supplementary risk of nosocomial transmission during hemodialysis. According to the Dialysis Outcomes and Practice Patterns Study, the medium HCV prevalence in dialysis patients is 13.5%, ranging from 2.6% to 22.9% [10]. In Romania, in 2015, the prevalence of HCV infection in the hemodialysis population was 27.3% [11]. KDIGO recommends HCV infection screening (using an immunoassay – anti - HCV antibodies, followed by HCV-RNA if the immunoassay is positive) at the time of initial evaluation of CKD, before initiation of dialysis and at the time of evaluation for KT. In dialysis centers, screening for HCV infection will be done every 6 months (more often if a new HCV infection is reported!) [4].

Several routes of transmission can explain the higher HCV prevalence in CKD patients: frequent healthcare procedures, blood transfusion, and shared use of dialysis equipment. According to KDIGO, the main preventive "hygienic precautions" for HCV transmission are: proper hand hygiene and glove changes, proper injectable medication preparation and administration practices, proper cleaning, disinfection of surfaces at the dialysis station, and adequate separation of clean supplies from contaminated materials [4]. It is considered that the isolation of chronic hemodialysis, HCV positive patients, and dedicated machines has no benefit for preventing infection transmission in the absence of good infection control practices [12]. In the last years, HCV prevalence has declined due to routine screening, follow-up and implementation of infection control practices in dialysis patients; however, the prevalence of HCV infection still remains higher in dialysis patients compared with the general population [13].

HCV Infection and CKD Outcome

HCV infection is associated with an accelerated progression of CKD to ESKD, requiring hemodialysis and KT. The presence of comorbidities (diabetes mellitus, hypertension) enhances progression to ESKD in HCV patients. It is an increased mortality in HCV – dialysis patients, related both with liver and cardiovascular diseases. A meta-analysis that included 23 studies with 574.081 patients on long-term dialysis, has demonstrated an association between HCV positive serologic status and increased risk of either liver or cardiovascular disease-related mortality among dialysis patients [14]. After KT, patients infected with HCV have an increased risk of graft loss and mortality [15].

Particularities of Liver Disease in HCV – CKD Patients

Interestingly, there are some studies suggesting that HCV patients with ESKD on hemodialysis have lower inflammation and fibrosis scores, comparing with those without CKD. The possible explanations are the passage of viral particles or the production of antiviral cytokines during the dialysis, or a weaker immune response that reduces HCV liver injury [16]. More than that, KT, in spite of immunosuppressive treatment, doesn't accelerate HCV related liver injury [17].

The evaluation of liver fibrosis in HCV patients with CKD is often challenging. On the one hand, biological evaluation (Fibrotest, APRI, FIB-4) tends to underestimate liver fibrosis, because of the attenuation of ALT and AST elevation in the presence of renal failure. Hemodialysis also induces a reduction in ApoA1, haptoglobin and bilirubin levels; these changes could alter the estimation of the fibrosis level indicated by FibroTest [18]. On the other hand, transient elastography (FibroScan) tends to overestimate the stage of liver fibrosis due to fluid overload. Liu *et al*. demonstrated that the area under the ROC curve is higher in hemodialysis patients evaluated by transient elastography compared with non- uremic patients in predicting fibrosis stage F2 and F3 [19].

DAA Treatment Particularities in CKD Patients

The goals of HCV therapy are: to cure the infection, to prevent liver – related and extra-hepatic complications, to improve quality of life, and to prevent virus transmission. Treatment of HCV infection with SVR achievement proved a survival benefit and a decrease in complications in all patients, including those with CKD. World Health Organization aims to achieve a 90% decrease in HCV infection incidence and a 65% decrease in HCV mortality by 2030. *Interferon – based with or without ribavirin therapy* had limited use in CKD patients due to low efficacy, severe side effects, anemia, and graft rejection in KT patients. DAAs, with efficacy rates exceeding 95% and an excellent safety profile, have

revolutionized HCV treatment, making HCV cure even in CKD patients a realistic goal.

Patients with CKD stage 1-3b (GFR > 30 mL/min/1.73m^2) can be treated with any DAA approved regimen. Protease inhibitors and NS5A inhibitors are metabolized mainly in the liver, so these can be used in CKD stage 4-5 and hemodialysis patients. There are three regimens used in patients with CKD G4-5: glecaprevir/pibrentasvir, elbasvir/grazoprevir and paritaprevir/ritonavir/ ombitasvir/dasabuvir (also known as PrOD or 3D regimen). EXPEDITION-4, a phase III trial in patients with stage 4 or 5 CKD treated with the combination of glecaprevir and pibrentasvir for 12 weeks, showed a SVR rate of 98% [20]. In the C-SURFER trial, 55 patients infected with HCV genotype 1b with stage 4 or 5 CKD, including 75% on haemodialysis, were treated with grazoprevir and elbasvir for 12 weeks. The SVR12 rate was 92% [21]. RUBY studies demonstrated the efficacy of PrOD regimen with or without ribavirin in genotype 1 HCV patients with stage 4-5 CKD, including dialysis patients [22]. Data from clinical studies was confirmed in many other real life settings.

Sofosvubir is eliminated mainly by the renal route, so the safety of sofosbuvir-based regimens has been questioned in patients with severe renal dysfunction (G4, G5, ESKD). More recently, there are many pieces of evidence that, in spite of initial recommendations, Sofosvubir can be safe and effective in patients with ESKD. In Li meta-analysis, 717 patients with CKD stage 4/5 (58.4% on dialysis) treated with sofosbuvir regimens across 21 studies had SVR 12/24 of 97%; serious adverse events were present in only 4.8% of the patients [23]. In November 2019, the US Food and Drug Administration approved the use of Sofosvubir in patients with GFR < 30 mL/min and dialysis population. As the HCV therapeutic recommendations in ESKD patients include protease inhibitors (grazoprevir, glecaprevir), drugs which are contraindicated in decompensated liver cirrhosis, the sofosbuvir - and velpatasvir-based therapy could be a solution for patients with advanced CKD and decompensated cirrhosis [24].

The choice of a therapeutic regimen in HCV patients with CKD depends on the viral genotype, severity of hepatic and kidney disease, comorbidities, and availability. There is no need for dose adjustment of HCV DAAs in patients with CKD, including ESLD. The recommendations of HCV treatment in CKD patients, according to the current guidelines, are resumed in Table **2** [4, 25, 26].

Table 2. Treatment recommendations in HCV patients with CKD [4, 25, 26].

CKD Stage	**HCV Treatment**		
G1–G3b	Any licensed direct-acting antiviral (DAA)-based regimen		
G4-5,	No cirrhosis/Compensated	**Glecaprevir/pibrentasvir** -all	Alternate regimens
including	Child-Pugh A cirrhosis	genotypes	***PrOD*** – genotype 1b (*add
hemodialysis	-	**Grazoprevir/elbasvir -**	ribavirine in genotype 1a)
-	-	genotype 1,4 (*EASL – only	***Daclatasvir/asunaprevir***
-	-	genotype 1b)	(genotype1)
-	Decompensated Child-	***Sofosbuvir/velpatasvir*** without	-
-	Pugh B or C cirrhosis	ribavirin for 24 weeks	

HCV Infection and Kidney Transplantation

HCV infection is associated with an increased mortality risk after KT. In the interferon-based regimens era, HCV treatment was possible only before KT. DAA regimens permit post-transplant treatment, even in HCV-positive donors.

In patients without cirrhosis or with compensated liver cirrhosis, therapeutic options are: glecaprevir/pibrentavir (all genotypes), sofosvubir/ velpatasvir (all genotypes), sofosvubir/ledipasvir (genotype 1, 4, 5, 6), elbasvir/grazoprevir (genotype 1,4), 12 weeks. DAA-experienced patients will be treated with a daily fixed-dose combination of sofosbuvir /velpatasvir/voxilaprevir, with or without ribavirin, 12 weeks [26]. Many studies demonstrated very good results (SVR – 98-100%) and very well tolerability [27, 28]. The main concern of post KT HCV patients is about drug-drug interactions between DAA and immunosuppressive therapy, resulting in graft rejection or toxicity. There are no interactions between DAA and Mycophenolate. Protease – inhibitors interact with calcineurin inhibitors and elbasvir-grazoprevir will not be administrated in patients taking cyclosporine; for this DAA regimen the level of tacrolimus needs to be closely monitorized [9]. The main drug-drug interactions between HCV - DAAs and immunosuppressants are presented in Table **3** [25].

Table 3. Drug-drug interactions between HCV – DAAs and immunosuppressants [25].

	SOF	**SOF/VEL**	**SOF/VEL /VOX**	**GLE/PIB**	**GZR/EBR**
Azathioprine	1	1	1	1	1
Cyclosporine	1	1	3	2	3
Mycophenolate	1	1	1	1	1
Sirolimus	1	1	2	2	2

(Table 3) cont.....

	SOF	SOF/VEL	SOF/VEL /VOX	GLE/PIB	GZR/EBR
Tacrolimus	1	1	2	2	2

1-No clinically significant interaction is expected; 2-Potential interactions which may require dosage adjustment, altered timing of administration, or additional monitoring; 3- These drugs should not be co-administered (more information on www.hep-druginteractions.org - University of Liverpool); DAAs, direct-acting antivirals; EBR, elbasvir; GLE, glecaprevir; GZR, grazoprevir; PIB, pibrentasvir; SOF, sofosbuvir; VEL, velpatasvir; VOX, voxilaprevir. The risks *vs*. benefits of treating patients with HCV before or after KT require individual assessment, depending on donor type (living or deceased), wait-list time, HCV genotype, the severity of liver fibrosis and center-specific policies [9, 25]. The management of HCV infection in KT candidates, according to KDIGO guidelines, is presented in Fig. (**1**) [4, 9].

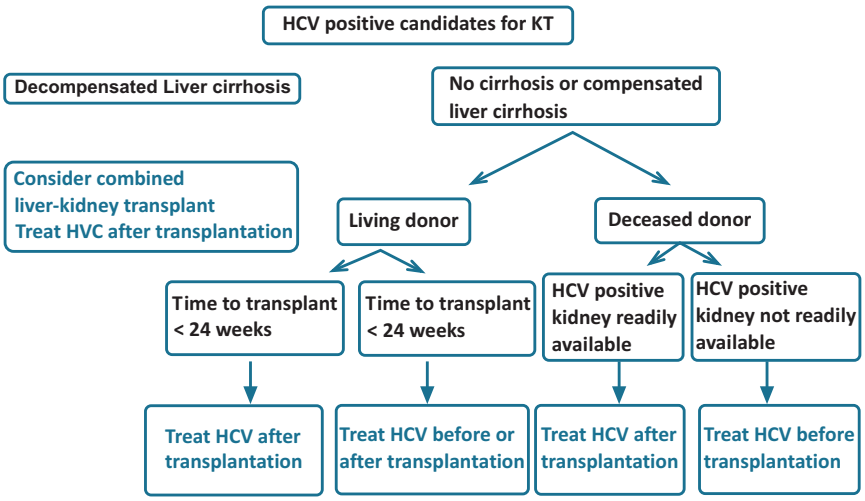

Fig. (1). Management of HCV infection in KT candidates, according to KDIGO recommendations KT: kidney transplant, HCV: hepatic C virus; KDIG: kidney disease: Improving Global Outcome.

One of the major challenges in the last few years is the possibility to transplant a HCV- positive kidney in a HCV-negative recipient with DAA treatment following transplantation. The positive consequences would be the increase of the organ supply, the shortening of the waiting period, and avoidance of long-term dialysis complications. On the other hand, there are concerns regarding the possibility of HCV fulminant hepatitis, rapid progression to cirrhosis, or failure of DAA treatment to cure the infection after transplantation. Some small studies (THINKER-1, EXPANDER-1) demonstrated encouraging results, but this approach needs to be explored further [4].

The field of HCV treatment in advanced CKD, including dialysis and KT patients, has continuously evolved. Preventive measures to reduce the risk of HCV transmission in the dialysis centers and the efficacy of DAA treatment made the elimination of virus C hepatitis in CKD patients possible.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 26

Advances in Imaging Diagnosis of Hepatocellular Carcinoma - the Place of Contrast Enhanced Ultrasound (CEUS)

Mirela Dănilă[1,*] and **Ana Maria Ghiuchici**[1]

[1] *Department of Gastroenterology and Hepatology, "Victor Babeș" University of Medicine and Pharmacy, 156, Liviu Rebreanu Bv, 300723, Timișoara, Romania*

Abstract: Hepatocellular carcinoma (HCC) is a primary malignant liver tumor that complicates advanced chronic liver disease, especially liver cirrhosis. Surveillance of this category of patients is mandatory for early detection of HCC and improved prognosis. Screening should be carried out by the abdominal US every 6 months with or without alpha-fetoprotein.

The diagnosis of HCC is confirmed by imaging methods that highlight the typical behavior of HCC: hyper-enhancement in the arterial phase and washout in the late phase. Imaging methods used for HCC diagnosis are Multi-detector computer tomography (MDCT), multi-phase nuclear magnetic resonance imaging (MRI), or contrast-enhanced ultrasound (CEUS).

LI-RADS algorithm is now one of the most used widely systems for the imaging diagnosis of HCC. It is a standardized system for technique, interpretation, reporting, and data collection for imaging (CT, MRI, and CEUS). The algorithm includes 8 categories with an increasing probability of HCC and malignancy with higher categories.

Studies that have attempted to validate this LI-RADS scheme for the diagnosis of HCC shown that LR-5 is highly predictive for HCC.

Keywords: Contrast-enhanced ultrasound, Diagnosis, Hepatocellular carcinoma, Liver cirrhosis, Liver Imaging Reporting and Data System, Magnetic resonance imaging, Multi-detector computer tomography.

[*] **Corresponding author Mirela Dănilă:** Department of Gastroenterology and Hepatology, "Victor Babeș" University of Medicine and Pharmacy, 156, Liviu Rebreanu Bv, 300723, Timișoara, Romania; Tel: +40256488003, Fax: +40256488003; E-mail: mireladanila@gmail.com

Ioan Sporea and Alina Popescu (Eds.)

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most frequent primary liver cancer, being the second most common cause of cancer death worldwide [1, 2]. The incidence of hepatocellular carcinoma (HCC) has been increasing in the last few years and is expected to increase until 2030 in some countries where the prevalence of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are raised [3].

Chronic liver diseases, especially liver cirrhosis, represent the major risk factor for hepatocellular carcinoma that accounts for 70% to 80% of the total liver cancer [4]. Most clinical practice guidelines recommend surveillance for early detection of HCC in this category of patients.

Hepatocarcinogenesis is a complex multistep process that includes the transition from regenerative nodules to hepatocellular carcinoma accompanied by changes in the blood supply of the nodules and malignant transformation consisting of gradually reducing the number of portal tracts while the number of unpaired arteries increases. In most cases, HCC is supplied mostly by the hepatic artery system, *via* abnormal unpaired arteries (a hypervascular tumor) [5].

This explains the characteristic enhancing pattern of HCC with hepatic arterial phase hyperenhancement and portal venous and/or delayed phases washout relative to the background liver on contrast enhanced imaging.

Imaging Diagnosis of Hepatocellular Carcinoma

Recent EASL guidelines recommend that the diagnosis of HCC in cirrhotic patients should be based on non-invasive criteria and/or pathology and in non-cirrhotic patients, diagnosis of HCC should be confirmed by pathology [6].

In clinical practice, all patients with liver cirrhosis and chronic hepatitis with advanced fibrosis included in the category of risk for HCC must be followed for early detection of HCC. Screening should be carried out by the abdominal US every 6 months with or without alpha-fetoprotein (AFP) [6]. The ultrasound sensitivity as a surveillance test ranges from 58 to 89%, with specificity greater than 90% [7]. Because the US's performance in early detection of HCC is highly dependent on the expertise of the operator and the quality of the equipment, the guidelines recommend that surveillance be performed by experienced personnel [6].

After US screening, liver nodules found must be characterized using imaging methods: multi-detector computed tomography (MD-CT) or multi-phase nuclear magnetic resonance imaging (MRI), or contrast enhanced ultrasound (CEUS) [6].

Multi-detector Computer Tomography (MD-CT) and Multi-phase Nuclear Magnetic Resonance Imaging (MRI) in the HCC Diagnosis

The noninvasive diagnosis of HCC using contrast enhanced imaging methods can be established only if the typical pattern is present. According to the European Association for the Study of Liver (EASL) guidelines, a single dynamic technique showing intense arterial uptake followed by a washout of contrast in the venous-delayed phases is valid to diagnose HCC [6]. Most guidelines recommend the diagnostic cut-off size of 1 cm.

These guidelines also recommend first-line imaging methods: multiphasic contrast-enhanced CT, multiphasic contrast-enhanced MRI, or gadoxetic-enhanced MRI. If the imaging method used is not typical, then another method will be used, including contrast enhanced ultrasound (CEUS) [6].

The MRI and CT sensitivity in HCC diagnosis was evaluated in a recent meta-analysis that included 19 studies [8]. This study showed a significant higher sensitivity for MRI with extracellular or with hepatospecific contrast over CT (82% *versus* 66%), but the specificity of MRI *versus* CT (91% *versus* 92%) was not different. For all imaging modalities, the results were better for HCC ≥2 cm, but not for HCC less than 2 cm in size. The study concludes that due to low to moderate quality of evidence and possible publication bias, the differences in pooled diagnostic performance are considered insufficient to definitively recommend MRI over CT.

In clinical practice, the choice between CT and MRI depends on patient safety preferences, local expertise, and possible contraindication, especially for MRI. Other MRI disadvantages are: higher cost, higher technical complexity, longer scan times, claustrophobia, increased tendency to the artifact. CT is more accessible, faster (has a short exposure time), but has the disadvantage of radiation exposure. For both diagnostic methods, renal insufficiency is the major limitation, because the kidneys eliminate most of the contrast agents used in CT and MRI.

LI-RADS Algorithm for CT and MRI

In order to provide standardization for HCC imaging diagnosis, LI-RADS algorithm was developed. The first version of LI-RADS was released in 2011,

supported by the American College of Radiology (ACR), and the major LI-RADS updates followed in 2013, 2014, 2017, and 2018 [9, 10].

New imaging criteria for HCC diagnosis called CT/MRI LI-RADS v2018 (Liver Imaging Reporting and Data System) [10] include: ***major imaging features***: nonrim arterial phase enhancement, tumor/observation size, nonperipheral washout, ***additional major feature*** enhancing capsule and threshold growth (≥50% increase in the size of a mass in ≤6 months); and also ***ancillary features*** [10].

CT/MRI LI-RADS algorithm defines eight diagnostic categories based on imaging. These categories are from LR-1-definitely benign lesions to LR--definitely HCC, LR-NC, LR-TIV, and LR-M [10].

LI-RADS v2018 algorithm changes the LI-RADS v2017 definition for LR5: a liver observation 10-19 mm in size with nonrim APHE and nonperipheral washout in patients at risk for HCC must be classified LR5. Using these new criteria for LR5 result in up-categorized of 40% of the nodules classified as LR4 by LI-RADSv2017 [11]. This study also showed that with the numerical increase of LI-RADS categories, the percentage of HCC in each category also increases, but no HCC or other malignancy was reported in the LR-1 category [11].

Other studies had demonstrated improved sensitivity and accuracy of the v2018 LR-5 category as compared with v2017 for the diagnosis of HCC [12 - 14].

After the HCC diagnosis, the disease's staging is essential to determine the outcome and planning of optimal therapy and include assessing tumor extension. For this purpose, CE-MRI or helical CT is also used. CT of the chest, abdomen, and pelvis is recommended to rule out an extrahepatic spread. Also, AFP level, liver function, portal pressure, and clinical performance status are mandatory to provide the best treatment management.

The new version of LI-RADS is now integrated into the AASLD 2018 HCC clinical practice guidance [15]. This algorithm also defines categories for assessing treatment response after loco-regional therapy for HCC.

Contrast Enhanced Ultrasound (CEUS) in the Diagnosis of Hepatocellular Carcinoma

US is the most widely used imaging technique for HCC surveillance in patients at risk, but US has no role in HCC characterization. The development of second generation ultrasound contrast media accompanied by dedicated software has

improved the diagnosis of ultrasound in the characterization of focal hepatic lesions.

Several second-generation contrast agents are available for CEUS examination. The most commonly used are: SonoVue/Lumason (Bracco Suisse SA, Geneva, Switzerland), Definity/Luminity (Lantheus Medical Imaging, Inc., North Billerica, MA, USA), Optison (GE Healthcare AS, Oslo, Norway), Sonazoid (GE Healthcare AS, Oslo, Norway).

CEUS permits real-time visualization of contrast-enhancement patterns of lesions during all three vascular phases (arterial, portal-venous, and late). The arterial phase starts 10 to 20 seconds after the contrast agent injection and lasts for 25 to 35 seconds approximately, following by the portal-venous phase that lasts for two minutes after contrast agent injection. The portal-venous phase is followed by the late phase, which lasts for 5 minutes (until the bubbles are cleared from the circulation). For HCC in the cirrhotic liver, the key feature is APHE, followed by washout with late-onset and mild degree [16].

The use of CEUS in clinical practice is supported by multiple studies highlighting both its performance in FLL characterization and malignant/benign differentiation. A large meta-analysis published by Friedrich-Rust, which included 8,147 FLLs, CEUS showed good performance in malignant *versus* benign differentiation (93% Se and 90% Sp) [17].

The large DEGUM study also shows that CEUS has high sensitivity, specificity, positive predictive value, and negative predictive value (95.8, 83.1, 95.4, and 95.9%, respectively) for differentiating benign *versus* malignant lesions. CEUS has been proved to be a sensitive method for the diagnosis of HCCs [18].

The first European Federation of Societies in Ultrasound in Medicine and Biology EFSUMB guidelines concerning the use of CEUS were published in 2004 [19], updated in 2008 [20], 2012 [21], and the last version in 2020 [22].

The EFSUMB guidelines formulated indications regarding the use of CEUS in the cirrhotic liver [19, 20]:

- to characterize all nodules found on routine US surveillance and to establish a diagnosis of HCC,
- when CT or MRI is inconclusive (in nodules not suitable for biopsy) or
- after inconclusive histology,
- for the selection of the most appropriate lesion for biopsy,
- for differentiating tumor in vein.

The guidelines recommend that before starting the CEUS examination is mandatory to review the patient's clinical history, laboratory data, and any prior imaging findings. Also, a systematic liver examination must be performed using B-mode and Doppler US.

Most hepatocellular HCCs exhibit a short hyper-enhancement in the arterial phase, but not all HCCs exhibit contrast washout in the portal or/and late phase, and this latter feature limits the sensitivity of CEUS in the diagnosis of HCC. More recent studies suggest that washout in HCC is of mild intensity and often occurs in the late phase (after 4–6 minutes) [23], and some well-differentiated HCC may show no washout at all [24].

Despite the good performance reported in the HCC evaluation, CEUS was dropped from the previous EASL and AASL guidelines based on CEUS' inability to differentiate between HCC and CCC [25].

CEUS LI-RADS in the Diagnosis of Hepatocellular Carcinoma (HCC)

CEUS LI-RADS algorithm was created to harmonize the interpretation of CEUS with that of CT and MRI and reduce diagnostic errors and inter-observer variability in patients at risk for developing hepatocellular carcinoma.

The first version of CEUS LI-RADS was released in 2016 by the ACR and then revised in 2017, and just as CT/MRI, LI-RADS is a standardized system for technique, interpretation, reporting, and data collection for contrast enhanced ultrasound exams in patients at risk for developing hepatocellular carcinoma [26, 27].

LI-RADS algorithm must be applied only for pure blood-pool agents —such as Lumason® (in the USA)/SonoVue® (outside the USA) and Definity® (in the USA, Canada)/ Luminity® (outside the USA, Canada), in patients at high risk for HCC with lesions visible at the precontrast ultrasound examination.

CEUS LI-RADS ***diagnostic categories*** are the same as in MRI and CT: CEUS LR-1 to LR5, CEUS LR-NC, CEUS LR-M, CEUS TIV [26, 27].

The major features that are used for categorization are:

-***Arterial phase hyperenhancement (APHE)***. In the arterial phase (10–20 to 30–45s) after the injection of contrast microbubbles, a liver mass visible on precontrast demonstrating a typical APHE pattern (not rim or peripheral discontinuous globular) should be characterized as a typical lesion of hepatocellular origin (CEUS LR-3, LR-4, or LR-5 depending on the size of the

lesion and the appearance in the portal/late phase).

-***Washout onset and washout degree.*** Hepatocellular carcinoma typically shows washout with late-onset (60 s) and of a mild degree, while non-hepatocellular lesions, including ICC, show early-onset (<60 s) and/or marked washout.

After CEUS examination and characterization of liver observation in the arterial, portal and late phase, in the next step, ***ancillary features*** can be applied to downgrade or upgrade the category. ***Ancillary features favoring malignancy*** (for HCC) are: nodule-in-nodule architecture, mosaic architecture, and other malignancy-definite growth. ***Ancillary features favoring benignity*** are: size stability ≥ 2 years and size reduction [26 - 28].

After the final characterization of liver observation/nodule, we can include the lesion in one of the following CEUS LI-RADS diagnostic categories [27, 28]:

-**CEUS LR-1** (definitely benign) can include: a cyst, a hemangioma with a typical enhancement pattern, or a hepatic fat deposition/sparing located around the gallbladder fossa and anterior to the right portal vein in segment 4 and with iso-enhancement in all phases (Fig. **1**).

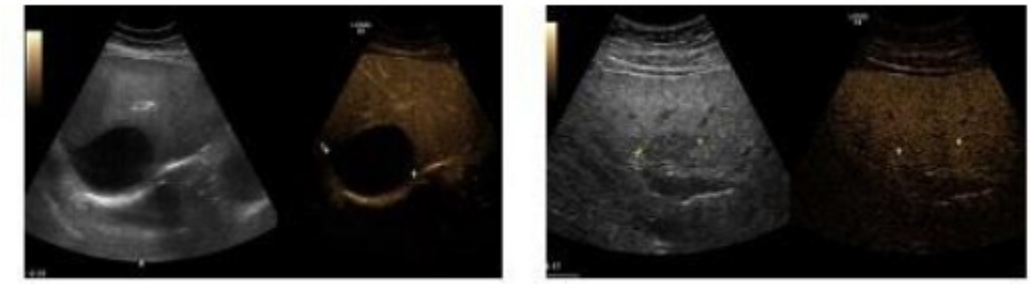

Fig. (1). CEUS LR-1 A. Cyst - an unenhanced area in all phases; B. fat sparing – isoenhancening in all phases.

-**CEUS LR-2** (probably benign) - a distinct is enhancing solid nodule < 10 mm, or a nonmasslike isoenhancing observation of any size, not typical hepatic fat deposition/sparing. If the isoenhancing nodule is ≥ 10 mm, categorize it as CEUS LR-3.

-**CEUS LR-3** (intermediate malignancy probability) - lesion <1cm with APHE and no washout of any type; a lesion<2 cm with no APHE and no washout of any type; a lesion<2 cm with no APHE, and late and mild washout.

-**CEUS LR-4** (probably HCC, but not 100%) - lesion≥1 cm with APHE and no washout of any type **or** a lesion <1 cm with APHE and late and mild washout **or** a lesion≥2 cm with no APHE and late and mild washout (Fig. **2**).

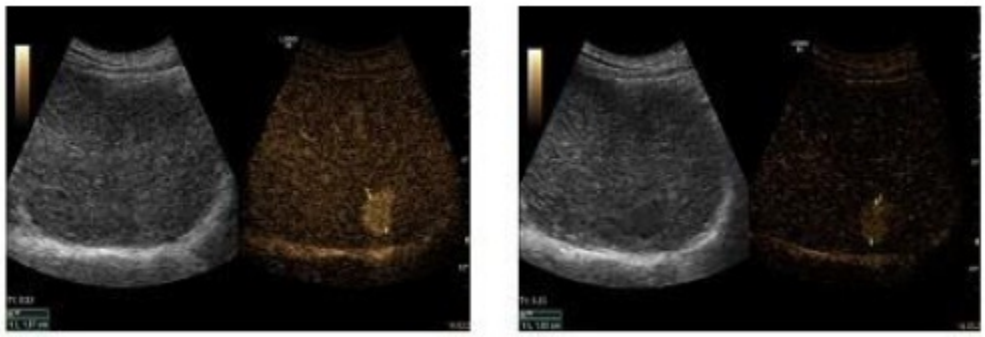

Fig. (2). CEUS LR-4 Baseline US image shows a slightly hypoechoic lesion sized 1.9 cm in segment VIII. At CEUS, the nodule is highly hypervascular during the arterial phase and with no washout in the late phase.

-**CEUS LR-5** (definitely HCC) - lesion ≥1 cm with APHE and mild and late washout (Fig. **3**).

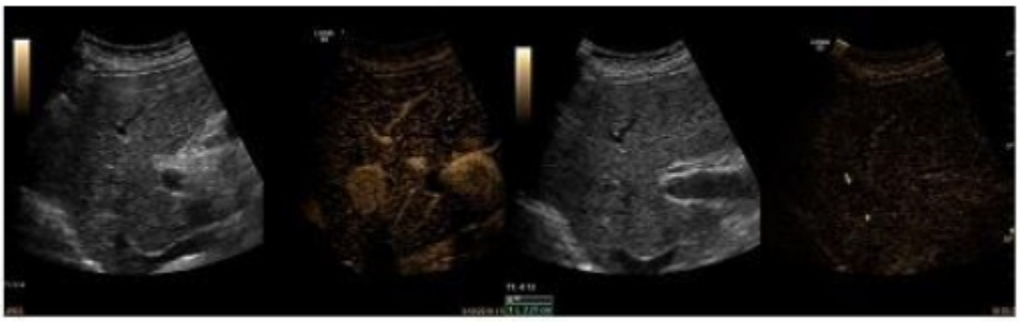

Fig. (3). CEUS LR-5 a nodule with arterial phase hyperenhancement and late and mild washout.

-**CEUS LR-M** (probably or definitely malignant, not HCC specific) - a lesion with rim APHE or early (<60s) washout or marked washout.

-**LR-TIV** (tumor in vein) refers to hyperenhancement of soft tissue within a vein in the arterial phase followed by washout in the late phase.

-**LR-NC-** cannot be categorized due to image degradation.

Even if they share the same algorithm, there are still differences between CEUS LI-RADS and CT/MRI LI-RADS [29]. The advantages of CEUS are:

- CEUS is a real-time imaging method and is more sensitive than CT or MRI for detecting APHE- the most important imaging feature for diagnosing HCC. In liver nodules categorized LR-3 and LR-4 at CT and MRI, CEUS is an excellent alternative imaging option to demonstrate APHE;
- vascular pseudo-lesions are rarely seen in CEUS examination. In liver cirrhosis, arterio-portal shunts are frequent, and these appear- in CT and MRI with APHE. These pseudo-lesions may be mistaken for true lesions on CT or MR;
- contrast agents used in CEUS examination are safe in patients with renal failure; multiple injections of microbubble contrast agents are allowed in the same examination if necessary for a more complete characterization of a lesion or to assess additional observations;
- CEUS does not use ionizing radiation as does CT. CEUS and is cheaper than CT or MRI;
- CEUS also has some potential limitations: CEUS is usually not suitable for the staging of hepatocellular carcinoma and is more operator-dependent than CT or MRI.

Several studies have attempted to validate this LI-RADS scheme for the diagnosis of HCC. In Terzy *et al*. [30] study, about 1000 liver nodules from 848 patients with chronic liver disease at risk of HCC were evaluated. The LR-5 category was 98.5% predictive of HCC with no risk of misdiagnosis for pure cholangiocarcinoma. Regarding the major concern that CEUS may misdiagnose ICC, the study shows that none of 519 LR-5 were pure ICC.

A recent prospective multicenter study funded by the DEGUM assessed the diagnostic accuracy of standardized contrast-enhanced ultrasound (CEUS) for the noninvasive diagnosis of HCC in high-risk patients. The authors compared standardized CEUS at the time of the examination (CEUS on-site) and two CEUS algorithms ESCULAP (Erlanger Synopsis for Contrast-enhanced Ultrasound for Liver lesion Assessment in Patients at risk) and CEUS LI-RADS. The sensitivity for the diagnosis of HCC was 94.2% (CEUS algorithm ESCULAP), 90.9% (CEUS on-site), and 64% CEUS LI-RADS algorithm ($p < 0.001$). All three modalities had high positive predictive values – around 90%. The study results showed that CEUS on-site diagnosis by an experienced examiner achieved an almost equal diagnostic accuracy compared to CEUS-based diagnostic algorithms [31].

In liver lesions found in cirrhotic patients, with inconclusive imaging findings, a biopsy with pathological examination can be performed for a definite diagnosis, depending on the size of the lesion, the intended treatment, and the stage of the disease.

Since radiological diagnosis is crucial for HCC, imaging studies must be performed in expert centers.

CONCLUSIONS

Contrast enhanced ultrasound for the diagnosis of HCC can help for a quick diagnosis, with some advantages over CT/MRI. CEUS LI-RADS diagnostic categories are useful for a better diagnosis.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this chapter contents have no conflict of interest.

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CHAPTER 27

Treatment of Intermediate Stage Hepatocellular Carcinoma – from Guidelines and Beyond

Zeno Spârchez[1,*] and **Iuliana Nenu**[1]

[1] *Institute for Gastroenterology and Hepatology "O.Fodor", University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania*

Abstract: Hepatocellular carcinoma (HCC) BCLC-B class is characterized by an extensive heterogeneity due to the wide range of liver function (Child Pugh A or B cirrhosis) and variable lesion number and size. With this regard, hepatologists must develop a better stratification of this HCC stage for patients to benefit from a better treatment allocation.

Trans-arterial chemo-embolization (TACE) procedure is the most widely used therapeutic option for intermediate stage HCC. One therapy is not beneficial unless clinicians might predict its outcome. Along these lines, several predictive factors for the TACE success have emerged such as mRECIST criteria, HAP and mHAP, Munich and CHIP score. The overall survival (OS) after the TACE procedure is around 16 months and in rigorous selected candidates, might increase the survival up to 3 years. Nevertheless, in some BCLC B patients, other therapies have proved their benefit compared to TACE. Resection and liver transplantation when technically possible is associated with an increased OS *versus* TACE. Moreover, astounding results have arisen from the combination of TACE with radiofrequency ablation. However, the literature fails to support the use of multi-kinase inhibitors in combination with TACE. Selective internal radiation therapy (SIRT) also known as radioembolization (TARE) induces fewer side effects and maintains a better tumoral control than TACE, but it is less available worldwide and is less cost-efficient.

In conclusion, navigating through all these treatment options, we believe that intermediate stage HCC has to be managed in a personalized way for each patient in order to have the best outcome.

Keywords: Hepatocellular carcinoma, Intermediate stage BCLC B, TACE.

[*] **Corresponding author Zeno Spârchez:** Institute for Gastroenterology and Hepatology "O.Fodor", University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania; Tel: 0040 745185153, Fax: 0040 264 433427; E-mail: zsparchez@yahoo.co.uk

Ioan Sporea and Alina Popescu (Eds.)

INTRODUCTION

BCLC B Subgroup and Beyond

The Barcelona Clinic Liver Cancer (BCLC) system grading was first presented more than 20 years ago, along its way has incorporated changes according to the clinical setting and treatment options and nowadays it still represents the cornerstone of hepatocellular carcinoma (HCC) classification [1, 2]. Since its original publication, it is acknowledged that BCLC-B class is defined by a subset of patients categorized as intermediate-stage HCC with multifocal disease confined to the preserved liver function, without vascular invasion and good performance status.

In theory, patients with BCLC-B stage are ineligible for curative treatment, but can benefit from trans-arterial chemo-embolization (TACE) as a standard of care [2]. According to the European Association for the Study of the Liver (EASL) the median survival for untreated patients at an intermediate-stage [BCLC-B – multinodular disease, good performance status (PS), without vascular invasion or extrahepatic spread] is around 16 months and in rigorous selected candidates, TACE can increase the survival up to 3 years [3].

On this subject, hepatologists and oncologists have agreed that BCLC-B class is characterized by an extensive heterogeneity due to the wide range of liver function (Child Pugh A or B cirrhosis) and variable lesion number and range. In order to limit the variability of TACE results worldwide, a sub-classification of intermediate stage HCC has been proposed by Bolondi (Table **1**) and later by Kudo (Table **2**) [4, 5]. Taking into account these subclassifications patients might benefit from a better treatment allocation as given below.

Table 1. Bolondi BCLC-B subclassification. TACE- transarterial chemo-embolization; LTx- liver transplantation; SIRT- selective internal radiation therapy.

BCLC-B Subclassification	***B1***	***B2***	***B3***	***B4***
Child-Pugh score	5-7	5-6	7	8-9
Beyond Milan within up-to-7 criteria	In	out	Out	out
ECOG PS	0	0	0	0-1
PVT	No	no	No	no
First treatment option	**TACE**	**TACE/ SIRT**		**BSC**
Alternative	LTx/ TACE+ ablation	Sorafenib	Trials TACE+Sorafenib	LTx

Table 2. Kinki score.

BCLC-B subclassification	***B1***	***B2***	***B3***	
Child-Pugh score	5-7	5-7	8,9	
Beyond Milan within up-to-7 criteria	in	out	Any	
Treatment	**curative**	**palliative**	**3a** **If up-to-7 leads to curative treatment**	**3b** **Out up-to-7 leads to BSC**

Clinicians have to bear in mind that a solitary nodule of HCC beyond 5 cm without vascular invasion and metastasis and without cancer-related symptoms might benefit from surgical resection if technically feasible and thus should be reclassified as BCLC-A [6]. Moreover, a poor outcome of treatment might be defined by an impaired performance status, refractory ascites, and events such as spontaneous bacterial peritonitis, hyponatremia or recurrent encephalopathy. In the absence of liver transplantation which is the only possible treatment, dismally the patient must be restaged as BCLC-D [7].

TACE Treatment Point of View

As a result of an exclusively arterial vascularization of HCC tumors and comprising the fact that the normal surrounding liver parenchyma is vascularized from branches of the portal vein, TACE and other image-guided transcatheter treatments were born in order to destruct arterial tumoral vessels and hence inducing tumor necrosis [8].

TACE procedure is based on intra-arterial infusion of a chemotherapy agent such as doxorubicin or cisplatin, frequently embedded in lipiodol as a vehicle to increase vulnerability to the drug. Furthermore, the tumoral blood vessels will be embolized with different agents such as gelatin sponge particles, metallic coils, polyvinyl alcohol, starch microspheres and autologous blood clots leading to an increased tumoricidal and ischemic effect [9, 10]. The five most common adverse effects reported are liver enzyme abnormalities (18.1%), fever (17.2%), hematological/bone marrow toxicity (13.5%), pain (11%), and vomiting (6%), which are related to the occurrence of postembolization syndrome. The overall mortality rate was reported less than 1% and is due to acute liver insufficiency [11].

Nevertheless, TACE therapy has its vicissitudes, the contraindications ruled out by Raoul *et al.* being listed in Fig. (**1**) [12].

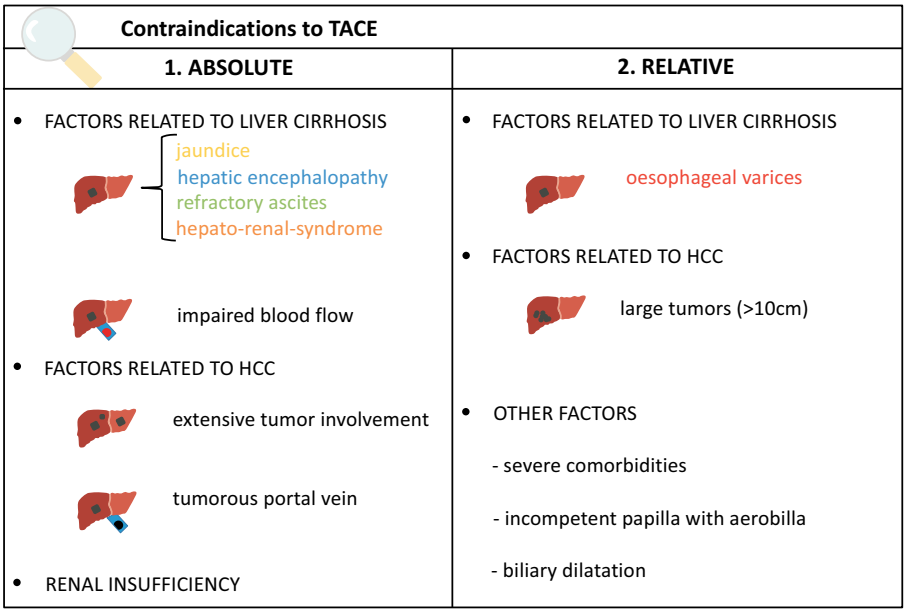

Fig. (1). List of contraindications to TACE treatment.

Drug-eluting beads (DEBs) have the potential to actively sequester a drug and subsequently release it slowly, in a controlled manner, after implantation within the tumor vessels. Along these lines, DEB-TACE hypothetically might augment the intensity and duration of ischemia in the target lesion with enhanced drug delivery and without significant side effects [13]. Nonetheless a meta-analysis that comprehended studies has brought to light that DEB-TACE is not superior compared to conventional TACE regarding OS. Although this technique is not available in many centers, might represent a paramount alternative if the beads will contain multikinase inhibitors or maybe immunotherapeutic agents.

As mentioned previously in the chapter, although TACE is the most widely used therapeutic option for intermediate stage HCC, a wide hiatus stands between guidelines and clinical practice. Clinicians have reported using downstaging therapies such as ablation, radioembolization, combination treatments with systemic therapies or even radical resection [14, 15]. Although TACE results might be distinctive across centers due to intermediate stage heterogeneity, it seems that liver function, nodule size, selectivity of embolization, emulsifying agents, and degree of treatment delivery play an important role in the treatment success [16]. Two randomized controlled trials and quite a few meta-analyses outlined the survival benefits of TACE compared to BSC [17, 18]. Although conventional TACE remains a palliative treatment method, it prolongs the survival up to 40 months. Likewise, a systematic review on more than 10.000

patients concedes that overall survival (OS) was 70.3% at one year, 51.8% at two years, 40.4% at three years, and 32.4% at five years with a median OS of 19.4 months (95% CI 16.2–22.6) [11]. Nonetheless, ten years ago, was published an intriguing meta-analysis questioning the performance of TACE, but the results were abandoned due to the bias of selection of numerous heterogeneous studies [19].

The success of the procedure is measured radiologically, by contrast, enhanced Computer Tomography (CE-CT) according to modified Response Evaluation Criteria in Solid Tumors (mRECIST). Radiologists characterize some important points to mention: response to therapy – either complete or partial, if the disease is stable or progressive under treatment, the potential appearance of new lesions, pleural effusions or ascites, lymph nodes in the porta hepatis vein and malignant portal vein thrombosis [18]. However, there is an uncertainty due to operator variability and the fact that the extent of necrosis sometimes might be overestimated when using mRECIST criteria. Unfortunately, some patients classified as having a complete response by these criteria have evidence of residual disease on pathological examination of surgical tissues after subsequent resection or transplantation [20, 21]. Nevertheless, studies favor assessing survival outcomes after loco-regional treatments and systemic therapies according to mRECIST response criteria [22]. One meta-analysis encompassing seven clinical trials brought to light what seems to be obvious, that a patient with a complete or a partial response has a better survival compared to those with stable or progressive disease [23].

One therapy is not beneficial unless clinicians might predict its outcome. Along these lines, several predictive factors for TACE success are listed and related to tumor burden (performance status, number, size, vascular invasion, and serum AFP levels), liver function (Child-Pugh score, serum bilirubin levels, and ascites) and associated comorbidities. Taking these clinical and serological factors into consideration, the HAP score was the first designed score to guide TACE treatment and it is based on bilirubin, albumin, AFP and tumor size as seen in (Table **3**) [24]. Patients with HAP C or D values might not benefit from an adequate TACE treatment.

Table 3. HAP score and modified HAP score. 4 stages comprise the HAP scale as the following scoring for each parameter. HAP A =0 points; HAP B =1 point; HAP C =2 points; HAP D >2 points. mHAP-II A =0 points; mHAP-II B =1 point; mHAP-II C =2 points; mHAP-II D=3-5 points. Alb- albumin; AFP- alphafeto protein; Bt - total bilirubin; tu- tumor.

HAP score	
Parameter	Scoring
Alb <36g/dL	1 point

(Table 3) cont.....

HAP score	
AFP > 400ng/mL	1 point
B_t > 17 µmol/L	1 point
TU size > 7cm	1 point

However, Park *et al*. proposed a modified HAP score eliminating bilirubin and adding portal vein involvement and mRECIST criteria response [25]. Unfortunately, the new score did not show any superiority compared to the HAP score in the clinical setting. However, Cappelli *et al.* also modified the HAP score (Table **4**) integrating tumor number, where a single tumor receives 0 points while two or more lesions receive 1 point and therefore having a better performance comparing to the other scores [26].

Table 4. HAP score and modified HAP score. 4 stages comprise the HAP scale as the following scoring for each parameter. HAP A =0 points; HAP B =1 point; HAP C =2 points; HAP D >2 points. mHAP-II A =0 points; mHAP-II B =1 point; mHAP-II C =2 points; mHAP-II D=3-5 points. Alb- albumin; AFP- alphafeto protein; Bt - total bilirubin; tu- tumor.

mHAP score	
Parameter	Scoring
Alb <36g/dL	1 point
AFP > 400ng/mL	1 point
B_t > 0.9mg/dL	1 point
TU size > 7cm	1 point
TU number ≥2	1 point

Another evaluation score comes from Germany, known as the Munich TACE score and depicts AFP, serum bilirubin, prothrombin concentration, creatinine, CRP, and tumor extension (Table **5**) [27]. The group evaluated their proposed score revealing an AUROC of 0.71 which was superior to the aforementioned scores [28].

Table 5. Munich-transarterial chemo-embolization (TACE) score. Alb- albumin; AFP- alphafeto protein; B_t - total bilirubin; CRP- C reactive protein; crea- creatinine.

Parameters	***Points***				
	0	**2**	**3**	**4**	**6**
AFP (ng/dL)	<35	-	35-999	-	≥1000
Bt (mg/dL)	<1.1	-	1.1-3.0	-	≥3.1
parameters	***Points***				

(Table 5) cont.....

Parameters	***Points***				
CRP (mg/dL)	<0.5	-	0.5-1.9	-	≥2
Tumor extension	Category A	-	-	Category B	-
Crea (mg/dL)	<1.3	≥1.3	-	-	-
Quick	≥75	<75	-	-	-

Ogasawara *et al.* have determined that patients carrying hepatitis C virus might represent a beneficial factor for receiving direct-acting antivirals against hepatitis C virus and slowing tumor development [29]. It is known as CHIP score – (Table **6**). However, given the fact that the design also encompasses Child-Pugh score makes it unreliable to clinicians. However, we must highlight the puzzle that encompasses whether to administer DAA to patients with HCV and intermediate HCC. Our group has tented some remarks according to Bolondi's subclassification [4]. Hence, for B1 patients DAA treatment should be initiated after TACE if the procedure is successful considering that these patients have the best OS [30]. For B2 patients we believe that the decision to start DAA therapy should be based on tumor response to TACE: treatment with DAA for those with complete response to TACE or reTACE, after adequate tumor control is documented. And last, for B3 and B4 patients probably DAA therapy might be initiated in the context of clinical trials [30].

Table 6. CHIP score.

Prognostic Factor	***Points***
Child Pugh Score	0
5	1
6	2
7	3
8-9	-
Number of Liver Tumors	0
1	2
2-7	3
8	-
HCV- RNA	0
positive	1
negative	-

Because the uncertainty of prognostic scores is not enough, a new dilemma has been brought to light. Under what circumstances the procedure has to be repeated and for how many times if tumor recurrence is present? Indubitably, major progression, extrahepatic metastasis or vascular invasion counteract the procedure. Moreover, impaired liver function and altered PS will hinder reTACE procedure. Last but not least, clinicians should bear in mind that doxorubicin is a potential cardiotoxic and should monitor cardiac activity when repeating TACE [31].

Forner and collaborators proposed a logic treatment algorithm illustrated below in Fig. (**2**) [32]. It seems that there is an unsteady balance between the advantages and disadvantages of retreatment. Clinicians must accept the given fact that the TACE procedure itself imbalances liver function and sometimes shortens the OS compared to patients that shifted to sorafenib treatment. In addition, it seems that sorafenib after procedure relapse is also superior in patients who have received one unsuccessful TACE compared to those who had three or more successive procedures [33]. Moreover, it must be kept in mind that the novel and in vogue immunotherapy agents might be superior to a reTACE treatment. If the philosophical dilemma is "to reTACE or not to reTACE" the answer stands in a personalized case-by-case manner.

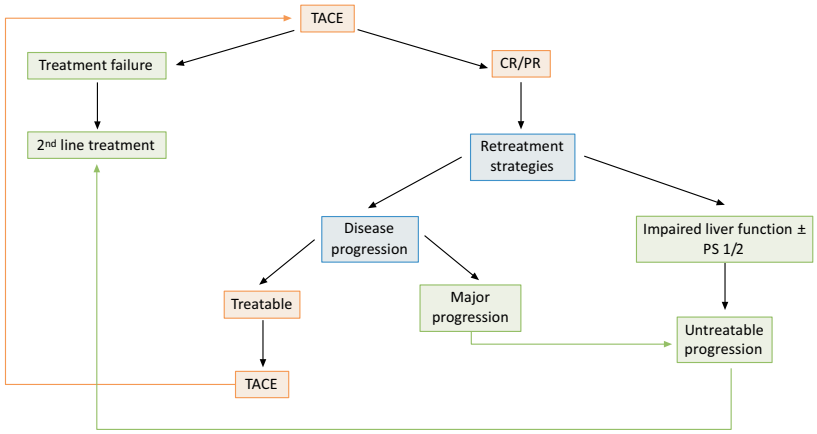

Fig. (2). TACE treatment follow-up algorithm (adapted from Forner 2014). CR- complete response; PR- partial response; PS- performance status.

Thinking Outside the Tace Box

Is there any room for ***surgery within intermediate stage HCC?*** is another dilemma that was long debated in the hepatology community. As stated before in the chapter, if technically feasible, it is recommended to perform surgery for solitary nodules larger than 5 cm. However, when possible, it is endorsed to measure hepatic venous portal gradient to select candidates without clinically

significant portal hypertension to prevent postoperatively liver failure. Studies concluded that surgery when technically possible is associated with an increased OS compared to TACE, 51.5% 3-year survival rate *vs* 18.1%; 43% 5-year survival *vs* 15%, $p<0.001$). The same assumptions are available for patients who underwent ***liver transplantation*** outside Milan criteria and inside up-to-7 score. Encouraging results were published by Kamo *et al.* on 56 patients with intermediate stage HCC who were transplanted and experienced a 5-year overall survival and recurrence rates of 88%/64%/58% and 22%/34%/44%, respectively [34]. Certainly, more advertising campaigns and more social entrepreneurship skills are necessary to raise awareness of organ donation to the general population.

Analogous to surgery, ***percutaneous ablation techniques*** have been proposed for intermediate stage HCC with impressive results regarding OS. Moreover, astounding results have arisen from the combination of TACE with radiofrequency ablation (RFA) [35]. A meta-analysis that included eleven studies and compared the impact of combined TACE plus RFA *versus* TACE only regimen in patients with intermediate stage HCC with diameter >5 cm, reported that the combined regimen had a higher survival rate *versus* TACE alone [36]. Although the published results so far are very encouraging, surgery, transplantation and percutaneous ablation seem to be available in the clinical trials or isolated cases. Hence, it is paramount to develop a better stratification of intermediate stage HCC for patients to benefit from the appropriate treatment.

Last but not least our attention was also focused on **combination treatments** between the combination of TACE and other treatment options. Probably the most debated combination in the hepatology community is between ***TACE and sorafenib***. A meta-analysis has reported intriguing results that the combination regimen was better than TACE alone in terms of time to disease progression, but in terms of OS it seems that there was no significant difference. While the combination has been proved its efficacy in Asian cohorts, it seems that for European ones does not bring any improvement [37, 38]. One explanation stands probably in the etiology of the cirrhosis, while in the East predominate viral hepatitis, in the West alcohol and obesity are the core of cirrhosis.

Selective Internal Radiation Therapy (SIRT) also known as ***radioembolization*** (TARE) is based on the intra-arterial injection of small microspheres (25–35 mm) loaded with the radionuclide yttrium- 90 (90Y) which emit high-energy, low penetration radiation that further induces tumor necrosis and thus is indicated for patients with locally advanced HCC [39]. Several reports concede encouraging results revealing a median survival time of 17.2 months for patients at intermediate stages and 10 months to 12 months for patients at advanced stages

with portal vein invasion [40, 41]. Compared to TACE, SIRT induces fewer side effects and maintains a better tumoral control. However, it is less available worldwide, more difficult to assess and the selection of patients is more rigorous. Instead, two RCTs compared SIRT with sorafenib and surprisingly revealed no significant difference in terms of OS [42, 43]. Although, SIRT was similar to sorafenib concerning OS with better quality of life it seems that is more advantageous than sorafenib in portal vein thrombosis [44].

The good news in the management of liver cancer is the fact that HCC is a radiosensitive tumor. Thus, external beam radiation therapy, including ***stereotactic ablative body radiotherapy*** (SABR), has been more and more used with success [45]. A meta-analysis published in 2018 by Rim *et al.* analyzed the data from 32 studies including 1950 patients with HCC and portal vein thrombosis, treated by SABR had an overall response rate of 70%, significantly better than the one achieved with SIRT. Almost half of the patients survived for at least 1 year. The most common clinically significant toxicity reported was platelet abnormality in 15% of the patients [46]. In 2018, Yoon published randomized data on 90 patients who received either Sorafenib or TACE-RT. 78% of the patients had multiple lesions, and the median tumor size was quite large (9.7cm). TACE-RT was associated with a significantly longer time to progression than sorafenib (31 *vs*. 11.7 weeks) and significantly better overall survival [47].

Immune Check-point Inhibitors bursted in many clinical trials with very encouraging results for advanced HCC [48]. Using own immune system to fight against tumoral cells was one of the most ingenious ideas in oncology and revolutionized patient care. If locoregional treatments such as TACE, SIRT and ablation induce a powerful immune response and release into the circulation huge amounts of tumor-associated antigens, why not combine them with immunotherapeutic agents [49]? Although it is at its beginning of these combined regimens, tremelimumab in combination with TACE for intermediate stage HCC revealed as expected a better OS and might be a ray of hope for the hepatology and oncology community [50].

HOW TO NAVIGATE THROUGH ALL OPTIONS

We find it helpful to add a short summation of the therapies used in intermediate HCC as following:

Therapy	*When to Consider*
1. **OLT**	HCC lesions out of Milan but within up-to-seven criteria Decompensated liver disease
2. **HR**	1 nodule larger than 5 cm, if technically approachable (peripheral location) * Assess HVPG if possible In combination with intraoperative ablation (a.e 6 cm resectable lesion in the left lobe+ 2.8 cm lesion in the center of the right lobe)
3. ***Ablation***	3-5 nodules, less than 3 cm Nodules up to 5cm (if larger than 4 cm MWA or combined with TACE) Combined with TACE in difficult to approach nodules/large nodules
4. **TACE**	Nodules <8 (10 cm) Preserved liver function
5. **TARE**	Larger nodules (>8-10cm) Malignant portal vein thrombosis Bridge to liver transplantation
6. **SABR**	Larger nodules (>10cm) Malignant portal vein thrombosis Macroscopic invasion
7. ***Systemic therapy***	Multiple and/or large nodules (unresectable/treatable by TACE) Vascular invasion/ distant metastasis In association with other therapies
8. ***Immunotherapy***	Failure to systemic therapy (second or third line) Clinical trials

Certainly, the not-so-distant future will bring to both patients and clinicians more, unique and brighter ways of treating hepatocellular carcinoma. Only by simple searching on https://www.clinicaltrials.gov/ and filling with the term "intermediate HCC" can be found 22 trials based on numerous novel approaches that are currently ongoing or have just finished enrolling patients.

We believe that the treatment of intermediate stage HCC needs a personal touch from a tumor board made by hepatologists, oncologists, surgeons and interventional radiologists.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 28

Direct-acting Oral Anticoagulants in Liver Cirrhosis: What is the Current Status?

Anca Trifan[1,*] and **Irina Gîrleanu**[1]

[1] *"Grigore T. Popa" University of Medicine and Pharmacy Iaşi, Institute of Gastroenterology and Hepatology, Iasi, Romania*

Abstract: In the last few years, the coagulation abnormalities associated with liver cirrhosis were better characterized, concluding that the patients with liver cirrhosis are predisposed to thrombotic or bleeding complications. Portal vein thrombosis is the most frequent thrombotic event, associated with liver cirrhosis. Atrial fibrillation is also a frequent comorbidity in patients with liver cirrhosis associated with higher risks of embolic complications, needing an anticoagulant prophylactic treatment. Direct-acting oral anticoagulants (DOACs), warfarin, unfractionated heparin or low weight molecular heparin are not always efficient in liver cirrhosis. According to recent studies, DOACs are relatively safe in Child-Pugh class A or B liver cirrhosis for the treatment of acute portal vein thrombosis or prevention of embolic events in patients associating atrial fibrillation. All DOACs are contraindicated in patients with Child-Pugh class C liver cirrhosis.

Keywords: Anticoagulation, Atrial fibrillation, Direct-acting oral anticoagulants, Liver cirrhosis, Portal vein thrombosis, Thrombosis.

INTRODUCTION

The role of anticoagulant (AC) treatment in patients with liver cirrhosis (LC) is still a debated subject. Patients with cirrhosis were considered to be naturally anticoagulated due to the decreased production of pro-coagulant proteins and platelets, combined with an increased international normalized ratio (INR). New data have shown that patients diagnosed with LC are at a concomitant risk of hemorrhagic and thrombotic events due to increased platelet aggregation, decreased fibrinolysis, and decreased synthesis of natural anticoagulants as protein C, protein S and antithrombin III (AT III) [1] (Fig. **1**).

[*] **Corresponding author Anca Trifan:** "Grigore T. Popa" University of Medicine and Pharmacy Iaşi, Institute of Gastroenterology and Hepatology, Iasi, Romania; Tel +40 722306020, Fax +40 232246611; E-mail: ancatrifan@yahoo.com

Ioan Sporea and Alina Popescu (Eds.)

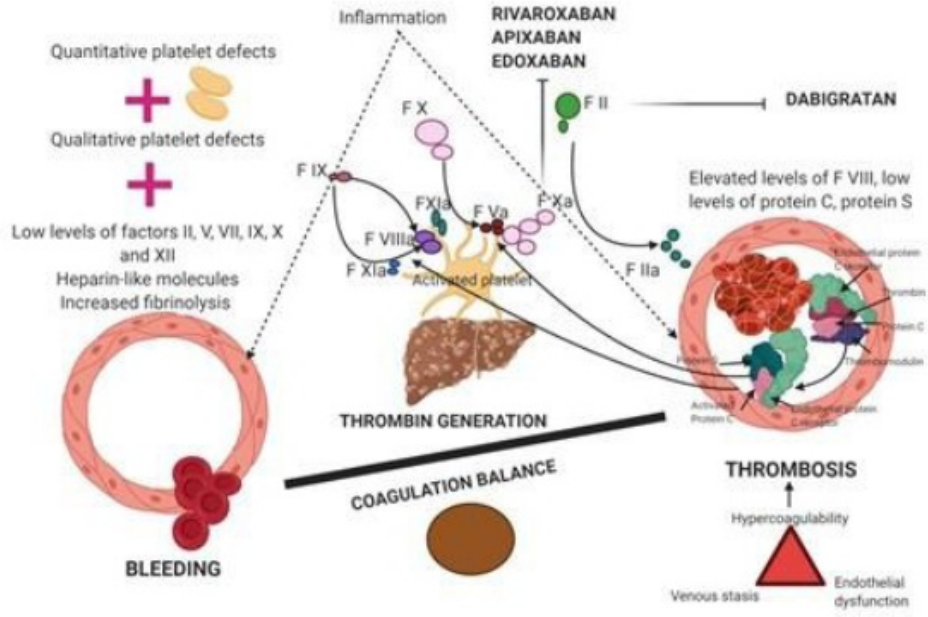

Fig. (1). Coagulation balance in patients with liver cirrhosis.

Multiple beneficial effects were attributed to AC in LC including decreasing decompensation rate or liver fibrosis [2]. A recent meta-analysis that evaluated survival rate and the antifibrotic effects of AC in animal models of liver cirrhosis concluded that the AC treatment could influence liver fibrosis, portal pressure and liver inflammation, with no impact on survival [3].

Direct Oral Anticoagulants in Liver Cirrhosis-real World Evidence

During the last decades, several indications of anticoagulation in patients with liver cirrhosis arise, starting with acute non-malignant portal vein thrombosis (PVT), preventing deep vein thrombosis (DVT) or thrombotic complications in non-valvular atrial fibrillation (AF) patients, to even prevent LC decompensation [4]. Many AC regimens were proposed and the studies were very inhomogeneous regarding this aspect [5]. There are four main direct-acting oral anticoagulants (DOACs) used frequently in our daily practice: rivaroxaban, apixaban or edoxaban (inhibitors of activated factor X) and dabigatran (inhibitor of thrombin). These anticoagulants are indicated in stroke prevention in non-valvular AF, venous thromboembolism (VTE) prophylaxis in patients after orthopaedic surgery, and the treatment of acute thromboembolic diseases [6]. The possibility of oral administration with no need of laboratory monitoring, and the mechanism of action not evolving the AT III make, in theory, the almost perfect anticoagulant

treatment for patients with LC, although we have to consider that both rivaroxaban and apixaban are in majority metabolized in the liver (67%) with half-lives between 5 hours and 12 hours [7]. Also, their concentration is depending on the plasma total protein level. Half of the total quantity of edoxaban is metabolized in the hepatocytes and has half-live of 10-15 hours [7]. Dabigatran is the DOACs with very low hepatic metabolism and his half-live [12-14 hours] is not influenced by the plasmatic proteins [7]. Also, dabigatran has a potent antidote – Idarucizumab (a monoclonal inhibitor antibody). Adexan *et al*fa is a recombinant modified human factor Xa protein and represents the antidote for the factor Xa inhibitors [8]. Idarucizumab has an intravenous administration, in a single dose with maximum effect [9]. Ciraparantag directly interacts with factor Xa inhibitors [10], but also with dabigatran, LMWH, and unfractionated heparin and at the moment there are ongoing studies evaluating its effect as an antidote for all the above anticoagulants. Also, gastric lavage soon after ingestion and hemodialysis in very severe cases could represent emergency therapeutic measures in dabigatran overdose.

Until now the ideal anticoagulant was not yet developed. In patients with cirrhosis, the efficacy of LMWH is decreased due to decreased levels of AT III, protein synthesized by the liver. The International Normalized Ratio (INR) is not correctly representing the real coagulation status in patients with LC and the INR could not be used to monitor the anticoagulant treatment (warfarin oracenocoumarol) [1]. DOACs would have theoretical advantages over antivitamin K antagonists (VKAs) or LMWH in cirrhosis and PVT [5].

DOACs have several advantages over VKA therapy, including oral administration, no need for frequent laboratory monitoring and low drug-drug interaction or food interactions. DOACs pharmacokinetics is represented in Table **1** [7, 11].

DOACs are indicated in non-cirrhotic patients for prevention or treatment of venous embolism, excepting patients with mechanical heart valves or those with antiphospholipid syndrome. Compared to VKA, DOACs do not need a dose adjustment and frequent laboratory testing and, most important, DOACs do not reduce protein C and S levels.

Table 1. DOACs hepatic metabolism and pharmacokinetics.

	Rivaroxaban	**Apixaban**	**Edoxaban**	**Dabigatran**
Mechanism of action	Factor Xa inhibition	Factor Xa inhibition	Factor Xa inhibition	Thrombin inhibition
Peak drug levels [C_{max}]				

(Table 1) cont.....

	Rivaroxaban	Apixaban	Edoxaban	Dabigatran
CP-A CP-B	↑ ↑↑	- -	↓ ↓	- ↓
Hepatic metabolism	++	+++	+++	+
INR influence				
CP-A CP-B	↑ ↑↑	↑ ↑	NR NR	↑ ↑

Abbreviation: CP- Child-Pugh class; DOACs- direct-acting oral anticoagulants; INR-International normalized ratio; NR- not reported.

Anticoagulant Treatment in Liver Cirrhosis: Indications

Including the recent data regarding the safety of the AC in patients with LC, this treatment is now indicated in patients with LC diagnosed with acute non-malignant PVT on the liver transplant awaiting list, cirrhotic patients with AF as a prophylactic treatment and the treatment and prophylaxis of DVT. The severe COVID-19 infection could represent an emergent indication of AC in patients with LC.

Non-malignant PVT Treatment

The early reports on the use of the DOACs in LC contained few case reports [12]. Intagliata *et al.* [13] was the first who evaluated the safety and the efficacy of DOACS in LC. He retrospectively described 20 patients with LC treated with apixaban or rivaroxaban for PVT, DVT or AF. The patients received 20 mg rivaroxaban or 10 mg apixaban (15 patients), and in 5 patients there was prescribed half of the initial recommended dose. The Child-Pugh (CP) class C patients were excluded from the study. Five percent of the patients treated with DOACs developed major bleeding complications with no fatal outcome. In this cohort, there were no drug induced liver injury, although considering the bleeding rate, larger prospective studies should confirm the safety of this treatment.

The Baveno VI consensus and some guidelines recommend anticoagulation primarily for PVT in patients on the liver transplant awaiting list and acute symptomatic PVT [14, 15]. The European Association for the Study of the Liver (EASL) clinical guidelines extended the indication for anticoagulation to all patients with LC and acute PVT [16].

Until now there are no clear data on the natural history of PVT and this fact raises doubts on the efficacy of the AC treatment. The studies that evaluated the efficacy of the AC reported a very low rate of spontaneous recanalization [17, 18].

Francoz *et al.* [19] reported no recanalization in the absence of AC, while more than 40% achieved total recanalization during AC. Senzolo *et al.* [20] described

thrombus progression in three quarters of patients that did not receive AC, compared to only 15% from the treated cohort. At the moment there are a few studies reporting the use of AC in patients with LC. Almost half of the patients achieved total recanalization after AC, while partial recanalization was observed in 15% – 35% of patients [19 - 22]. The small number of patients is one of the most important problems that needs to be soon overcome. The bleeding complications- gastrointestinal [variceal bleeding, post ligation ulcer, peptic ulcers], intracerebral hemorrhages, epistaxis and hematuria were the most frequent hemorrhagic complications [19, 20, 22]. Considering all these data, the DOACs appeared to be a more simple and safer alternative to anticoagulation in cirrhosis patients. The studies including DOACs treatment in patients with LC and PVT are summarized in Table **2**.

Table 2. Anticoagulation with DOACs in patients with liver cirrhosis and portal vein thrombosis.

Author, Year	Type	Country	No Patients	Treatment	Follow-up	Side Effects	Outcomes
Naymagon *et al*, 2020 [23]	Retrospective, cohort	USA	18	Rivaroxaban Apixaban Dabigatran	15 months	Major bleeding 3-16.7%	Complete resolution- 10-55,6%
Ming-Hua *et al*, 2020 [24]	Prospective, cohort	China	40	Rivaroxaban Dabigatran	6 months	Major bleeding 2-5%	Complete resolution- 11-27.5
Jones *et al*, 2020 [25]	Retrospective, cohort	USA	42	Apixaban Rivaroxaban Edoxaban Dabigatran	6 months	Major bleeding 8-19.1%	-
Scheiner *et al*, 2018 [26]	Retrospective, cohort	Austria	10	Apixaban Rivaroxaban Edoxaban Dabigatran	9.2 months	Major bleeding 1 variceal bleeding-10%	Complete resolution- 2-20%
Hanafy *et al*, 2018 [27]	Randomized controlled trial	Egypt	40	Rivaroxaban	1 year	0	Parial/complet resolution 39-97.5%
Nagaoki *et al*, 2018 [28]	Retrospective, cohort	Japan	20	Edoxaban	6 months	Major bleeding 3-15%	Complete resolution 18-90%
De Gotardi *et al*, 2017 [29]	Retrospective, cohort	Spain	22	Apixaban Rivaroxaban Dabigatran	15 months	Major bleeding 5-22.7%	-
Intagliata *et al*, 2016 [13]	Retrospective, cohort	USA	20	Apixaban Rivaroxaban	3 years	Major bleeding 1-5%	-

In the VALDIG study, major bleedings with DOACs discontinuation were seen in only 0.71% of patients without cirrhosis and 2.7% of patients with LC [29]. Intagliata *et al.* [13] demonstrated that two thirds of the CP class A or B LC patients received DOACs for PVT treatment, although they did not report the recanalization rate. They also described the PVT recurrence during the AC treatment, considering that other mechanisms of thrombosis could be involved in this special population. Considering the efficacy of VKAs, Hanafy *et al* [27] compared in a clinical trial rivaroxaban with warfarin in 80 patients with virus C compensated LC. The recanalization rate was 85%, significantly higher compared to 45% in patients treated with warfarin. Also, the DOACs patients had a higher survival rate and lower gastrointestinal hemorrhages. Edoxaban and warfarin were compared in LC patients with PVT by Nagaoki *et al.* [28], concluding that edoxaban is an effective AC treatment, although the majority of the gastrointestinal bleeding events were associated with the edoxaban treatment (15% *vs* 7%).

Recently, Mohan *et al.* demonstrated that the rate of treatment response with AC was 66.7% and compared with the control group of only 26%. The subgroup analysis, the treatment response rate was higher in patients receiving DOACs (76.7% compared to 60.7% for LMWH and 66% for VKAs) [30]. These results indicate that AC treatment for PVT in patients with LC has more benefits than no treatment.

A recent meta-analysis that aimed to investigate the safety and efficacy of AC in patients with LC and PVT [31], concluded that early AC treatment should be supported in patients with LC and acute non-malignant PVT. According to the subgroup analysis regarding the type of AC regimes, the recanalization rates were higher in DOACs alone compared to VKAs, fondaparinux, and LMWH alone. Another single center study reported a higher rate of PVT recanalization after DOACs treatment [32].

Both, European Medicines Agency (EMA) and Food and Drug Administration (FDA) recommend Child-Pugh class as a method to evaluate LC severity and to guide the DOACs treatment. FDA [33] and EMA [34] do not recommend DOACs in patients with Child-Pugh class C LC. Rivaroxaban is contraindicated in Child-Pugh class B LC. Apixaban, dabigatran and edoxaban can be used in patients with Child-Pugh class A or B LC with adjusted doses. Acute renal insufficiency, chronic kidney disease, drug-drug interactions and history of bleeding events should be thoroughly evaluated before DOACs treatment initiation. In patients with long-term AC the ratio between risks and benefits should be periodically and continuously evaluated.

Atrial Fibrillation and Liver Cirrhosis

Atrial fibrillation is one of the most frequent clinical arrhythmias. The long-term DOACs treatment is recommended in AF patients according to the thrombotic risk in order to prevent ischemic events. Impaired liver function secondary to LC represents one of the most significant risk factors for bleeding in AF patients treated with AC [35]. Considering all this data, the experience of AC treatment in cirrhotic patients with AF is limited. Moreover the guidelines make no clear statement regarding the AC for embolic events prevention in cirrhotic patients with AF [35]. The appropriate dose of DOACs in patients with AF and LC remains to be demonstrated. The pieces of evidence for the efficacy of DOACs in preventing ischemic events in patients with AF and LC are scanty as most of the randomized controlled trials excluded the patients with LC.

A recent retrospective cohort study, confirmed that DOACs could be safely administrated in Child-Pugh class A or B cirrhotic patients diagnosed with non-valvular AF or DVT. They also demonstrated that the prophylaxis with DOACs in LC has the same efficacy as warfarin prophylaxis [25]. Furthermore, DOACs were associated with a reduction in the overall mortality (pooled HR 0.77, 95% CI 0.61-0.96).

In a meta-analysis recently published, DOACs were associated with a lower risk of hemorrhagic complications, intracerebral hemorrhage and ischemic stroke, with no reduction in the risk of gastrointestinal bleeding in cirrhotic patients compared with warfarin. The results were not influenced by the DOACs dosage. More over, in the LC group the bleeding risk and also the ischemic risk was lower in patients receiving DOACs compared with warfarin. Of all DOACs, dabigatran and apixaban reduce the risk of hemorrhagic complications, including gastrointestinal bleeding. At present, two studies that evaluated the safety of DOACs compared to warfarin in AF and LC were published [36, 37].

Until now, the dose of DOACs for preventing ischemic events in patients with LC and AF is not determined. The VALDIG consortium demonstrated that reduced-dose DOACs therapy has the same rates of bleeding complication compared to regular-dose in patients with LC [29]. Moreover, a recent meta-analysis, including patients with LC and AF, demonstrated the same efficacy for the reduced-dose of DOACs compared with the regular-dose [30]. There are limited data regarding the efficacy of the reduced-dose compared to regular-dose of DOACs in patients with LC. The data reported until now suggests that lower doses of DOACs may be safer and with the same efficacy in patients with LC and short term treatment.

All four DOACs are contraindicated in patients with Child-Pugh class C LC, and these data are according to the US FDA recommendation for DOACs [33]. Even if DOACs significantly reduce the risk of major hemorrhagic events, ischemic stroke, and intracerebral hemorrhage with no statistically significant effect on the risk of digestive tract bleeding compared to warfarin, the dabigatran or apixaban seems to be safer regarding bleeding complications in AF patients with mild to moderate LC. Low-dose DOACs, dabigatran or apixaban could represent a safer alternative for long-term AC in AF patients and LC.

DVT in Patients with Liver Cirrhosis

The incidence of DVT in patients with LC is not lower compared to those without LC [38, 39]. Moreover, the reports including large population cohorts demonstrated that patients with LC had an increased risk of developing VTE [40, 41]. The data were confirmed by a meta-analysis that demonstrated a higher risk of DVT in patients with LC compared to controls [42]. Despite this risk, prophylactic AC for DVT in hospitalized patients with LC is not routinely used [43]. No studies demonstrated, until now, that the prophylactic AC in patients with risk factors for DVT is more harmful in the context of LC and all the cirrhotic patients should receive at least mechanical DVT prophylaxis during hospitalization [44].

Recently, Naqvi *et al* [45] demonstrated that there is no major difference in bleeding rates between DOACs and warfarin groups in patients with LC that received AC for DVT, including patients with Child-Pugh class C LC, although these patients were more prone to bleeding as compared to those with Child-Pugh class A or B LC. A recent meta-analysis clearly demonstrated the efficacy of DOACs as compared to VKAs in patients with LC and DVT [46]. Also, in patients receiving DOACs, it was demonstrated a reduction of DVT progression with no pulmonary embolism. All these pieces of evidence sustain the routine DVT prophylaxis in admitted patients with LC [47]. In patients with low bleeding risk, DOACs could represent a safe method for VTE prophylaxis. For the rest of the patients mechanical DVT prophylaxis should be utilized.

Anticoagulation in COVID-19

Coagulopathy and disseminated intravascular coagulation could represent complications in severe COVID-19 infection. The development of microthrombosis in the small pulmonary vessels could aggravate the respiratory dysfunction. Endothelial dysfunction and secondary antiphospholipidic syndrome development secondary to COVID-19 sepsis are the main factors contributing to thrombotic events, developed in patients with LC [48] and the prophylactic anticoagulant treatment should be included in the therapeutic protocol [49]. The

AC treatment in LC should be based on the evaluation of risk factors as sepsis induced coagulopathy score ≥ 4. Anticoagulant treatment with LMWH (enoxaparin) or DOACs (rivaroxaban 10 mg daily) in Child-Pugh class A cirrhotic could be used in severe COVID-19 cirrhotic patients. In decompensated LC, before starting AC treatment, all the patients should have an endoscopic evaluation in order to diagnose esophageal varices. The prophylactic esophageal band ligation is indicated before AC, although this could postpone the AC [50].

Direct-acting Anticoagulant Treatment in Decompensated Liver Cirrhosis

The indication of AC in patients with compensated LC is completely different compared to decompensated LC. Recently, Mort *et al*, found high rates of hemorrhagic complications in patients with decompensated LC treated with DOACs and this risk was not associated with DOACs dose, LC severity or laboratory parameters, confirming the fact that other factors may be associated with the risk of bleeding, which are not discovered until now [51]. The use of DOACs in decompensated LC is challenging, with low grade available evidence. Sharma *et al*, found that dabigatran has the same efficacy and hemorrhagic risk as VKAs in patients with Budd-Chiari syndrome and decompensated LC [52]. The recent literature does not certainly establish the role of DOACs in the treatment of thrombotic events in decompensated cirrhotic patients, and larger clinical trials are needed to confirm the safety of DOACs in Child-Pugh class A or B LC.

FUTURES CHALLENGES

An ideal AC treatment for thrombotic events in cirrhotic patients is not yet discovered. Although there are no randomized controlled trials to confirm the use of DOACs in LC, these drugs are recommended off label for PVT treatment. All the trials designed to confirm DOACs safety in LC should have end-points mortality, major bleeding complications and decompensation rates.

During the last few years, many advances were made to understand the mechanism of thrombosis in patients with LC even a lot of the puzzle pieces are still missing.

The DOACs could represent an innovative treatment in LC, easy to be administrated, with no need of closely monitoring and with pleiotropic effects including in liver fibrosis development. Moreover, the safer profile of DOACs may reduce the risk of bleeding, particularly, in Child Pugh B class patients maintaining a good level of protection from thrombotic events.

DOACs are indicated for the treatment of acute PVT thrombosis in Child-Pugh class A or B liver cirrhosis patients on the awaiting liver transplant list. These

patients will receive the AC treatment until liver transplantation. For the patients with PVT and no indication for liver transplantation, DOACs are indicated, if acute symptomatic PVT is diagnosed or the patient is diagnosed with AF or VTE. It has to be mentioned that all DOACs are contraindicated in patients with Child-Pugh class C liver cirrhosis.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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CHAPTER 29

Latest Data on the Epidemiology, Pathological Classification, and Staging of the Combined Hepatocellular Carcinoma-Intrahepatic Cholangiocarcinoma

Monica Acalovschi[1,*]

[1] *Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, Romania*

Abstract: Combined hepatocellular carcinoma–intrahepatic cholangiocarcinoma (cHCC–CCA) is a primary liver cancer with features of both hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA). This combined tumor represents 1% of all primary liver cancers, but recent studies have shown its increasing incidence and incidence-based mortality. The risk factors (identifiable in about 30% of the cases) are similar to those of HCC and CCA: cholestatic liver diseases, hepatobiliary flukes, toxins, liver cirrhosis of any etiology, and metabolic diseases such as obesity and diabetes mellitus. The first pathological classifications of cHCC-CCA described three types of tumors: collision, transition and intermediate tumors. Intermediate tumors develop from a cell intermediate between the hepatocyte and biliary epithelial cell. The 4th WHO classification of digestive system tumors (2010) was the first one to report cHCC-CCA as a distinct entity, with two main subtypes: classical type and cHCC-CCA with stem-cell features. The collision type was no longer accepted. In the 5th WHO classification (2019), the tumors of the subtype with stem cell features were re-categorized as either HCC or iCCA. Due to the cHCC-CCA mixture of phenotype characteristics, the staging criteria have been also controversial. Presently, the cHCC-CCA tumors are staged by a similar algorithm as for iCCA: the TNM staging of HCC is used for clinical applications and prognosis, and the SEER staging is used for epidemiological studies. The growing interest in molecular research, genetic biomarkers identification, diagnosis and staging of these combined tumors will eventually lead to the development of effective therapeutical approaches.

Keywords: Combined tumor, Epidemiology, Hepatocellular carcinoma, Intrahepatic cholangiocarcinoma, Pathology, Tumor staging.

[*] **Corresponding author Monica Acalovschi:** Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, Romania; Tel. +40 264595649, Mobile: +40 722559970; E-mail: monacal@umfcluj.ro

Ioan Sporea and Alina Popescu (Eds.)

INTRODUCTION

Combined (mixed) hepatocellular carcinoma–intrahepatic cholangiocarcinoma (cHCC–CCA) is a rare primary liver carcinoma, an independent entity sharing features of both hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA). The cHCC-CCA is an aggressive disease, with increasing incidence and a poor prognosis. Due to its rarity, the clinical, diagnostic, therapeutic, and prognostic characteristics of cHCC-CCA have not been entirely defined and are under constant research, as for all other types of cholangiocarcinomas (CCAs). The interest in this heterogeneous class of tumors, difficult to be diagnosed and with limited therapeutic options available, has been proved by the numerous publications in recent years, including the recent Expert European Consensus Statement (Cholangiocarcinoma 2020: the next horizon in mechanisms and management) [1]. Here we will review some of the topics related to cHCC-CCA.

Epidemiology

Epidemiology of Cholangiocarcinomas

Cholangiocarcinomas (CCAs) are a group of malignancies with pathologic features of biliary tract differentiation. They have a heterogeneous anatomical location and pathology. Intrahepatic CCA (iCCA) arises from small intrahepatic bile ducts (above the second-order bile ducts), while perihilar (pCCA) and distal (dCCA) tumors arise from extrahepatic and large intrahepatic ducts, being anatomically extrahepatic CCAs. The three entities have distinct epidemiology, pathogenesis and management requirements. Presently, there is a growing interest in establishing the prevalence, risk factors, diagnosis and staging of these tumors, and identifying better therapeutic options.

Extrahepatic CCAs represent the most common type of CCA accounting for more than 80% of cases. Their incidence has remained stable or slightly declined during the past decades. Conversely, the iCCA, the second most frequent primary hepatic malignancy, has an increasing prevalence. The data of the Surveillance, Epidemiology, and End Results (SEER) registry for the interval 1973 to 2012 demonstrated an increasing iCCA incidence, and a stable incidence of extrahepatic CCAs [2]. Intrahepatic CCA may frequently be misdiagnosed as the metastasis of a cancer of unknown primary (CUP), therefore the authors analyzed in parallel the CUPs incidence. They observed a dramatic decrease during this time interval and suggested that improved clinical distinction between the two entities might have contributed to the apparent increase in iCCA incidence. Using the data of the SEER registry, Petrick *et al*. [3] evaluated the incidence of iCCA and HCC for a period of 25 years (1992 – 2016). They found increasing rates for

iCCA, due to better investigation possibilities, and an overall increased incidence of CCAs.

The most recent data comes from a systematic review and meta-analysis of 53 epidemiological studies published both in Western and Asian countries between 2008 and 2019 [4]. The incidence of primary liver cancers increased during this interval with an annual percentage change (APC) of +2.6 for HCC and a higher APC (+4.3) for iCCA. The increase occurred mainly in Western countries, whereas trends decreased in the Asian region, although still remaining high.

Epidemiology of the Combined HCC-CCA

Intrahepatic CCA accounts for about 15% of all the primary hepatic malignancies [5]. The cells-of-origin of iCCAs are cholangiocytes, peribiliary glands and hepatic stem/progenitor cells. The hepatic progenitor cells have the potential to differentiate into either hepatocytes or cholangiocytes, depending on the damaged cell population, and represent the origin of cHCC-CCA. Intrahepatic CCA shows several histological variants (conventional, *i.e*. large-duct type and small-duct type, cholangiolocarcinoma and rare variants). The small-duct type and cholangiolocarcinoma occur more often in chronic viral liver disease and cirrhosis.

The cHCC-CCA is a very rare tumor, which shows features of both hepatocellular and biliary epithelial differentiation. The presence of cholangiocarcinoma elements in the tumor could be confirmed with cytokeratin 19 (CK19) and cytokeratin 7 (CK7) staining by immunohistochemistry. Generally, reports on these tumors were published decades ago mostly as small patients series or case reports. The first analysis of the prevalence of cHCC-CCAs in a series of patients with primary liver cancers was published by Allen and Lisa in 1949 [6], who found a prevalence of 14.2%. Later studies indicated a lower incidence. Taguchi *et al*. [7] mentioned a prevalence of 6.3% of the combined tumors among primary liver cancers.

In a SEER registry (1973-2003) comprising 22,583 patients with intrahepatic tumors, 282 patients had combined tumors (1%), 2,935 (13%) had iCCA and the remainder (85.7%) had HCC. In this study, the combined tumors had the poorest prognosis compared with the other tumors and the authors concluded that, when deciding therapy, “the combined tumors should be considered neither HCC nor CCA” [8]. A 1% prevalence of the combined tumors was also found by Berquist *et al*. [9], who retrospectively reviewed a population of 106,103 patients registered with primary liver cancers in another database, the US National Cancer Data Base (NCBD) (1998-2011). Most patients had HCC (90,499 - 85%), 14,463 (14%) had iCCA and 1,141 (1%) had combined tumors.

While keeping the proportion of 1% among primary liver cancers, the cHCC-CCA had a steadily increasing incidence, from 0.26 per 1,000,000 individuals in the year 2000 to 0.59 per 1,000,000 individuals in the year 2014 in the SEER database [10]. The derived APC was 3.84%, and more alarming was a sustained increase (APC 4.59%) of the cHCC-CCA incidence-based mortality during this interval.

Risk Factors for Intrahepatic CCA and Combined HCC-CCA

Many risk factors are common for all CCAs, independent of their site in the biliary tract (Table **1**). Most of them are associated with inflammation and cholestasis. Intrahepatic CCA also shares some risk factors with HCC, such as hepatitis B and C infection, non-alcoholic fatty liver disease, alcohol consumption, liver cirrhosis of any etiology, type 2 diabetes mellitus and obesity [11, 12]. In spite of the long list of risk factors, most CCAs arise in the absence of any predisposing risk factors, especially in Western countries.

Table 1. Risk factors for intrahepatic cholangiocarcinoma.

Cholestatic Liver Diseases	**Primary Sclerosing Cholangitis**
-	Fibropolycystic liver disease
-	Congenital hepatic fibrosis
-	Caroli disease
-	Choledochal cysts
Liver cirrhosis	Any etiology, including NAFLD
Biliary lithiasis	Hepatolithiasis
Infections	Hepatobiliary flukes: *Opisthorchis viverrini, Clonorchis sinensis*
-	Hepatitis B, C
-	Recurrent pyogenic cholangitis
Inflammatory disorders	Inflammatory bowel disease
-	Thyrotoxicosis
Toxins	Alcohol
-	Tobacco
-	Thorotrast
-	Chemical toxins – dioxins, vinyl chloride, nitrosamines
Metabolic diseases	Diabetes mellitus
-	Obesity
-	Non-alcoholic fatty liver disease (NAFLD)

A meta-analysis of the studies performed both in countries of high and low prevalence of hepatobiliary cancers showed a 10-20-fold increase in the ratio to develop iCCA for patients with cirrhosis of any etiology relative to patients without cirrhosis [13]. But in contrast to HCC, iCCA more often develops in a non-cirrhotic liver.

The prevalence of some of the common risk factors for HCC and iCCA is presently in transition. Chronic HBV and HCV infections are declining in many regions due to the public health measures. HBV infection is preventable by vaccination, and a long-term viral suppression could be achieved in patients with HBV-hepatitis. HCV has been removed from the blood supplies, and HCV infection is largely curable with DAA therapies.

The incidence of primary sclerosing cholangitis (PSC), an important risk factor for CCAs in Western countries, did not significantly change during the last few decades [14]. The AGA Best Practice Advices do not recommend surveillance for CCA in PSC patients with small-duct PSCs or those younger than age 20. However, they recommend surveillance for HCC by ultrasound, computed tomography, or magnetic resonance imaging, with or without α-fetoprotein, every 6 months, in all PSC patients having liver cirrhosis [15].

In the context of a better control of viral B and C infections, the increased risk of HCC and incident iCCA should probably be related to the worldwide increase in the prevalence of metabolic disorders (obesity, type II diabetes mellitus) and NAFLD. These factors have been shown to contribute to the increasing rates of liver cancer in many lower-risk countries. The NAFLD-related cirrhosis has a 1%-3% HCC risk per year, and has been included in the updated AGA Clinical Practice recommendations [16]. The growing obesity and diabetes epidemics suggest that primary prevention of obesity and diabetes treatment will be essential for reducing the incidence of liver cancer [17].

Considering the common risk factors for both iCCA and HCC, it could be postulated that a combined HCC-CCA tumor has similar risk factors. From a clinical perspective, surveillance strategies for preventing HCC might also improve the outcomes for iCCA and cHCC-CCA. However, because in less than 30% of the CCAs, a specific factor could be identified, there is no chance for a successful targeted surveillance in a population with predisposing conditions.

Pathological Classification of cHCC-CCA

The first pathological classification was that of Allen &Lisa [6], followed by Goodman *et al*. [18] and Taguchi *et al*. [7]. Three types of pathological aspect of the cHCC-CCA tumors were identified, with small changes in the description:

- Type I (collision tumors) (type A in the Allen &Lisa classification): "coincidental" occurrence in the same liver: HCC and iCCA develop independently and separately;
- Type II (transition tumors) (type B in Allen &Lisa classification), the largest group: contiguous tumors with intermediate differentiation and visible transition between HCC and iCCA: one tumor appears first, then it changes in the other type, completely, or incompletely;
- Type III (intermediate tumors) (type C in Allen &Lisa classification): the tumor develops from a cell intermediate between the hepatocyte and biliary epithelial cell and the cancer cells are able to be evaluated as either HCC or iCCA (progenitor cell / HPC / bipotential hepatic stem cells, CK7 + and CK19 +) and differentiate with both components. The tumor cells are almost indistinguishable from iCCA or HCC.

In 1989, the Liver Cancer Study Group of Japan classified cHCC-CCA into the same three subgroups: double cancer, combined type, and mixed type [19].

Xue *et al*. recently observed in their series of 133 patients with combined HCC-CCA tumors divided according to Allen &Lisa classification, that type B and C tumors are also distinct tumor subtypes in terms of their genetic expression: they found that type B tumors resemble more with iCCA, and type C tumors resemble more with HCC [20].

The 4th WHO classification (2010) first reported cHCC-CCA as a distinct entity and identified two main subtypes: the "classical" type, and the cHCC-CCA with stem-cell features. Three variants were described for the cHCC-CCA with stem-cell features: a "typical" subtype, an "intermediate cell" subtype, and a "cholangiolocellular" subtype. The term collision tumors (type A and type 1, respectively, in the first classifications, *i.e*. different foci in the same liver) was no longer accepted.

In the 5th WHO classification (2019) of tumors of the digestive system (Table **2**), the "classical" form of cHCC-CCA persisted. The cell of origin of the classical cHCC-CCA would be a single form of bipotent hepatic progenitor cell capable of final differentiation into either hepatocytes or cholangiocytes. However, the subtype cHCC-CCA with stem cell features, present in the 4th WHO classification, was no longer accepted. Some of the cHCC-CCA subtypes with stem cell features have been recategorized as either HCCs or iCCAs. Distinctive diagnostic terms for intermediate cell carcinomas and cholangiolocarcinomas (previous cholangiolocellular carcinoma subtype) were recommended [21].

Table 2. 5th WHO histological classification system of combined primary liver cancers (2019) (modified after Kim TH *et al*. 2020) [22].

Descriptive Classification	5th WHO Classification System (2019)
HCC	HCC
Classical cHCC-CCA	cHCC-CCA
cHCC-CCA with "typical" stem/ progenitor cell features	*Omitted
cHCC-CCA with "intermediate" stem/progenitor cell features	Intermediate cell carcinoma - No strong consensus as to whether or histological pattern of cHCC-CCA
Cholangiolo-predominant carcinomas with HCC and ICC	cHCC-CCA-CLC - Categorized under cHCC-CCA when HCC components are present
Cholangiolo-predominant carcinomas with ICC component	cCCA-CLC - Categorized under ICC when CLC is admixed with conventional ICC
Classic CLC (> 80% of tumor consists of CLC)	CLC - Categorized under ICC when CLC is present alone
ICC	ICC

*present in the 4th WHO classification
Abbrev. ICC: intrahepatic cholangiocarcinoma; HCC: hepatocellular carcinoma; cHCC-CCA: combined HCC-CCA; CLC: cholangiolocarcinoma.

The definition and diagnosis of the cHCC-CCA could be established as hepatocytic or cholangiocytic differentiation within the same tumor by histopathological examination using hematoxylin and eosin (H&E) staining [22]. Within the cHCC-CCA tumor, the CCA component shows mucin-producing glandular structures within stroma, whereas HCC differentiation is characterized by Mallory-Denk bodies, bile canaliculi and a trabecular growth pattern. However, there are still no objective criteria for evaluating the amounts of specific components required for pathological diagnosis in the patients with biphenotyping tumors, and no specific cut-off values establishing a pathological diagnosis. And this might affect the accuracy of the systematic studies of primary liver cancers other than HCC and iCCA [21].

The *Guidelines for the diagnosis and management of intrahepatic cholangiocarcinoma* recommend specific immunostaining for detecting markers of HCC or progenitor cells for distinguishing cHCC-CCA from iCCA tumors only, if this information will change management" (Recommendation B1) [23].

Staging of the Combined HCC-CCA

Clinical Diagnosis and Imaging Characteristics

The clinical diagnosis of cHCC-CCA in an early stage is difficult due to the anatomical location and growth patterns as well as the lack of specific symptoms. Most tumors are diagnosed by chance as an intrahepatic mass tumor. Preoperative biopsy of a nodule could be misleading, because it could reveal mainly one of the components of the tumor (CCA or HCC). The tumors could be diagnosed only in advanced stage disease based on clinical symptoms, often similar for both HCC and CCA. The Cancer Antigen 19–9 (CA 19–9) is the primary serum biomarker used in the diagnosis of CCAs [24]. However, a smaller proportion of patients with cHCC-CCA tumors have CA 19-9 elevation than patients with iCCA, and this might help in the distinction of tumors preoperatively [9].

More often, iCCA and cHCC-CCA have been detected in the liver during screening, being suspected to be HCC in patients with chronic hepatitis or liver cirrhosis. The final diagnosis was based on the pathological examination of the surgical specimens removed by liver resection or transplantation.

The imaging characteristics could be preoperatively suggestive for diagnosis. CT is considered the standard imaging method for the preoperative assessment of iCCA. MRI has similar accuracy to CT for diagnosis and staging. The most frequent imaging patterns displayed by iCCA on both CT and MRI are arterial peripheral rim enhancement with progressive homogeneous contrast agent uptake until the delayed or stable uptake during late dynamic phases. When gadoxetic acid is used, the washout should be read in the portal phase instead of in delayed phases to prevent misclassification between HCC and iCCA in a cirrhotic liver. CT and MRI have comparable performance in the detection of primary and satellite iCCA lesions, but CT imaging is superior for the detection of vascular enhancement and, thus, assessment of resectability [23, 24]. The *Guidelines for the diagnosis and management of intrahepatic cholangiocarcinoma* consider that assessment of resectability as well as of arterial and venous invasion, essential for tumor staging, are best performed using CT and/or MRI (Recommendation A1) [23].

More controversial is the use of CEUS, particularly in the setting of underlying chronic liver disease: iCCA exhibits, similar to HCC, a homogeneous arterial hyperenhancement followed by venous washout in almost 50% of patients. An ultrasonographic case-control study confirmed that some CEUS imaging features of cHCC-CCA, HCC, and iCCA overlap. In these cases, the combination of CEUS features with tumor markers (simultaneous elevation of α-fetoprotein and CA 19-9) is helpful in diagnosing the combined tumors [25].

Tumor Staging

Different staging systems have been proposed in the last decades to categorize either HCC or iCCA. Most of these staging systems are meant to provide both prognostic information and therapeutic guidance: the Barcelona Clinic Liver Cancer (BCLC) algorithm, the Hong Kong Liver Cancer Staging System for HCC, and the TNM classification and other staging systems in the case of iCCA [26]. Until the 7th edition of the AJCC/UICCA staging, there was no distinct staging for iCCA. The further 8th edition changed some of the TNM categories in iCCA, trying to more accurately stratify prognosis in order to better guide treatment decisions [27].

Do the existing staging systems for primary liver cancer apply to cHCC-CCA? The staging criteria for cHCC-CCA are controversial, due to their mixture of phenotype characteristics, and therefore it is not clear how they should be treated. Until very recently, no staging systems specific for cHCC-CCA were proposed. Combined HCC-CCA is presently staged by TNM and by SEER staging. Both staging systems are based for cHCC-CCA on the same staging algorithm as for iCCA (Table **3**). The TNM for HCC staging system should be prioritized for clinical applications in predicting cHCC-CCA prognosis [9, 28]. The epidemiological studies to date are using the SEER Program of the National Cancer Institute: localized cancer is limited to the anatomical site of origin without spread, regional cancer is limited to the nearby draining lymph nodes, tissues or organs by direct extension, and distant cancer has spread to distant non-continuous parts of the body [10, 22].

Table 3. TNM *versus* SEER staging of combined HCC-CCA.

TNM stage	Tumor	Node	Metastasis	SEER General Staging System
IA	T1a	N0	M0	Localized
IB	T1b	N0	M0	-
II	2	N0	M0	-
-	-	-	-	Regional
IIIA	T3	N0	M0	-
IIIB	T4	N0	M0	-
-	Any	N1	M0	-
IV	Any	Any	M1	Distant

TNM classification: T1 solitary tumor with no vascular invasion (T1a ≤ 5 cm and T1b >5 cm), T2- a solitary tumor with vascular invasion or multiple primary

tumors (irrespective of vascular invasion), T3 - the primary tumor perforates the visceral peritoneum and, T4 - tumor involves local extrahepatic structures by direct invasion. N1 - regional lymph node metastases, M1 - distant metastatic disease. In the SEER general staging system for tumors such as cHCC-ICC, some TNM stage II tumors may be classified as localized and others as regional [22].

Combined HCC-CCA is a rare primary liver carcinoma showing differentiation toward hepatocellular and cholangiocellular carcinoma and demonstrating a great heterogeneity in terms of morphopathology. Epidemiological data indicates an increasing incidence of all primary liver cancers, including the combined tumors. Progress in molecular research, genetic biomarkers identification, diagnosis and staging represents the aspiration for the development of efficient approaches for treating these tumors.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 30

Endoscopic Therapy in Cholangiocarcinoma

Marcel Tanțău[1,*]

[1] *Department of Gastroenterology, 3rd Medical Clinic, Cluj-Napoca, Romania*

Abstract: Cholangiocarcinoma is an aggressive tumor with a poor prognosis. In its early stages, the diagnosis is difficult and mostly incidental, for example during routine abdominal ultrasound we may see some indirect signs like biliary tree dilatation and rarely an intrabiliary three hypoechogenic lesion (extrahepatic cholangiocarcinoma) or focal hypoechogenic mass (intrahepatic cholangiocarcinoma).

The prognosis of the patients with metastatic and advanced unresectable extrahepatic cholangiocarcinoma is very poor. More than 50% of patients with jaundice are inoperable at the time of the first diagnosis.

The development of new minimally invasive techniques provides these patients a chance to symptoms relief, symptoms that sometimes impair the treatment (like jaundice), and a better quality of life.

Endoscopic treatment in patients with obstructive jaundice ensures bile duct drainage in preoperative or palliative settings. Relief of symptoms (pain, pruritus, jaundice) and improvement in quality of life are the aims of palliative therapy. Stent implantation by endoscopic retrograde cholangiopancreatography is generally preferred for long-term palliation. There is a vast variety of plastic and metal stents, covered or uncovered. The stent choice depends on the expected length of survival, quality of life, costs, and physician expertise.

Keywords: Biliary stents, Cholangiocarcinoma, Cholangioscopy, Endoscopic drainage, Endoscopic retrograde colangiopancreatography.

INTRODUCTION

Cholangiocarcinomas (CCAs) have a very high mortality rate worldwide [1]. Due to clinical asymptomatic behavior in the early stages in most of the cases, the lack of a standardized protocol for screening for early-stage disease and the limitations of using CA19-9 as a cancer marker, the diagnosis is delayed in most of the patients [1]. The ability to achieve a definite cytopathological or histopathological

* **Corresponding author Marcel Tanțău:** Department of Gastroenterology, 3th Medical Clinic, 19-21 Croitorilor Street,400162, Cluj-Napoca, Romania; E-mail: matantau@gmail.com

Ioan Sporea and Alina Popescu (Eds.)

diagnosis in patients with suspected CCA remains at 26–80% [1 - 4]. CCAs are divided into 3 types: intrahepatic CCAs (iCCAs), distal CCAs (dCCAs) and perihilar CCAs (pCCCAs) or Klatskin tumors. The majority of CCAs are perihilar CCAs (60-75% of cases). Distal CCAs are present in 15%to 25% of cases and intrahepatic CCAs account for 5% to 15% of cases [3, 4]. Magnetic resonance imaging (MRI) plus magnetic resonance cholangiopancreatography (MRCP) is the preferred imaging modality as it can assess resectability and tumor extent with a high accuracy [3, 4]. Endoscopic ultrasound (EUS) and fine needle aspiration guided by EUS is a useful technique in diagnosis and staging of CCAs (Figs. **1** - **3**) and should be always taken into consideration for CCAs clinical management.

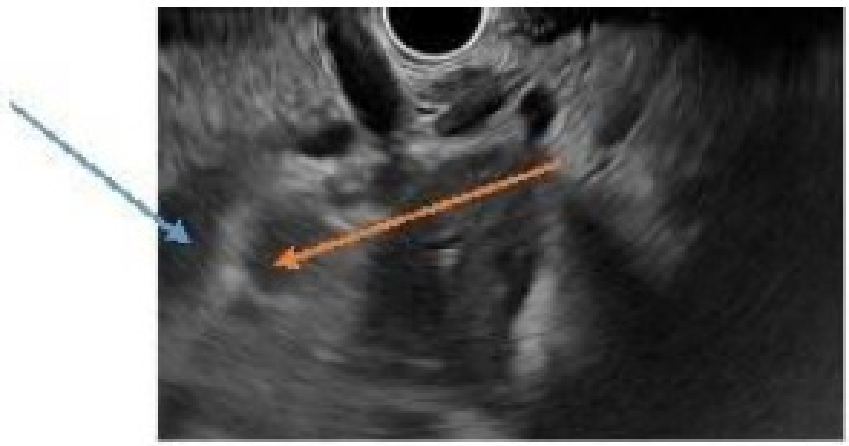

Fig. (1). Upper endoscopic ultrasound. Klatskin tumor. A hypoechoic tumoral mass (blue arrow) at the level of hepatic hilum can be seen. In the center of the tumor was placed a biliary stent (orange arrow).

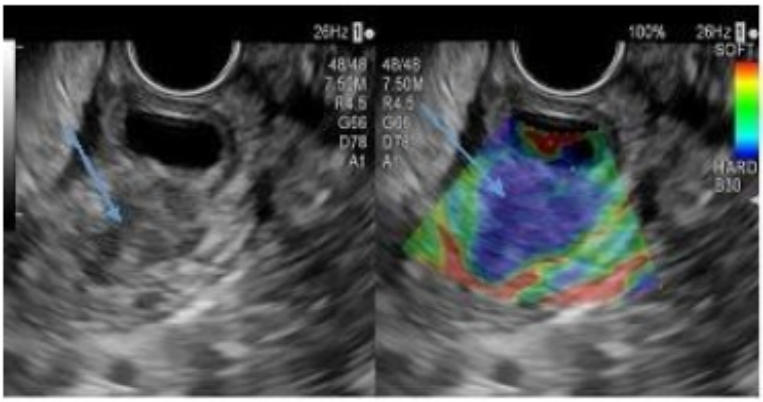

Fig. (2). Upper endoscopic ultrasound. Distal cholangiocarcinoma. On the left, a dilated distal common bile duct and a hypoechoic tumoral mass (blue arrow) can be seen. On the right is an image of the elastography examination with the tumoral mass colored in blue (hard tissue) (blue arrow).

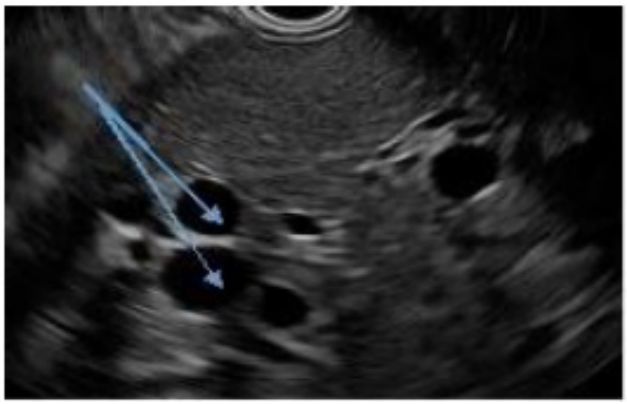

Fig. (3). Upper endoscopic ultrasound. The liver with dilated intrahepatic biliary ducts (blue arrows).

Also, in patients with obstructive jaundice, intraductal ultrasonography may be useful for the assessment of bile duct strictures and local tumor staging [5]. Peroralcholangioscopy (POC) allowing direct visualization of the biliary tract with targeted biopsy of suspicious lesions has shown to be a useful diagnostic procedure in the evaluation of biliary strictures (Figs. **4** and **5**).

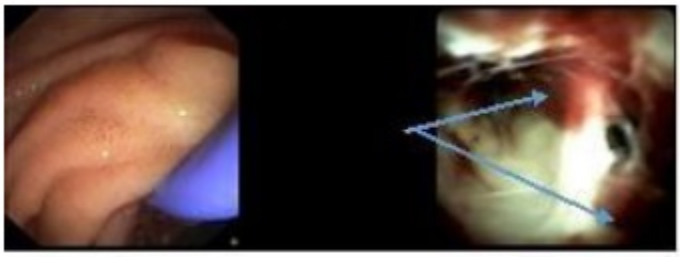

Fig. (4). On the left-endoscopic view, the Vater ampulla is accessed by cholangioscopy (in blue). On the right-cholangioscopic view with a tumoral mass at the level of the hilum (Klatskin tumor) (blue arrows).

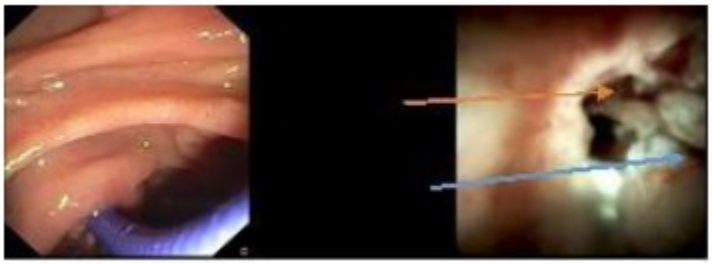

Fig. (5). On the left- endoscopic view, the Vater ampulla is accessed by cholangioscopy (in bleu). On the right-cholangioscopic view with a tumoral mass at the level of the hilium (Klatskin tumor)(blue arrow), the right biliary duct is invaded (orange arrow).

Surgery is the only curative treatment for both intrahepatic and extrahepatic CCA, but it is possible in only a minority of the patients. The goal is R0 resection. More than fifty percent of the patients with jaundice are inoperable at the time of first diagnosis. Locally advanced, unresectable CCA includes patients with macroscopic residual disease following resection, categorically unresectable disease at presentation or locally recurrent disease after potentially curative treatment. The prognosis of these patients is poor with a median survival time of < 6 months [6]. Relief of symptoms (pain, pruritus, jaundice) and improvement in quality of life are the aims of palliative therapy.

Additional treatment measures in CCA may include the following: stenting, radiofrequency ablation (RFA), photodynamic therapy (PDT), radiation therapy, chemotherapy. Stents can be placed *via* endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous transhepatic cholangiography (PTC) to relieve the biliary obstruction. Stenting may relieve pruritus and improve the quality of life. Using endoscopic retrograde cholangiopancreatography (ERCP) unilateral or bilateral plastic or metallic stents can be provided. RFA and PDT are effective in restoring biliary drainage and improving the quality of life in patients with nonresectable disseminated cholangiocarcinomas. Local radiotherapy combined with metallic stent placement is a new and efficient method in advanced cholangiocarcinoma.

Palliation of Obstructive Jaundice

Endoscopic treatment of CCA with obstructive jaundice ensures bile duct drainage in preoperative or palliative settings [7]. Endoscopic procedures are the preferred palliative treatment options for patients with advanced or unresectable CCA. In patients with advanced hilar CCA, endoscopic biliary drainage *via* ERCP is more difficult than those with distal CCA [1]. If the transpapillary approach failed, other procedures can be considered: percutaneous transhepatic biliary drainage, endoscopic ultrasound-guided biliary drainage, especially hepaticogastrostomy or locoregional therapies including trans-luminal photodynamic therapy and radiofrequency ablation [1].

Preoperative Biliary Drainage

There is some controversy in literature, whether preoperative biliary drainage should be accomplished prior to laparotomy for patients with obstructive jaundice [8 - 10]. In distal CCA, preoperative bile duct drainage is not always necessary and might be associated with an increased risk of cholangitis and postoperative infectious complications [7, 11].

Acute cholangitis, sepsis, bilirubin > 10 mg/dl, scheduled neoadjuvant therapy, the need for extensive hepatic resection are the indications for preoperative biliary drainage. The goal is to reduce peri- and postoperative complications [7, 12]. Cholestasis, liver dysfunction and biliary cirrhosis can develop rapidly with unrelieved obstruction and may influence postoperative morbidity and mortality after surgery [8, 13]. The definitive operation is deferring until bilirubin levels are less than 2 to 3 mg/dl.

There are different data regarding the benefits of preoperative biliary drainage in jaundiced patients with perihilar CCA. A meta-analysis of 11 studies showed no difference in the death rate or length of postoperative stay with and without preoperative biliary decompression, overall postoperative complication rate and infectious complication rates were adversely affected by preoperative drainage as compared with surgery without preoperative biliary drainage [13]. In cases of percutaneous transhepatic drainage, some studies reported the catheter tract recurrence rates up to 6% [11, 14, 15], the median time of recurrence being months.

Palliative Biliary Drainage

The relief of symptoms (pain, pruritus, jaundice) and improvement in the quality of life are the goals of palliative therapy. Radiotherapy, photodynamic therapy, local ablation and embolization are the nonsurgical local therapy which can prolong the time to local failure (macroscopically positive margins only) or palliate local symptoms, pain or jaundice (unresectable or recurrent disease). For unresectable CCA, the guidelines recommend endoscopic bile duct drainage as the first approach.

Stenting

Stent implantation by ERCP should be the standard procedure (Figs. **6** and **7**). Placement of a stent is generally preferred for long-term palliation. It has similar rates of successful palliation and survival and less morbidity compared with the surgical approach [16, 17].

The extent of decompression that is necessary to restore the sufficient bile flow while avoiding the risk of bacterial cholangitis, the optimal approach to placement of the stents, and the use of plastic or metal uncovered/covered stents are the major issues of biliary endoscopic stenting [1, 3, 18]. The goal of palliative drainage is to drain more the half of the biliary tree according to the Asia Pacific consensus, although it has been showed that jaundice may be clinically improved if only a quarter of the liver is drained [19]. Also, target stenting using previous superior imaging methods is preferred to be performed by a financial point of

view [19]. In cases of cholangitis, drainage of all suspected or infected intrahepatic segmental branches should be performed [1, 20]. In complex and difficult cases, a multimodality biliary drainage (transpapillary drainage in combination with percutaneous transhepatic biliary drainage) should be considered. Rendezvous technique, anterograde percutaneous transhepatic biliary drainage (PTBD) and transluminal stenting through stomach, duodenum or jejunum walls are the possible procedures using endoscopic ultrasound drainage (EUS-BD) in these cases. This approach can be performed even when a passage of a wire through a biliary stricture is not possible [21]. The technical success rate of PTBD is 60% -90%, morbidity rate 18%- 67% [22]. In some difficult cases, an external drainage may be required and the life quality of the patient may be decreased. EUS-BD technical success varies from 70% to 100% and the rate of possible complications goes up to 77% [23]. The technical success rates are similar in most studies, but a higher incidence of complications for PTBD than EUS-BD.

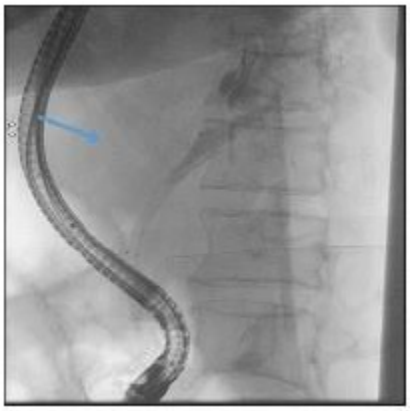

Fig. (6). Radiologic view. A metallic stent for a hilar cholangiocarcinoma can be seen (blue arrow). The stent is partially open.

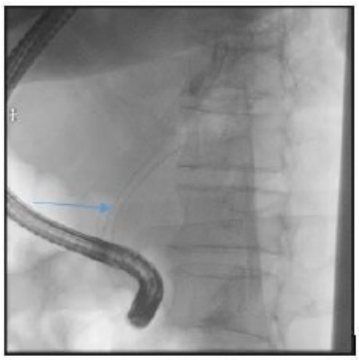

Fig. (7). Radiologic view. A metallic stent for a hilar cholangiocarcinoma can be seen (blue arrow). The stent is completed openly.

Regarding the biliary drainage *via* ERCP, in most cases, unilateral stent placement will be adequate because only 25 to 30% of the liver needs to be drained to relieve jaundice [29]. However, unilateral drainage alone may not relieve jaundice completely and may increase the risk of cholangitis due to contrast medium injection into the undrained bile ducts [18]. Unilateral stenting is technically easier and less expensive than bilateral stenting, with reintervention for stent dysfunction also being considerably easier. Previous studies have demonstrated bilateral stenting to be associated with longer stent patency as compared to unilateral stenting [30].

Some studies have shown a higher survival in patients with bilateral biliary drainage with a longer stent patency *versus* unilateral biliary stenting [31, 32] and no significant differences between unilateral and bilateral self-expandable metallic stents (SEMS) regarding the technical success or complications [32]. Another retrospective study [33] showed repeat endoscopic biliary drainage for stent occlusion was required more frequently in the unilateral plastic stent than in bilateral plastic stents (80.9% *vs* 34.2%, $P < 0.001$) as well as a significant difference in the cumulative stent patency period between unilateral and bilateral PS ($P = 0.0004$, hazard ratio [HR] = 2.24) as well as between unilateral and bilateral SEMS ($P < 0.0001$, HR = 3.69). These results highlight the superiority of bilateral stenting. However, several study results have similarly supported the superiority of unilateral stenting [30, 34, 35].

Endoscopic biliary drainage can be performed using plastic or expandable metal stents (SEMS). There are a large variety of plastic and metal stents, covered or uncovered. While some studies showed benefits of metallic stents regarding the successful drainage and early complication rate, stent patency and survival [36 - 40], a systematic review concluded that neither stent type offered a survival advantage [41]. The decision to use one *versus* another should be guided by the expected length of survival, quality of life, costs, and physician expertise. Usually, SEMS should be considered for patients with a life expectancy of >3 months [18]. Plastic (polyethylene) stents are inexpensive, effective, can be easily removed or exchanged. The major disadvantage is a higher rate of occlusion by sludge and/or bacterial biofilm with cholangitis development and the necessity of multiple ERCPs. Instead, metal stents have a longer patency (approximately 8 to 12 *versus* 2 to 5 months) [18], higher costs and may not be removable. The high occlusion rate of plastic stents (average 42%) can be reduced by changing the stents every three to six months. Another way is to wait for a complication before changing the stent since many patients will die before the stents will obstruct. The preferred approach for patients who are expected to live beyond a few months is to replace the plastic stent with a metal one as soon as it is feasible [18]. Several trials showed that patency rates are not higher for covered stents, despite showing

significantly less tumor ingrowth. Tumor overgrowth and stent obstruction by debris and biliary sludge may be the cause [18]. Usually, in distal malignant biliary obstruction, the uncovered metal stents are used in patients with an intact gallbladder. For patients who have undergone prior cholecystectomy, the choice of a covered *versus* uncovered stent is individualized given the location and geometry of the stenosis. Patients with extrinsic compression may be adequately treated with an uncovered stent, while those with intrinsic and/or papillary tumors may benefit from a covered stent in an attempt to minimize tumor ingrowth. Covered metal stents are only used for distal biliary strictures because, with hilar tumors, deployment may inadvertently result in the occlusion of a major hepatic duct [18].

The stent-in-stent technique (Y stenting) and the side-by-side technique are other two endoscopic techniques for biliary drainage in CCA. By using the Y-stent technique, studies have demonstrated an 86.7% technical success rate and a 100% functional success rate regardless of the stent type [20]. For side-by-side stenting technique in hilar CCA, Lee *et al.* [29] reported a 91% technical success rate and a 100% functional success rate with no statistically significant difference between stent patency and median survival of the 8-mm and 10-mm groups. Another study [42] reported the use of a small (6-mm) introducer SEMS for bilateral biliary stenting for both techniques with no difference in terms of technical success, procedural time, rate of stent revision, and revision success rate.

The reported rate of stent dysfunction following hilar CCA biliary drainage was 45–57% due to tumor in-growth, tumor overgrowth, or stent migration. Given the fact that SEMS may be successfully revised in the majority of cases and that the second SEMS has a higher patency compared with plastic stents [43], it seems that SEMS is the best choice in cases of SEMS dysfunction.

Guidelines recommend prophylactic antibiotics in patients with placed plastic or metal stent for long-term palliation of obstructive jaundice after the first episode of cholangitis [1, 21]. In 5-10% of cases, endoscopic biliary drainage by ERCP will fail or will be incomplete. In these cases, multi-modality drainage should be considered [1].

Percutaneous *versus* Endoscopic Approach

Several studies have shown a higher rate of successful palliation of jaundice and lower rates of cholangitis in the percutaneous approach rather than the endoscopic approach of biliary drainage in patients with malignant hilar obstruction (proximal CCA/gallbladder cancer) [44 - 46]. Bile leaks and bleeding are more frequent and morbidity and mortality are higher [47]. Percutaneous stents are usually left to open drainage externally to the body, less comfortable for the patient. Thus, an

initial endoscopic attempt at drainage is usually preferred, if possible [18]. Another technique is the combination of ERCP with percutaneous drainage.

Endoscopic Ultrasound-guided Biliary Drainage

The use of endoscopic ultrasound-guided biliary drainage (EUS-BD) is feasible for a left system drainage procedure in patients with advanced CCA who failed transpapillary drainage [48, 49]. For hilar CCA, the procedure of choice is EUS-guided hepaticogastrostomy, which allows left system access only. EUS-BD is performed in the same session, allows immediate internal biliary drainage. It is less invasive given that it affords a more accurate control as well as more access sites to the bile duct than the classical alternatives of PTBD or surgery [50]. EUS-guided hepaticogastrostomy (HG) was first reported in a Billroth II patient with unresectable pancreatic cancer and failed ERCP due to tumor infiltration of the papilla [50]. After the identification of the biliary duct, the technique goes on with puncture and dilatation by EUS and a stent is placed across the bile duct into the digestive lumen. Literature data showed a 94% success rate and a 90.2% success rate. Peritoneal bile leakage and cholangitis are the frequent possible complications [50]. Early migration or the clogging of the plastic stents may lead to cholangitis [51, 52]. Bile peritonitis and biloma are more frequent in transmural SEMS placement [52]. Only a case of death was reported [53]. However, most of the complications are mild and can be conservatively treated. By combining an uncovered metal stent with a covered metal stent inside, the risk of leakage is minimized. The uncovered stent is initially deployed to provide anchorage and prevent migration. The covered stent is inserted coaxially and dropped in the first stent. In some cases, hepaticogastrostomy may be associated with a metal stent placement [52]. A fully covered SEMS [54] or a double pig-tail stent through the expanded SEMS may be used to prevent stent migration [55]. The advantages of EUS-HG over rendezvous or antegrade stent insertion are particularly relevant in patients with prior duodenal or biliary SEMS who experience recurrent biliary obstruction [56]. In cases of failure of all interventional options, surgical bypass should be considered as a last rescue procedure and typically it is performed only during an unsuccessful attempt at resection or it may be necessary in jaundiced patients in whom stenting is not possible due to tumor location.

CONCLUSIONS

Currently, endoscopic treatment in patients with CCAs and jaundice remains the first choice of biliary duct decompression, either preoperatively or with a palliative purpose. Endoscopic approach and EUS-guided biliary drainage are the preferable methods for jaundice palliation and they may increase the quality of

life and long-term survival in patients with locally advanced, unresectable or recurrent disease.

CONSENT FOR PUBLICATION

Not Applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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CHAPTER 31

Telemedicine in Hepatology, is it Time to Move Forward?

Ion Rogoveanu[1] and **Bogdan Silviu Ungureanu**[1,*]

[1] *Department of Gastroenterology, University of Medicine and Pharmacy of Craiova, Romania*

Abstract: Telemedicine has been suggested as a potential alternative for specific medical situations and has even been embedded in some countries in their medical systems. Due to current time challenges, its involvement might be embraced more rapidly, since medical consultations have become difficult due to the COVID-19 pandemic. Patient's access to medical care might be hampered, thus, telemedicine might offer new opportunities for both the medical system as well as patients. Healthcare technology is under continuous evolution and the medical care is taking part not only with specific therapeutic medical devices, but also with remote medical information and monitoring. Patients suffering from chronic liver disease require personalized management plans according to their clinical and biological disease evolution, thus new alternatives should be considered for isolated locations. This may help us fight new global challenges that may surface in the years to come. In this chapter, we have discussed the current status of telemedicine and its implementation for the various liver diseases over the years.

Keywords: Electronic consultation, Hepatitis C, Liver disease, NAFLD, Store and forward, Telemedicine.

INTRODUCTION

Nowdays, when we feel utterly defenseless in front of the COVID-19 pandemic, the use of special medical care, although strongly associated with improved survival rate in patients with liver pathologies, is not always feasible or in accordance with each individual`s need in protection against the virus. Conventional approach regarding the healthcare services (one-to-one meeting between the patient and the medical personnel) is becoming rather difficult. The subject of telemedicine has not been exploited enough, creating knowledge gaps that were meant to be filled in this short amount of time given by lockdowns worldwide. Telemedicine is an innovative method that offers care remotely, using

* **Corresponding author Bogdan Silviu Ungureanu:** Department of Gastroenterology, University of Medicine and Pharmacy of Craiova, Romania; Tel: 0351 443 500; E-mail: bogdan.ungureanu@umfcv.ro

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different types of electronic communication, with great advantages for all the parties involved, such as physicians, patients or even hospitals or governments.

Defined by the American Telemedicine Association (ATA), telemedicine is the exchange of medical information from one site to another *via* electronic communication to improve a patient`s clinical health status [1]. On the other hand, *TeleHealth* offers a more broadly general concept and includes other forms of devices to communicate and even remote patient monitoring. Because of the pandemic, telemedicine became an indispensable way to provide clinical care, used for the monitoring of patients, self-management plans, treating different conditions and even for educational purposes, limiting the exposure of patients and medical practitioners. This path of care delivery is needed to be embraced and integrated more efficiently in our routine, as new technologies develop, offering a wider range of medical procedures to be made and highlighting the inefficient resource utilization for diverse acts for which telemedicine promises alternatives.

According to a report from 2017, from 15 different leading causes of death in the United States, which were accountable for almost 80% of total deaths, chronic liver diseases and cirrhosis were on the eleventh place, climbing a place in the ranking report compared to 2016 [2]. Responsible for approximately 2 million deaths in one year worldwide [3], liver diseases ask for new strategies regarding medical care, quality of life and survival rate improvement.

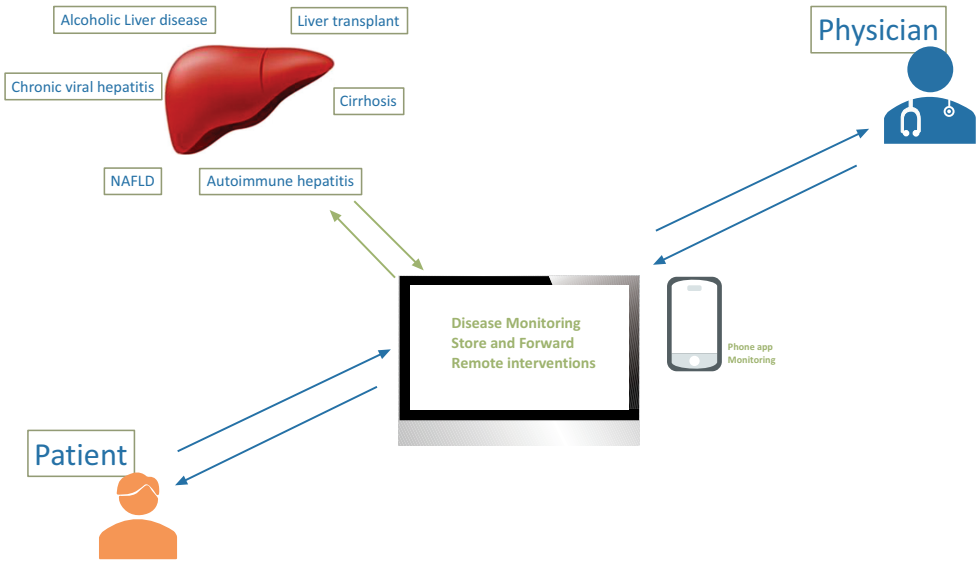

Fig. (1). Telemedicine concept in liver disease.

Electronic Consultation and Monitoring

Electronic consultation, although a term used for decades, has expanded, including a wide variety of means such as video, email, phone calls or even multimedia messages, used both by healthcare workers and by patients, facilitating the medical practice in inconvenient times for a proper traditional consultation. Telemedicine embraces an increase in its use in daily practice as technology improves and as access to a computer or a smartphone became almost universal. A study made by the Pew Research Center`s Internet and American Life Project found that in 2013 almost 91% of adults owned a mobile phone [4]. As patients become more connected to the digital sphere, it feels like a natural service to be addressed, looking for a more comfortable and easy access to a basic need.

HCV

With almost 71 million people suffering from chronic HCV infection, the prevalence remains high, with an estimated 1,75 million new individuals infected annually, highlighting the continuous rise of this disease [5]. Telemedicine has been used for many years for hepatitis C in rural and impracticable populations and one durable example is the ECHO program, Extension for Community Healthcare Outcomes, which targets specialized primary care providers to help develop skills through problem-based learning with video conferences to subspecialty health practitioners. This method is an effective tool to initiate treatment of HCV in incarcerated patients or those who are located in remote regions. The study also shows that there is no difference in SVR in patients who underwent telemedicine consultation or the traditional clinic visit. Adverse situations were estimated as lower in the ECHO program, compared to the on-site visit, 6.9% *versus* 13.7% [6], giving a new perspective regarding the management of hepatocellular carcinoma and cirrhosis. Collaboration with other medical specialties through telemedicine is another point of interest. Ensuring direct antiviral therapies may have adverse drug reactions, especially skin lesions, which may require a rapid dermatology consultation. Involving a teledermatology service would allow a unique collaborative model and may be the starting point for other potential therapies [7].

More studies emphasized that telehealth is as useful in managing HCV therapy as face-to-face consultation and sustained virologic response rates are not different (93% telehepatology *vs* 89% specialty care p=203) [8]. Thus, access to modern therapies may have similar outcomes and serve as valid options alternatives. Another study by Schulz *et al*. [9] suggests that by their telehealth use for HCV treatment, they saved a median of 634 km of patient travel which is definitely a

saving in costs for patient's expense. A program that integrated hepatitis C services (IHCS) in Melbourne Australia focused on providing healthcare services in smaller cities through telehealth consultations. Even though technical difficulties were encountered in less than 10% of consultations, the SVR was rather similar. Moreover, the authors also suggested that in these situations, telehealth has an important role in reducing the carbon footprint by avoiding extensive travelling for an on-site consultation, and this may be extended on a larger scale.

Other institutions such as correction facilities or prisons benefited from HCV treatment through telemedicine [10, 11]. Prisons are known to be major points for HCV infection and also lack of specialized therapies since is difficult to transfer inmates to hospital clinics. Telemedicine surpassed these barriers and improved both communications with prison physicians as well as direct access to the patients. In an observational study performed in Madrid, 163 patients were found positive for HCV infection. The telemedicine consultations were performed once a week in special conditions by a close-up camera and also by accessing laboratory and radiology findings. The SVR obtained were no different than other regions of Spain, and when there were failures in patient monitoring continued for the second course therapy. They also measured the degree of satisfaction, which was considered high, especially since they had no case of reinfection during the program.

Risk population groups, such as opioid abusers may benefit from telemedicine therapy and monitoring if HCV is associated. A pilot study [12] on 45 HCV infected opioid user disorder on methadone were treated with telemedicine-based encounters and despite the fact they were skeptical of the results, after finishing the program they were more confident in following such programs.

Besides using local physicians or nurses, a collaborative practice agreement with pharmacists may serve as a key instrument for HCV therapy in difficult to reach places [13, 14]. Monitoring the "cascade of care" for HCV patients may be done with the help of the pharmacist which can use the telehealth option for additional information. Thus, more patients may benefit from viral therapy and the goals promoted by the World Health Organization to eradicate HCV by 2030 may be achieved.

HBV

Hepatitis B is a common liver infection with a great potential of a life-threatening condition, affecting in 2015 an estimated number of 257 million people worldwide. As the prevalence of HBV is dependent on socioeconomic status, having a higher incidence in low-income countries, a proper method to combat the

transmission is through education and vaccination programs. Mother to child transmission and in between persons are the two main factors of spreading the virus and the idea of promoting an easier access to the means of protection and screening programs is an inexpensive and efficient measure that should be taken by the governments. Teleconferences or even electronic consultations could have a great impact on the high rates of infection with hepatitis B virus, preventing the unsafe practices. Furthermore, detection of HBV infection and the use of antiviral therapy among patients with chronic infection were associated with a lower risk of developing hepatocellular carcinoma, emphasizing the need of early diagnosis and treatment, which could be efficiently made using telemedicine or simple video calls, in order to perform a face-to-face consultation.

While most of the telehealth programs are similar to HCV infection, for Hepatitis B, chronic patients require follow-ups regularly every six months, as the infection can have multiple courses, progressing with complications in different phases of the disease [15]. While the need for a consultation is imperious, unfortunately patients do not comply with the guidelines proposed by specialists, or cannot simply conform to the recommendations made by the doctor because of their lack of access to medical care. By using electronic consultations, patients reduce the wait time and travel expenses for a face-to-face consultation. In order to facilitate the communication between the health care provider and the patient, a mobile texting application called HepTalk, designed especially for hepatitis B care, was used as a secure channel which proved a great potential in education, engagement and improvement of their access to health. HepTalk provided effective and secure communication, fast response and reduced patient expenses, not being limited by any geographic boundaries or by the lack of transportation means [16].

Non-Alcoholic Fatty Liver Disease (NAFLD)

NAFLD is a pathology characterized by histological alterations with lobular inflammation and hepatocyte ballooning, associated with a rapid progression of fibrosis, which affects approximately one billion individuals worldwide [17]. Along with metabolic comorbidities, such as obesity, hyperlipidemia and type 2 diabetes, NAFLD is becoming a major cause of liver disease-related morbidity and mortality [18]. Despite pharmacological discoveries, healthy choices regarding daily life remain the best approach for therapy. The frequency of metabolic risk factors in NAFLD expresses the unhealthy life-style and requires a better management of nutrition and creates a terrain for screening, making electronic consultation not only a follow-up requirement, but also a method to carry this public health problem through prevention. App stores for both Android and Apple gained popularity for weight loss applications as more and more people

recognize that dietary intake and physical activity are key factors for healthy bodies.

Although liver biopsy is still the gold standard for detecting and staging fibrosis in NAFLD, ultrasonography (US) remains the preferred method given the fact that is non-invasive, with no risks and low costs. Online consultations could offer the opportunity for less experienced doctors to collaborate with specialists and interpret the findings of ultrasonography, establishing the best suited steps for the management of the disease in either rural or impracticable regions. Fibrosis progression occurs at a rate of 0.09 stage/year[3], which is a sluggish move to cirrhosis, giving time for both the medical personnel and the patient to counteract the disease with lifestyle changes and efficient medication.

Surveillance, as well as monitoring programs for NAFLD require a first evaluation based on ultrasound and fibrosis score to assess the disease. For example, a study on 700 patients has focused on monitoring individual fibrosis parameters over a 5 years period [19]. The web-based group was introduced to a Cloud/Saas e-learning platform by individual user-id and password. The main reasons they chose this option were either distance or job-related reasons. The main objective was to achieve long term results by implementing sessions of group counselling which consisted of a questionnaire and a motivation to change especially on their diet and habitual physical activity. While interaction was not always available, patients had the option to centralize food diaries and send them by e-mails. After a two years follow-up when compared to the other group that went for regular visits with the physician, there were no notable differences. The main disadvantage of this type of monitoring remains the fact that many patients were lost during the follow-up. However, the ones that agreed to continue with the program obtained a 10% weight loss, and the biological status improved in time. Challenges still prevail for monitoring programs in NAFLD since they require engagement and high motivation due to high periods of time to obtain results. Telehealth programs are an option, but they should be well addressed, with an easy-access platform and also interactive to maintain the patient's motivation.

A small population randomized Asian study [20], introduced a mobile app nBuddy (Nutritionist Buddy) to monitor dietary and lifestyle changes of 55 patients with NAFLD. A remote support of 6 months was done by the mobile app, after a single face-to-face meeting. The app consisted of step monitoring, which increased gradually each week, a weight tracking system to assess weight loss progression and food choices each day to ensure diversity and healthy diet. All of these were maintained with automated reminders. The results were significant with improvement on the baseline characteristics.

However, even though NAFLD status could be monitored through apps or web-based programs, it still depends on the patient's motivation and their understanding of the disease.

Autoimmune Hepatitis

As a chronic disease, autoimmune hepatitis (AIH) requires long-life management for both therapy and disease evolution. All patients should visit their physician for biochemical remission or relapse assessment. This might be done by involving technology and avoiding the patient's presence within the medical clinic. A study performed in Turkey during the COVID-19 pandemic involved 46 AIH patients that were monitored electronically by forwarding their blood tests, by live-video interaction used to assess patient's characteristics and also by text messaging as general reminders [21].

Alcohol-associated Liver Disease

Alcohol consumption is often associated with liver diseases, since it exacerbates liver injury. Heavy drinkers are more prone to develop cirrhosis, rather than NAFLD patients and also mortality is higher when alcoholic hepatitis is involved. As specialized medical care is concentrated in urban areas and heavy alcohol consumers inhabit rurally or out of reach places, telemedicine promotes a way of access for this type of population. Electronic consultation is a possible resource for this group of patients, but healthcare providers should be aware of the limitations of this method. Alcohol consumers who did not have a prior interaction to the digital sphere and who may not be as prepared to the use of a monitoring application as alcohol consumers who are smartphone users for a while. These type of patients would probably give worse results during a consultation, rather than the ones already familiar with technology although at an equal state of health [21].

Most of the available studies are based on data storage on available patients with alcoholic liver disease or are focused on alcohol abstinence and patient's behavioral monitoring. Alcohol relapse may be delayed or even avoided if other means of monitoring are embedded, such as mobile apps with different reminders and sessions that focus on patient psychological status. In an ideal matter, using a digital intervention might help patients that are unable to address a physician to identify a potential risk of relapse and also become a predictive model for alcohol use. On the other providing digital support material, might also be a solution, since timeline reminders *via* e-mails, SMS technology, or app notifications might be introduced to enhance the telehealth necessary environment to aid the patients.

Nonetheless this might be even more relevant, if patients require a liver transplant intervention, and the patient needs to be in close contact with the transplant team [22 - 25].

One of the major objectives of possible digital interventions on patients with alcoholic liver disease is to provide effective support so that abstinence is reached. Miniaturized sensors may offer related data on measuring alcohol consumption, however, they are difficult to be accepted by patients and require time to be inserted in clinical care.

Chronic Liver Disease Complications

Telehealth offers various options for patients with the chronic liver disease while focusing on the same objective which is delaying complications. Disease progression might be remotely monitored by using phone apps or tablets on weight gain or treatment adherence. Similar to heart failure disease, where telemonitoring offers more opportunities, even in cost savings between 5000 and 50.000 $ per year, per patient, cirrhotic patients could also benefit more from teleconsultation or telemonitoring [26]. Either wireless blood pressure monitors, pulse oximeters and different scales are efficient in chronic liver disease complication identification. Infections, bleedings, fluid overload may be preventable if disease monitoring is ensured. For example, a mobile app "Patient Buddy App" [27] focuses on medication adherence which it is of utmost importance in cirrhosis monitoring, weight gain along with sodium intake and may help prevent readmission of decompensated patients. Also, smartphone implementation of different scales, such as the Stroop test for hepatic encephalopathy is an important addition to this field [29, 30].

Table 1. Available apps for medical use, with validation studies.

Mobile App	Disease	Objective
Patient Buddy [27]	May be adapted to several diseases. Used for cirrhosis monitoring	Compliant secure patient communications, adherence monitoring and real-time data analysis
EncephalApp [28]	Covert hepatic encephalopathy	Assess hepatic encephalopathy through stroop test
PGHD*Connect* App [31]	Cirrhosis – ascites monitoring	Smartphone app in facilitating outpatient ascites management. The app is connected *via* Bluetooth to a scale
Nutritionist Buddy (nBuddy)	NAFLD	Lifestyle intervention on weight loss monitoring in NAFLD patients

Liver Transplant Evaluation

After kidney, the liver is the second most common solid organ being transplanted globally, still with a level of fulfillment of the needs of less than 10%. Considering this low rate, it is desirable to take measures in order to improve the donor pool and the post-transplantation care in other centers than expert ones due to their high volume of demands and decreased accessibility. Telemedicine helps reducing expenses and time, which patients need to invest in order to have a post-liver transplant consultation. Patients recognize the advantages of a virtual meeting with the healthcare provider, such as less time off from work, saving travel time and travel expenses and the satisfaction of not needing to ask for a caregiver to take time off in order to assist them to their appointments.

Electronic consultation should be implemented in liver-transplant centers in order to relieve congestion, improve patient flow and reduce wait times. Patient satisfaction is for sure another reason for using telemedicine, while the benefits are obvious, both time and cost-effective.

After adopting strict lockdown measures within the COVID-19 pandemic, healthcare providers adapted to telemedicine in order to offer liver transplant patients the remote consultation much needed, making them able to triage the ones who needed physical examination, in this way lowering the virus spread rate and protecting the patients [32].

Telecytopathology

Rapid on-site evaluation (ROSE) of fine needle aspiration for hepatic lesions helps the patient in receiving a prompt diagnosis and also improves the diagnosis accuracy since the pathologist may communicate directly with the physician who performed the procedure. This on-site evaluation process also guides the clinical management more rapidly being more cost-effective. However, this process is not available in every clinic, thus collaboration should be well designed. Performing this process by using live video images and direct interpretation by a cytopathologist located in a different location might be an alternative.

Many studies have focused on dynamic telecytopathology guided ROSE on different organs, especially the thyroid and pancreas. Nous *et al.* used this process to assess liver lesions. While a radiologist performed the procedure and the slides, the cytopathologist located in a different location helped by means of video imaging and voice communication systems by providing a preliminary diagnosis. After assessing 178 lesions on either US or CT guided FNA an accuracy rate of 94.4% was obtained. Even more, when the diagnosis was difficult, the cytopathologist could ask for more additional material [33].

Store and Forward Concept

The store and forward concept is used in telemedicine as a manner of storing the patient information, which is meant to be analyzed by a medical doctor, specialized in the specific disease of the patient. This is a practical method in which the patient receives a special management, in the online environment, in a cost and time efficient way [34].

Although this method holds significant potential, the concept needs to diminish the associated challenges, such as security and privacy concerns. In a manner to curtail the mortality and morbidity regarding liver pathologies, advanced and early diagnosis require an exact timeline of the disease. Perhaps by fully organizing medical data through telehealth, a well-developed store and forward system might be introduced.

Remote Monitoring Interventions

Remote monitoring refers to the tracking of multiple patient parameters, which do not usually require a special medical education, such as blood pressure, heart rate, weight, parameters that can be transmitted through a telephone. The interpretation is made by professionals, but empowering the idea of digital interaction, can offer a new perspective in patients` vision, being as effective as a traditional consultation. Using digital diagnostic tools, telemedicine promises a wider addressability and accessibility. The use of a smartphone monitoring application is feasible especially for cirrhotic patients in which the weight is representative for the accumulation of abdominal fluid, ascites. Thus, analyzing daily weight could be engaged in this manner for a better disease management [35, 36].

Smartphone applications can detect different changes in the behavior of patients that could predict the liver-related events, such as the sleep cycle or social interactions. The use of this type of app regarding remote monitoring would allow early detection of warning signs in liver diseases and would offer the medical practitioners an accurate prediction for hospitalization, reducing the admission rate for the patients and the associated costs [37].

CONCLUSIONS

The COVID-19 pandemic radically changed our routine healthcare, as guidelines show that face-to-face interaction is not recommended. Chronic patients are the ones suffering most from the lack of follow-ups and continuous monitoring, especially in a population with precarious education. Telemedicine is evolving beyond phone calls regarding triage, expanding to remote monitoring, teleconsultations, conferencing for educational purposes and continuously

improving care between visits. Definitely that telemedicine will soon educate patients and also medical personnel, providing alternatives to the traditional healthcare system worldwide, not only as a method to prevent the increasing level of contagiousness of the COVID-19 infection, but also as a future approach for the high demand of medical service in different centers.

It is unavoidable that the use of technology in medicine will develop gradually in order to fulfill the needs of patients as a natural flow of evolution and consumerism. Despite promising evolving technologies in health, multiple challenges continue to keep in place the decision to rely more on them. Boundaries regarding liability concerns, technical difficulties or personal restraints will slowly decrease the importance as governments and private healthcare providers will realize the advantages in the use of telemedicine compared to the risk associated factors and high costs needed for healthcare delivery.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 32

Pathologies of the Peritoneum, Mesentery and Diaphragm

Lucian Negreanu[1,*]

[1] *Internal Medicine I-Gastroenterology Department, Emergency University Hospital of Bucharest & UMF Carol Davila Bucharest, Romania*

Abstract: Pathologies of the peritoneum, mesentery and diaphragm are uncommon, making their diagnosis more challenging. We present the main issues in diagnosis and treatment.

Peritonitis represents acute inflammation of the peritoneum that can be caused by perforation, inflammation or gangrene of an intra- or retroperitoneal structure. The most frequently encountered peritoneal tumours are metastases originating in gastrointestinal, ovarian, lung, pancreatic and breast adenocarcinomas. Lymphomas can primarily or secondary affect the peritoneum.

There are two main categories of diseases affecting the mesentery: diseases that start from the mesentery (which can also affect neighbouring organs) and diseases that originate in neighbouring organs.

The most encountered hernias of the diaphragm are those occurring through the oesophageal hiatus, but there can also be congenital hernias (oesophageal, Morgagni and Bochdalek) or through post-traumatic defects. As in all other organs, primary diaphragmatic tumours can be classified as benign (cyst and lipomas) or malignant (rhabdomyosarcoma and fibrosarcoma), with other types of primary tumours than those aforementioned being very rarely seen.

Keywords: Ascites, Diaphragmatic hernias, Mesentery, Peritoneal tumours, Peritonitis, Pseudomyxoma peritonei, Tuberculosis.

[*] **Corresponding author Lucian Negreanu:** Internal Medicine I-Gastroenterology Department, Emergency University Hospital of Bucharest Splaiul Independentei Nr. 169, Sector 5, Bucharest, Bucuresti, Romania; E-mail: negreanu_99@yahoo.com

Ioan Sporea and Alina Popescu (Eds.)

GASTROENTEROLOGICAL PATHOLOGIES OF THE PERITONEUM

Peritonitis

Secondary peritonitis (surgical peritonitis) represents acute inflammation of the peritoneum that can be caused by perforation, inflammation or gangrene of an intra- or retroperitoneal structure. Surgical therapy is often required, although more recently, several antibiotics have been shown to be beneficial in acute diverticulitis and appendicitis. Without treatment, the natural evolution is SIRS, a septic shock that can eventually be lethal [1].

The most common causes of secondary peritonitis are peptic ulcer, appendicitis, diverticulitis and gallbladder disease (although sterile bile leakage can be tolerated even in large volumes). Internal haemorrhage (ovarian cysts or tubal pregnancy rupture) can be a non-infectious aetiology; blood is highly irritative to the peritoneum, and the clinical presentation is similar [2].

The most common microorganisms (74%) responsible for secondary peritonitis are mixed aerobes and anaerobes, the most frequently encountered being *E. coli, enterococci, Clostridioides spp. and B. fragilis*. Fungal supra-infection is related to poorer outcomes, as is the presence of haemoglobin, barium, devitalised tissues or bile, all of which have the capacity to interfere with the immune response [1].

The cardinal signs of secondary peritonitis are intense abdominal pain and abdominal guarding. Pain can be absent or diminished in several situations, such as intoxication with ethanol, polyneuropathy, elderly patients and patients on glucocorticoids, NSAIDs or immune suppressants. Ascites can evolve without pain if the peritoneum is not involved in inflammation. Several signs can be noticed in the initial phase: fever (>37.5 C), abdominal immobility, tachycardia (that can be in response to pain, not to SIRS), the absence of hepatic dullness or rebound tenderness (Blumberg sign). Iliopsoas and obturator signs along with extensive rectal and pelvic examination can be helpful in identifying possible abscesses.

The hallmark of laboratory tests in this condition is leucocytosis with a left shift (immature WBCs/band cells), a lack of which might signify that the bone marrow is exhausted. Haemoconcentration, metabolic acidosis, prerenal azotaemia and Gram-negative septicaemia are also associated [3]. Ultrasound can help identify lesions such as large fluid collections, abscesses, bile duct enlargement and occasional pancreatitis. Abdominal and pelvic CT scans are the gold standard for diagnosis, as they can identify causes that will not necessitate surgical therapy (*e.g.*, diverticulitis).

Although laboratory and imaging studies can be helpful, the diagnosis can be confirmed only by laparoscopy or laparotomy when purulent fibrinous peritonitis is found. If the effluent has >500 WBCs/mm^3, positive Gram staining or higher than normal serum bilirubin or amylase, then the probability for secondary peritonitis is 90%.

The main therapeutic resources in secondary peritonitis are fluid resuscitation (recommended monitorisation in an ICU setting), broad-spectrum antibiotics and laparotomy or laparoscopy. Vasopressors should be avoided until the intravascular volume is replaced [4]. Empirical therapy with a broad-spectrum beta-lactam associated with aminoglycoside or 3rd/4th generation cephalosporines and metronidazole should be started, although in every case, a tailored therapy should be used if an antibiogram is available.

Surgical therapy should not be postponed and will need to be targeted to the cause, peritoneal toilet and prevention of recurrence [5]. The prognosis is variable, with poorer outcomes in elderly patients and those who develop MODS before the development of clinical manifestations of peritonitis. The mortality rate ranges from 10% in appendicitis and perforated duodenal ulcers to 50% in postoperative peritonitis, with an average of 14% [1].

Other Types of Peritonitis

Spontaneous bacterial peritonitis is a condition associated with ascites caused by cirrhosis or nephrotic syndrome. Its definition and treatment are currently highly protocolised [1].

Continuous ambulatory peritoneal dialysis is a frequent cause of bacterial peritonitis. It is estimated that this condition occurs 1,4 times/year in the peritoneal dialysis population. Most frequently, the isolated microorganism is *S. epidermidis* together with other commensal skin flora (secondary to poor patient education in regard to sterile dialysis techniques). Rarely, *M. tuberculosis* can be found (see below). Presentations include abdominal tenderness and pain, hypotension, diarrhoea, polydipsia, cloudy effluent with more than 100 neutrophils/mm^3 and the presence of microorganisms on Gram staining [4]. Antimicrobial therapy with vancomycin and 3rd generation cephalosporins should be started, but if there is a possibility to review an antibiogram, then it is recommended to follow that result. Heparin addition to the dialysis bag might lower the risk of postinfectious peritoneal adhesions.

Tuberculous peritonitis is considered a rare disease, but there is recrudescence, especially in immune-compromised patients, and it is increasingly an isolated disease, with only 20% of cases having pulmonary urogenital tuberculosis.

Symptoms are non-specific, with abdominal pain and ascites being encountered in 50-60% of cases. The diagnosis of peritoneal tuberculosis should be ruled out in every cirrhotic patient with ascites. Sometimes it is hard to differentiate this illness from peritoneal carcinomatosis, as both conditions usually present with lymphocytic ascites but the latter without fever. Cytology and sometimes diagnostic laparoscopy can be necessary to establish a definitive diagnosis. Ninety percent of the patients had an elevated protein level of more than 2.5 g/L in the ascitic fluid and a leukocyte count >150/μL with >80% lymphocytes. Serum levels of CA 125 can be elevated, which can also make ovarian neoplasia hard to differentiate at first glance. If there is no associated cirrhosis, high levels of adenosine deaminase in the ascitic fluid are pathognomonic for TB. Laparoscopy might be useful for diagnostics, showing millimetric deposits on the peritoneum in 95% of cases. Eight weeks of four-drug therapy followed by 4 months of isoniazid/rifampicin regimen are necessary, and sometimes secondary schemas are used [6].

Patients with AIDS can develop bacterial, fungal, parasitical or viral peritonitis. Additionally, AIDS-related malignancies (Kaposi sarcoma and non-Hodgkin lymphoma) can metastasize in the peritoneum. Clinical manifestations include tenderness and pain, fever, anorexia, weight loss, and ascites. The approach is generally medical, but in some cases with gut involvement, surgical treatment is needed.

Fitz-Hugh-Curtis syndrome (perihepatitis) is caused by *N. gonorrhoeae* or *Chlamydia spp.* and is due to bacterial insemination through the fallopian tubes [7]. It manifests with fever, right upper quadrant pain, hepatic friction rubs and inflammatory ascites. Laparoscopy reveals adhesions from the abdominal wall or diaphragm to the liver.

Other rare causes of primary peritonitis include fungi (mostly *Candida* spp.), parasites, starch peritonitis (which is due to the contamination of peritoneum with glove powder, treated with glucocorticoids), connective tissue diseases (lupus, polyarthritis and scleroderma) and familial Mediterranean fever (FMF). This condition affects mainly patients of Jewish origin and has variable genetic transmission, most frequently autosomal recessive (the gene is located on chromosome 16). The clinical manifestations include episodes of intense abdominal pain in the presence of ascites. The crisis takes 24-72 hours to resolve, but some patients can have symptoms up to ten days or present with chronic symptoms that diminish with older age. The pain is intense in the epigastric region and then becomes more diffuse. Prodromal symptoms include nausea, diarrhoea, arthralgia, altered general status and fatigue. Diagnosis is often difficult (family history, or other immune manifestations, such as pleurisy, arthritis, and

amyloidosis). The drug of choice for the crisis is colchicine, which is also effective to prevent the crisis if administered when prodromal symptoms start. If left untreated, FMF can provoke fatal renal amyloidosis [1]. The clinician must be aware that a crisis of abdominal pain in such patients might also mask cholecystitis, appendicitis or bridle occlusive syndrome, especially if colchicine treatment seems ineffective. Preventive appendicectomy is recommended.

Peritoneal Tumours

The most frequently encountered peritoneal tumours are metastases originating in gastrointestinal, ovarian, lung, pancreatic and breast adenocarcinomas. Lymphomas can primarily or secondary affect the peritoneum. Their main finding, although not specific, is ascites. Other manifestations are weight loss, early satiety and abdominal pain. With the exception of ovarian metastasis, the prognosis is poor, with the main therapeutic resources being large-volume paracentesis (as diuretics are generally not effective) [8] and surgical cure combined with chemotherapy and other newer approaches such as hyperthermic intraperitoneal perfusion or VEGF inhibitors.

Pseudomyxoma peritonei is a rare condition (found in approximately 2 out of 10,000 laparotomies) that is most frequently found during the exploration of an ascitic syndrome, ovarian tumour, acute appendicitis or incidentally during an imaging exam or laparotomy. The classification is not exclusive. Its primary origin site can be the appendix, other gastrointestinal mucinous tumours or the ovary [9]. The patient can be asymptomatic or present with painless distention of the abdomen or ascites. The potential for malignancy is variable, and the treatment involves hyperthermic perfusion with cytoreductive surgery.

Primary peritoneal tumours include benign (cyst or lipomatosis) and malignant (mesothelioma) lesions.

Peritoneal cysts are rare and can recur, and their main features are abdominal pain or non-specific symptoms related to their mass effect. Pelvic lipomatosis can be seen mostly in black men and can cause urinary tract obstruction, hypertension, and, rarely, gastrointestinal symptoms [10]. In most patients, the approach is conservative, as the lesions will not evolve. Transrectal ultrasound and pelvic CT scan or MRI can be of help in differentiating pelvic lipomatosis from liposarcomas.

Approximately 1/4 of mesotheliomas occur in the peritoneum, and most of them are linked with exposure to the asbestos. The prognosis of mesotheliomas is bleak; symptoms are highly non-specific, ranging from dyspepsia, abdominal pain, and constipation to fever, a palpable abdominal mass, cachexia and ascites.

Men are more frequently affected. Diagnosis is difficult, with coexisting thoracic asbestosis in 50% of cases. Ascitic fluid is an exudate that is sometimes haemorrhagic, with malignant mesothelial cells evident in 5% of cases. Laparotomy might be necessary for a definitive diagnosis. Serum osteopontin is used to distinguish between pleural mesothelioma and asbestosis without mesothelioma. As these are malignant tumours, the approach is based on surgery combined with chemo- and/or radiotherapy [1].

Pathologies of the Mesentery

There are two main categories of diseases affecting the mesentery: diseases that start from the mesentery (which can also affect neighbouring organs) and diseases that originate in neighbouring organs.

In Crohn's disease, the manifestations on the mesentery are directly related to the degree of inflammation of the intestinal mucosa; mesenteric adipocytes are probably major sources of TNF-α, which increases the expression of other proinflammatory cytokines [11]. Chronic transmural intestinal inflammation affects fatty tissue, leading to hyperplasia, and the affected mesenteric segment, leading to local fibrosis. There is an evidence that the thickening of the mesentery may be correlated with a more severe course of the disease and with more recourse to surgical therapy. Currently, there is a trend towards a complete and periodic evaluation of the patient using biomarkers and colonoscopy to capture mucosal inflammation and using MRI to evaluate the degree of inflammation of the mesentery.

In *mesenteric ischaemia,* both vascular sources (arteries and veins) of the mesentery can be involved, with obstruction causing lesions at the intestinal level and at the level of the mesentery itself. Divided into acute mesenteric ischaemia and chronic ischaemia, both types have similar clinical pictures that include abdominal pain - usually periumbilical, as their main symptom, which occurs postprandially most often, and which, depending on the type of ischaemia and the type of vessel affected, may have different intensity. In cases of venous obstruction and chronic arterial obstruction, patients will present with mild to moderate colicky pain, which occurs postprandially. In the case of an acute arterial obstruction, the pain is severe and continuous, with or without association with food intake. Accompanying signs and symptoms might be present, such as haematochezia, ileus, bloating, pseudodiarrhoea and systemic signs from tachycardia, hypotension or fever to shock. The prognosis is bleak in the absence of surgical, radiological or pharmacological revascularization treatment. In recent years, a tendency to establish vascular emergency centres in tertiary hospitals has led to a better prognosis and survival of such patients.

A *mesenteric volvulus* appears after the twisting of an intestinal loop around the mesenteric axis. Causes of mesenteric volvulus are congenital defects of rotation or attachment of the mesentery or secondary to an obstacle to intestinal peristalsis (bridles, tumours or an inflammatory process as in retractile mesentery). The clinical manifestations are similar to those of mesenteric ischaemia, and the therapeutic treatment is almost always surgical.

Mesenteric sclerosis is characterized by chronic inflammation, fibrosis and necrosis of the mesentery. Lipodystrophy and mesenteric panniculitis (Weber-Christian disease) make up the spectrum of this pathology. The aetiology is largely unknown - there are links between other autoimmune inflammatory pathologies (sclerosing cholangitis, rheumatoid arthritis, and thyroiditis) or intra-abdominal surgery, abdominal tuberculosis, vascular insufficiency or the administration of certain drugs. Clinically, this illness manifests with abdominal pain, nausea, vomiting, and a palpable abdominal mass but also with general manifestations such as weight loss, anorexia and fever. Chronic inflammation requires therapy based on corticosteroids and various types of immunosuppressants. Favourable evolution was observed with the administration of cyclophosphamide, colchicine, azathioprine and pentoxifylline, although these are anecdotal reports [12].

Mesenteric adenitis is a non-specific, self-limiting inflammation of the mesenteric lymph nodes secondary to a viral infection (adenoviruses, *Epstein-Barr* virus and HIV) or bacterial infection (*Yersinia, Salmonella, Shigella,* and *Mycobacterium*). It is more common in children, and is often difficult to differentiate mesenteric adenitis from acute appendicitis. Physical examination, ultrasonography and computed tomography are used to differentiate between these two pathologies. Complications with mesenteric adenitis are rare because it is a benign and self-limiting disease. If complications occur, they may be due to electrolyte imbalance, lymph node abscesses, peritonitis or sepsis but also to ischaemic colitis caused by a mass effect on mesenteric vascular structures [13].

Mesenteric phlegmon is a complex inflammatory process that affects multiple segments of the intestinal tract and affects the mesentery. It is often found in diverticular disease, Crohn's disease, and complicated appendicitis. The most common treatment is surgical.

Mesenteric tumours are uncommon lesions. They may be cystic or solid and may be clinically malignant (extremely rare) or benign. Mesenteric tumours are found in all age groups. Most of the time, the diagnosis is made radiologically. Frequently asymptomatic for a long time, they can manifest through a mass effect

on neighbouring structures. Pain is the main symptom, initially manifesting as deep and poorly localized discomfort.

Mesenteric cysts represent rare malformations of the abdominal cavity and are more commonly seen in children. Their aetiology is unknown, but abnormalities of the lymphatic vessels of the mesentery may play a role. The clinical picture of mesenteric cysts is very non-specific, with clinical manifestations related to large cyst dimensions; in this case, the cyst can be palpated or can cause asymmetry of the abdomen. Intestinal obstruction, an inflammation of the cyst, intracystic haemorrhage and rupture are rare complications [14]. The cysts are curable by surgical treatment, which consists of their enucleation or en block resection of the cyst with the portion of the mesentery involved.

D*esmoid tumours of the mesentery* are fibroblastic proliferations that locally invade the apparent mesentery; they do not metastasize but have a high degree of recurrence. They are frequently associated with familial adenomatous polyposis, multiparity and postsurgical scars. Although they are described as being histologically benign, their infiltrative growth pattern and local extension can cause mesenteric vascular obstruction and visceral involvement. Surgical excision and radiotherapy remain treatments of choice [15].

Liposarcomas are local malignancies with an increased risk of recurrence and metastasis. They are phenotypically similar to and have the same therapeutic response as gastrointestinal stromal tumours (GISTs).

Mesenteric lymphoma is the most common cause of mesenteric malignancy, and non-Hodgkin's lymphoma is the most frequent [16]. It is a disease of the mesenteric lymph nodes that is localized or disseminated. The clinical presentation of mesenteric lymphoma is similar to that of other mesenteric tumours: abdominal pain or discomfort, a palpable abdominal mass, fever of unknown primary cause, and weight loss.

Carcinoid tumours of the mesentery are most frequently metastases of intestinal carcinoid tumours affecting the lymph nodes that appear enlarged in volume, usually with a diameter greater than 2 cm. Cases of severe malabsorption syndrome associated with extensive mesenteric carcinoid tumours have been reported in the literature. This type of disease may be accompanied by extrinsic complexion and occlusion of the mesenteric arterial blood supply with segmental ischaemia or bowel infarction.

Gastroenterological Pathologies of the Diaphragm

Hernias of the Diaphragm

The most encountered hernias of the diaphragm are those occurring through the oesophageal hiatus, but there can also be congenital hernias (oesophageal, Morgagni and Bochdalek) or those that occur through post-traumatic defects [17].

Most hernias involve the stomach, and their mechanism is gastric sliding (or type 1) passing through the phrenoesophageal membrane. Most of these cases are age-related, as the aforementioned membrane deteriorates over time [18]. Weight gain and high intra-abdominal pressure [as in abdominal compartment syndrome] can also be conditions that are associated with this type of hernia. It is estimated that approximately 90-95% of the hiatal hernias found on barium radiographs are of this type 1 [19]. Most sliding hernias are asymptomatic, but some of them can provoke GERD, with the typical signs and symptoms associated, including heartburn, regurgitation and response to proton pump inhibitors and diet. Large type 1 hernias can produce discomfort in the chest and epigastrium, dysphagia or even dyspnoea in several cases [20]. Iron-deficiency anaemia is nearly three times higher in this population [21], and this phenomenon can be partially explained by the appearance of Cameron ulcers at the site of the lesion, which can provoke upper gastrointestinal bleeding (overt or obscure). The diagnosis is easy to make through barium oesophagogram, CT scan or upper digestive endoscopy. If asymptomatic, type 1 hiatal hernias do not require treatment. It is very important to explain to the patients that although the presence of a hernia might be involved in their reflux symptoms, it is not necessarily so (*i.e.*, one can have GERD without a hernia or have a hernia without GERD). The indication for surgical treatment is made by the size of the hernia and severity of symptoms (recurrent anaemia, for example). Careful and thorough exploration must be performed preoperatively with endoscopy, pH monitoring and manometry. The most common procedure is Nissen or Toupet laparoscopic fundoplication [17].

Type 2 hiatal hernias or paraesophageal hernias are those that will most likely involve patients who already have a defect of the hiatus. These hernias are characterized by the fact that the gastroesophageal junction remains in position and the stomach is sliding all around it. There are cases in which the entire stomach and the omentum, spleen and colon are also herniated [17]. Gastric volvulus is seen frequently in this type of hernia. Rarely, these patients remain completely asymptomatic, and half of them suffer from GERD. Other non-GERD symptoms that can arise are vague postprandial discomfort and shortness of breath, and some of the patients will also encounter chronic gastrointestinal haemorrhage [22]. In the case of gastric volvulus, acute pain and retching will

occur, with a high possibility of evolving to a surgical emergency. Simple chest radiography and CT scans are the most efficient ways to diagnose this lesion. Upper digestive endoscopy can be difficult to perform if there is also gastric volvulus, but the suspicion can be raised by CT scan (pneumatosis, a thickening or lack of filling of the wall with contrast). Almost always, paraesophageal hernias should be referred for surgery. Although we should perform pH-metry and manometry, those explorations are difficult to perform in the case of type 2 hernias [23]. The mixed type of hiatal hernia, combining both elements of sliding and paraesophageal mechanisms, is called a type 3 hiatal hernia.

Congenital diaphragmatic hernias are due to defects of fusion of the several key components from which the diaphragm is formed (the septum transversum, mesentery of the oesophagus, retroperitoneal membranes and muscle of the chest wall). This type of lesion can occur through the oesophageal hiatus, sternocostal foramina (Morgagni hernia) or lumbo-costal foramina (Bochdalek hernia) [24]. The incidence of these hernias is approximately 1 in 2000-10000 live births, and most of them (80%) are left-side Bochdalek-type hernias [25]. Some Morgagni hernias, which are more prone to appear in the right hemithorax, are diagnosed in adulthood, even if they are congenital. Prenatal ultrasound can identify these lesions before birth. Large hernias will also imply pulmonary hypoplasia. In the Bochdalek type, the hernia's presentation can vary from neonatal death by pulmonary failure to asymptomatic finding in an adult. In the late childhood and adult stages, Bochdalek-type hernias can manifest with symptoms related to the herniation of viscera [the stomach, spleen, transverse colon, omentum], with half of them presenting with incarceration. Other findings are similar to those seen in hiatal hernias, such as dyspnoea, malaise, dysphagia, and constipation. Morgagni hernias, on the other hand, will most likely become a clinical issue for patients in adulthood. Their clinical manifestations will be similar to those mentioned above for hiatal and Bochdalek hernias, including the risk of incarceration. They can be diagnosed through imaging investigations, such as lateral-view chest radiography or chest CT scans [25]. The treatment of symptomatic congenital diaphragmatic hernias is surgery [24].

Post-traumatic hernias are caused in the majority of cases (80%) by blunt trauma, and the rest are caused by penetrating trauma and are less severe. The shockwave caused by a blunt trauma can cause large defects, most often seen on the left side of the diaphragm [17], and through negative pressure found inside the thorax that can cause large hernias, not only immediately but also in time. This type of lesion can facilitate the herniation of the stomach, colon, omentum, spleen, kidney or small bowel [26]. It is estimated that approximately 5% of patients with multiple traumatic injuries will also have various degrees of diaphragmatic injury [17] and thus are prone to diaphragmatic hernias. Their symptoms are mainly in the area of

respiratory and/or abdominal distress, with some of these patients manifesting with a latency of even a decade. Penetrating injury between the 4th intercostal space and umbilicus should raise the concern of diaphragmatic trauma. Chest radiography will be able to identify hernias in approximately 50% of cases, and CT scans are more sensitive in this regard [27]. In selected cases, diagnostic laparoscopy can be performed. Acute post-traumatic hernias will be approached through laparotomy or through open chest surgery. Chronic hernias lack a hernial sack and are associated with severe adhesions; they will be best approached simultaneously from the chest and abdomen in an open approach.

Diaphragmatic Primary Tumours

As in all other organs, primary diaphragmatic tumours can be classified as benign (cysts and lipomas) or malignant (rhabdomyosarcoma and fibrosarcoma); types of primary tumours other than those aforementioned are very rarely seen [28].

Diaphragm cysts of mesothelial origin can present with non-specific abdominal complaints. Imaging studies such as ultrasound (thin-walled cystic structure), CT or MRI scans can sometimes establish the diagnosis incidentally in an asymptomatic patient. The approach should be conservative, as regression or complete resolution can be obtained without intervention. Surgical or percutaneous approaches are recommended only if these patients are symptomatic [29].

Bronchogenic cysts are cysts lined with ciliated pseudostratified columnar epithelium. Rarely seen in the diaphragm, they are most often asymptomatic but rarely can present with hiccups, cough, pain or discomfort. Imaging studies such as chest radiographs, ultrasound (hypoechoic lesion), CT scans or MRI can provide additional information about the lesion. As little is known about their natural history, it is recommended that these cysts be approached surgically, through laparotomy, VATS or laparoscopy.

Lipomas of the diaphragm are asymptomatic in the majority of cases. Only large lipomas will develop cough, pain or even dyspnoea. The CT scan is the diagnostic tool of choice (density of lesion similar to adipose tissue).

Although very rarely found in the diaphragm, the most frequent type of malignant tumour is rhabdomyosarcoma – embryonal type (58%). Most of the time, it is asymptomatic, but some patients present with cough, dyspnoea, dysphagia or an abdominal mass. Even if a CT scan can be of help, the diagnosis is histological by image-guided biopsy. The recommended therapy is complete surgical resection with adjuvant chemotherapy and radiotherapy [30].

CONCLUSIONS

Pathologies of the peritoneum, mesentery and diaphragm are rare and must be known to be diagnosed when encountered in clinical practice.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 33

Overview of Iron Products in Gastroenterological Anemia

Dan Ionuț Gheonea[1,*] and **Carmen Nicoleta Oancea**[2]

[1] *Department of Gastroenterology, University of Medicine and Pharmacy of Craiova, Romania*

[2] *Analytical Chemistry Department, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, Romania*

Abstract: The gastrointestinal tract is the site of iron absorption and also the most common localization of hemorrhage. The cause of iron deficiency anemia (IDA) is often chronic blood loss. One liter of blood contains approximately 500 mg of iron. Despite the representative increase in the absorption rate, the loss in this case cannot be compensated and the body's iron reserves decrease. Iron deficiency leads to disruption of hemoglobin synthesis: iron deficiency anemia.

The etiology of iron deficiency anemia can be widely categorized into: decreased iron uptake (malabsorption due to gastrointestinal disease or surgery, inadequate diet) and increased iron use/loss (blood donation, pregnancy, acute/chronic blood loss, rapid growth during childhood, menses). IDA can be the first sign of celiac disease, gastritis and occult GI malignancy.

The first choice treatment (after finding and disposal of the cause of the bleeding) consists of the oral administration of Fe II compounds. It can take several months to replenish iron reserves. Oral administration, however, has the major advantage that it is difficult, even impossible to overload the body with iron, because the absorption is regulated through an intact mucosa (enteral blockage). Only when adequate oral replacement is not possible, parenteral administration of iron compounds is indicated. There are potential side effects: administration of persistent pain at the injection site (i.m. administration) and facial flushing, hypotension, anaphylactic shock (i.v. administration).

Keywords: Anemia, Blood loss, Hemoglobin, Hemorrhage, Iron.

INTRODUCTION

Iron is an important component of myoglobin and hemoglobin and many enzymes involved in redox reactions and energy supply. It plays an essential role in both

* **Corresponding author Dan Ionuț Gheonea:** Department of Gastroenterology, University of Medicine and Pharmacy of Craiova, Romania; E-mail: digheonea@gmail.com

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the storage and transport of oxygen and oxidative metabolism as well as in growth cells and proliferation. Most plasma iron is intended for bone marrow erythropoiesis. The absorption of dietary iron from the duodenum is an elaborate process, controlled by different proteins and it is determined by the need for iron in the body, the concentration of iron in the intestinal lumen and anatomical cell wall integrity.

Iron deficiency is a dominant cause of anemia, which affects over half a billion people around the world. The greatest iron deficiency is manifested in newborns and children and may be caused by abnormal absorption of iron from the gastrointestinal tract, by reduced bioavailability, which can be altered by increasing gastric pH, the presence of inhibitors or disturbance of intestinal structure (celiac disease, Crohn's disease). The gastrointestinal tract is the site for iron absorption and also the most common localization of hemorrhage. Other causes of bleeding are gastroduodenal ulcer, hiatal hernia, gastric parasite infestation and Helicobacter pylori infection.

Women, infants, children and adolescents need iron to develop muscle mass. A baby has 70-80 mg of iron per kg at birth, of which 2/3 is iron hemoglobin. Iron ingested in food is present in various forms. Trivalent Fe^{3+} is virtually unabsorbable in the small intestine, and bivalent Fe^{2+} is much better absorbed. Absorption is especially effective in the form of heme (present in hemoglobin and myoglobin). In the cells of the intestinal mucosa these iron complexes (hemoglobin, myoglobin) are very well absorbed and represent the physiological source of iron, before the appearance of iron-enriched foods.

IRON DEFICIENCY ANEMIA AND ASSOCIATED CONDITIONS IN GASTROENTEROLOGY

The cause of iron deficiency anemia (IDA) is often chronic blood loss. One liter of blood contains approximately 500 mg of iron. Despite the representative increase in the absorption rate, the loss in this case cannot be compensated and the body's iron reserves decrease. Iron deficiency leads to the disruption of hemoglobin synthesis: iron deficiency anemia.

The etiology of iron deficiency anemia can be widely categorized into: decreased iron uptake (malabsorption due to gastrointestinal disease or surgery, inadequate diet) and increased iron use/loss (blood donation, pregnancy, acute/chronic blood loss, rapid growth during childhood, menses). IDA can be the first sign of celiac disease, gastritis and occult GI malignancy.

GI bleeding is a usual cause of IDA, whether the bleeding is chronic or acute. Patients may present signs like blood in their stools or just maroon-colored stools,

but symptoms like the above mentioned are often unrecognized. GI bleeding can occur at any location within the GI tract and can be associated with a variety of lesions. IDA is prone to occur in patients taking chronically nonsteroidal anti-inflammatory drugs or aspirin. By endoscopic evaluation of the GI tract, the site can be visualized for those with angiodysplasia or other structural contusions.

Esophagitis and Hiatal Hernia

One of the established causes of iron deficiency anemia is gastric bleeding in hiatal hernia or Cameron lesions. There has been reported an incidence from 8% to 42%, with a moderate of 20%, of IDA for types of hernia [1]. Suggested causes of hernia that are related to iron deficiency anemia are erosions, gastro-esophageal acid reflux and mechanical trauma plus esophagitis [1]. Even if during the endoscopy, there are no lesions visible, a large hiatal hernia can still be a possible cause of iron deficiency anemia with unidentified etiology. Treating and preventing recurrences of IDA can properly be made with proton pump inhibitor (PPI), even in larger hiatal hernia [1].

Nonvariceal Upper GI Bleeding

A retrospective study recently made acknowledged that more than 85% of the patients accepted by the hospital with nonvariceal acute upper gastrointestinal bleeding (disorder associated with a rate of mortality of 3% to 15%), were anemic at the time of release [2]. There are rare studies analyzing the risks associated with anemia and the clinical impact after nonvariceal acute upper gastrointestinal bleeding, but one of them reported that patients that had Hb values $\geq$ 10 g/dL got two-fold lower risks of mortality and re-bleeding than patients that had Hb values $\leq$ 10 g/dL [3]. A placebo-controlled trial, established the clear benefit given by oral and intravenous iron supplementation on patients with iron deficiency anemia after nonvariceal acute upper gastrointestinal bleeding. Regarding iron stores, they were restored with intravenous iron supplementation more effectively than by oral iron administration. However, only 16% of the patients that were anemic at the time of discharge from the hospital with nonvariceal acute upper gastrointestinal bleeding received a suggestion of oral iron supplementation, but intravenous iron administration was not considered.

NSAID-associated Blood Loss

The GI injury can include bleeding that can often result in hospitalization. The upper and lower GI injuries can be associated with nonsteroidal anti-inflammatory drugs (NSAIDs) administrations. From low aspirin doses as well as NSAIDs to $\geq$ 1800 mg/d aspirin doses, they increase mean fecal blood loss from 0,5 mL/d ($\geq$ 2.5 mg iron loss/d) to $\geq$ 5 mL/d (*i.e.*, $\geq$ 2.5 mg iron loss/d). Long term use of

COX-2 inhibitors (cyclooxygenase-2), even they are correlated with fewer gastrointestinal injuries than NSAIDs, may require the concomitant recommendation of associated medication for anemia, in case of GI injuries induced or small intestinal injuries [4].

Gastric Antral Vascular Ectasia (GAVE) and Portal Hypertensive Gastropathy (PHG)

In patients with liver cirrhosis, PHG and GAVE can cause GI blood loss, even if they are distinct entities. PHG can also exist in non-cirrhotic patients, and its management is established by diminishing hepatic venous pressure and iron supplementation and/or blood transfusions. GAVE was first described in a patient with chronic iron deficiency anemia. The management of this disease comprises endoscopic interventions and surgical procedures, but also the iron supplementation or even blood transfusions.

Autoimmune Atrophic Gastritis (AIG)

AIG, first described in 1909, is a progressive inflammatory condition that leads to reduced or absent acid production (achlorhydria or hypochlorhydria) and it is associated with 20-30% of iron deficiency anemia refractory to oral iron products [5]. Studies have recently revealed that impaired iron absorption has a key factor in the lack of gastric acidity.

Helicobacter Pylori Gastritis

It is well known that IDA is an extra gastric manifestation of H. pylori infection and that over 50% of refractory IDA patients have active H. pylori infection [5]. Two meta-analyses of several interventional and observational trials showed that the eradication of *H. pylori* reverses IDA [6, 7]. Many national guidelines recommend for the treatment of IDA with unknown origin the eradication of H. pylori. In patients with H. pylori and IDA, the eradication therapy using bismuth was revealed to be more effective for increasing iron stores and hemoglobin than PPI-based triple therapy as first line choice. Studies showed that H. pylori increases the evolution of inflammation, dysplasia and the development of adenocarcinoma in an IDA environment.

Bariatric Surgery

Bariatric procedures may lead to IDA as a result of reduced iron absorption or intestinal bleeding. Iron absorption is affected due to diminished gastric acid secretion, postoperative intolerance for red meat or exclusion from the alimentary tract of the duodenum. The incidence of IDA after bariatric surgeries ranges from

12% to 50%, depending on the types of interventions, postsurgical follow-up periods and patient populations [8]. In a study in which 959 patients underwent laparoscopic gastric bypass, 51.3% had iron deficiency and 6.7% were with intravenous iron therapy recommendation [9]. Oral iron preparations have low tolerance following bariatric surgery, so that intravenous iron supplementation is the preferable option [8]. A study involving 280 patients treated with ferric carboxymaltose (FCM) after bariatric surgery showed promising results [10].

Celiac Disease

Celiac disease affects 1% of the population. It is a common chronic inflammatory condition of the gastrointestinal system, with a well-established relationship with IDA [11]. Between 32% and 69% of patients with celiac disease presents anemia and 80% of these are also iron-deficient [12]. Blood loss and impaired iron absorption are pathological contributors to anemia in celiac disease. Nutritional deficiencies may be a causative factor. The low absorption rate of nutritional iron in a gluten-free diet leads to slow or lacking recovery from IDA (6-12 months until the recovery from anemia), and half of the patients remain with iron deficiency after 1-2 years. Patients with the celiac disease receive intravenous iron treatment immediately, without starting with oral iron administration and after intolerance or non-response to switch on intravenous iron treatment [13].

Intestinal Failure (IF)

IF results from surgical resection, dysmotility, congenital defect, obstruction and it is represented by the inability to maintain micronutrient balance, electrolyte, and protein energy. In patients with intestinal failure, total parenteral nutrition (TPN) is recommended until partial or full recovery of enteral nutrition (EN). During the transition from TPN to EN and even after that, ID is the most common deficiency of micronutrients, with an incidence of 60%-80% for iron deficiency and 30-40% of IDA [14]. Iron dextran has been revealed to be compatible at an amino acid concentration > 2% with lipid-free solutions [15].

GI Cancers

In patients with colorectal cancer (CRC), anemia and IDA have an incidence of 50%-60% [16]. Malignant polyps are associated with higher incidence by greater blood loss than benign polyps. In gastrointestinal stromal tumors (GIST) and especially in pediatric GIST, IDA (85% with symptomatic anemia) is the most frequent clinical data [17]. Also imatinib, which is the standard treatment for metastatic GIST, has a side effect, anemia. Since the guidelines focus on the surgical follow-up for colorectal cancer and significant blood loss may result after CRC surgery, studies were made with allogeneic blood transfusion (ABT) used

perioperatively. ABT involves certain risks and significant costs, therefore a controlled trial using preoperative i.v. ferric carboxymaltose comparative with oral iron administration as preoperative anemia therapy is ongoing [18].

Diverticular Disease

Diverticular disease is a common cause of lower gastrointestinal bleeding and has a prevalence of 30%-50% of massive lower gastrointestinal bleeding cases. Information on the incidence of IDA in patients with diverticulitis is lacking. IDA has been found more frequent in patients with acute bleeding or in elderly patients [19].

Angiodysplasia

Angiodysplasia may cause severe gastrointestinal bleeding, being a clinical condition that involves thin-walled, fragile vascular malformations [20]. It has been found that angiodysplasia is present in 60% of elderly patients (over 60 years old), and it accounts for up to 40% of obscure gastrointestinal bleeding cases and up to 5% of known GI bleeding cases [21]. Angiodysplasia has a high prevalence of re-bleeding therefore often results in chronic IDA [22]. If blood loss exceeds 10 mL/day, intravenous iron preparations can be considered as a valuable treatment option [22].

Intestinal Parasitic Infections

Parasitic infections, notably *T. trichiura* has been found to be closely correlated with iron deficiency anemia, causing dysentery and bleeding by invading the mucosa of the large intestine.

Restorative Proctocolectomy

Pouchitis is a common complication of restorative proctocolectomy and it is associated with IDA due to impaired iron absorption and mucosal bleeding [23]. Studies have shown that oral iron can therapeutically be used, and in the case of patients that are unresponsive or intolerant, intravenous iron treatment can correct the anemia.

Chronic Hepatitis and Liver Conditions

Due to acute or chronic gastrointestinal hemorrhage which appears among patients with chronic hepatitis and liver disease, 75% have IDA as a consequence [24]. Impaired blood coagulation determined by lower thrombocyte number, reduced synthesis of blood coagulation factors lead to increased risk of bleeding in hepatocellular disease. After initial treatment with solutions of human albumin,

gelatin-based colloids or red blood cell transfusion, oral or intravenous iron therapy is recommended to treat IDA caused by chronic blood loss in cases of chronic liver disease. Recently, the direct-acting anti-virals (DAAs) used by WHO guidelines, such as boceprevir (BOC) or telaprevir (TVR) used as triple combination therapy [abandoned today] has been found to raise IDA up to 20% compared to peginterferon/ribavirin treatment (30% patients have grade 1 anemia and 10% have grade 2 anemia) [25 - 29]. Second generation DAAs, including sofosbuvir (SOF), simeprevir (SMV), ledipasvir (LDV) and daclatasvir (DCV), approved in combination, offer a shorter treatment duration, significantly greater cure rates and lower incidence of anemia [30].

Non-alcoholic Fatty Liver Disease

One-third of adult patients with NAFLD (non-alcoholic fatty liver disease) are iron deficient [31]. Dysmetabolic iron overload syndrome (DIOS) is correlated with 50% of the cases of NAFLD.

Studies on pediatric patients with NASH (non-alcoholic steatohepatitis) based on hepatic gene expression, hypothesized that: upregulation of hepcidin, due to elevated expression of transferrin receptor II, lead to impaired duodenal iron absorption and a reduced erythropoietic activity (a typical feature of anemia) appear due to decreased level of transferrin receptor I in NASH patients.

Inflammatory Bowel Disease (IBD)

The two entities, ulcerative colitis and Crohn's disease, have a risk of deficiencies of vitamins and minerals due to long-term intestinal mucosa inflammation and low oral intake. Anemia has a higher incidence as extra-intestinal complication of IBD. IDA is a significant and costly complication of IBD, due to its potential effect on increasing hospitalization rates and affecting the quality of life and the ability to work. In IBD, it is inadequate to determine the iron status using common biochemical parameters alone. Oral iron therapy in IBD has been found to have extensive GI side effects and may be associated with disease exacerbation, therefore current guidelines suggest using parenteral iron preparations.

TREATMENT OF IRON DEFICIENCY

The first choice treatment (after finding and disposal of the cause of the bleeding) consists of the oral administration of Fe II compounds. It can take several months to replenish iron reserves. Oral administration, however, has the major advantage that it is difficult, even impossible to overload the body with iron, because the absorption is regulated through an intact mucosa (enteral blockage).

Oral Supplements

The most frequent iron salts administered orally are fumarate ferrous $(HCOO)_2$ Fe, ferrous sulfate ($FeSO_4$) and ferrous gluconate $(C_6H_{11}O_5)_2$ Fe. The standard preparation used in IDA is iron sulfate. Other salts, such as gluconate ferrous and iron (II) fumarate are as efficient as ferrous sulfate when equivalent amounts of elemental iron are administrated, but do not offer any therapeutic advantage and they are more expensive [32].

The amount of elemental iron is the key in establishing the appropriate dose to be administered for iron preparations. Ferrous fumarate contains 33%, ferrous gluconate contains ~ 12 and ferrous sulphate contains 20% elemental iron. For adults with iron deficiency, 50 to 100 mg of orally administered elemental iron, 3 times a day, is usually the proper dose.

Oral iron formulas may have side effects like nausea, vomiting, abdominal pain, diarrhea, dark stools or constipation. These effects are dependent on the dose and, excepting the dark stools, usually disappear by continuing therapy. To reduce intolerance, lower doses may be given initially and the medicine can be administrated before or even better after meals. When it is used as medication ferrous fumarate or gluconate the side effects are much less irritating than taking ferrous sulphate. Also, when taking another dosage form (*e.g.* syrup) gastrointestinal tract intolerance may be reduced. To increase compliance, the delayed-release and enteric-coated iron preparations have been developed. They are not, however, as well absorbed as the nonenteric-coated supplements [33].

Patients should be extra careful when taking the tablets or capsules because ferrous sulfate can affect the lining of the esophagus producing an acidic solution once it is dissolved (pH <3). The patient should be advised to take capsules or tablets sitting or in an upright position with at least 200 mL of liquid and avoid lying in a horizontal position for at least 15 minutes after a dose.

Liquid formulations are extremely useful for young children, elderly or immobilized patients in bed, who have esophageal compression or delayed esophageal transit times due to age or the disease. Liquid preparations can generate tooth staining, which can be largely avoided by mixing with water or fruit juice [34].

Brushing your teeth with baking soda or 3% hydrogen peroxide is beneficial to remove existing stains. If the taste is a problem, the liquid preparation can be mixed with juice fruits or milk to increase palatability.

Various preparations have been prepared for prolonged release dosage forms, in an attempt to improve compliance, as well as to reduce gastrointestinal irritation, which is often associated with iron administration. However, the maximum absorption of iron in these delayed preparations occurs in the duodenum and proximal jejunum, while in the distal jejunum iron is only minimally absorbed.

It has not yet been determined how long oral treatment with iron should be given until the body gains resistance and needs to move from oral administration to an intravenous one. In a randomized, controlled study, a 10 day period was required until oral administration of iron was replaced with intravenous treatment with carboxymaltosis or iron sulphate [three times a day for 6 weeks] at women with postpartum anemia. Pinsk *et al*. [35] treated their patients with a therapeutic dose of 6 mg/kg [oral administration] for 3 months, with no effect on the value of red blood cell indices or hemoglobin. In the context of anemia iron refraction, it is reasonable to resort to i.v. therapy for at least 3 months, with careful monitoring of laboratory values.

Regarding possible interactions: iron absorption is inhibited by antiacids. The combination with vitamin C (ascorbic acid) to protect Fe^{2+} from oxidation to Fe^{3+} is justified, but practically not necessary.

Parenteral Supplements

Fe dextrans are indicated for i.m. administration. If it is decided to replace oral administration of iron supplements with parenteral administration (intramuscular or intravenously), i.m. dose of iron should be limited to 2 mL (100 mg Fe per injection). Therefore, at maximum 20 injections are required for iron therapy [36].

Only when adequate oral replacement is not possible, parenteral administration of Fe^{3+} compounds is indicated. With iron deposits in the tissues (hemosiderosis), there is a risk of overdose. Transferrin binding capacity is limited and free Fe^{3+} has high toxicity. Therefore, Fe^{3+} complexes are administered, which can be phagocytosed by macrophages or donate directly to Fe^{3+} transferrin, allowing iron to be incorporated into the ferritin deposit. There are potential side effects: administration of persistent pain at the injection site (i.m. administration) and facial flushing, hypotension, anaphylactic shock (i.v. administration).

Iron Dextran (INFeD or DexFerrum)

Iron dextran represents a colloidal solution. It is a complex of ferric oxyhydroxide with polymerized dextran. It is recommended for patients with documented ID (iron deficiency) when oral iron therapy is inadequate or unsatisfactory. Even if iron dextran has the advantage of a total-dose infusion (one administration), it has

been associated with a higher prevalence of side effects: arthralgia, myalgia, hypotension, abdominal pain, nausea. In the beginning, all new patients should receive a 25 mg test dose and they should be monitored for 1 h after the test dose. The prevalence of side effects with DexFerrum and INFeD is 9.7% *versus* 5.4%. Iron dextran may be used intravenously by infusion [the rate should not exceed 50 mg/min] and is the only parenteral iron product that can be used by the i.m. route, but it is undesirable due to the multiple side effects: staining of the skin, pain on injection and especially unpredictable absorption and delivery of iron [37].

Sodium Ferric Gluconate Complex in Sucrose Injection (Ferrlecit)

Ferrlecit for injection has a route of administration intravenous injection or venous infusion. A dose of 125 mg administrated by i.v. injection over 10 min is the standard dose, and at patients on dialysis or with chronic kidney disease the 125 mg dose needs to be repeated in 8 doses. Studies revealed that it is safe to administrate ferric gluconate to iron dextran-sensitive patients, but the intolerance to ferric gluconate was higher in iron dextran-sensitive patients than in the tolerant ones [38]. The 250 mg dose of ferric gluconate administrated i.v. over 1h has been found to be well tolerated and safe [39, 40].

Iron Sucrose Injection (Venofer)

Venofer is a complex of iron hydroxide sucrose in water, which is administrated by i.v. injection or infusion. It is recommended to be administrated in doses of 100 mg i.v. over 5 min [should not exceed 20 mg per minute], 1-3 times weekly until it reaches 1000 mg. Studies showed that iron dextran-sensitive patients were successfully treated with Venofer, with doses of 200-300 mg i.v. over 2h and being safe and well tolerated.

Ferric Carboxymaltose (Ferinject)

Ferinject is a relatively new i.v. preparation with physiological osmolality and a near-neutral pH. It can be administrated in a shorter time period (15 minutes) and in higher single doses (1000mg). Ferric carboxymaltose has been demonstrated to be efficient in the treatment of IDA in trials with inflammatory bowel disease patients, heavy bleedings and chronic kidney disease patients. Studies showed increases in haemoglobin level greater in patients treated with ferric carboxymaltose than in patients treated with iron dextran and equivalent to those treated with iron sucrose (IS). In an analysis of cost *versus* benefit, the higher cost of ferric carboxymaltose may be offset by greater efficacy compared to iron dextran administration and by savings in staff time compared to iron sucrose (one infusion *vs* 4-6 in IS).

CONCLUSIONS

Iron deficiency anemia is associated with many conditions in gastroenterology. Recognition and treatment of this condition is useful for the gastroenterological/ hepatological patient.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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Ioan Sporea

He is Professor of Gastroenterology and Hepatology, Ph.D., Department of Gastroenterology and Hepatology of the "Victor Babeș" University of Medicine and Pharmacy Timișoara, senior attendant of Gastroenterology and Internal Medicine, an expert in general ultrasonography according to the multilevel classification of SRUMB, a fellow of the EBGH. Since 2017 he is a member of the Romanian Academy of Medical Sciences. He is the coordinator of the Regional Centre of Research in Advanced Hepatology of the Romanian Academy of Medical Sciences. He is Past President of the Romanian Society of Gastroenterology and Hepatology (2012-2014) and Past President of the Romanian Society of Ultrasound in Medicine and Biology (SRUMB). During 1999-2002 he was a member of the Executive Board of EFSUMB (European Federation of Societies for Ultrasound in Medicine and Biology), during 1999 - 2005 member in the Educational Committee of EFSUMB, and between 2007-2011 he was Honorary Treasurer of this society. He is the President of the WFUMB (World Federation of Ultrasound in Medicine and Biology) Center of Education (COE) Timi?oara, and since 2017 he is Director of the WFUMB COE task force Europe. He coordinated several courses and scientific sessions in Romania and abroad, mainly regarding gastroenterology and ultrasound. He is a member of several editorial boards (i.e., Ultraschall in der Medizin, Medical Ultrasonography, Journal of Gastrointestinal and Liver Diseases, World Journal of Gastroenterology). He is the author and co-author of more than 270 original papers published in medical journals. He coordinated and participated in numerous research projects.

Alina Popescu

She is Professor of Gastroenterology and Hepatology, Ph.D., Department of Gastroenterology and Hepatology of the "Victor Babeș" University of Medicine and Pharmacy Timișoara, senior attendant of Gastroenterology and Internal Medicine, an expert in general ultrasonography according to the multilevel classification of SRUMB. She is President of the Romanian Society of Ultrasound in Medicine and Biology (SRUMB) for 2020-2022. She is a member of the Executive Bureau of EFSUMB (European Federation of Societies for Ultrasound in Medicine and Biology), of the Education and Professional Standards Committee of EFSUMB, and the World Federation of Ultrasound in Medicine and Biology eLearning Task Force. Between 2013-2015 she was Secretary of the Romanian Society of Gastroenterology in Medicine and Biology. She is the Secretary of the WFUMB (World Federation of Ultrasound in Medicine and Biology) Center of Education (COE) Timi?oara. She took part in the organization of several courses and scientific meetings in Romania and abroad, mainly in gastroenterology and ultrasound. She is a member of Medical Ultrasonography and Journal of Gastrointestinal and Liver Diseases editorial boards. Her scientific achievements are materialized in more than 160 papers published in medical journals.