GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

HABILITATION THESIS

FUNCTIONAL OR ORGANIC DIGESTIVE DISORDER?
New insight in diagnostic and therapy in neurogastroenterology

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CONTENTS

REZUMAT

Teza de abilitare cu titlul: **"Afecțiuni digestive funcționale sau organice? Elemente noi de diagnostic și tratament in neurogastroenterologie"** include cele mai importante rezultate ale activității personale științifice, profesionale și academice care au fost obținute după susținerea titlului de Doctor în medicină (din 1999 pînă în prezent). Aceste studii sînt concentrate pe 2 direcții importante de cercetare:

1. Cercetări priviind boala de reflux gastroesofagian cu manifestări extradigestive;
2. Cercetări privind sindromul de intestin iritabil.

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

Secțiunea I: cuprinde prezentarea realizărilor științifice, academice și profesionale din perioada postdoctorală;

Secțiunea II: include planurile viitoare de dezvoltare incluzînd noi teme de cercetare;

Secțiunea III: este alcatuită de lista de referințe bibiografice.

La începutul **secțiunii I** este prezentată o scurtă sinteză a activității profesionale, academice și științifice desfășurate pe parcursul întregii cariere, punîndu-se accent pe elementele sale originale. Se poate urmări astfel, imaginea de ansamblu a activității pe care am desfășurat-o pe o perioadă de aproape 30 de ani în domeniul gastroenterologiei și medicinii interne în cadrul Universității de Medicină și Farmacie "Grigore T. Popa" din Iași și a Institutului de Gastroenterologie si Hepatologie.

Activitatea științifică inclusă în această teză de abilitare se axează în primul rînd pe cercetări efectuate în domeniul Neurogastroenterologiei, elaborate în continuarea studiilor desfășurate și incluse în teza de doctorat susținută în 1999, avînd ca subiect **EPIDEMIOLOGIA, DIAGNOSTICUL ȘI TRATAMENTUL SINDROMULUI DE INTESTIN IRITABIL**. Cercetările personale desfășurate în domeniul Neurogastroenterologiei au început practic în anul 1994 cînd am beneficiat de o bursă de cercetare în Marea Britanie sub conducerea Prof David Thompson. Activitatea personală de cercetare s-a putut desfășura datorită existenței unui mediu propice pentru cercetarea științifică, realizată prin eforturile îndelungate ale domnului Prof Dr Carol Stanciu, membru de onoare a Academiei Române. Printre importantele sale realizări s-a aflat și întemeierea primului laborator de motilitate digestivă din România.

Activitatea mea științifică include un numar de: 28 de articole incluse în Thompson ISI Web of Science Core Colection (dintre care 14 autor principal și 14 articole coautor), 18 articole

în extenso incluse în ISI proceedings (6 autor principal și 12 coautor), 41 de rezumate ISI (29 autor principal și 12 coautor), 72 articole indexate BDI (PubMed) (dintre care 28 ca autor principal si 44 coautor), precum și un număr important de cărți și capitole de cărți științifice.Aceste articole beneficiază de un numar de 165 citări în ISI Web of Science Core Collection cu index Hirsh de 8 și respectiv 751 citări în Google Scholar cu index h 13.

În prima secțiune, care include realizările științifice din perioada postdoctorală se evidențiază cele două direcții majore de cercetare: **Boala de reflux gastroesofagian cu manifestări extradigestive și respectiv Sindromul de Intestin Iritabil**.

Capitolul privind fenomenele extradigestive ale bolii de reflux gastroesofagian (BRGE) include un review al datelor stiințifice în domeniu precum și rezultatele unor studii privind caracteristicile bolnavilor cu aceasta afecțiune, rezultatele unui studiu privind posibila asociere a BRGE cu fibrilația atrială, privind relația dintre boala de reflux gastroesofagian și disfonia care poate să apară la persoane care au profesii cu utilizare intensă a vocii. Capitolul include și cercetări privind importanța factorului alimentar în asocierea bolii de reflux gastroesofagian cu sindromul dispeptic și despre utilitatea folosirii medicației de protecție mucosală în tratamentul bolii de reflux gastro-esofagian.

Capitolul privind sindromul de intestin iritabil, începe cu un review ce cuprinde date noi despre afecțiunea menționată. Capitolul include studii privind importanța dietei în sindromul de intestin iritabil, aspectele fenotipice ale bolnavilor, importanța suprapopulării bacteriene în această afecțiune, modul în care o relație de încredere dintre medic și pacient este importantă pentru evoluția favorabilă a bolnavilor, precum și un studiu privind modul în care accesul și consultarea internetului influențează evoluția sindromului de intestin iritabil.

Secțiunea a II-a are ca obiect prezentarea viitoarelor direcții personale de cercetare. Aceste proiecte vor include continuarea studiilor în domeniul formelor extradigestive ale bolii de reflux gastroesofagian și a sindromului de intestin iritabil, precum și direcții noi de cercetare. În domeniul bolii de reflux gastroesofagian, cercetările vor include studii care vor evalua modul in care detecția pepsinei la nivelul tractului digestiv superior ar putea ajuta la diagnosticul acestor forme de boală. În domenul sindromului de intestin iritabil, studiile vor fi axate pe evaluarea, din punct de vedere genetic, a bolnavilor, a modului în care microbiota poate determina apariția simptomatologiei, precum și pe cercetarea modului în care relația pacient – medic poate modifica evoluția boli.

Direcții noi de studiu vor include cercetarea modului în care imagistica prin terraherz poate fi utilă în diagnosticul precoce al cancerului gastric, noi studii privind etiopatogenia dispepsiei funcționale precum și stabilirea unui algoritm rațional de diagnostic și tratament în contextul apariției unor noi metode de diagnostic. Studii și cercetări sociologice vor fi incluse pentru evaluarea impactului social al bolilor funcționale digestive dar și a bolilor inflamatorii intestinale, ambele boli cu evoluție cronică.

Dezvoltarea bazei materiale de cercetare în domeniul neurogastroenterologiei este esențială atît pentru desfășirarea acestei activități cît și pentru activitatea cotidiană, pentru asistența medicală a bolnavului și pentru o educație de calitate a studenților și rezidenților. Din păcate,

pentru acest domeniu, fondurile sînt cu totul deficitare, investițiile lipsind de ani de zile. Identificarea de granturi, inclusiv granturi structurale care să includă dotarea cu aparate și dispozitive medicale și de cercetare, va fi o prioritate pentru viitor.

Neurogastroenterologia este prin esența sa un domeniu de graniță în care studiile științifice se pot efectua doar dacă există o buna colaborare între cercetători dedicați, cu diverse competențe, din diferite domenii de activitate. Astfel, cercetările pe care le-am efectuat s-au putut desfășura doar printr-o colaborare multidisciplinară. Va fi necesară continuarea colaborăririi cu colegii din departamentele de microbiologie, genetică, imunologie, imagistică - radiologie, epidemiologie, farmacologie.

Secțiunea III include lista de referințe bibliografice utilizate pe parcursul tezei de abilitare.

ABSTRACT

The Habilitation thesis: **FUNCTIONAL OR ORGANIC DIGESTIVE DISORDER? NEW INSIGHT IN DIAGNOSTIC AND THERAPY IN NEURO-GASTROENTEROLOGY** summarizes the most important scientific, professional and academic achievements after completion of the studies included in the Doctoral thesis (1999). The thesis is focused on two directions:

1. Studies on Extradigestive Gastrointestinal Reflux Diseases;
2. Studies on Irritable Bowel Syndrome.

The thesis is elaborated in accordance with The National Council for the Attestation of University Titles and Certificates (CNATDCU). It consisted of three main sections:

Section I: Presents the scientific, academic and professional achievements of the post-doctoral period.

Section II: Includes future development and new research plans.

Section III: Includes the list of references.

At the beginning of the first section is presented a brief synthesis of personal scientific, academic and professional activity. A period of almost 30 years of activity in the field of Gastroenterology and Internal Medicine at the University of Medicine and Pharmacy "Grigore T Popa" from Iasi and the Institute of Gastroenterology and |Hepatology is presented as a broad picture.

The scientific activity included in this thesis is focused mainly on studies in the field of Neurogastroenterology, conducted after completion the PhD thesis (1999). The topic of my PhD Thesis was: 'Epidemiology, Diagnosis and Therapy of Irritable Bowel Syndrome'. The beginning of my studies and also my interest in Neurogastroenterology started in 1994 when I held a position of visiting research fellow at the University of Manchester (UK), working under the supervision of Prof David Thompson. However, this interest in Neurogastroenterology was possible due to the efforts of Prof Carol Stanciu, Honorary Member of Romanian Academy who developed the first Digestive Motility Lab in Romania, allowing a success framework for scientific research.

My scientific activity consists of a number of 28 papers included in Thompson ISI Web of Science Core Collection (14 papers as main author and 14 as co-author), 18 ISI proceedings papers (6 as main author and 12 as co-author), 41 abstracts (29 main author and 12 as co-author), 72 BDI papers (PubMed) (28 main author and 44 co-author). The papers lead to 165 citations in ISI Web of Science Core Collection with Hirsh Impact Factor of 8 and 751 citations in Google Scholar with h index of 13. My scientific activity included an important number of books and chapters in several scientific books.

The **section I** includes the results of several scientific studies developed after my PhD and is organized in two main directions: Studies on Extradigestive Gastrointestinal Reflux Diseases (GERD) and Studies on Irritable Bowel Syndrome.

The section focused on Extradigestive GERD starts with an up-to-date review of the field. The section includes studies on demographic, clinical, biological and endoscopic features of these patients, on the atrial fibrillation and sympathovagal balance in patients with Gastrointestinal Reflux Disease and on the environmental factors associated with dysphonia in professional voice users. The section also includes studies on the role of diet in the overlap between GERD and functional dyspepsia and studies on the mucosal protective compounds in GERD.

The section on the Irritable Bowel Syndrome is starting with a review of newly published data. Personal studies on several topics are included in this chapter, such as: studies on the relationship between diet and Irritable Bowel Syndrome, IBS phenotypes, bacterial overgrowth and its relationship with IBS. The section includes also studies on the relationship of trust between doctor and patient and the impact of the internet on this relationship.

In **section II** are presented the plans for future studies. It includes new studies on Extradigestive Gastroesophageal Reflux Disease, new studies on Irritable Bowel Syndrome and also new research directions. Related to GERD, new directions will include studies on the importance of detecting pepsin as a marker for diagnosis extradigestive GERD. Related to Irritable Bowel Syndrome, new directions will include genetic studies, studies on microbiota and future studies on how doctor patient-relationship may affect the evolution of the disease.

New research direction will be developed in the future. These will include the evaluation of Therahertz imaging role in the early detection of gastric cancer, studies on the etiology of functional dyspepsia and also on new elements for the diagnostic of functional dyspepsia. Studies on the social aspect of functional digestive but also on inflammatory bowel diseases will be done to better characterized and put into the life context these chronic diseases.

Development and upgrade of the medical infrastructure in neurogastroenterology is essential for high quality research. It is also essential for daily clinical practice and teaching undergraduates and fellows in gastroenterology. Unfortunately the investments in this infrastructure was very poor in the last years. Identifying funding sources including research and structural grants is essential for future positive results.

Neurogastroenterology is a new field developed at the boundary of several fields. Positive development will be possible only in multidisciplinary teams. It is essential to continue the good relationship with colleagues from several departments such as: microbiology, genetics, immunology, Imagistics-radiology, epidemiology and pharmacology.

Section III of the habilitation thesis includes the list of bibliographic references used for the elaboration of the thesis.

OVERVIEW OF PERSONAL PROFESSIONAL, ACADEMIC AND SCIENTIFIC ACHIEVENTS

The habilitation thesis entitled: **"FUNCTIONAL OR ORGANIC DIGESTIVE DISORDER? New insight in diagnostic and therapy in neurogastroenterology"** presents the main directions of research that followed after defending with success my PhD Thesis. Before starting my PhD I was accepted for one year (between 1993 and 1994) as Visiting Clinical Research fellow in gastroenterology at Hope Hospital, Salford-Manchester, UK under the supervision of Prof David Thompson. My fellowship studies on small bowel motility opened my interest in the field of neurogastroenterology. After my return, I started my doctoral studies, coordinated by Prof Carol Stanciu, in the same field and I defended my PhD Thesis entitled: "Irritable Bowel Syndrome – Epidemiology, Diagnostic and Therapeutic Aspects" in 1999.

Scientific research in neurogastroenterology was the most important and performing topic in the activity of Iasi Gastroenterology Center starting from the'70. The world recognized activity in the field was led by Professor Carol Stanciu, honorary member of the Romanian Academy. Due to his activity in 2004 the Institute of Gastroenterology and Hepatology Iasi was recognized as CENTER OF EXCELENCE IN GASTROENTEROLOGY AND HEPATOLOGY by the governing bodies (CNCSIS).

My PhD Thesis included several studies about Irritable Bowel Syndrome (IBS) that are still valid and cited today. It started with an epidemiological study on the prevalence of IBS and showed that 14.49% of general population have IBS. Also, factors influencing the motor and sensory responses of the human small-intestine to distention were studied and showed that duration, length and speed of distention are important factors. Anorectal manometry studies on IBS patients and controls were included and showed that IBS patients tend to have lower sensibility threshold and also that anorectal manometry may help revealing diseases that may explain IBS symptomatology. Follow-up of the IBS diagnosed patients revealed that the management is sub-optimal, almost half of patients maintaining or even worsening symptomatology.

After my PhD studies I followed my interest in clinical research in gastroenterology particular in neurogastroenterology and digestive endoscopy. Medical research implies important human resources and quality infrastructure. Personal research activity in neurogastroenterology imposed the necessity of developing a good and competitive interdisciplinary research team.

I was honor to include in this team: Prof. Dr. D Cobzeanu, Assoc. Prof. Dr. I Palade (ENT) lecturer Dr. Lavinia Caba, Prof. Dr. Vlad Gorduza (Genetics), lecturer Dr. Aida Bădescu, Prof. Dr. Smaranda Iancu (Microbiology), Dr. Ioan Chirila, Prof. Dr. Doina Azoicăi, Assoc Prof. Dr. F. Petrariu (Epidemiology and Public Health), Assoc. Prof. Dr. Mariana Floria (Cardiology), Assoc. Prof Dr. Liviu Oprea (Family Medicine, Public Health), Lecturer Psychologist Tudor Rotaru, Prof. Dr T. Mihaescu (Pneumoftiziology). Fruitful and efficient collaboration was done also with important teams from Romania, namely Cluj: Prof. Dan Dumitrașcu – Cluj and also

from abroad: Prof. R. Tutuianu (Bern), Prof. Beate Niesler (Heidelberg), Prof. A. Bradenoor, Dr. R. Timmer (Amsterdam, Utrecht), and Prof. Q. Aziz (London).

During past years my scientific activity consisted in important number of publications: 6 books, 27 book chapters, 47 ISI articles and proceedings papers, 72 papers included in PubMed with ISI Hirsh index 7 and Google scholar h index 13.

My main interest in research, in all these years are related to the field of Neurogastroenterology with two main areas: Extradigestive Gastroesophageal Reflux Diseases (GERD) and Irritable Bowel Syndrome.

Extradigestive GERD patients were studied looking for the demographic, clinical, biological and endoscopic features of the patients, evaluating the possible relationship between GERD and the atrial fibrillation, the environmental factors associated with dysphonia in professional voice users. The role of diet in the overlap between GERD and functional dyspepsia and the role of mucosal protective compounds in GERD were also studied.

The Irritable Bowel Syndrome was studied looking for the relationship between diet and Irritable Bowel Syndrome, IBS phenotypes, bacterial overgrowth and its relationship with IBS. We also studied the relationship of trust between doctor and patient and the impact of the internet on this relationship.

The studies on these research topics were able to be fulfilled due to the fact that I was personal involved in the following grants: BMBS COST Action BM1106 The Genes in Irritable Bowel Syndrome Research Network Europe (GENIEUR) 2012-2016 and also as Clinical Research Coordinator in the POSDRU/159/1.5/S/133377 Project: “Coordination of Doctoral and Post-Doctoral Research in Chronic Diseases”

Ongoing research interest and activities are continuing in the field of Digestive Endoscopy having published several papers in the field. Recent personal studies are focusing on gastric cancer and on new possibilities for early diagnosis such as Terahertz Spectroscopy and Imaging. Studies was partial supported by the NanoTerraplasia - cod: PN-III-P2-2.1-PED-2016-1598 2017-2018 research Grant.

Being a member of University staff since 1991 my activities included not only research activities but also teaching and clinical duties.

TEACHING ACTIVITY

My didactic activity has a duration of 28 years. All these years, I worked only at the University of Medicine and Pharmacy “Grigore T Popa” Iasi. During this period, I passed through the necessary steps in the university career from assistant professor in training in 1991 to professor in 2017. The didactic tasks varied according to my didactic position. It included teaching undergraduates from the Romanian and also English program medical semiology (III rd. year) and gastroenterology (Vth year). I was also invited as lecturer to teach research methodology (III rd. year) and to be involved in postgraduate teaching. I was honored to be invited by the students to give lectures at their CONGRESSIS meetings on topics related to gastroenterology.

The postgraduate education consisted in training gastroenterology fellows in gastroenterology, internal medicine, general medicine, rheumatology and dermatology. Since 2018, I am one of the academics delegated to coordinate the gastroenterology training program at the University of Medicine and Pharmacy "Grigore T Popa" Iasi.

During years, my didactic activity included lecturing for Clinical Epidemiology Master Degree Course and also for Doctoral Studies. A postgraduate course in Diagnostic and Therapeutic Techniques in Gastroenterology was proposed and delivered with success for gastroenterologists, internal medicine and family doctors for a period of years since 2005. Various other continuous medical education programs on several gastroenterology topics were also part of my didactic activity. My academic career was acknowledged by my peers, being elected to be member of the Council of the Faculty of Medicine in 2016 and 2020, member of the Senate of the University of Medicine and Pharmacy "Grigore T Popa" Iasi.

CLINICAL CAREER

Clinical activity represents an important part of daily activities. From 1994 I become Specialist in Internal Medicine and from 1997 Specialist in Gastroenterology. Since 1999 I am Consultant in Medicine and from 2001, also Consultant in Gastroenterology. I have formal training, and license for: "General Ultrasonography" from 1999, "Diagnostic Digestive Endoscopy" from 2002 and "Therapeutic Digestive Endoscopy" from 2010. My major clinical training was at the University Hospital "St Spiridon' from Iasi, at the II-nd Medical Clinic Gastroenterology, also called later: Institute of Gastroenterology and Hepatology.

Struggling for good quality training was an important characteristic of my clinical career, especially in the early years. It was done in several important gastroenterology centers from Europe. In 1996-1997 I received a bursary from France Foreign Ministry at General Hospital Beziers –France for training in gastroenterology and gastrointestinal endoscopy (Dr Olivier Duhamel); in 1998 bursary entitled: "Oxford Hospitality Scheme" at the Gastroenterology Unit of Radcliff Hospital, Oxford, UK also for training in gastroenterology and inflammatory bowel disease (Prof Derek Jewel), in 2000 Bursary from the Netherlands Society of Gastroenterology at "St Antonius Ziekenhuis" Nieuwegein (Dr Robin Timmer) and in 2001: European Society of Gastrointestinal Endoscopy Postgraduate Grant - at Hospital Val d'Hebron, Barcelona, Spain (Prof Armengol-Miro). During my training I received cutting edge knowledge in gastroenterology, neurogastroenterology and digestive endoscopy allowing me to learn the correct and modern management of gastroenterology patients.

My clinical career was acknowledged by my peers, being invited to be a member of the Experts Committee for Gastroenterology of the Ministry of Health in Romania, Experts Committee of the Romanian College of Physicians. I was also elected Vice-President of the College of Physicians from Iasi.

SECTION I.
SCIENTIFIC ACHIEVEMENTS

I. EXTRADIGESTIVE GASTROESOPHAGEAL REFLUX DISEASE

I.1. STATE OF THE ART

Gastroesophageal reflux disease (GERD) is considered nowadays a disease characterized by esophageal mucosal lesions or troublesome symptoms due to abnormal gastro-esophageal reflux. Extra digestive GERD is a recognized form of gastroesophageal reflux diseases according to 2006 Montreal Consensus. In this document, it is stated that chronic cough, chronic laryngitis, asthma, dental erosions, especially on the lingual and palatal tooth surfaces are significantly associated with GERD. However, it stated that GERD is rarely the sole cause involved in ethiopathology of these conditions (Vakil et al., 2006)**.** On the opposite, it is unclear whether GERD has a significant causal or exacerbating role in the pathogenesis of sinusitis, pulmonary fibrosis, pharyngitis, or recurrent otitis media or for triggering apneic episodes in patients with obstructive sleep apnea (Vakil et al., 2006).

Another term used in conjunction with GERD is laryngopharyngeal reflux disease (LPR). It is also known as supraesophageal reflux disease, atypical gastroesophageal reflux disease (GERD) or reflux laryngitis (Ford, 2005). GERD and LPR are considered to be part of a spectrum within the same disease. However, they are different clinical entities produced through patho-physiological mechanisms which may not be similar. Koufman launch the name of laryngopharyngeal reflux disease in the ‘90 and is now a frequent recognized clinical condition. Laryngopharyngeal Reflux Diseases (LPRD) is often used by otolaryngologists to describe laryngeal findings of irritation in patients with chronic throat symptoms, associated with gastroesophageal reflux (Saritas Yuksel and Vaezi, 2012). In the USA, during a period of 11 years, the number of ENT consultations for clinical conditions related to GERD increased by over 300% and the prescription of proton pump inhibitor (PPI) also multiplied about 14 times.

Epidemiology

The prevalence of GERD symptoms reported from worldwide population-based studies, (however with large geographic variation) is considered to be 13% (Eusebi et al., 2018). It is estimated that 4-10% of ENT patients have GERD. At the same time, LPR was identified in more than 50% of dysphonic patients (Campagnolo et al., 2014). In the last decades, the prevalence of GERD and LPR has increased dramatically. Using a statistical model in an analysis of 17 studies, El-Serag (El-Serag et al., 2010) showed that the average increase rate of reflux disease since 1976 has been 4% per year (P <0.0001).

Pathophysiology

At least two mechanisms are suggested to be involved in the pathophysiology of LPR. One mechanism may be related to the direct injury of the esophageal and pharyngolaryngeal mucosa by the gastro-duodenal content, with or without microaspiration in the respiratory airways. The larynx is an organ about 100 times more sensitive than the esophagus and it is at high risk of injury due to its immediate proximity to the digestive system (Koufman, 2002). Thus, only a small amount of gastric content is sufficient to cause lesions of the larynx. The second mechanism that is incriminated in the pathophysiology of LPR is the tracheal - bronchial reflex mediated by the vagal nerve, produced by the acidification of the distal esophagus, and which determines cough, throat clearing and laryngospasm (Koufman, 2002). The vocal folds are covered by stratified squamous epithelium which creates a barrier against external and internal factors, including reflux. Erickson and Sivasankar conducted a study using transepithelial electrical resistance (TER) for assessing the permeability of the epithelium of the vocal folds. They found that the exposure of vocal folds to refluxate determines the decrease of TER, making them more susceptible to injury (Erickson and Sivasankar, 2010). It has also been demonstrated that carbonic anhydrase type III plays a protective role of the epithelium of the larynx, as it regulates cellular pH when the larynx comes in contact with acid by producing bicarbonate (Gill et al., 2005). Ford (Ford, 2005) showed that this enzyme was absent in 64% of patients with LPR. The peptic injury was also associated with depletion of other key protective proteins such as E-cadherin and the stress proteins (e.g. Sep 70) (Gill et al., 2005).

Clinical manifestations of Laringo-Pharingeal Reflux (LPR)

Despite the association between GERD and LPR, the diagnosis of reflux laryngitis remains a difficult task. First, a complete and accurate anamnesis is needed for correct diagnosis. According to the study published by Koufman two decades ago, we should discriminate in clinical practice between GERD and LPR as distinct clinical entities (Koufman, 2002). The symptoms of LPR are diverse and many physicians may not link these symptoms to LPR. The most common manifestations of LPR are dysphonia/ hoarseness, throat clearing or pain, chronic cough and globus (Voineag et al., 2011; Cobzeanu et al., 2012). However, it is known that these symptoms are nonspecific to LPR and can also be found in other diseases such as postnasal drip syndrome, exposure to different allergens or environmental irritants like smoke or vocal abuse. Because these symptoms are not exclusive due to LPR, the clinicians should rely on a combination of manifestations and diagnostic tools such as laryngoscopy exam, impedance-pH monitoring and empiric trial of proton pump inhibitors (PPI) to establish an accurate diagnosis. It is important to emphasize that patients with LPR do not usually present typical GERD symptoms (heartburn and regurgitation), leading to increase difficulty in diagnosis (Ford, 2005).

Diagnostic methods.

Laryngoscopy

The diagnosis of **reflux laryngitis** is usually performed by the ENT surgeons. Transnasal fiberoptic laryngoscopy is the first line method to be used by otolaryngologists to diagnose LPR.

Laryngoscopic exams are essential for excluding neoplasms, but their role in detecting GERD associated laryngitis remains uncertain. There is high variability among ENT surgeons evaluating the same laryngeal signs. The specificity of the laryngeal signs of LPR is still debated in the literature. The most frequent laryngoscopic lesions that have been found to be related to GERD were edema and erythema, particularly in the posterior region. These abnormalities were the main findings used by different researchers for the diagnosis of LPR, while ulcers and ventricular obliteration, nodules, polyps and leukoplakia were reported to be less useful. In another study, the presents of pseudo-ulcus was found to have 70% sensitivity and 77% specificity in patients with LPR (Belafsky et al., 2002). In 2007, Vavricka et al. evaluated the prevalence of specific laryngeal lesions thought to be GERD related, both in patients with documented reflux and normal subjects (Vavricka et al., 2007). They found that the prevalence of laryngeal lesions was the same in both groups. Only posterior pharyngeal wall findings showed a statistically significant higher prevalence in GERD patients as compared to the control group. Furthermore, the study of Hicks et al. revealed that the most common signs seen in patients suspected of LPR were also found in healthy, asymptomatic subjects (Hicks et al., 2002). To improve the diagnostic accuracy, Belafsky et al. developed the Reflux Finding Score (RFS) based on the presence and severity of the lesions found in laryngoscopy (Belafsky et al., 2002). This scoring system including the most common laryngoscopic lesions has reasonable reproducibility and is useful to evaluate the effectiveness of therapy in patients with LPR. However, Rafii et al. reviewing patients already known with laryngopharyngeal reflux revieled that these patients have in fact other laryngeal causes for dysphonia (Rafii et al., 2014). The most common findings were vocal fold paresis (29%) and muscle tension dysphonia (14%). Therefore, the present recognized recommendation is that the diagnosis of reflux induced laryngitis should not be made alone on laryngoscopy findings.

Upper digestive endoscopy

Upper digestive endoscopy has excellent specificity for the diagnosis of GERD in the presence of an erosive esophagitis, but only one third of patients with GERD symptoms and even fewer after treatment with PPIs have erosive esophagitis (Gralnek et al., 2006). However, it is important to notice that, although upper endoscopy is necessary to rule out esophageal cancer, it is not a specific diagnostic test for LPR. Indeed, it has been reported that up to 80% of LPR patients have normal upper digestive endoscopy. Red flags that implies the necessity of early endoscopy include dysphagia, bleeding, chest pain, weight loss or dyspnea.

Ambulatory 24 H pH impedance-monitoring.

Ambulatory 24 H pH impedance-monitoring is a considered nowadays the gold standard for the diagnosis of GERD including extradigestive forms (Gyawali et al., 2018). Esophageal pH-impedance monitoring is a method that detects both the anterograde and retrograde movements of the refluxate and evaluates the physical (liquid, gaseous or mixed) and chemical (acid or non-acid) nature of the refluxate (Barboi et al., 2015; Saritas Yuksel and Vaezi, 2012). However, the sensitivity and specificity of ambulatory pH monitoring for diagnosing GERD in

patients with extraesophageal reflux symptoms is far from perfect (Campagnolo et al., 2014). In the presence of esophagitis, the sensitivity and specificity of this method is higher (up to 100%), but in patient without esophagitis, the sensitivity is dropped to about 70% (Hirano et al., 2007). The availability of multichannel intraluminal impedance and pH monitoring (MII-pH) seems to perform better in the diagnosis and management of extraesophageal manifestations of GERD than pH-testing alone (Carroll et al., 2012).

Another technology used to detect acid involved in LPRD is Dx-pH measurement system (Respiratory Technology Corp., San Diego, CA). The device is measuring pH in liquid or aerosol droplets (Sun et al., 2009; Saritas Yuksel and Vaezi, 2012,). However, this tool has limited worldwide daily clinical use.

Pepsin testing

The pepsin test (PepTest) is a non - invasive, quick and inexpensive tool for the diagnosis of GERD. It consists of a lateral flow device that uses 2 monoclonal antibodies to human pepsin to detect the presence of pepsin in saliva. The results can be read in 5-15 minutes. Because of the benefits and ease of application, a positive salivary pepsin test in a patient suspected of having LPR can be a cost effective, accurate and alternative diagnostic method. Yuksel et al. (Saritas Yuksel et al., 2012) found sensitivity and specificity of 87%, a positive predictive value of 85% and a negative predictive value of 68% for the pepsin test in the diagnosis of LPR. In a very recent cross-sectional study, *in vitro* pepsin detection tests were compared to the 24-hour double probe pH monitoring results for patients with suspected LPR (Ocak et al., 2015). The authors found a sensitivity and specificity of the pepsin detection test of 33% and 100%, respectively. A positive predictive value of 100% was recorded (Barboi et al., 2015) (Saritas Yuksel and Vaezi, 2012b). However, recently published meta-analysis of good quality papers that included patients with GERD but not with laringopharingeal reflux showed that sensitivity, specificity, NLR and PLR were 0.60 (95% CI 0.41-0.76), 0.71 (95% CI 0.51-0.86), 0.56 (95% CI 0.34-0.93) and 2.1 (95% CI 1.1-4.1) respectively. It concluded that Pepsin testing (PepTest) may have only moderate diagnostic value for GERD, being less successful that was anteriorly believed (Guo et al., 2018). However, new meta-analysis and systematic reviews for extradigestive GERD would be useful to be developed.

PPI diagnostic test

The therapeutic test with 3 months double dose PPIs is considered by many clinicians as a reasonable practical clinical approach (Koufman et al., 2002). However, the role of diagnostic PPI trial is still controversial. A meta-analysis of randomized studies has demonstrated no benefit of PPIs vs. placebo in diagnosing GERD related laryngitis (Koufman et al., 2002; Qadeer et al., 2006). However, the current recommendation is for the empirical trial in case of patients with concomitant typical symptoms of GERD (Ford, 2005). The advantage of this diagnostic tool seems to be cost-effectiv, with convenient use, good sensitivity, but with low specificity (Saritas Yuksel and Vaezi, 2012, Barboi et al., 2015).

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I.2. GASTROESOPHAGEAL REFLUX DISEASE - EXTRADIGESTIVE MANIFESTATIONS: DESCRIPTION OF DEMOGRAPHIC, CLINICAL, BIOLOGICAL AND ENDOSCOPIC CHARACTERISTICS

I.2.1. INTRODUCTION

Apparently a banal disease, Gastroesophageal Reflux Disease (GERD) has a significant morbidity, consuming human and material resources and burdening health system budget. The worldwide prevalence of GERD symptoms is considered to be 13% ranging from 10% in Sotheast Asia, Canada and France to more than 25% in South Asia and Southeast Europe (Eusebi et al., 2018). Advancing age is associated strongly with complications such as erosive esophagitis, strictures, Barrett and adenocarcinoma (Thukkani and Sonnenberg, 2010). Obesity is a major risk factor for GERD symptoms with odds ration of 1.73 (Eusebi et al., 2018). Also, H Pylori infection may influence the presence of GERD symptoms (Richter and Rubenstein, 2018).

Data published in 2013 by Francis et al (Francis et al., 2013) showed that in USA the expenses for the management of extradigestive GERD are comparable with the cancer spending.

Patients with GERD may be asymptomatic, but also may present heartburn, regurgitation, and less specific dysphagia, chest pain, nausea, vomiting, hiccups, burping and odynophagia. Sensitivity and specificity for symptom based diagnosis is considered suboptimal ranging between 63% to 67% for sensitivity and 63% to 70% for specificity (Richter and Rubenstein, 2018). This suboptimal value of GERD symptoms was showed by the Diamond study (Dent et al., 2010).

Upper gastrointestinal endoscopy is considered the most useful diagnostic test in GERD management (Kahrilas et al., 2017). However, non-esophagitis GERD is very common in this disease. Nowdays, ambulatory 24 H pH impedance-monitoring is considered to be the gold standard for the diagnosis of GERD, showing better accuracy comparing with 24 H pH measurements (Gyawali et al., 2018).

These methods are used also for the diagnosis of extradigestive GERD. According to the Montreal classification the GERD established associations are: the reflux cough syndrome, chronic laryngitis, bronchial asthma and dental erosions. There are also proposed associations such as pharingitis, sinusitis, idiopathic pulmonary fibrosis and recurrent otitis media (Vakil et al., 2006).

There are limited data on extradigestive GERD in Romanian patients.

The aim of this study was to evaluate extradigestive GERD patients in terms of demographic, clinical, biological and endoscopical features.

I.2.2. MATERIAL AND METHOD

A prospective case-control study including 137 patients referred to the Iasi Institute of Gastroenterology and Hepatology Iasi from otolaryngology, cardiology and pneumology services in the interval July 2014 - September 2015 was performed. The study included adult patients previously diagnosed with asthma, non-cardiac chest pain or chronic laryngitis in which GERD

was also suspected. Written informed consent for the participation in the study was obtained. Patients with prior gastric or esophageal surgery, malignant tumors, specific esophageal motility disorders (achalasia, scleroderma, myopathies), alarm signs (hematemesis, jaundice, unexplained weight loss), psychiatric disorders, pregnant women, peptic ulcer, active tuberculosis or other acute lower respiratory tract infections, unstable arrhythmias or myocardial infarction in the last 6 months were excluded from the study.

Demographic data, personal history of diseases and smoking were recorded in individual files. All patients were tested for Helicobacter pylori antibodies by ELISA. Knowing that obesity is a risk factor for GERD, the cholesterol and triglycerides serum levels were analyzed and body mass index (BMI) was calculated in all patients. Obesity was considered present if BMI was greater than 30kg/m^2.

Based on the presence/absence of typical symptomatology (heartburn or regurgitation), the patients were investigated in order to diagnose or exclude GERD. The patients presenting typical manifestations underwent a therapeutic test with double-dose PPI (Pantoprazole 40 mg BID), while the patients not presenting the classical symptoms were assessed by upper digestive endoscopy (UDE) for the detection of the lesions of esophagitis or hiatus hernia. In some cases biopsies were taken and submitted to anatomopathological exam for diagnosing Barrett's esophagus. Patients without esophageal lesions were further assessed by impedance-pH metry in order to confirm GERD.

Data were statistically analyzed using SPSS 18.0. The used parametric tests were: student's t- test for comparing the means, χ^2-test for comparing frequency distribution and Pearson's test correlation coefficient. A p value < 0.05 was regarded as statistically significant.

I.2.3. RESULTS

According to the predominant extradigestive manifestation, the patients were assigned into 3 groups: group I included 94 patients with chronic laryngitis, group II-24 patients with asthma and group III-19 patients with non-cardiac chest pain.

Demographic features. Both non-cardiac chest pain group and chronic laryngitis group showed a slightly tendency towards females (68.4% and 52.1%, respectively), while asthma patients were more likely to be men (58.3%), the majority (78.1%) living in urban areas. The study patients were homogenous in terms of gender (p=0.218): 47.4% were males and 52.6% were females (fig. 1).

The age of reflux laryngitis patients ranged from 20 to 83 years, with a mean of 50.33±15.10 years and a median of 50 years. In the asthma group the mean age was slightly lower than in group I (50.25 ±14.14 years) and the median was also 50 years. The age of non-cardiac chest pain patients ranged between 44 and 74 years, with a median of 59 years and a mean age of 58.84±8.33 years, which is significant higher compared to the other two groups (p=0.05) (tab. I). The age > 50 years induced an estimated risk of GERD four times higher in the pseudoangina group (p=0.012).

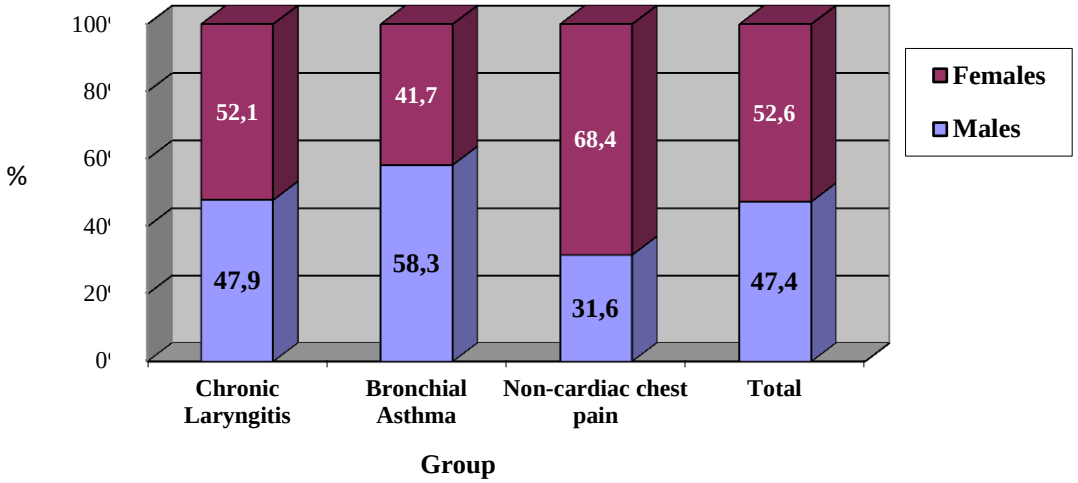

Fig. 1. Distribution of gender

Table I. Study groups characteristics

Study group (Lots)	N	Mean age	Standard deviation	Std. error	Confidence interval		Min	Max	p F_{ANOVA} test
					- 95%CI	+95%CI			
I	94	50.33	15.10	1.56	47.24	53.42	20	83	**0.050**
II	24	50.25	14.14	2.89	44.28	56.22	18	77	
III	19	58.84	8.33	1.91	54.83	62.86	44	74	
Total	137	51.50	14.41	1.23	49.06	53.93	18	83	

Mean age was lower in males presenting asthma and laryngitis (p=0.002).

Of the 137 patients enrolled, 1 was under 20 (0.7%) and 3 (2.2%) were over 80 years of age. In group I the maximum frequency was between 40-49 years (26.6%), in group II-between 50-59 years (33.3%) and in group III-between 60-69 years (47.4%) (p=0.026) (fig. 2).

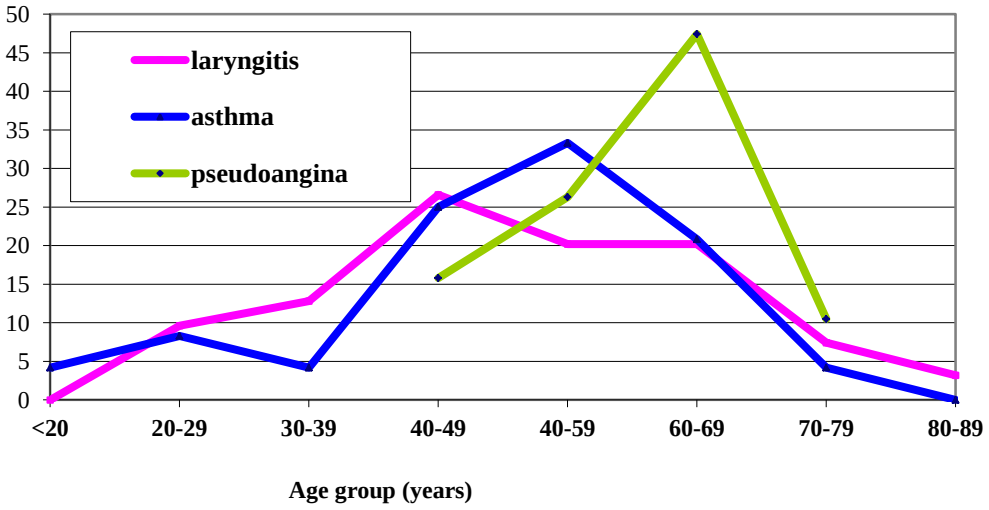

Fig. 2. Study group characteristics - age

Clinical features. Typical reflux symptoms were more frequent in asthma subjects (54.2%), but there were no significant statistical differences between this group and laryngitis (39.4%) and non-cardiac chest pain patients (36.8%) (p=0.384) (Fig. 3).

The highest frequency of obese patients was found in the pseudoangina group (52.6%) and the lower in the laryngitis group (16%), with significant statistical differences (p=0.002); the overall percentage of obese people was 23.4%.

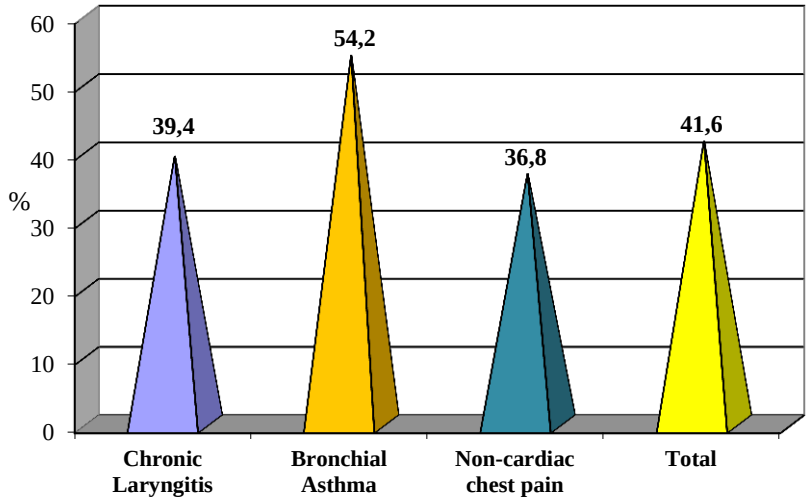

Fig. 3. Distribution of typical symptoms by study groups

According to smoking status, the patient's distribution by study groups was homogenous. Although the group with pseudoangina recorded the lowest rate of smoking patients (5.3%), there were no statistical differences between reflux laryngitis (18.1%) or GERD-related asthma patients (25%) (p=0.234).

Biological features. Dyslipidemia was found in 57.9% of patients presenting non-cardiac chest pain, which is a significantly higher in comparison with the other two groups (24.5% for laryngitis and 37.5% for asthma) (p=0.013).

Helicobacter pylori infection was identified in 52.6% of all GERD patients, without any significant statistically differences between study groups; fewer cases of infection with Helicobacter pylori were identified in the pseudoangina group (p=0.619).

Endoscopic features. Esophagitis was found more frequently in patients with non-cardiac chest pain (84.2%) compared to the other two groups (79.8%, 75% respectively) (p=0.115). Statistical analysis showed Barrett's esophagus in only 6.6% of GERD patients, but with no significant differences between the study groups (p=0.457). Hiatus hernia occured in 63.2% of pseudoangina patients and in only 36.2% and 41.7% of patients with laryngitis and asthma, respectively (p=0.094).

I.2.4. DISCUSSION

Our study is one of the few studies on extradigestive GERD conducted in Romania. It is known that GERD with or without extradigestive manifestations is found in approximately equal proportions in both genders, with a slight tendency towards males (Nilsson et al., 2004), (Cobzeanu et al., 2012). In our study there was a gender homogeneity of the study groups, with a slightly higher frequency of women in the group with non-cardiac chest pain.

GERD can occure at any age, but it is most commonly diagnosed after 40 years (Johnson and Fennerty, 2004),(Isolauri and Laippala, 1995). The age of our patients showed wide variations in the three study groups. The mean age was 50 years both in patients with laryngitis and asthma, but in the pseudoangina group the mean age was significant higher (58 years), suggesting that age is a risk factor for this category of GERD patients. The highest frequency of young patients was recorded in the group with laryngitis, while older patients were most numerous in the group with pseudoangina.

In the literature (Garcia Rodriguez et al., 2008), (Beedassy et al., 2000), (Festi et al., 2009) it is postulated that smokers, obese and dyslipidemic patients are more likely to have GERD. In our study, a quarter of asthma patients were smokers, with a 1.57 times higher relative risk of developing GERD. It appears that non-smoking status may be a protective factor in patients with pseudoangina, but this result can not be extrapolated to general population. We found obesity in less than a quarter of extradigestive GERD patients, of which half were in group III. The highest percentage of patients with dyslipidemia was found in the group with non-cardiac chest pain. The relative risk of developing GERD in these patients was 3 times higher.

It is well known that typical symptoms of reflux (heartburn/regurgitation) may be absent in patients with extradigestive GERD in upto 50% (Saritas Yuksel and Vaezi, 2012), (Voineag et al., 2011). Up to 40%-60% of patients with Bronchial Asthma and GERD evidenced with pH-impedance may not exhibit typical esophageal symptoms (Sandur et al., 2014). Similar results were obtained, however, an increased proportion of patients experiencing both typical and extradigestive manifestations were in the asthma group.

However, there are currently no investigations that are good enough to diagnose safely extradigestive GERD. Three months therapeutic trial with double dose PPI has a recognized diagnostic value. However, non-response to the therapeutic trial does not exclude GERD as a causative factor due to the presence of weakly acidic and biliary reflux. Upper digestive endoscopy is the first technique that a gastroenterologist is using to confirm/exclude GERD. The presence of esophagitis does not mean that GERD may cause extradigestive symptoms in a specific patient. Also, many patients are currently taking PPI before endoscopy and the esophagitis may be cure at the moment when endoscopy is done (Ghisa et al., 2019).

Many patients were referred from the ENT surgeon due to abnormalities observed at laringoscopy. However, laryngeal signs attributable to reflux can be found in up to 86% of healthy subjects. Also, a high level of inter and intra-observer variability is recognized for these lesions (Vaezi and Sifrim, 2018).

Lesions of esophagitis were observed in a high percentage of our patients (62.8%), especially in those with pseudoangina, result that is not consistent with other studies (Dickman et al., 2007), (Koufman et al., 2002). Barrett's esophagus has been identified in a minority of cases regardless of dominant extradigestive manifestation. Hiatus hernia, known as a contributing factor in GERD (Noble, 2013), was present in comparable percentages in the laryngitis and asthma group, exceeding 50% of cases only in the pseudoangina group.

Literature data referring to the association between *Helicobacter pylori* infection and GERD are contradictory. Some studies, (Loffeld et al., 2000; Fallone et al., 2000; Koike et al., 2001) support the protective role of this bacteria in patients with GERD, while other studies (Wu et al., 2002; Rubenstein et al., 2014), () postulate that *Helicobacter pylori* infection is a favorable factor in GERD. In our patients, *Helicobacter pylori* infection was identified in just over half of the cases, the lowest frequency being in the pseudoangina group. This result would suggest that in this category, *Helicobacter* infection may play a protective role in the development of GERD (RR = 0.61). However, the results cannot be extrapolated to the general population.

I.2.5. CONCLUSIONS

Our study supports the idea that extradigestive manifestations of GERD are frequent and heterogeneous. There are differences between patients with GERD and chronic laryngitis, asthma and non-cardiac chest pain in terms of demographic, clinical, biological and endoscopic features. We demonstrated that extradigestive manifestations correlate with smoking, obesity and dyslipidemia, as contributing factors. Typical GERD symptoms are absent in about half of extradigestive GERD patients, making the diagnosis being sometimes difficult. It is obvious that *Helicobacter pylori* infection influences the evolution of GERD, but is still not clear if it is in a positive or a negative way.

I.3. ATRIAL FIBRILLATION AND GASTROESOPHAGEAL REFLUX DISEASE

I.3.1. INTRODUCTION

Atrial fibrillation (AF) is the most common arrhythmia in medical practice. Its incidence has risen in countries with rapidly aging populations (January et al., 2014). It reaches the highest values (about 20%) in a population older than 60 years. In the last two decades, an increase of 13% of the incidence was noted. Over 6 million Europeans suffer from AF and due to aging, its prevalence is estimated to become at least double in the next 50 years (Camm et al., 2010; Roman et al., 2014).

Atrial fibrillation occurs when structural and/or electrophysiological abnormalities (electrical, mechanical and structural remodeling of left atrium) alter atrial tissue to promote abnormal impulse formation and / or propagation. Beginning with this step AF begets AF, through remodeled atrial myocardium. Left atrial remodeling may be caused by diverse pathophysiologic mechanisms and AF may represent a final common phenotype for multiple disease pathways.

In a large majority of patients with AF the background condition is represented by arterial hypertension, obesity or diabetes mellitus. These were reported as substrate for left atrial remodeling. In addition, patients diagnosed with idiopathic AF have most frequently suffered from insidious coronary artery disease than healthy controls (Calo and Martino, 2012). Most common risk factors for both AF and GERD are obesity and aging. In addition both AF and GERD are associated with other pathologies like sleep apnea or diabetes mellitus. In AF, similar with other cardiac arrhythmias, three elementary electrophysiological mechanisms, alone or in combination, may be involved in the initiation and perpetuation of arrhythmia. These are: enhanced or abnormal automaticity, triggered activity and reentry (Floria et al., 2015).

Gastroesophageal reflux disease (GERD) is a frequent benign disorder of the upper gastrointestinal tract (Vakil et al., 2006). Due to the close positioning of the esophagus and the atria and their similar autonomic innervations, it has been proposed that GERD could be associated with the occurrence of AF. Sympathovagal imbalance seems to be involved both AF and GERD occurrence (Roman et al., 2014). Hiatus hernia, esophagitis and dilated left atrium seems also to be involved in this association through a mechanical effect or inflammatory process. In spite of the fact that the association between GERD and AF is supported by clinical and experimental studies, this relationship is still considered controversial (Floria et al., 2015). Most studies on the association between AF and GERD are based on retrospective data from national registries or self-reporting questionnaires (Floria et al., 2015, Roman et al., 2014). These studies did not evaluate autonomic imbalance in these patients. Heart rate variability (HRV) is a non-invasive tool that is very useful in the assessment of imbalance in autonomic tone (1996). HRV decreases when sympathetic activity is predominant; conversely, HRV increases when parasympathetic activity is predominant (Pumprla et al., 2002). Therefore, it represents an important tool for the assessment of the cardiovascular autonomic nervous system. Several methods are available for the analysis of HRV, most frequently used being in the time and

frequency domains. Also, there are limited prospective data about autonomic (sympatho-vagal) imbalance and the risk of arrhythmias in patients with GERD.

The aim of the study was to compare prospectively the heart rate variability (HRV) parameters in the time and frequency domains in patients with and without GERD and diagnosis of AF based on 24-hour electrocardiographic (ECG) Holter monitoring. In addition, we aimed to analyze: 1) the relative risk (RR) of AF in patients with GERD and 2) parameters of left atrial structural remodeling (left atrial size) determined by transthoracic echocardiography.

I.3.2.MATERIAL AND METHOD

Study Population

Patients with Gastroesophageal Reflux Disease (GERD) and /or Atrial Fibrilation (AF) were successively included by a joint team consisting of a gastroenterologist and a cardiologist if they met the inclusion and exclusion criteria of the study.

Inclusion criteria: patients older than 18 years, without any prior gastroenterological evaluation (naïve patients), with symptoms suggesting GERD defined as mild symptoms of heartburn and/or regurgitation at least twice per week or moderate/severe symptoms occurring on more than 1 day per week that were perceived as "troublesome" by patients (according to the Montreal definition) (Pumprla et al., 2002; Vakil et al., 2006).

Exclusion criteria: patients younger than 18 years; patients with any type of documented valvular AF or under anticoagulation treatment; patients with chronic gastrointestinal diseases such as inflammatory bowel disease and celiac disease; patients with any valvular disease of more than mild intensity, thyroid disorders, previous myocardial infarction, transient ischemic attack, or stroke; patients who refused to be included; patients with a pacemaker/defibrillator, inflammatory disorders or under immunosuppressive therapy, active neoplasm, dementia, or other neurological or psychiatric disabling pathology; and all patients on non-steroidal anti-inflammatory drugs (including acetylsalicylic acid at a dose of greater than 100 mg/day) at enrollment and during the preceding 30 days. Proton pump inhibitors treatment was interrupted 8 weeks before inclusion in the study. Antiarrhythmic drugs in patients with AF were not stopped during the study (due to ethical reasons).

The diagnoses of non-valvular AF and GERD were defined according to current guidelines (January et al., 2014, Vakil et al., 2006). Therefore, non-valvular AF referred to patients without mitral stenosis or artificial heart valves (January et al., 2014). GERD was diagnosed by the gastroenterologist clinically. The diagnosis of GERD was determined on the basis of clinical symptoms (according to the Montreal Consensus) (Chong et al., 2019, Vakil et al., 2006): mild symptoms of heartburn and/or regurgitation at least twice per week or moderate/severe symptoms occurring on more than 1 day per week that were perceived as "troublesome" by patients. All patients underwent a gastroenterological and, after that, a cardiological assessment.

They underwent upper gastrointestinal endoscopy, 24-hour ECG monitoring, and echocardiography, even if they were asymptomatic.

After a detailed medical history, the following clinical parameters were noted: age, sex, the presence of obesity (defined as a body mass index [BMI] of higher than 30 kg/m^2), dyslipidemia, hypertension, diabetes mellitus, heart failure, ischemic heart disease, and peripheral arterial disease.

Upper gastrointestinal endoscopy was performed only by one experienced gastroenterologist (VD) using Olympus Exera CV-160 endoscope 48–72 hours. The presence of hiatus hernia (as a condition favoring GERD), esophagitis, and Barrett's esophagus was noted.

Echocardiographic measurements were performed using two-dimensional transthoracic echocardiography with a Sonoscape SSI 8000 ultrasound machine (Providian Medical Equipment LLC, OH, USA). Diastolic left ventricular function (by the E/A ratio and E/Em ratio) and left atrial size were assessed as markers of left atrial structural remodeling. All measurements were made by an experienced operator only.

HRV parameters in the time and frequency domains were recorded by 24-hour ECG Holter monitoring using a two-channel tracker (EC-2H 2-Channel, Cardiospy, Labtech Holter ECG System, Hungary). Individuals went about their normal daily activities. In time-domain analysis the standard deviation of normal-to-normal (N-N) intervals (SDNN; ms) was determined. In the frequency domain, the frequency spectrum was assessed using a fast Fourier transform to determine the low-frequency (LF)/high-frequency (HF) ratio as an indicator of sympathovagal balance of the autonomic nervous system. All intervals of less than 200 ms and greater than 2000 ms were rejected as being artifacts. All HRV data were collected and interpreted by an experienced electrophysiologist.

In this study experiments were performed in compliance with the ethical principles of the University Ethics Committee (approval number 2005/2014). Informed consent was obtained from each patient before inclusion in the study. This study conforms to the Declaration of Helsinki.

Statistical Analysis

Categorical data are presented as frequencies and percentages; continuous variables are expressed as the mean±standard deviation. Categorical, ordinal, and numerical variables were compared between groups using the χ^2, Cochran, and Wilcoxon rank sum tests, Kruskal–Wallis, and Anova test, respectively. All statistical tests were two-tailed and performed with Statistical Package for the Social Sciences 15.0 software (SPSS, Inc., Chicago, IL, USA). A p-value of less than 0.05 was considered as statistically significant.

I.3.3.RESULTS

Between July 2014 and February 2015, 135 patients were prospectively and successively included: 61 patients with GERD (study group I) and 74 patients without GERD (control group II); 41% versus 46% were male, ages were 61.5±9 versus 58±9 years, and BMI values were 28.8±4 kg/m^2 versus 29±4 kg/m^2 (all p-values were greater than 0.05).

Clinical data for both groups are presented in table II. No significant differences were found in clinical parameters between the GERD patients and controls. The frequencies of AF were 33% and 39% in groups I and II, respectively (p=0.52). Patients with GERD had an RR of AF of 1.17 (95% confidence interval [CI] 0.78–1.75; p=0.34). No significant differences were found between group I and group II based on medication (tab. III).

Table II. Clinical parameters and RR in patients with GERD (group I, n=61) versus those without GERD (group II, n=74)

Parameter	Group I n (%)	Group II n (%)	Odds ratio (95% CI)	Relative risk (95% CI)
Male gender	25 (41.0)	28 (37.8)	1.14 (0.57-2.28)	1.07 (0.74-1.56)
Age≥60 years	38 (62.3)	34 (45.9)	1.94 (0.97-3.88)	1.45 (0.98-2.14)
Obesity	24 (39.3)	33 (44.6)	0.81 (0.38-1.70)	1.13 (0.77-1.65)
Dyslipidemia	43 (70.5)	58 (78.4)	0.66 (0.28-1.54)	1.24 (0.84-1.84)
Hypertension	43 (70.5)	58 (78.4)	0.66 (0.28-1.54)	1.24 (0.84-1.84)
Diabetes mellitus	11 (18.0)	11 (14.9)	1.26 (0.46-3.44)	0.89 (0.56-1.41)
Heart failure	17 (27.9)	18 (24.3)	1.20 (0.53-2.79)	0.91 (0.60-1.36)
Ischemic heart disease	19 (31.1)	15 (20.3)	1.78 (0.76-4.20)	0.74 (0.51-1.09)
Peripheral arterial disease	2 (3.3)	1 (1.4)	2.44 (0.17-69.8)	0.68 (0.30-1.54)
Atrial fibrillation	20 (32.8)	29 (39.2)	0.76 (0.35-1.63)	1.17 (0.78-1.75)

All p values were greater than 0.05 (Student's t-test)

Table III. Patients with GERD versus those without GERD

Treatment	Group I (n=61)		Group II (n=74)		P	OR(IC95%)	RR(IC95%)
	N	%	n	%			
Statins	14	23.0	19	25.7	0.841	0.86 (0.39-1.91)	0.92 (0.59-1.44)
Omega3 FA	2	3.3	4	5.4	0.689	0.59 (0.11-3.35)	0.73 (0.23-2.30)
ACE inhibitors	9	14.8	9	12.2	0.800	1.25 (0.46-3.38)	1.13 (0.68-1.86)
Sartans	14	23.0	18	24.3	0.852	0.93 (0.42-2.06)	0.96 (0.61-1.50)

Comparative data for the ECG Holter, echocardiography, and upper gastrointestinal endoscopy parameters of the two groups included in the study are presented in table IV.

The mean value of SDNN was statistically significantly lower in patients with GERD than in those without GERD: 97.6±13.7 ms versus 139.9±44.6 ms (p=0.001). In terms of the frequency-domain parameters of HRV, the mean value of the LF/HF ratio was lower, but statistically non-significant, in patients with GERD than in those without GERD, with values of 0.75±0.17 and 0.76±0.24, respectively (p=0.930).

The E/A ratio was statistically significantly different in patients with GERD versus those without GERD (0.97±0.40 versus 1.31±0.67; p=0.007). There was no statistically significant difference in terms of left atrial area (as a marker of left atrial structural remodeling) in patients with GERD versus those without GERD (25±5.4 cm^2 versus 25±5 cm^2; p=0.781).

Table IV. Comparative data for ECG, echocardiography, and upper gastrointestinal endoscopy parameters in patients with GERD (group I) versus those without GERD (group II)

Parameter	GROUP I (n=61)	GROUP II (n=74)	p value (Student's t-test)
ECG Holter			
SDNN[+] (ms)	97.6±13.7	139.9±44.6	0.001
LF/HF ratio[++]	0.75±0.17	0.76±0.24	0.930
Echocardiography			
E/A ratio[§]	0.97±0.40	1.31±0.67	0.007
E/Em ratio[§§]	8.1±2.3	7.8±2.9	0.592
Left atrial area (cm^2)	25±5.4	25±5	0.781
Upper Gastrointestinal Endoscopy			
Esophagitis (%)	52.5	10.8	0.001
Hiatus hernia (%)	18.0	17.6	0.999

[§]E/A ratio: ratio of E-wave velocity to A-wave velocity; [§§]E/Em ratio: ratio of E-wave velocity to Em velocity; [++]LF/HF ratio: low-frequency/high-frequency ratio; [+]SDNN: standard deviation of normal-to-normal (N-N) intervals.

We found 32 patients with esophagitis among patients with GERD and only 8 patients among those without GERD (odds ratio (OR) =9.61, 95% CI 3.74–22.15; RR=2.62, 95% CI 1.86–3.68; p=0.0019). Hiatus hernia was not statistically significantly more frequent in patients with GERD than in those without GERD (11 versus 13 patients; OR=1.03, 95% CI 0.43–2.50; RR=1.02, 95% CI 0.63–1.65; p=0.999). None of the patients had esophagitis more severe than Los Angeles class A. Only one patient was diagnosed with Barrett's esophagus (in the GERD group).

Patients with AF and GERD

A comparison between the ECG Holter, echocardiography, and upper gastrointestinal endoscopy parameters in patients with AF + GERD (n=36) and AF - GERD (n=39) versus patients with sinus rhythm (SR) + GERD (n=25) and SR - GERD (n=35) is presented in table V.

In patients with AF the mean value of SDNN was statistically significantly lower in those with GERD than in those without GERD, with values of 114±58 ms and 273±100 ms, respectively; p=0.001.

In terms of frequency-domain parameters of HRV, there were no differences in the mean value of the LF/HF ratio between the four groups (p=0.749). In the presence of AF, the mean value of the LF/HF ratio was higher in those with GERD than in those without GERD, with values of 0.71±0.16 and 0.69±0.17, respectively; however, the p-value was statistically non-significant (p=0.862).

The parameters of left ventricular diastolic dysfunction such as the E/A ratio, E/Em ratio, and left atrial area were statistically significantly different in the four groups of patients. There was a statistically significant difference in terms of left atrial area (as a marker of left atrial

structural remodeling) in patients with AF+GERD versus those with AF-GERD, with values of 25.8±5.1 cm^2 versus 27.3±5.1 cm^2 (p=0.04).

Table V. Comparative data for ECG, echocardiography, and upper gastrointestinal endoscopy parameters in patients with AF versus SR depending on the diagnosis of GERD

Parameter	AF+GERD n=36	AF–GERD n=39	SR+GERD n=25	SR–GERD n=35	p value Anova test
ECG Holter					
$SDNN^{+}$ (ms)	114±58	273±100	88±53	146±33	0.001
LF/HF $ratio^{++}$	0.71±0.16	0.69±0.17	0.76±0.24	0.72±0.17	0.749
Echocardiography					
E/A $ratio^{§}$	1.02±0.49	1.65±0.87	0.94±0.34	1.10±0.42	0.001
E/Em $ratio^{§§}$	8.7±2.3	8.5±3.9	7.3±2.1	7.2±1.3	0.046
Left atrial area (cm^2)	25.8±5.1	27.3±5.1	23.9±5.8	22.9±3.4	0.002
Upper Gastrointestinal Endoscopy					
Esophagitis (%)	50.0	7.7	56.0	14.3	0.001*
Hiatus hernia (%)	19.4	15.4	16.0	20.0	0.942*

*Kruskal–Wallis Test. AF+GERD: patients with AF and GERD; AF-GERD: patients with AF without GERD; SR+GERD: patients with SR with GERD; SR-GERD: patients with SR without GERD.
§E/A ratio: ratio of E-wave velocity to A-wave velocity; §§E/Em ratio: ratio of E-wave velocity to Em velocity; $^{++}$LF/HF ratio: low-frequency/high-frequency ratio; $^{+}$SDNN: standard deviation of normal-to-normal (N-N) intervals.

Esophagitis was diagnosed in 16 patients with AF+GERD and 2 patients with AF-GERD (RR=8.53, 95% CI 2.14–34.0; p=0.001). Esophagitis was statistically significantly more frequent in patients with GERD irrespective of the presence of AF or SR. Hiatus hernia was not more frequent in patients with GERD than in those without GERD independently of the presence of AF or SR. Hiatus hernia was confirmed in 23.3% of patients with AF+GERD and 9.4% of patients with AF-GERD (RR=2.49, 95% CI 0.71–8.75; p=0.251).

I.3.4.DISCUSSION

Cardiovascular involvement in GERD has been limited studied in the literature. One of the main pathophysiological mechanisms in paroxysmal AF is the presence of a trigger (Calkins et al., 2012). The development of AF requires a trigger and an anatomic or functional substrate capable of both the initiation and perpetuation of AF. Haissaguerre et al. (Haissaguerre et al., 1998) have observed that AF is often triggered by an ectopic beat arising from the pulmonary veins. This observation induced the electrophysiologist community to refocus its attention on the posterior wall of the left atrium with the pulmonary veins and on the autonomic innervation of that region. Gastroesophageal reflux could be a trigger for AF via sympathovagal imbalance (Reddy et al., 2013).

Another cornerstone in the pathophysiology of AF is the presence of a substrate or a substantially modified atrial myocardium owing to structural, electrical, and mechanical remodeling of the left atrium. The appearance of a trigger could alter the substrate. However, left atrial remodeling, which is a cornerstone of AF, may be a final common phenotype of multiple disease pathways caused by diverse pathophysiological mechanisms. It may be important to identify subtypes of AF and not to consider AF as a single disease (Dewland et al., 2015). This idea is increasingly encountered when discussing AF as an inhomogeneous disease (Dewland et al., 2015).

In this study, there were no differences between risk factors for AF (hypertension, ischemic heart disease, and diabetes mellitus) in patients with GERD versus those without GERD. In addition, there was no statistical difference in the percentage of patients with AF in the GERD group in comparison with the group without GERD. Thus, AF was not significantly more frequent in patients with GERD. At the moment of publishing our results, only one study, which was published by Bunch et al. (Bunch et al., 2008) based on a self-reporting questionnaire including more than 5.000 patients, concluded that the presence of GERD, after the exclusion of other risk factors, did not involve higher risk of AF. Our study also suggests that AF is not significantly more frequent in patients with GERD. Comparing with Bunch et al study (Bunch et al., 2008), we assessed autonomic balance via parameters of HRV and left atrial structural remodeling in these patients. However, in 2019 Lu Xu et al (Xu et al., 2019) our paper togheter with other 6 papers were included in a systematic review and meta-analysis. The summary adjusted relative risks (RRs) AF-induced GERD and GERD-induced AF were 1.54 (95% CI, 1.08-2.17) and1.06 (95% CI, 0.86-1.31), respectively. The subgroup analysis showed that the associations were not significantly modified by sample size, study design, age, or geographic area. The authors recognized the limitations of the meta-analysis due to limited number of studies and subjects, studies design and also publication bias.

Although diastolic dysfunction was more frequent in the GERD group, left atrial area, as a marker of chronic diastolic dysfunction, was not greater in this study group. When left atrial remodeling was analyzed in the four subgroups of patients, left atrial area was statistically significantly larger in patients with AF than in those with SR, irrespective of the presence of GERD. Thus, left atrial remodeling seems to be related to AF but not to GERD. Therefore, it is difficult to suspect left atrial remodeling as a possible substrate for AF in patients with GERD, in spite of the speculation that a dilated and palpitating left atrium may induce compression or irritation in the neighboring lower esophagus.

Time-domain methods are more frequently used in comparison to frequency-domain methods when short-term recordings are used to investigate HRV. SDNN is the most representative parameter of HRV in the time domain. A value of SDNN of less than 50 ms is considered indicative of high risk; a value between 50 and 100 ms indicates moderate risk, whereas a value of over 100 ms is considered normal (Pumprla et al., 2002). A low value of SDNN indicates low HRV. A reduction in HRV has been reported in several cardiovascular and non-cardiovascular diseases (Pumprla et al., 2002). A decrease in HRV has received increasing

attention as a prognostic indicator of risk associated with a variety of chronic diseases, behavioral disorders, mortality, and aging (Pumprla et al., 2002). In our study, a lower value of SDNN suggests lower HRV in patients with GERD and implicitly, an increased risk of arrhythmias. In addition, the mean value of SDNN represents a moderate risk of arrhythmia in patients with GERD; in those without GERD, the mean value of SDNN implies no risk of arrhythmia.

The LF/HF ratio is a marker of imbalance between the sympathetic and parasympathetic systems. An increase in this ratio reflects dominance of the sympathetic system, whereas a decrease indicates dominance of the parasympathetic system. In this study, the lower value of the LF/HF ratio in patients with GERD than in those without GERD signifies dominance of the parasympathetic nervous system, although this was statistically non-significant. However, by analysis of the subgroups, in patients with AF we observed a higher mean value of the LF/HF ratio in patients with GERD; this could signify a decrease in parasympathetic activity, which was probably due to other mechanisms such as heart failure, although this was statistically non-significant. Present data are still speculating on possible mechanisms which may lead to atrial fibrillation as consequence to GERD. These mechanisms could be the autonomic inbalance with increase vagal tone and altered LF/HF ratio, mechanical influence and the local inflammatory process (Maruyama et al., 2019).

Esophagitis was more frequent in patients with GERD, as was expected. It seems that the pattern of autonomic function differs depending on the presence of erosive esophagitis (Montenero et al., 2005). The LF/HF ratio appears to be significantly lower in patients with non-erosive GERD compared with those with erosive GERD (Chen et al., 2006). However, in comparison with patients with non-erosive GERD, autonomic tone in patients with endoscopically confirmed esophagitis (even without symptoms) is lower. Probably, the structural state of the esophagus is important in the status of autonomic function (not symptomatology), (Lee et al., 2004).

Obesity is associated with both AF and GERD. Body fat seems to be associated with HRV (Hillebrand et al., 2015). In this study obesity in terms of BMI was not different between the study and control groups. However, the RR of GERD induced by obesity was 1.13 (95% CI 0.77–1.65).

To date, we know that autonomic neuropathy in GERD patients may have a mixed character (Dobrek et al., 2005). Dobrek et al. (Dobrek et al., 2004) showed one year previously that an impairment in parasympathetic activity was associated with GERD. In addition, it was also the primary factor contributing to the pathophysiological mechanism of GERD, owing to modulation of the activity of the vagus nerve, which plays an important role in maintaining the physiological function of the lower esophageal sphincter (Blaut et al., 2001).

Study Limitations

It is difficult to interpret the parameters of HRV without taking into account all cardiovascular comorbidities and other daily factors. However, in our study there were no

differences regarding cardiovascular diseases between patients with GERD and those without GERD. Antiarrhythmic drugs can reduce HRV, but antiarrhythmic treatment was not interrupted because of ethical reasons. The assessment of HRV before and after treatment with proton pump inhibitors, not only in the absence of this treatment, could bring new information, because therapy with proton pump inhibitors may have proarrhythmic or antiarrhythmic effects (Linz et al., 2017, Milovanovic et al., 2015). A larger study population will be necessary.

Sympathovagal balance seems to be disrupted in patients with GERD, with dominance of the parasympathetic system and an increased risk of arrhythmias, although AF was not significantly more frequent in these patients.

I.3.5. CONCLUSIONS

Our study showed that AF is not significantly more frequent in patients with GERD. According to the data from our study, sympathovagal balance seems to be disrupted in patients with GERD; in this group of patient's dominance of the parasympathetic system could be involved.

I.4. GASTROESOPHAGEAL REFLUX DISEASE AND DYSPHONIA IN PROFESSIONAL VOICE USERS

I.4.1. INTRODUCTION

Data on the Gastro-Esophageal Reflux Disease (GERD) implication in the genesis of the laryngeal lesions and the occurrence of disphonia are extensively presented in the recent medical literature. GERD may causing irritation and/or mucosal inflammation of larynx through direct contact or neuro-mediated reflex (Ghisa et al., 2019). Approximately 4-10% of patients with ENT diseases may have symptoms induced by reflux (Ahmed et al., 2006). GERD is widely spread in the general population. As much as 15-20% of the population may present at least once a week symptoms evoking gastro-esophageal reflux the patients with laryngitis and dysphonia, symptoms of reflux are extremely frequent. Also, 73% of those patients may show symptoms of GERD and 50% may have pathologic pH measurements (Drug et al., 2005; Cobzeanu et al., 2012). This represents a much higher incidence of reflux symptoms than in the general population.

A recent study made in patients with GERD indicated a prevalence of symptoms suggesting concomitant laryngeal diseases in 10.4% of cases (Cobzeanu et al., 2012). Dysphonia, and also other multiple symptoms such as throat clearing, sore or burning throat, cough, pain with swallowing, oropharingeal heartburn, voice fatigue, excessive phlegm and globus may be present secondary to GERD (Ghisa et al., 2019).

Moreover, evidence that suggests GERD involvement in laryngitis comes also from therapeutic trials. After administration of proton pump inhibitors, ENT conditions may improve in some patients (Sataloff et al., 2010). The treatment of reflux in those patients also improves voice objective parameters (Sataloff et al., 2010). However, data on the efficacy of proton-pump inhibitors (PPI) for treating laryngopharingeal reflux are conflicting. Older meta-analysis and systematic reviews by Karkos PD, Wilson JA. (Karkos and Wilson, 2006) and Qadeer MA et al (Qadeer et al., 2006) evidenced that PPI therapy did not show any difference in symptom response. Recent meta-analysis showed a positive effect of PPI in treating laryngopharyngeal reflux if the duration of treatment is for 3-6 month (Guo et al., 2016).

A particular aspect is represented by voice alteration in patients with high vocal strain, such as, actors, priests and professional singers (Voineag et al., 2011).

The aim of the study was to evaluate the possible association between dysphonia and gastroesophageal reflux disease in professional voice users patients.

I.4.2. MATERIAL AND METHOD

A number of 50 subjects (age 43 +/- 11.5, 26 males – 52% and 24 females - 48%) professional voice users (teachers, actors, singers, priests) with disphonia were prospectively included between 1st January 2012 and 1st October 2012 based on referral to ENT Department of University Hospital" St Spiridon" from the occupational/GP doctor.

A standardized questionnaire was delivered in order to reveal the presence of concomitant ENT symptoms and also of gastroesophageal reflux and respiratory symptoms. The predisposing factors which may influence the appearance of dysphonia, vocal abuse during physical exercise, the presence of environmental and individual factors associated with gastroesophageal reflux were also investigated in the questionnaire.

The including criteria in the study were the following:

- patients with intense daily vocal use due to profession such as: teachers, actors, singers, priests;
- more than 3 months duration of dysphonia (chronic diysphonia);
- absence of laryngeal benign or malignant tumors;

Patients with the following criteria were excluded:

- upper airways infections less than one month before inclusion;
- personal history of anti-reflux surgery;
- medication that could alter the esophageal motor function or the acid secretion (anticholinergics, sedatives, prostaglandins, calcium channel blockers, potassium, antibiotics, NSAIDs).

All patients were examined by an ENT surgeon and included a laryngoscope examination.

Statistical analysis

In order to check the relevance of our data we used data specific parametric and non-parametric tests. We used SPSS for statistical analysis. Data are presented as Mean +-SD and percentage. We used multivariate analysis ANOVA to investigate multiple correlations.

I.4.3. RESULTS

The study included 50 cases (10 actors, 40 singers, 10 teachers and 10 priests (fig. 4) with demographic data presented in fig. 5 and fig. 6.

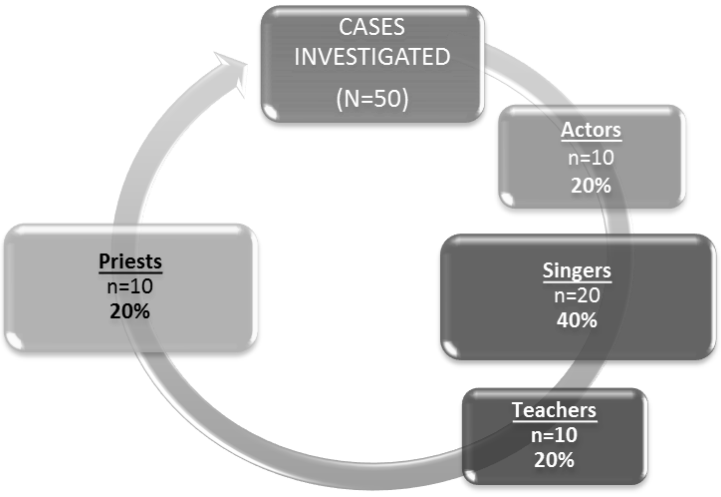

Fig. 4. Type of professional voice users

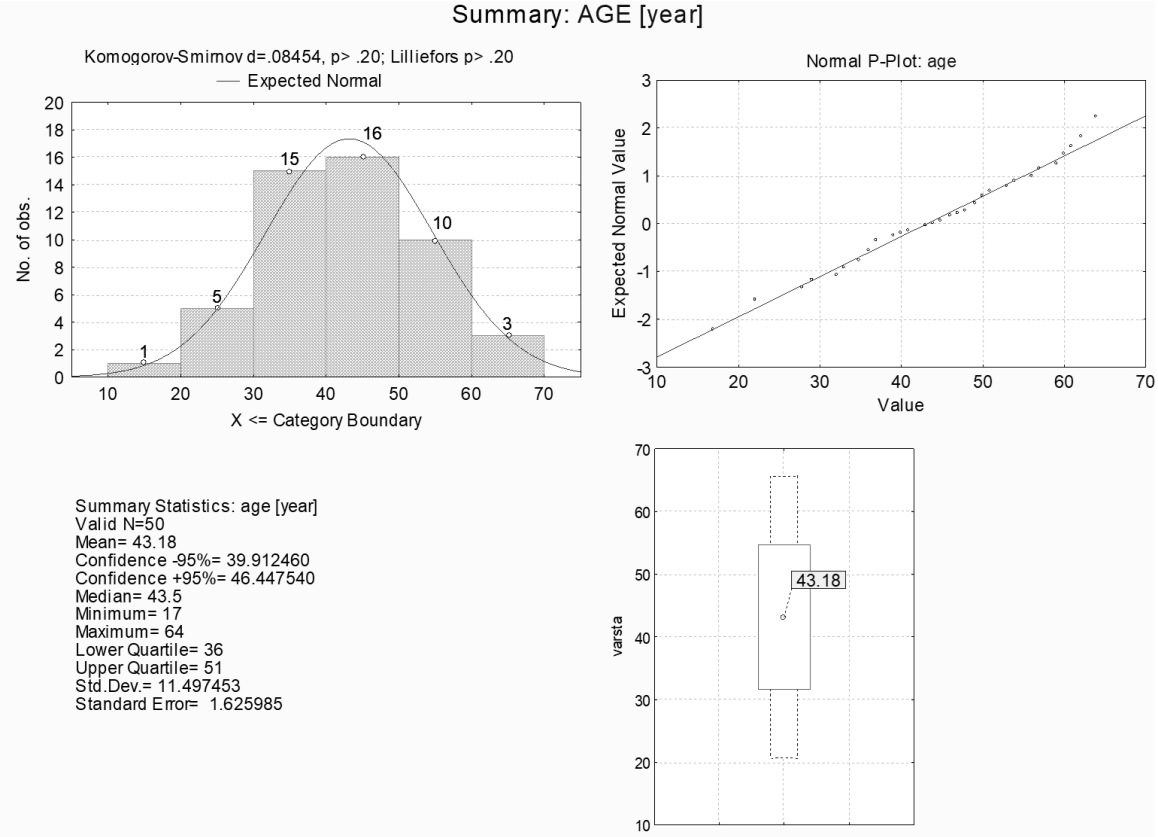

Fig. 5. Distribution of age

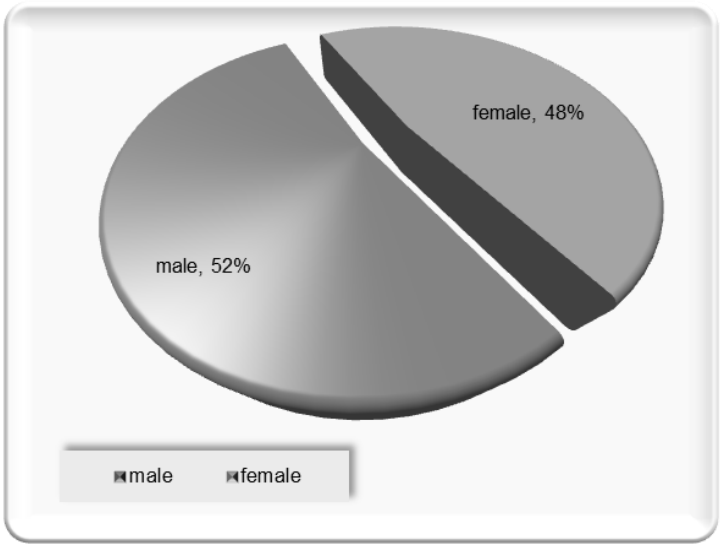

Fig. 6. Distribution of gender

Patient's ENT, digestive and respiratory symptomatology is presented in table VI and figure 7. Patients included in the study presented more often heartburn (70%) or regurgitation (24%) suggesting that GERD is more often present in patients with dysphonia than in the general population. Also, almost all (90%) of patients with dysphonia had respiratory symptoms (chronic cough). Our results are consistent with recent data, however showing a higher association between dysphonia, GERD and respiratory symptoms.

Table VI. Distribution of cases according to symptoms

	Symptoms	Number of cases	%
GERD	Heartburn	35	70%
	Regurgitation	12	24%
ENT Symptoms	Globus Histericus	12	24%
	Foreign body sensation	15	30%
	Hoarseness	50	100%
	Fonasteny	17	34%
RESPIRATORY Symptoms	Irritating cough	45	90%
	Total cases	**50**	

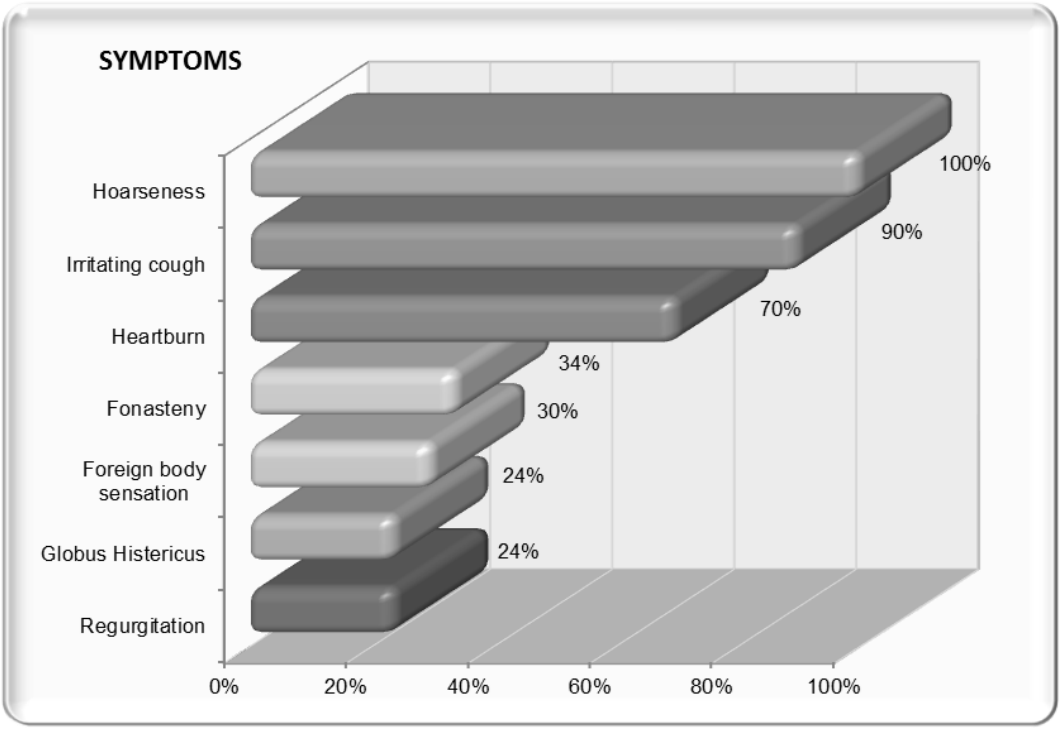

Fig. 7. Distribution of cases according to symptoms

Results on the laringoscopy examination are presented in table VII and figure 8. As it is showed in the table 2, congestion of vocal cords and edema of posterior comissure was the most prevalent lesion (66%). Reincke edema (16%) leukoplakia (8%) interarytenoid pachydermy (6%) and granuloma (4%) was often frequent present.

Table VII. Laryngeal lesions

	Number of cases	%
Edema post. comissure + congestion vocal cords	33	66%
Granuloma	2	4%
Reinke Edema	8	16%
Pachyderminteraritonoidiala	3	6%
Leukoplakia	4	8%
Total cases	**50**	

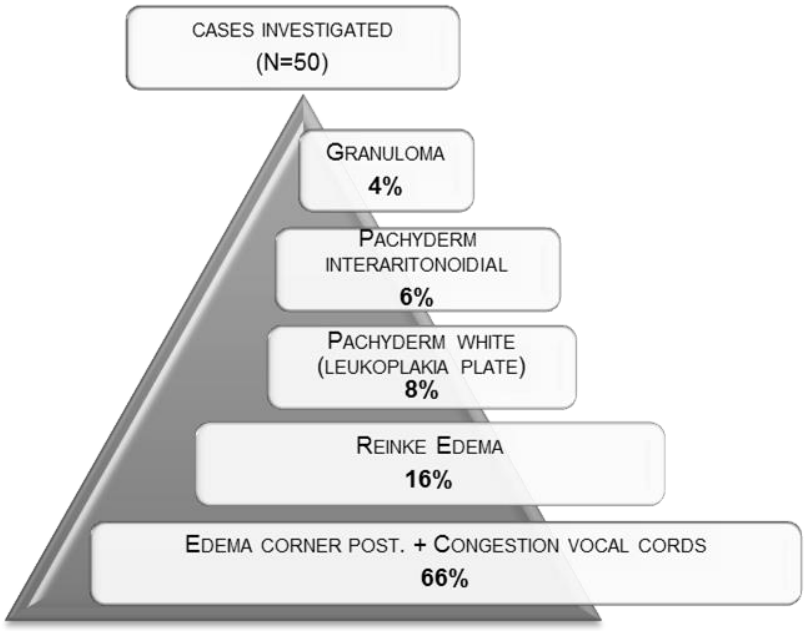

Fig. 8. Laringeal lesions

Age may influence the type of laryngeal lesions present in these patients according to data presented in figure 9.

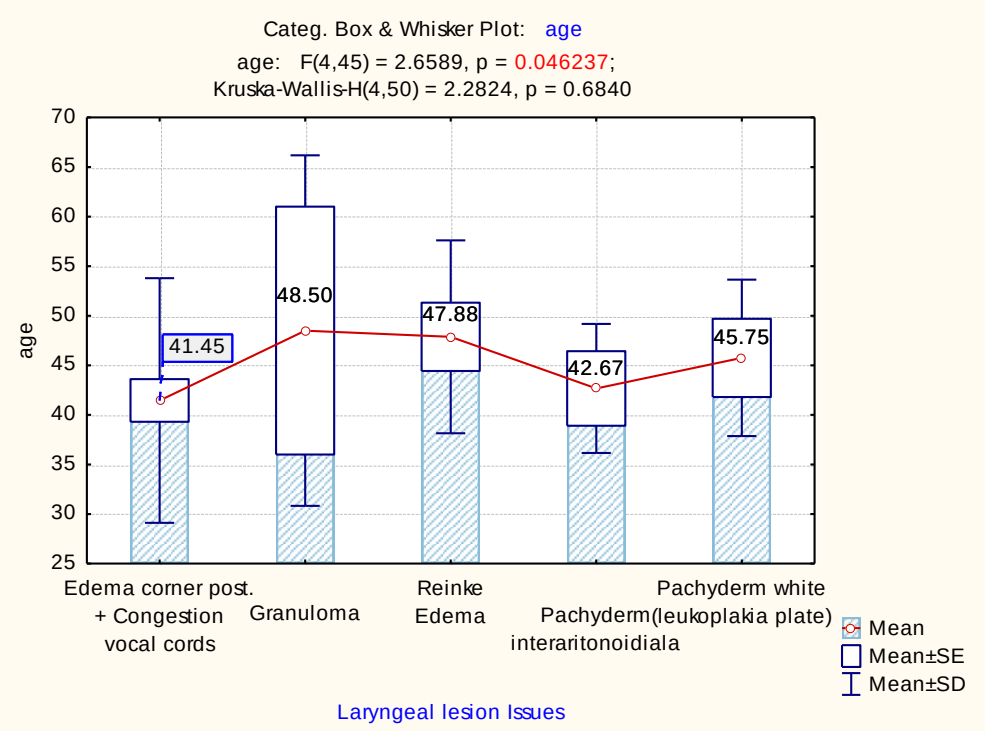

Fig. 9. Distribution of cases based on Laryngeal lesions

As showed in table VIII multivariate analysis of risk factors vs laryngeal lesions showed a positive association between smoking, alcohool and age with the presence of laringeal symptoms.

Multivariate analysis of risk factors versus laryngeal lesion

Table VIII. Multivariate analysis of risk factors versus laryngeal lesion

Multiple correlations	Estimated value
Multiple correlation coefficient	0.56598
Multiple R^2	0.3203334
F	11.044100
p (95%CI)	0.0410636
Std.Err. of Estimate	1.336667

Partial correlation	Coefficient correlation (Beta)	Std.Err. (Beta)	B	Std.Err. B	t(135)	p *95% interval confidence*
Intercept			-0.049032	1.437483	-0.034109	0.972948
Smoking	2.292841	0.171074	0.583952	0.004904	3.711780	0.009414
Alcohol	2.141565	0.148643	0.431650	0.017328	2.952378	0.034622
Age	1.145309	0.208269	0.358741	0.008419	2.397699	0.048912
Coffee	-0.139261	0.233156	-0.003662	0.006131	-0.597287	0.553448
Gender	0.083683	0.160985	0.222238	0.427533	0.519815	0.605858
Spices	-0.094598	0.241612	-0.002881	0.007359	-0.391530	0.697339

I.4.4. DISCUSSION

It is generally recognized that dysphonia is frequently associated with Gastroesophageal Reflux Diseases. More than that, professional voice users with dysphonia often may present gastroesophageal reflux symptoms and also respiratory complains. A correct diagnostic and therapeutic attitude in these patients involves a multidisciplinary team (including otolaryngologist, gastroenterologist and pneumologist). Our study showed that 94% of professional voice users with dysphonia had symptoms suggesting GERD. 70% of them had heartburn. In this population with dysphonia, associated respiratory symptoms are also common. However, 90% of them are associating irritating cough.

Laryngeal lesions were common in the study population. Laryngoscopy revealed in 66% edema of posterior comissure and congestion of vocal cords.

Multivariate analysis showed a positive association between smoking, alcohol, age but also inappropriate feeding habits (spicy food, alcohol intake, coffee consumption) and the presence of laryngeal lesions. Due to high associating rate between dysphonia and GERD one may speculate that these factors are important for both conditions in professional voice users.

A correct diagnosis of GERD in this group of patients is difficult. It requires modern and accurate investigations: flexible endoscopic laryngoscopy, upper gastro intestinal endoscopy (UGIE), pH / impedance monitoring.

The edema and erythema of the vocal folds and the edema of the interarytenoid area are the most frequent laryngeal changes that demonstrate, with statistical significance, that the gastro-esophageal reflux is involved in the laryngeal pathology. In the absence of GERD symptoms, upper gastrointestinal endoscopy may reveal esophagitis as a complication of GERD. In the absence of esophagitis, pH-impedance has clear indication and it is considered to be gold standard for the diagnostic of GERD.

I.4.5. CONCLUSIONS

All of the investigated groups (actors, priests, singers, and teachers) due to the specificity of their profession (intense voice use, physical effort during speech in some cases) and the association with different behavioral and environmental factors, presented a high rate of GERD related inflammatory laryngeal lesions.

I.5. DIET AND THE OVERLAP BETWEEN GASTROESOPHAGEAL REFLUX DISEASE AND FUNCTIONAL DYSPEPSIA

I.5.1. INTRODUCTION

Upper gastrointestinal symptoms are highly prevalent among people worldwide. The prevalence of upper gastrointestinal symptoms was 44.9% in USA (Camilleri et al., 2005) and 38% in European countries. It was highest in Central and East European countries and was closely associated with socioeconomic factors (Haag et al., 2011). Functional digestive disorders are considered to represent up to 50% of medical consultations in gastroenterology (Chang, 2004), and epidemiological studies suggest that there is a considerable overlap between these disorders (Hori et al., 2009).

Gastroesophageal reflux disease (GERD) is define according to Montreal definition and clasification by the presence of esophageal mucosal lesions or troublesome symptoms due to abnormal gastro-esophageal reflux (Vakil, van Zanten et al. 2006). Functional dyspepsia (FD) is define by current guidelines (including ROME IV) as a complex and multifactorial condition characterized by a broad spectrum of symptoms centered in the gastroduodenal region (Pesce et al., 2020).

Gastroesophageal reflux disease (GERD) is increasingly prevalent worldwide, particularly in the Western world, where reflux symptoms have a prevalence of up to 40% in population-based studies. Approximately 20%–30% of the general population presents with dyspepsia which has not been investigated (Tack et al., 2006).

Dyspepsia may also overlap with GERD, suggesting common pathogenic mechanisms (Fujiwara et al., 2011), (Ohara et al., 2011). In current practice, an over-diagnosis of GERD and under-diagnosis of functional dyspepsia was reported (Pleyer et al., 2014).

Recent papers highlighted the role of diet in dyspepsia and GERD, but its role in pathogenesis remains uncertain and under-studied. Although many patients recognize the impact of certain food in symptom occurrence, few population-based studies evaluated the role of diet in dyspepsia or GERD (Bhatia et al., 2011). However, the overlapping symptoms within the diagnostic criteria of the two entities (GERD and functional dyspepsia) may be linked to the consumption of certain foods (Feinle-Bisset and Azpiroz, 2013; Boettcher and Crowe, 2013).

The aim of our study was to update the prevalence data for functional dyspepsia and GERD and for the overlap of these diseases and to evaluate the type of diet associated with them.

I.5.2. MATERIAL AND METHOD

Population

A randomly chosen representative sample of the adult general population living in an urban area was invited for an interview in the family doctor's office. The study was conducted in a medical center which serves 18,000 people in an area of the city of Iasi, in North East Romania. The sample size and demographic characteristics were calculated to be representative using Epi

Info™ 3.5.2 software (Centers for Disease Control and Prevention (CDC); Atlanta, USA). We selected 250 subjects from family doctor's patient lists using a randomization function in Microsoft Excel™ software (Microsoft Corporation; Redmond, Washington, USA). The family doctors invited the selected subjects by telephone for an interview in their offices. The inclusion criteria were 18–79 years of age and a resident in this urban area. There were no exclusion criteria.

Measures

In the family doctor's office, an interview-based questionnaire was administered to all subjects to diagnose gastrointestinal disorders and to evaluate eating habits and frequency of food intake. General practitioners (GPs) were instructed to diagnose functional dyspepsia and GERD using Rome III and Montreal criteria, respectively (Talley et al., 2008; Tack and Talley, 2013; Vakil et al., 2006).

Socio-demographic factors and general medical history were also included in the interview together with an objective evaluation of obesity (the GPs measured the height and weight of subjects). Age, gender, and educational level were studied as demographic factors. The educational level was categorized into three classes: low (no school or elementary school only), medium (high school), and high (college or university). Health and health-related behaviors were investigated, such as smoking (classified as "current smokers" and "non-smokers"), physical activity (classified as "physically active" if the activity was performed at least weekly and "physically inactive" for a lower period), self-perceived stress (using a 3-point Likert-scale: "high, medium, or low level"), and general well-being (using a 5-point Likert scale: "very good, good, acceptable, poor, or very poor condition"). Subjects were considered overweight or obese if the body mass index (BMI) was between 25 kg/m2 and 29.9 and ≥30.0 kg/m2, respectively.

A food frequency questionnaire based on the validated European Prospective Investigation of Cancer (EPIC) protocol was designed to reveal the regular intake over a long period (Pasanisi et al., 2002; Slimani et al., 2002; Riboli et al., 2002). Consumption frequencies were noted as "never or rarely", "monthly", "once a week","several times a week","once a day," and "several times a day." We also investigated the individuals' eating habits (including a daily breakfast, the number of meals and snacks a day, the use of home prepared food, and eating in a hurry).

Statistical analysis

All data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 17.0 for Windows (SPSS Inc.; Chicago, IL, USA). We used the mean for parametric characteristics and median for non-parametric or ordinal variables. To characterize the frequency of food consumption in the population studied, we used the median as the cutoff point, and the group was divided into two categories of consumers (less than median frequency and equal to or more than median frequency). An initial Spearman's correlation test and cross-tabulation analysis were performed. These analyses examined whether there was any association between referral patterns, personal history of illness, eating habits, food consumption frequency, and other associated conditions. Finally, we used multivariate analysis for risk factors that were significant in univariate analysis, and we calculated odd ratios (ORs) and 95% confidence intervals (95%

CIs) for significant predictors of functional dyspepsia and GERD derived from the initial analysis. A value of $p<0.05$ in both analyses was considered to be relevant for our statistics.

The study was approved by the Ethics Committee of the University of Medicine and Pharmacy, and informed consent was obtained from all subjects.

I.5.3. RESULTS

1. Prevalence of functional dyspepsia and GERD

During a period of 4 months (January–April), 184 subjects (106 women and 78 men, mean age 49.4 years) participated in the study. The participation rate was 73.6%. Functional dyspepsia was present in 7.6% (3.8% for women and 12.8 % for men, $p<0.05$) of patients, and GERD was present in 31.0% (33.0% in women and 28.2% in men, $p>0.05$) of patients (fig. 10). In total, 25.9% of GERD subjects were diagnosed with functional dyspepsia. Also, 92.9% of functional dyspepsia subjects were diagnosed with GERD. The overlap of the two diseases was 22.4% among subjects with upper gastrointestinal disorders, and in our sample, 7.1% of participants received both diagnoses.

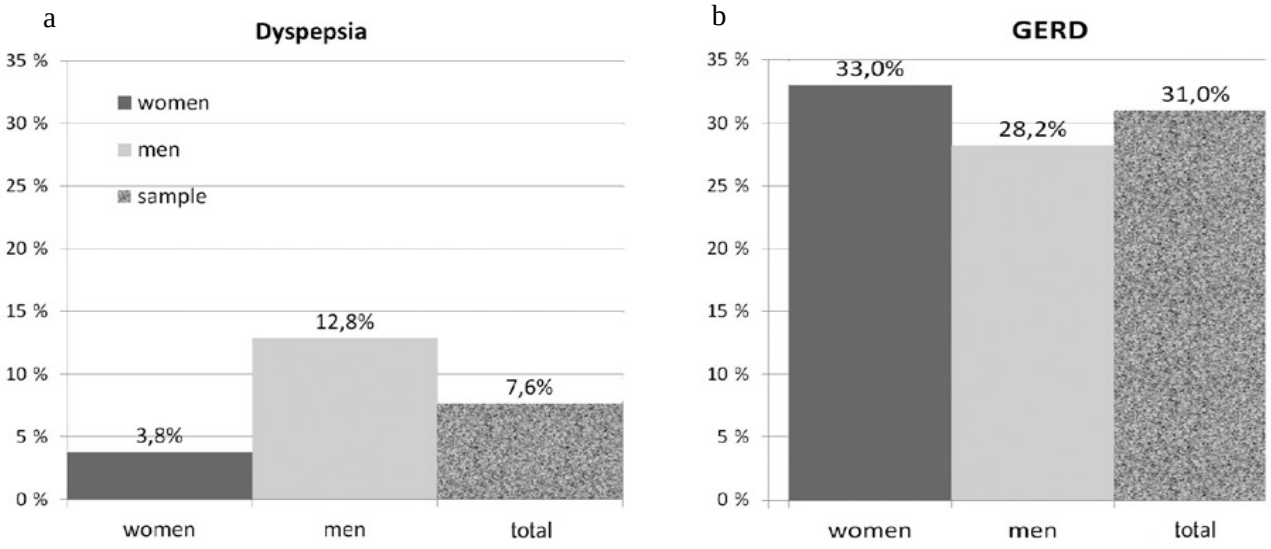

Figure 10. a, b. Prevalence of functional dyspepsia (a) and GERD (b) according to gender

The age distribution (tab. IX) indicated an increased prevalence of functional dyspepsia for subjects above the mean age of the sample (11.7% vs. 2.5%, $p<0.05$). The prevalence of GERD also increased with age ($r=0.938$, $p<0.05$).

The educational level of the subjects significantly influenced the prevalence of functional dyspepsia in the general population ($p<0.05$). The prevalence of functional dyspepsia was 2.6% among high, 9.5% among medium, and 16.6% among low levels of education. The prevalence of GERD was 27.3% among high, 32.9% among medium, and 40.0% among low levels of education ($p>0.05$) (fig. 11).

Table IX. Prevalence of functional dyspepsia and GERD in different age *groups*

		Prevalence	
Age (years)	**GERD**		**Dyspepsia**
20–29	20.8%		0.0%
30–39	26.5%		2.9%
40–49	25.0%		4.2%
50–59	35.4%		14.6%
60–69	36.8%		13.2%
70–79	37.5%		0.0%

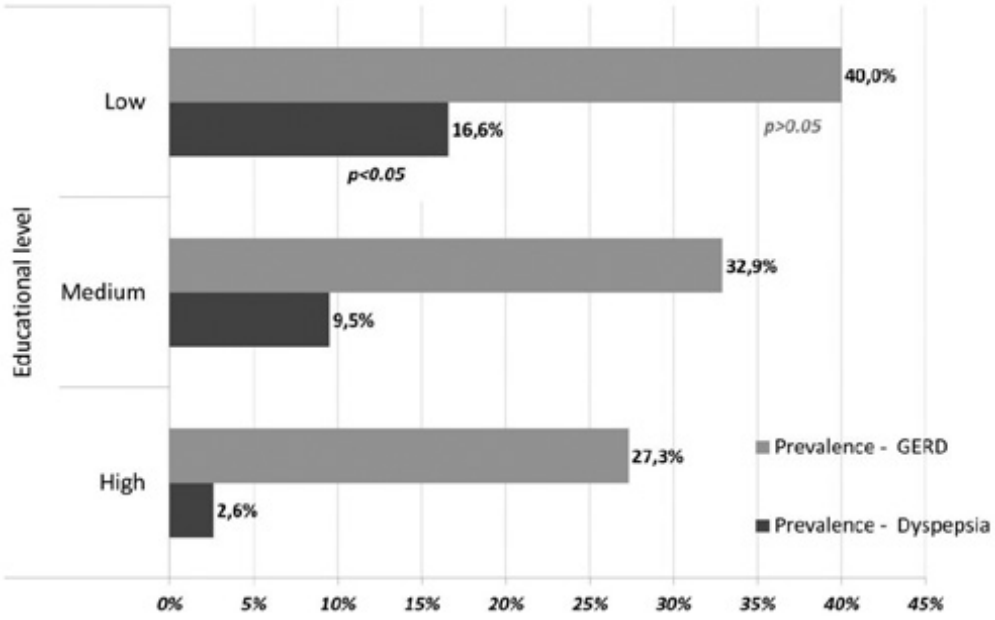

Figure 11. Educational level and prevalence of functional dyspepsia and GERD.
GERD: gastroesophageal reflux disease

2. Functional dyspepsia, GERD, and health-related behaviors/conditions

Smoking

Smoking was not associated with functional dyspepsia or GERD (p>0.05): 35.7% of dyspeptic patients were smokers vs. 26.5% of non-dyspeptic subjects (p>0.05); a similar situation was observed in the case of GERD (22.8% vs. 29.1%, p>0.05).

Physical activity

The majority of subjects were physically inactive: 71.4% of patients with functional dyspepsia, 63.8% of non-dyspeptic subjects (p>0.05), 73.7% of GERD patients, and 60% of non-GERD subjects (p>0.05).

Stress

The perception of stress was not significantly associated with functional dyspepsia. High and medium levels of stress were perceived in 21.4% and 78.6% of functional dyspepsia patients, respectively, whereas high and medium levels of stress were perceived in 13.6% and 75.1% of non-dyspeptic subjects, respectively ($p>0.05$). However, stress was associated with GERD. High and medium levels of stress were perceived in 12.3% and 86% of GERD patients, respectively, whereas high and medium levels of stress were perceived in 15.6% and 70.6% of non-GERD subjects, respectively ($p=0.025$). A very good general well-being was perceived only in non-dyspeptic subjects (15.4% vs. 0%, in dyspeptic patients) and was more frequent in non-GERD subjects than in GERD patients (18.1% vs. 6.4%, $p=0.06$).

Obesity

In the sample studied, 48.4% were overweight and 21.2% were obese. The presence of overweight and obese subjects was not significantly different in dyspeptic (85.7%) and non-dyspeptic subjects (68.2%) ($p>0.05$). However, GERD was more frequently present in overweight subjects (35.9%) than in subjects with normal weight (19.6%) ($p<0.05$).

3. Food consumption frequency and eating habits

The median frequency of food consumption in the studied population is presented in figure 12. Using the median as a cutoff point, we analyzed the frequency of food consumption among subjects with or without functional dyspepsia and GERD (tab. X).

Dyspeptic patients consumed canned food significantly more frequently; all of them consumed canned food (fish, meat, or vegetables) at least monthly. Grain cereals ($p=0.05$) and alcoholic beverages were consumed at least weekly (OR=5.58, 95% CI=1.58–25.74, $p=0.004$).

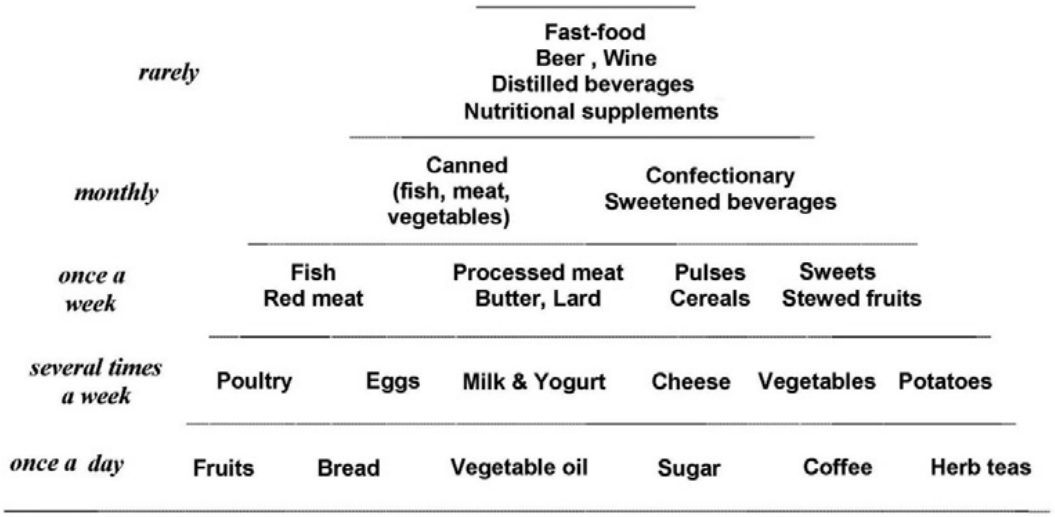

Figure 12. Median frequency of food consumption.

Table X. Median frequency of food consumption among subjects with or without functional dyspepsia and GERD

Food frequency consumption		Non-dyspepsia n=170		Dyspepsia n=14		p*	Non-GERD n=127		GERD n= 57		p*
		No.	%	No.	%		No.	%	No.	%	
Pork	Less than once a week	79	46.5%	4	28.6%	0.196	59	46.5%	24	42.1%	0.583
	At least once a week	91	53.5%	10	71.4%		68	53.5%	33	57.9%	
Beef	Less than once a week	135	79.4%	8	57.1%	0.054	103	81.1%	40	70.2%	0.100
	At least once a week	35	20.6%	6	42.9%		24	18.9%	17	29.8%	
Poultry	Once a week or less	26	15.3%	3	21.4%	0.545	25	19.7%	4	7.0%	0.029
	At least several times a week	144	84.7%	11	78.6%		102	80.3%	53	93.0%	
Processed meat	Once a week or less	73	42.9%	3	21.4%	0.116	61	48.0%	15	26.3%	0.006
	At least several times a week	97	57.1%	11	78.6%		66	52.0%	42	73.7%	
Fish (fresh)	Less than once a week	61	35.9%	3	21.4%	0.275	51	40.2%	13	22.8%	0.022
	At least once a week	109	64.1%	11	78.6%		76	59.8%	44	77.2%	
Fish (canned)	Rarely	77	45.3%	0	0.0%	0.001	73	57.5%	4	7.0%	<0.001
	At least monthly	93	54.7%	14	100.0%		54	42.5%	53	93.0%	
Canned food	Rarely	58	34.1%	0	0.0%	0.008	55	43.3%	3	5.3%	<0.001
	At least monthly	112	65.9%	14	100.0%		72	56.7%	54	94.7%	

Food frequency consumption		Non-dyspepsia n=170		Dyspepsia n=14		p*	Non-GERD n=127		GERD n= 57		p*
		No.	%	No.	%		No.	%	No.	%	
Eggs	Once a week or less	43	25.3%	3	21.4%	0.748	32	25.2%	14	24.6%	0.927
	At least several times a week	127	74.7%	11	78.6%		95	74.8%	43	75.4%	
Milk	Once a week or less	34	20.0%	1	7.1%	0.239	33	26.0%	2	3.5%	<0.001
	At least several times a week	136	80.0%	13	92.9%		94	74.0%	55	96.5%	
Cheese	Once a week or less	21	12.4%	2	14.3%	0.837	20	15.7%	3	5.3%	0.047
	At least several times a week	149	87.6%	12	85.7%		107	84.3%	54	94.7%	
Butter, lard	Less than once a week	67	39.4%	3	21.4%	0.183	61	48.0%	9	15.8%	<0.001
	At least once a week	103	60.6%	11	78.6%		66	52.0%	48	84.2%	
Vegetable oil	Less than once a day	32	18.8%	3	21.4%	0.811	24	18.9%	11	19.3%	0.949
	At least once a day	138	81.2%	11	78.6%		103	81.1%	46	80.7%	
Potatoes	Once a week or less	48	28.2%	3	21.4%	0.584	40	31.5%	11	19.3%	0.087
	At least several times a week	122	71.8%	11	78.6%		87	68.5%	46	80.7%	

Food frequency consumption		Non-dyspepsia n=170		Dyspepsia n=14		p*	Non-GERD n=127		GERD n= 57		p*
		No.	%	No.	%		No.	%	No.	%	
Vegetables with 10% carbohydrate (carrots, onions, beet	Once a week or less	25	14.9%	2	14.3%	0.966	21	16.5%	6	10.5%	0.287
	At least several times a week	145	85.3%	12	85.7%		106	83.5%	51	89.5%	
White bread	Less than once a day	17	10.0%	1	7.1%	0.729	10	7.9%	8	14.0%	0.193
	At least once a day	153	90.0%	13	92.9%		117	92.1%	49	86.0%	
Grain bread / pasta	Once a week or less	47	27.6%	2	14.3%	0.277	45	35.4%	4	7.0%	<0.001
	At least several times a week	123	72.4%	12	85.7%		82	64.6%	53	93.0%	
Corn flour***	Less than once a week	23	13.5%	0	0.0%	0.141	21	16.5%	2	3.5%	0.013
	At least once a week	147	86.5%	14	100.0%		106	83.5%	55	96.5%	
Grain cereals	Less than once a week	73	42.9%	0	0.0%	0.005	69	54.3%	8	14.0%	0.038
	At least once a week	97	57.1%	14	100.0%		58	45.7%	49	86.0%	
Sugar	Less than once a day	70	41.2%	5	35.7%	0.689	52	40.9%	23	40.4%	0.940
	At least once a day	100	58.8%	9	64.3%		75	59.1%	34	59.6%	
Sweets	Less than once a week	84	49.4%	7	50.0%	0.966	66	52.0%	25	43.9%	0.309
	At least once a week	86	50.6%	7	50.0%		61	48.0%	32	56.1%	

Food frequency consumption		Non-dyspepsia n=170		Dyspepsia n=14		p*	Non-GERD n=127		GERD n= 57		p*
		No.	%	No.	%		No.	%	No.	%	
Confectionary (cakes, cream, ice-cream)	Rarely	59	34.7%	5	35.7%	0.939	55	43.3%	9	15.8%	<0.001
	At least monthly	111	65.3%	9	64.3%		72	56.7%	48	84.2%	
Stewed fruit	Less than once a week	81	47.6%	6	42.9%	0.730	72	56.7%	15	26.3%	<0.001
	At least once a week	89	52.4%	8	57.1%		55	43.3%	42	73.7%	
Alcoholic beverages (beer, wine, distilled beverages)	Less than once a week	103	60.6%	3	21.4%	0.004	81	63.8%	25	43.9%	0.011
	At least once a week	67	39.4%	11	78.6%		46	36.2%	32	56.1%	
Carbonated sweetened beverages	Rarely	77	45.3%	4	28.6%	0.226	67	52.8%	14	24.6%	<0.001
	At least monthly	93	54.7%	10	71.4%		60	47.2%	43	75.4%	
Coffee	Less than once a day	36	21.2%	0	0.0%	0.055	31	24.4%	5	8.8%	0.013
	At least once a day	134	78.8%	14	100.0%		96	75.6%	52	91.2%	
Herb teas	Less than once a day	71	41.8%	6	42.9%	0.937	61	48.0%	16	28.1%	0.011
	At least once a day	99	58.2%	8	57.1%		66	52.0%	41	71.9%	
Fast-food (hamburger, hot-dog, chips, pretzels)	Never /rarely	91	53.5%	7	50.0%	0.799	80	63.0%	18	31.6%	<0.001
	At least monthly	79	46.5%	7	50.0%		47	37.0%	39	68.4%	

Also, GERD patients consumed canned food (13.6, 4.46–57.5, p<0.001), grain cereals (p<0.05), and alcoholic beverages (2.24, 1.18–4.27, p=0.011) significantly more frequently. They consumed the following foods more frequently: fresh fish (17.65, 6.47–60.45, p=0.022), processed meat (2.57, 1.31–5.22,p=0.005), milk (9.57, 2.56–61.32, p<0.001), cheese (3.34, 1.03–14.69, p=0.047), animal fat (butter, lard) (4.89, 2.26–11.37, p<0.001), vegetables with a low content of carbohydrates (3.76, 1.62–9.66, p=0.002), pulses (12.4, 5.38–31.8, p=0.001), confectionary (4.04, 1.87–9.41, p<0.001), stewed fruit (3.64, 1.85-7.4,p<0.001), carbonated sweetened beverages (3.4, 1.71–7.02,p<0.001), coffee (3.34, 1.28–10.2, p=0.013), herb teas (2.35,1.21–4.72, p=0.011), and fast food (3.66, 1.89–7.62, p<0.001).

There were no statistical differences regarding the consumption of the following types of

food: red meat, poultry, eggs, vegetable oil, potatoes and vegetables with a high content of carbohydrates, white bread, fruits, sugar, and sweets.

None of the eating habits we investigated was significantly related to dyspepsia or GERD.

4. Predictors of functional dyspepsia and GERD

Using a multivariate regression analysis to reduce confounding factors, the predictors for dyspepsia were low educational level (22.44, 3.36–150.1, p=0.001), consumption of canned food (2.38, p<0.05), and alcoholic drinks at least weekly (5.4, 1.23–23.61, p=0.025).

The predictors for GERD were advanced age (1.086, 1.052– 1.122, p<0.001) and the use of canned food (13.94, 3.61–53.98, p<0.001) or fast food (4.646, 1.773-12.177, p=0.002).

The predictors of overlap between GERD and functional dyspepsia were advanced age (1.057, 1.012–1.105, p=0.013) and the consumption of canned food (2.82, p<0.05).

I.5.4. DISCUSSION

Prevalence

In our study, the prevalence of functional dyspepsia was 7.6%. In other studies, the prevalence of dyspepsia varied according to country and the definition used, i.e., from 1.8% to 57.0%, and was higher in women (OR 1.24; 95% CI 1.13–1.36) and smokers (Ford et al., 2015). The overall pooled prevalence of uninvestigated dyspepsia in a very recent meta-analysis of 100 separate study populations was 20.8%. The greatest prevalence was found when a broad definition for dyspepsia (29.5%) or upper abdominal or epigastric pain or discomfort (20.4%) were used (Ford et al., 2015). The prevalence for functional dyspepsia may vary from 20% to 40%. In USA, functional dyspepsia was 29.2% and 15% if subjects with GERD were excluded. Approximately 20%–30% of the general population presents every year with uninvestigated dyspepsia (Tack et al., 2006).

The estimated prevalence in Romania is 20%–30 %, half being considered functional. However, there are no conclusive epidemiological studies, and it is clearly under-reported. At the Ministry of Health, records can be found with a prevalence of 60–70/100,000 inhabitants in 2002–2003 (Pop et al., 2012).

The prevalence of GERD varies worldwide for unknown reasons, but genetic differences, difference in the *Helicobacter pylori* prevalence, and lifestyle factors such as obesity might be an influence. The highest population-based prevalence is reported from Europe (23.7%) and USA (28.8%) (Ronkainen and Agreus, 2013). In our sample, using Montreal criteria, the prevalence of GERD was higher. The older age and the high percentage of overweight participants may explain this high GERD prevalence.

In our sample, the overlap of functional dyspepsia and GERD was 22.4%. In a recent review, the prevalence of dyspepsia was 27%, and this overlapped partially with GERD (from 10% to 66%, depending on the diagnostic criteria used for each) (Ford et al., 2010; Ohara et al., 2011). More studies suggest common pathogenic mechanisms with other functional digestive disorders (Wang et al., 2008; Ford et al., 2010; Fujiwara et al., 2011).

The overlap of functional dyspepsia with GERD can also be explained by the inability of

GPs to discriminate between the two entities. The term "dyspepsia" has been confusing in the past. Patients do not use the term and physicians have variable interpretations, minimizing its usefulness (Tack et al., 2006; Talley et al., 2008). The difficulty in differentiating between dyspepsia and GERD symptoms was also reported; a recent paper revealed an over-diagnosis of GERD and under-diagnosis of functional dyspepsia in a US community. Actually, only 62.9% of subjects reporting GERD symptoms were correctly diagnosed with GERD, and only 12.5% of subjects reporting dyspepsia were correctly diagnosed (Pleyer et al., 2014). In our unpublished data, looking for recent symptoms, we found that heartburn (epigastric pain) was frequently present in both diseases, and this suggests the same idea and explains the overlap.

Socio-demographic factors

Many studies show a high prevalence of dyspepsia in women. In our study, the higher prevalence of functional dyspepsia in men than in women could be explained by the large overlap with GERD. Also, in our region, the presence of *H. pylori* is more common, particularly in men (Klitorakis and Stanciu, 2010). As in other studies, the prevalence of GERD increased with age, obesity, physical inactivity, a low education level, and with stress (Saberi-Firoozi et al., 2007; Bhatia et al., 2011; Cela et al., 2013), but we did not observe an association with smoking.

Food

The possible contribution of food and dietary habits as a cause or exacerbating factor of dyspeptic symptoms represent a relatively new area for evidence-based research. Despite frequent reports by patients that their symptoms are often related to food ingestion, this association has not been formally assessed.

Dietary assessments have frequently implicated fatty foods in symptom induction, and these findings are supported by laboratory-based studies, particularly the demonstration that patients with functional dyspepsia more often experience symptoms after intra-duodenal infusions of fat than glucose. Some studies suggest that food intolerance has no remarkable influence on food pattern and nutritional status in most functional dyspepsia patients. Further studies on the potential role of dietary factors as a cause of dyspeptic symptoms are required to establish whether dietary therapies have any place in the management of functional dyspepsia (Feinle-Bisset et al., 2004). Although GERD can have anatomical explanations, there may be a relationship between the presence of symptoms and food because of food allergies (Semeniuk and Kaczmarski, 2008; Trikha et al., 2013).

A very frequent consumption of some foods was related to GERD and functional dyspepsia. Bhatia et al. reported that the consumption of non-vegetarian and fried foods, aerated drinks, tea, and coffee were associated with GERD, and using multivariate analysis, the consumption of non-vegetarian food was independently associated with GERD symptoms. In a Chinese study, routine usage of greasy food was considered a significant independent risk factor for non-erosive reflux disease (Du et al., 2007).

Using a multivariate regression analysis, to reduce confounding factors, the predictors for functional dyspepsia were educational level, consumption of canned food, and alcoholic drinks. The predictors for GERD were age and use of canned food or fast food.

Canned foods appear as a predictor in both illnesses. Although the consumption of canned food was not so popular in the studied group (median frequency was monthly), it was significantly correlated with the presence of disease. Some components of cans (food additives, pH, and tin) may possibly determine digestive symptoms by certain mechanisms (intolerance, interference with medication, etc.).

Tin is present in low concentrations in most canned foods and beverages; the highest levels are found in products when plain uncoated internal surfaces are used. A limited number of case reports of acute gastrointestinal disorders after consumption of food containing high concentrations (700 ppm or above) of tin have been reported, but there is little evidence for an association between the consumption of food containing tin at concentrations up to 200 ppm and significant acute adverse gastrointestinal effects (Boogaard et al., 2003; Blunden and Wallace, 2003).

Limitations, drawbacks, and shortcomings

The study design, based on the invitation to the medical ceter of the selected subjects, may have influenced the results, presenting to the doctor mainly those who had symptoms in the last period.

A cross-sectional study cannot establish causality but only a relationship between the studied elements. A correlation can have several possible explanations. Frequent consumption of a particular food may positively or negatively influence the presence of disease; for example, canned food, alcoholic drinks, or processed meat for upper gastrointestinal disorders. Also, the disease can lead to a certain lifestyle or diet, sometimes in compliance with dietary recommendations or due to the subjects preconception about the protective role of food in diseases (they may frequently use herb teas or grain cereals). Both factors may be dependent on a third factor; for example, age or educational level, which influenced both the eating or lifestyle and the presence of disease. To reduce confounding factors, we used a multivariate regression analysis.

Unfortunately, we could not assess what types of cans were consumed. This has become a topic for future research.

I.5.5. CONCLUSIONS

This survey, conducted in an urban population from Romania, using interviews in a doctor's office, revealed a 7.6% prevalence of functional dyspepsia using Rome III criteria and a 31.0% prevalence of GERD using Montreal criteria, and it showed that the overlap of the two diseases was 22.4%.

Both diseases occurred at an increased rate in subjects who were older and who had a low educational level, and they were associated with the consumption of canned food, grain cereals, and alcoholic beverages.

The mechanisms by which diet influences gastrointestinal disorders are not fully elucidated, but the findings suggest the need for extensive research and specific strategies tailored to each specific population to promote healthy eating and life- style habits.

I.6. THE TREATMENT OF GASTROESOPHAGEAL REFLUX DISEASE- THE ROLE OF MUCOSAL PROTECTIVE COMPOUNDS

I.6.1. INTRODUCTION

Gastroesophageal reflux disease (GERD) is known to be the result of gastric content reflux in the esophagus, leading to troublesome symptoms that alter the quality of life (QoL) or determine complications (Moayyedi and Talley, 2006). Patients with increased esophageal acid exposure and those with GERD symptoms trigered by acidic reflux episodes but with normal esophageal acid exposure will respond to gastric acid suppression, such as proton pump inhibitors (PPIs) and H2 blockers. However, gastric acid suppression with PPIs and also H2 blockers are not always efficient (Boeckxstaens et al., 2014) even in these cases. Obviously, patients with non-acid reflux and patients with functional heartburn (i.e. normal esophageal acid exposure and no correlation between reflux events and reflux symptoms) will be more resistant to PPIs (Boeckxstaens et al., 2014), (Scarpignato, 2012).

Majority of GERD patients are treated empirically, based on the suggestive symptomatology, without further investigation. In the majority of cases the treatment implies acid suppression with PPI or with a histamine 2 receptor antagonist (H2RA) (Modlin et al., 2009). PPIs are currently the most effective drugs for healing Erosive Esophagitis (EE) and for symptoms elevation (Khan et al., 2007). However, PPIs may have side effects and also there may be some drug interactions which need to be considered especially in the elderly.

Very often, the reflux symptoms are intermittent. Automedication is also common in GERD patients.

Mucosal protective drugs (sucralfate, guaiazulene, alginates, and antacids), are developed decades ago, but there are currently used in several forms of GERD: patients with mild GERD symptoms (less than once a week) or in patiens with severe forms as associated medication with PPI (Yadlapati and DeLay, 2019).

The aim of this paper was to study the Romanian expert's opinion on the indications and therapeutic value of these non-anti-secretory drugs in the treatment of GERD. The paper is constructed as a useful tool for primary care physicians and for specialists.

I.6.2. MATERIAL AND METHOD

A systematic literature search was performed using the following terms: "sucralfate and reflux", "guaiazulene and reflux or gaiazulene and reflux", "dimethicone and reflux", "alginate and reflux" and "antacids and gastroesophageal reflux" in PubMed data base from the beginning until October 2015. The articles included were published in English, French or German, and the studies were conducted on humans. References of relevant articles were manually searched to find papers not returned by our search strategy. The initial search included 864 papers. After excluding the irrelevant papers, 47 papers (including meta-analyses and systematic reviews) were included for this paper.

Based on available data in the literature, several statements were developed regarding the use of these drugs in GERD, and were sent by e-mail to all experts authors, who were asked to

vote using a six-point Likert scale: 1) strongly agreement, (2) agreement with minor reservation, (3) agreement with major reservation, (4) disagreement with minor reservation, (5) disagreement with major reservation, and (6) strongly disagreement. Consensus for a statement was defined as > 70% strongly agreement or agreement with minor reservations. The level of evidence and grade of recommendation were discussed between the authors, using the Oxford evidence criteria (tab. XI). The strength of the evidence was classified according to the GRADE system. Quality of evidence was graded as high, moderate, low and very low (Atkins et al., 2004).

Table XI. Grade of recommendation according to Oxford criteria Grade of recommendation

A	Consistent level 1 studies
B	Consistent level 2 or 3 studies or extrapolations from level 1 studies
C	Level 4 studies or extrapolations from level 2 or 3 studies
D	Level 5 evidence or troublingly inconsistent or inconclusive studies of any level

"Extrapolations" are where data is used in a situation that has potentially clinically important differences than the original study situation

I.6.3. RESULTS

Use of antacids in GERD

The most widely practiced management strategy for symptomatic GERD is to reduce gastric acidity, and thereby the exposure of esophageal mucosa to gastric acid during episodes of reflux (Boeckxstaens et al., 2014). One option to reduce gastric acidity is the use of antacids, mainly for mild GERD symptoms. Antacids may contain a combination of magnesium trisilicate, aluminium hydroxide or calcium carbonate, with clear effect on reducing gastric acidity and peptic activity. In addition, aluminium containing antacids accelerate the healing of gastroduodenal ulcerations, due to a cytoprotective activity resulted from the enhancement of natural mucosal defense mechanisms. This dose dependent protective activity remains after acidification, i.e. after loss of acid neutralizing capacity. The active component of aluminium containing antacids is Al(OH)3 which activates the nitric oxide system, and subsequently increases the mucosal microcirculation (Konturek et al., 1992). Some studies have reported that aluminium containing antacids increased prostaglandin formation in gastric mucosal tissues and enhanced secretion of prostaglandins into the gastric lumen (Gasbarrini et al., 1990). These results were not confirmed in other studies (Lambrecht et al., 1993).

Antacids may provide relief of heartburn within 5 minutes, but their effect is very short (30 to 60 minutes). Three randomized controlld trials (RCTs) compared antacids (aluminum hydroxide/magnesium carbonate, and magnesium/aluminum hydroxide) with placebo (Graham et al., 1983; Weberg and Berstad, 1989; Simon et al., 1995). In one double-blind crossover RCT in 47 patients with reflux esophagitis, antacids reduced the global symptomatic scores, the episodes of regurgitation and episodes of heartburn during night and day (Weberg and Berstad,

1989). Simon et al. compared antacid (113 patients) and placebo (111 patients) and concluded that antacid relieved 62% of heartburn episodes, while placebo relieved 41% of heartburn episodes ($p<0.05$). Antacids provided more rapid and more frequent relief than placebo (OR = 1.57 vs. 1, $p<0.003$). One third of patients in the antacid group and 43% in the placebo group used rescue antacids ($p<0.05$) (Simon et al., 1995). The third trial included only 32 patients and found no significant difference in heartburn frequency or severity between an antacid (Maalox) and a placebo (Graham and Patterson, 1983).

In the meta-analysis of Tran et al., 4 trials comparing antacids (578 patients) and placebo (577) were analyzed. Subjective improvement after 2 and 4 weeks of treatment was evaluated. There was a trend favoring antacids over placebo, but the combined absolute benefit increase of antacids over placebo was only 8% (95% CI: 0-16%, $p=0.06$). The combined relative benefit increase was 0.11 (95% CI: 0.03-0.20), and number needed to treat was 13 (95% CI: 6-250) (Tran et al., 2007).

Several studies showed that in NERD patients, antacids seemns to be at least as effective as H2RAs for up to 3 hours (Konturek et al., 2007), (Holtmeier et al., 2007), (Xiao et al., 2013). One large double-blind, 3-fold cross - over RCT (including 562 patients with GERD symptoms) comparing a single dose of 1000 mg of hydrotalcite (aluminium– magnesium-hydroxide–carbonate)10 mg of famotidine or placebo (as on-demand treatment of heartburn) showed that hydrotalcite significantly decreased heartburn severity after 10 minutes (when compared with placebo), and had better efficacy at 30 minutes and 3 hours after intake, in comparison with famotidine or placebo (Holtmeier et al., 2007). Another study concluded that hydrotalcite is a good option for on-demand therapy for NERD patients, due to its cost-effectiveness (1/3 of the costs using esomeprazole on-demand) and rapidity of action (Xiao et al., 2013).

When used excessively and for long periods of time, side effects of some antacids seem to be significant. Aluminum containing antacids may cause constipation and binds phosphate in the gut, leading to hypophosphatemia, metabolic bone disease, neurotoxicity, and anemia. Several side effects (such as hypercalcaemia, milk–alkali syndrome or constipation) may also be observed with calcium-antacids, but not at the recommended doses. Overdose/repetitive use of magnesium-containing antacids may cause hypermagnesaemia, including fatal cases (Tytgat et al., 2003).

Regarding the use of antacids in pregnant women, the data comes mainly from the experts' consensus and reviews. A recent meta-analysis identified only one trial that evaluated the effect of magnesium and aluminum hydroxide plus simethicone (liquid and tablet) compared with placebo, for relieving heartburn in pregnancy. Women who received the treatment reported complete heartburn relief more often than women receiving no treatment or placebo (RR 1.85, 95% CI 1.36-2.50), without a clear difference in the rate of side effects (RR 0.63, 95% CI 0.21 to 1.89) (Phupong and Hanprasertpong, 2015). From the experts' consensus, calcium or magnesium containing antacids are preferred, because there are data showing that women with an adequate calcium intake are at a reduced risk of developing hypertension and pre-eclampsia during pregnancy (Villar and Belizan, 2000). Magnesium sulfate supplementation reduces the risk of eclampsia by 50% compared with placebo (Altman et al., 2002). Both supplementation with

calcium and magnesium (absorbed from antacids) are considered to have beneficial effects in pregnant women, and are therefore preferred in the treatment of heartburn in pregnancy. The antacids should be used 'on demand' and the recommended doses should not be surpassed.

Based on these studies, the recommendations are summarized in table XIII.

Table XII. Recommendations of the Romanian Society of Neurogastroenterology on the antacids use in GERD

Statement	Experts agreement	Level of evidence	Grade of evidence	Grade of recommendation
Antacids are slightly superior over placebo in treating heartburn	91.6%	2a	Low	B
Antacids may be used to rapidly relief uncontrolled GERD symptoms	91.6%	2b	Moderate	B
Antacids may be used as over-the counter drugs, to treat mild or infrequent reflux symptoms, especially in NERD patients who choose to self-medicate	83.3%	2b	Low	B
Antacids (calcium or magnesium-containing antacids) may be used in pregnant women with GERD symptoms.	91.6%	4	Very low	C
In pregnant women, there is no clear difference in the rate of side effects between antacids and placebo.	83.3%	4	Very low	C

GERD: Gastroesophageal reflux disease, NERD: Non-erosive reflux disease

Use of raft-forming agents in GERD

One of the concepts in GERD pathogenesis, mainly in the pathogenesis of postprandial reflux, is that of the gastric acid pocket. This is actually the entrence of the stomach, which is becoming highly acidic after a meal (Beaumont et al., 2010). The presence of this gastric acid pocket has become an attractive therapeutic target.

Alginate, a polysaccharide derived from seaweed, binds water in the acid pocket and forms a viscous gel, displacing the acid pocket distally, below the diaphragm in 70% of patients (Rohof et al., 2013). The bicarbonate added to alginate is converted to carbon dioxide which forms bubbles which are trapped within the gel and convert it to a lighter substance that can rise to the surface of gastric contents and float (thus the name "raft-forming agent") (Lambert et al., 1990). The exact mechanism of action of alginate-antacid combination in GERD is not well established. The common assumption is that alginate-antacid combination creates a mechanical barrier to acid reflux as it moves into the esophagus ahead of acidic gastric content and prevents reflux (Boeckxstaens et al., 2014).

Recent data showed that, although alginate-antacid reduced distal esophageal acid exposure it did not influence the number of reflux events (acid or weakly acidic). Therefore, it seems that alginate–antacid effect relates to displacement and neutralization of the postprandial

gastric acid pocket, rather than preventing reflux (De Ruigh et al., 2014). On the contrary, another small study that compared pre and postprandial esophageal pH-multichannel impedance parameters, showed that alginate decreased significantly the number of acid reflux events, the percentage time pH<4.0 and the height of proximal reflux compared with baseline (Zentilin et al., 2005). Alginate preparations are extensively studied, and are available in Romania, marketed as Gaviscon.

Alginate/antacid combination versus placebo

We identified three placebo-controlled trials assessing alginates efficacy in GERD. The most recent (2015) and largest RCT on this subject included 1107 GERD patients [intention to treat (ITT) population, 1073]: 536 patients in the Gaviscon Double Action (DA) group and 537 patients in the placebo group. Reflux disease questionnaire (RDQ) symptom score before and after a 7-day treatment was used to assess the efficacy of Gaviscon DA. There was a substantial placebo response. However, Gaviscon DA was superior to placebo in reducing RDQ score for GERD dimensions (from 2.51 at baseline to 1.25 after treatment for Gaviscon DA, and from 2.50 to 1.46 for placebo, p<0.001). Ninety percent of patients in DA group, and 84% in the placebo group experienced symptom improvement (Sun et al., 2015).

Another RCT included 110 GERD patients. Gaviscon DA decreased reflux symptoms and the overall RDQ symptom score. The overall treatment response was higher in the Gaviscon group than in the placebo group (4.1 vs. 1.9, p<0.001) (Thomas et al., 2014). The third double-blind parallel-group RCT included 100 patients with GERD (ITT population, 94) and showed that sodium alginate was assessed as superior to placebo, both by investigators and patients at week two and week four (Chatfield, 1999).

In the meta-analysis of Tran et al. (2007), four RCTs were analyzed (including 146 patients in the treatment group and 138 patients in placebo group). The combined absolute benefit increase of alginate/antacid combination versus placebo was 26% (CI 95%: 12-41%, p<0.0001), and the relative benefit increase was 0.60 (95% CI: 0.25-0.91). The NNT was 4 (95% CI: 2-9) (Tran et al., 2007).

Alginate antacids versus antacids

Several studies compared the clinical efficacy and safety of alginate-antacid and antacids alone. Studies from the 70's showed that alginate and standard antacid were equally effective in reducing the number of heartburn episodes(McHardy, 1978) and determined clinical improvement in the majority of patients (Scobie, 1976).

One open-label, prospective, randomized, parallel group clinical trial included 203 GERD patients, and randomized 191 patients (only patients with symptoms in the run-in period) to a 14-day treatment with either drug. Sodium alginate was faster than magaldrate (hydroxymagnesium aluminate complex) in relieving GERD symptoms. The duration of action and the efficacy were slightly better for alginate; 81.6 % of patients in the sodium alginate group and 73.9% of patients in the magaldrate group reported total disappearance of symptoms, but without reaching statistical significance. Two events in the sodium alginate group (diarrhea and nausea) were

considered to be potentially drug-related (Giannini et al., 2006).

Another study randomized 134 NERD patients to receive alginate-antacid or antacid (Nacid®) for a 6 weeks period and showed a greater reduction in the severity ($P < 0.0001$) and frequency ($P = 0.0015$) of heartburn at 6 weeks, and a lower frequency of heartburn, regurgitation and belching at 3 weeks. The improvement in the QoL was more remarkable in the alginate-antacid group. The adverse events were similar in both groups, two patients in the alginate-antacid group reporting constipation (Lai et al., 2006).

Alginate/antacids versus PPIs

Several studies compared the efficacy of sodium alginate with PPIs. One study that included 195 NERD patients, compared alginate suspension (20 ml three times a day) with omeprazole (20 mg/day) and showed that 53.3% of patients achieved adequate heartburn and regurgitation relief compared to 50.5% of patients in the omeprazole group ($p=0.175$) after 4 weeks of treatment. More than 86% of patients graded the overall satisfaction using one of these treatments as 'good' or 'very good'. The authors concluded that sodium alginate was as effective as omeprazole for symptomatic relief in NERD patients (Chiu et al., 2013).

Adding sodium alginate to omeprazole may increase the rate of NERD patients with a complete resolution of heartburn. A study that included 76 NERD patients reported a complete resolution of heartburn in 56.7% of patients in combination group vs. 25.7% in omeprazole group alone. The heartburn-free days also increased in combination group (Manabe et al., 2012).

In another study, the authors compared the short-term efficacy (at day 7 and day 14) of Gaviscon 10mL, 4 times a day (120 patients) with omeprazole 20mg/day (121 patients) in a randomized double- blind, double-dummy trial. With both treatments the mean time to onset of the first 24-h heartburn-free period after initial dosing was 2 days (± 2.2). The omeprazole group had a higher mean number of heartburn-free days by D7 (3.7 ± 2.3 days vs.3.1 ± 2.1; $p = 0.02$), and a better relief of pain ($p=0.049$) (Pouchain et al., 2012).

Alginates in pregnancy

Few studies have investigated the safety and efficacy of alginates in the treatment of heartburn in pregnancy. Half of the 150 pregnant women instructed to take Gaviscon 5-10 ml when necessary reported rapid heartburn relief (within 10 minutes), and 88% rated drug's efficacy as "very good". No safety concerns for the mother or the fetus were reported in this study (Lindow et al., 2003). In another study, 50 pregnant women (during the 2nd and 3rd trimester of pregnancy) with gastroesophageal reflux symptoms were treated for 1 month with Gaviscon (2 tablespoons after each meal and at bedtime). All symptoms improved after 1 month; 98% of women considered that the drug is efficient, and no side effects were noted (Uzan et al., 1988). Some adverse effects of alginates were reported, mainly related with the antacid included in the preparation (i.e. magnesium trisilicate or sodium bicarbonate). Therefore, some authors suggested that alginates combined with these antacids should be avoided during pregnancy due to adverse events if used long-term and at high doses (Richter, 2005).

Alginate-antacid combination is superior both over placebo and antacids alone in alleviating heartburn in GERD patients. This combination proved similar efficacy as compared to a single dose omeprazole, especially in NERD patients. Alginate- antacid can be added to PPI treatment in case of persistent symptoms, leading to a further improvement of symptoms (tab. XIII).

Table XIII. Recommendations of the Romanian Society of Neurogastroenterology concerning the use of alginate-antacid combination in gastroesophageal reflux disease

Statement	Experts agreement	Level of evidence	Grade of evidence	Grade of recommendation
Alginate-antacid is superior over placebo in the treatment of GERD symptoms	100%	1b	Moderate	A
Alginate-antacid combination can be used to manage mild symptoms of reflux, especially in NERD patients	100%	2b	Low	B
Alginate-antacid combination is more effective than antacids for the treatment of symptoms in NERD patients	100%	2b	Low	B
Alginate–antacid combination is as effective as Omeprazole 20 mg/day in patients with mild GERD symptoms, especially in NERD patients	83.3%	2b	Low	B
Alginate-antacid combination may be used in pregnant women with heartburn	100%	3b	Very low	B
Alginate-antacid combination may be used to treat persistent reflux symptoms despite acid suppressant therapy	83.3%	2b	Very low	B

GERD: gastroesophageal reflux disease, NERD: Non-erosive reflux disease

Use of Sucralfate in GERD

Sucralfate is a salt of sucrose sulfate and aluminium hydroxide, which binds to the mucosa creating a physical barrier that blocks the diffusion and interaction of hydrochloric acid, pepsin or bile salts and esophageal mucosa (Wang et al., 2013). The affinity of sucralfate for inflamed mucosa is explained by the formation of polyvalent bridges between the negatively charged sucralfate polyanions and positively charged proteins present in mucosal lesions (Gorget, 1985). It also has cytoprotective properties attributed to the fact that sucralfate increases the local levels of fibroblast growth factors and induces a rise of prostaglandins in the mucosa, thus inducing mucosal healing (Candelli et al., 2000). Sucralfate (1g qid, for 3 months) normalizes esophageal acid clearance rate suggesting that once the inflammation subsides, esophageal motor function is restored (Elsborg, 1987). In addition, sucralfate absorbs pepsin and bile salts, resulting in a comprehensive defense against several aggressive factors (Gorget, 1985).

Several RCTs argued the superiority of sucralfate versus the placebo in alleviating GERD symptoms. We identified four RCTs, with variable doses (1g bid to 1g qid) and durations of treatment (6, 8 or 12 weeks) that proved some benefit of sucralfate over placebo in improving

GERD symptoms and endoscopic aspect of esophageal mucosa. However, in two of these studies statistical significance was not achieved. One study (141 NERD patients, 1g sucralfate gel bid for 6 weeks), showed a "good" or "excellent " overall response in 45% of patients in the active group, compared with 22% in the placebo group ($p<0.001$) (Simon et al., 1996). In another study (36 patients with severe EE), sucralfate (1g after meals and 2g at bed time) or placebo were added to cimetidine (300mg qid) for 12 weeks. The combination of cimetidine and sucralfate was superior to cimetidine alone in improving daytime heartburn and overall endoscopic outcome, without statistical significance regarding endoscopic healing. In another two studies (that included 68 and 138 patients, respectively) the proportion of patients with symptomatic or endoscopic improvement was higher after sucralfate treatment, but not significantly when compared with placebo (Williams et al., 1987), (Carling et al., 1988). A meta-analysis published in 1987 (including 51 double blind RCTs) concluded that sucralfate was better than placebo in improving esophagitis endoscopic lesions. More recently, in 2005, a meta-analysis confirmed the superiority of sucralfate over a placebo as the maintenance therapy of GERD (Donnellan et al., 2005).

Sucralfate seems to be equally efficient with H2RAs in improving GERD symptoms, and also in determining mucosal healing. At the end of the 80's it was considered a safe alternative to H2RAs in GERD patients. We identified 8 studies (in adults) that compared sucralfate and ranitidine or cimetidine, the majority of them in EE, the endpoint of the trials being mucosal healing. In one study, monotherapy with sucralfate in milder forms of EE was comparable with the combination of sucralfate during the day and ranitidine after dinnertime (Vermeijden et al., 1992). In another study, endoscopic healing was observed in 47% on sucralfate (suspension 6g/day) and in 31% of patients receiving ranitidine (150mg bid), and healing or endoscopic improvement was observed in 81% of patients on sucralfate vs. 64% of patients on ranitidine ($p> 0.05$). Heartburn and acid regurgitation were relieved in similar proportions in both groups (Bremner et al., 1991). Complete healing of esophageal erosions with sucralfate varied from 19.4% (Schotborgh et al., 1989) to 60% . In all these studies, the duration of treatment ranged between 8 weeks and 6 months, so that tachyphylaxis commonly seen with H2RAs sometimes after only 2 weeks was not taken into account, and could partly explain the noninferiority of sucralfate.

There is conflicting data regarding the role of sucralfate in preventing the recurrence of reflux esophagitis. A double-blind RCT followed for 6 months 88 patients treated with sucralfate (2g bid) and 93 with placebo and reported a relapse rate of esophagitis of 31% in sucralfate group vs. 65% in the placebo group ($p<0.001$) (Tytgat et al., 1995). Another study reported no difference between sucralfate and placebo in terms of relapse rate of esophagitis during long-term treatment (Schotborgh et al., 1989).

The efficacy of sucralfate vs. alginate/antacid was compared in one study. Both treatments significantly improved symptoms after 6 weeks (in 70% of patients) and esophagitis healed in 53% of patients treated with sucralfate, and 34% of patients treated with alginate/antacid ($p> 0.05$) (Laitinen et al., 1985).

Data about the use of sucralfate in pregnancy is scarce. A RCT included 42 pregnant women and reported symptoms improvement in over 80%, without maternal or fetal adverse events. In a surveillance study, among 185 newborns exposed to sucralfate in the first trimester, 5 birth defects were observed, whereas 8 were expected (Richter, 2005). In a meta-analysis (Phupong and Hanprasertpong, 2015), only one study with sucralfate in pregnancy was included (n= 65). Women in the sucralfate group experienced more often complete relief of heartburn compared to women who received advice on diet and lifestyle choices (RR 2.41, 95% CI 1.42 to 4.07). The evidence on side effects rate between the two groups (RR 1.74, 95% CI 0.07 to 41.21) was not clear (Phupong and Hanprasertpong, 2015). Sucralfate is a FDA category B drug. As sucralfate is poorly absorbed from the gastrointestinal tract, current guidelines and our recommendation is that sucralfate can be used in pregnant women who experience GERD symptoms.

Sucralfate can decrease bioavailability of certain drugs (i.e. fluoroquinolones, digoxin), including some PPIs. Recommending patients to dose their PPI correctly (i.e. 30– 60 min before any meal of the day) (Scarpignato, 2012) and to use sucralfate after each meal (i.e. at least 1 hour after PPI ingestion) diminishes the interaction between the two drugs.

Sucralfate relieves GERD symptoms, can induce mucosal healing and can prevent to some extent recurrent esophagitis when used as maintenance therapy (tab. XIV).

Table XIV. Recommendations of the Romanian Society of Neurogastroenterology concerning the use of sucralfate in gastroesophageal reflux disease

Statement	Experts agreement	Level of evidence	Grade of evidence	Grade of recommendation
Sucralfate is superior over placebo in alleviating GERD symptoms	100%	2a	Low	B
Sucralfate is as efficient as H2RAs in improving GERD symptoms, and in promoting mucosal healing	91.6%	2b	Moderate	B
Sucralfate can be used as maintenance therapy (after healing of EE with PPIs) in order to prevent relapse of esophagitis	83.3%	2b	Very low	B
Sucralfate can be used in pregnant women with GERD symptoms	100%	2b	Low	B

GERD: gastroesophageal reflux disease, H2RA: histamine 2 receptor antagonist, EE: erosive esophagitis

Use of guaziaulene-dimethicone in GERD

Pepsane®, a combination guaiazulene (4mg)-dimethicone (3g), is currently marketed in Romania with two major indications: GERD and dyspepsia. Azulenes are widespread in nature, one of their prime sources being algae. Studies on animal models showed that azulenes have antioxidant activity, interact with membrane lipids, reduce histamine levels, and thus acid secretion in the stomach. They also have anti-inflammatory (Rekka et al., 2002) and anti-oedema

action, and increase the blood flow in the mucosa (Yano et al., 1990). Dimethicone and simethicone (polydimethylsiloxane) are substances with anti-foam action, transforming small gas bubbles in larger ones which are easier to move and eliminate. This mechanism is poorly documented, but several studies showed that simethicone reduces gas related dyspeptic symptoms, and that dimethicone has a gastroprotective effect (Bergmann et al., 1989) .

Our search identified four studies related with these two compounds. Two studies referred to dimethicone (Ogilvie and Atkinson, 1986), (Smart and Atkinson, 1990)and both showed a potential benefit of adding dimethicone to antacids for improving both macroscopic and microscopic esophageal appearances. The combination dimethicone / antacid gel was compared with a simple antacid gel in a double-blind trial in 45 patients with reflux esophagitis. Thirty eight patients completed the 8-week course of therapy. Both treatments reduced pain scores at 4 and 8 weeks. There was a tendency for the dimethicone/antacid group to better improve esophageal inflammation (Ogilvie and Atkinson, 1986). The other trial compared the efficacy of dimethicone/antacid vs. alginate/antacid in 53 patients with GERD (Smart and Atkinson, 1990). The results nearly reached statistical significance, possibly due to a type II error.

Another study was a double blind RCT of guaiazulene and dimethicone vs. placebo and effects on QoL (Leplege et al., 2005). This study was part of a phase III trial published in 2003 by Ruszniewski et al., and we contacted the manufacturer for the final article. The trial included 233 patients with moderate GERD symptoms and either NERD or grade 1 esophagitis (Savary-Miller classification). The patients were randomized to receive either Pepsane® or placebo after each meal, for 28 days. The primary endpoint of the study was a 50% reduction in the global symptomatic score, and was achieved in 54.1% of patients from the Pepsane® group, vs. 41.1% of patients in the placebo group at 14 days ($p= 0.07$). The secondary endpoints were: physicians assessment of efficacy (Pepsane® vs. placebo at 14 days - 66.7% vs. 51.7 %, $p<0.02$, but not at 28 days); global efficacy of the treatment ("clear improvement" in 52% of patients in Pepsane® group vs. 37% of patients in placebo group, $p<0.05$); tolerance and side effects were comparable between the two groups. Pepsane® significantly improved the scores of quality of life (QoL) in three dimensions. After 4 weeks of treatment, QoL scores in the Pepsane® group were similar with those observed in the general population, but remained significantly lower in the placebo group (Leplege et al., 2005).

In an open trial involving 118 dyspeptic patients, including patients with GERD, the treatment with guaiazulene- dimethicone combination reduced the proportion of patients with heartburn, from 66% at the beginning of trial to 14% after one month of therapy. The therapeutic effect of this combination was very rapid (less than 20 minutes) in 82.2% of patients.

Even though these trials do not allow a definite conclusion on efficacy, they showed that Pepsane® rapidly relieves heartburn in a high proportion of patients, and improves the QoL. It could be a therapeutic option in patients with symptomatic NERD (tab. XV)

Table XV. Recommendations of the Romanian Society of Neurogastroenterology concerning the use of guaiazulene- dimethicone in gastroesophageal reflux diseas

Statement	Experts agreement	Level of evidence	Grade of evidence	Grade of recommendation
Guaiazulene-dimethicone is slightly superior over placebo in the control of GERD symptoms	91.6%	2b	Very low	B
Guaiazulene-dimethicone is more effective than placebo in improving quality of life in GERD patients	91.6%	2b	Very low	B
Guaiazulene-dimethicone rapidly relieves heartburn	91.6%	4	Very low	C

GERD: gastroesophageal reflux disease

I.6.4. CONCLUSIONS

Several therapeutic options besides PPIs are available for GERD: antacids, alginate-antacids combinations, sucralfate and guaiazulene-dimethicone. These drugs offer a rapid relief of symptoms. Sucralfate promotes mucosal healing and to a minor extent prevents recurrence of esophageal erosions. Given the limited absorption from the digestive tract, these preparations can also be used during pregnancy. The use of these drugs might also reduce the costs of treating this chronic disorder. Based on the current knowledge, mucosal protective compounds cannot replace PPIs in the treatment of GERD, but can be useful in mild cases or in PPI-refractory GERD, either alone or in combination with PPIs. There are some data suggesting that long-term use of these drugs might delay symptom relapse and prolong remission, but this remains to be proven.

II. IRRITABLE BOWEL SYNDROME

II.1. STATE OF THE ART

Irritable bowel syndrome (IBS) is considered to be a functional bowel disorder. The most recent largely recognized definition and diagnostic criteria for IBS was established in 2016 and published by the Rome IV working group (Palsson et al., 2016), (Tack and Drossman, 2017). Rome IV definition for the IBS is referring to recurrent abdominal pain associated with defecation or a change in bowel habits (Tack and Drossman, 2017). Diagnostic criteria for Irritable Bowel Syndrome implies the presence of recurrent abdominal pain, on average, at least 1 day per week in the last 3 months, associated with 2 or more of the following criteria: 1. Pain is related to defecation; 2. Pain is associated with a change in frequency of stool; 3. Pain is associated with a change in form (appearance) of stool. Diagnostic criteria have to be fulfilled for the last 3 months with onset of symptoms at least 6 months before diagnosis.

Epidemiology.

The global prevalence of IBS is considered to be 11.2% (95% confidence interval: 9.8% - 12.8%), based on a large meta-analysis published on 2012 by Lovell, on 80 studies (Lovell and Ford, 2012). However, the prevalence may vary widely from 5% to 65% according to definition and population included (Kay et al., 1994). In Romania reported prevalence for IBS ranged 14.5% to 19.1% (Drug et al., 2000) (Chirila et al., 2012).

Pathogenesis.

During years, multiple etiological factors have been related with the occurrence of IBS symptoms. Abnormal intestinal motility, visceral hypersensitivity and altered brain-gut interaction, increased mucosal permeability, abnormal intestinal flora, psychosocial distress, diet and also genetic factors have been implicated in pathogenesis of IBS (Adriani et al., 2018).

Gut abnormal intestinal motility related to abdominal symptoms compatible with IBS was first described by Thompson in 1978 (Thompson et al., 1979). Since then, multiple motor abnormalities have been described at different gut level. However they failed to show specific abnormalities related to IBS (Drossman et al., 1997). Visceral hypersensitivity was confimed as a pathogenic factor involved in IBS and proposed as diagnostic marker (Ritchie, 1973). It is now considered, together with gut motor abnormalities to be related to serotonin (5-HT) and its effects on 5-HT3 and 5-HT4 receptors, altered brain-gut interaction and increased mucosal permeability (Adriani et al., 2018). Dysbiosis is reportedly related to IBS (Chong et al., 2019), with decrease of composition and activity of Lactobacilli and Bifidobacteria (Bellini et al., 2014), increase in Bacteroides (Tap et al., 2017) and decrease in microbial diversity (Carroll et al., 2012). Alteration of mycobiota in IBS was also reported (Botschuijver et al., 2017).

Diet has been linked by patients with the occurrence of IBS symptoms. The basis for this relationship between diet and IBS symptoms could be related through altered microbiota,

intolerance to FODMAP or non-celiac gluten sensitivity, abnormal permeability, local inflammation and brain – gut interaction (Chong et al., 2019).

The brain-gut axis (GBA) is a bidirectional communication system between the brain and the gut. Recent studies support the involvement of abnormal function of brain-gut axis in IBS but also in functional GI disorders. Due to its role, functional GI disorders were reconsidered by ROME IV experts to be alterations of brain gut axis (Drossman, 2016).

Genetic studies have focused on the relationship between the IBS clinical phenotype and the genotype but also on endophenotype-genotype association. In the first type of studies, a large number of people from different geographic areas have to be included. In this way we can minimized the effect of environmental or other unknown factors (Camilleri et al., 2008). Over 60 candidate genes in IBS were studied, targeting the pathophysiological pathways: synthesis, transport and serotonin reuptake activation of immune mechanisms of intestinal mucosal inflammation and neuropeptide signaling, nociception, bile acid synthesis, intestinal secretion (Saito, 2011, Gazouli et al., 2016). Heritability in IBS was estimated to be 22-57%. Positive family history of the disorder is a possible predictor and is associated with a twofold to threefold higher risk in relatives of IBS patients (Saito, 2011; Waehrens et al., 2015).

Other studies have focused on pharmacogenetic variations in the treatment of IBS, important also for future personalized treatment. These studies identified genetic variants in neurotransmitter pathways, that may play a role in mediating the effects of placebo induced analgesia (Kaptchuk et al., 2010; Hall et al., 2015).

The most studied pathway for IBS symptoms involves receptors and serotonin transporter genes. The region of promotor gene SERT (serotonin transporter) contains a polymorphism 5HTTLPR (5-HT transporter lenghth polymorphic region), consisting of the deletion / insertion of the 44 bp allele yielding S (short) and one L (long). SS/SL genotypes are associated with low levels of transcription of the gene product SERT, which indicates a weak serotonin reuptake (Gazouli et al., 2016, Wang et al., 2012). The correlation between IBS and 5HTTLPR is contradictory. There are genetic studies that have not indicated an association, while other studies have suggested a significant association between 5HTTLPR and IBS subtypes or symptoms (Van Kerkhoven et al., 2007, Yeo et al., 2004). Colluci's study suggests a significant association of the genotypes LS and SS with the severity of the symptoms of IBS, but not with IBS subtype (IBS-D, IBS-C, IBS-M) (Colucci et al., 2013). S allele was shown to be associated also with depression, anxiety, increased sympathetic tone, decreased parasympathetic tone, increased anxiety / neuroticism and increased levels of cortisol (Gazouli et al., 2016, Farmer et al., 2013, Yuan et al., 2014). Rs25531 is a SNP associated with IBS, but also with psychiatric disorders. The polymorphism 5HTTLPR-rs25531 analysis identified several subtypes of genotypes allele L (LG / LG, LG / LA, LA / LA) and the allele LG seems functional similar with S allele (Kohen et al., 2009, Jaworska et al., 2016, Uher and McGuffin, 2008). The study of polymorphisms in genes encoding various subtypes of serotonin receptors have identified a number of positive associations. 5-HT receptors are very well represented at the gastrointestinal level. Five families from seven of 5-HT receptors are expressed in the intestinal mucosa - 5-HT1, 5-HT2, 5-HT3, 5-HT4 and 5-HT 7 – also with functional implications (Takaki et al., 2014).

Rs1062613 polymorphisms (in *HTR3A* gene) and rs 62625044 (in *HTR3E* gene) have been associated with IBS-D in both females and males (Gu et al., 2015, Kapeller et al., 2008). Study of rs1176744 polymorphism in the gene *HTR3B* showed that activation in various brain regions is different between the subjects with genotype AA and AC / CC, with various effects on symptoms (Fukudo et al., 2009). Rs6766410 polymorphism in the gene for *HTR3C* show positive association with IBS-D homozygous genotype CC (Kapeller et al., 2008). It is noteworthy to mention that the associations between SNP polymorphisms in the gene *HTR2A*, *HTR3E, SLC6A4* and IBS / IBS subtypes were confirmed by genome-wide association studies (GWAS) (Ek et al., 2015, Mulak, 2013).

A recent meta-analysis, showed that among the genes involved in immune function, *TNFSF15* (tumor necrosis factor superfamily member 15) was associated with IBS. Polymorphism rs4263839 in the gene *TNFSF15* was associated with IBS by modulating the differential proinflammatory and / or antibacterial response so the gene *TNFSF15* seems to be involved in increasing the cellular immune response and the production of cytokines (Gazouli et al., 2016, Zucchelli et al., 2011, Czogalla et al., 2015).

Some of the polymorphisms of the genes responsible for the neuronal function have been shown to be associated with IBS and IBS subtypes both by association studies and by GWAS.

The endocannabinoid system is known to be involved in secretion, perception, motility and anti-inflammatory activity of the gastrointestinal tract, implicated also in the development of functional gastrointestinal disorders, including IBS (Drug, 2015).

The existence of genetic markers associated with IBS could be in future a good diagnostic tool, but may also assist in the management and personalized therapy. In this context, between 2012 and 2016, the GENIEUR project (Genes in Irritable Bowel Syndrome in Europe), an EU COST funded project, created a network of interdisciplinary scientists with its main role to identifie the genetic factors involved in the etiopathogenesis of IBS. Our University was involved in this project with the active participation of gastroenterologist, genetician, microbiologist and an epidemiologist (www.genieur.eu, Boeckxstaens et al., 2016).

Diagnosis

In 1978 Manning notice that in IBS some symptoms were more commonly present than in organic diseases (Manning et al., 1978). Since than, other new diagnostic criterias have been proposed.

Presently, largelly recognised are the Rome IV criteria which implyies the presence of recurrent abdominal pain, on average, at least 1 day per week in the last 3 months, associated with 2 or more of the following criteria: 1. pain is related to defecation 2. pain is associated with a change in frequency of stool 3. pain is associated with a change in form (appearance) of stool. These criteria have to be fulfilled for the last 3 months, with symptom onset at least 6 months before diagnosis (Mearin et al., 2016).

IBS patients are also sub- classified according to stool consistency in IBS with diarrhea (IBS-D), IBS with constipation (IBS-C), mixt IBS (IBS-M) and unclassified IBS (IBS-U). Stools should be hard in more than 25% of bowel movements in IBS-C, and respectively loose in IBS-

D. For IBS-M loose stools should be >25% of stools and hard stools >25% of stools. In IBS-U loose stools should be <25% of stools and hard stools <25% of stools (Mearin et al., 2016).

In order to fulfill the diagnostic criteria, a clinical history, physical examination and also limited number of investigation related to the clinical context have to be done. The presence or absence of the alarm symptoms such as weight loss, digestive bleeding, anemia and palpable tumor leads to different clinical scenarios. In the absence of alarm symptoms the clinical history should include information on diet, medication, psychosocial context, family history of cancer or other diseases which may explain the symptoms. The correct physical exam of these patients should include also the rectal examination. Complete blood count and C-reactive protein have to be done in all patients. Based on the clinical context and especially based on the clinical form of IBS, the following tests should be done: thyroid function tests, serology for celiac disease, scintigraphic evaluation (75SeHCAT test) for bile acid malabsorption, breath tests for carbohydrate malabsorption, stool analysis for infections, parasites (stool culture, ova and parasite exam) and also tests for inflammation (calprotectin). Colonoscopy should be performed in the absence of alarm symptoms in case of family history of colorectal cancer or of onset of symptoms after age of 50 and also for microscopic colitis. Abdominal US and upper GI endoscopy should be performed according to the clinical context (Mearin et al., 2016), (Adriani et al., 2018).

Abdominal CT and also MRI are more and more used in IBS patients. However, they still have to be used in the clinical context for the exclusion of abdominal neoplasm or inflammatory bowel disease even some suggestive MRI abnormalities have been revealed in IBS patients (Kavanagh et al., 2018).

Treatment

Optimal doctor-patient relationship is essential for the management of IBS patients. It is important for the doctor to understand correctly the patient's symptoms and his concerns. For the patient, is important to understand the role of investigations, to understand that there is no clear marker for IBS but also to have correct expectation from the medical treatment. It is also important to understand why sometimes the patient needs additional help from dietetition, psychologist or psychiatric specialist.

Dietary advice is given to the patient according to a standard IBS recommendation form, initially by the gastroenterologist. Standard specific dietary written recommendation is done initially. The gastroenterologist should consider in the recomandations possible dietary intolerance such as lactose, fructose intolerance or even non-celiac gluten sensibility. Fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) are incompletely absorbed carbohydrates found in wheat, fruits and vegetables, sorbitol, and some dairy and may cause IBS symptoms. FODMAPs restriction could lead to symptom relief in IBS patients, but long term restriction is not considered to be healthy. It is probably the role of a specialist person such as the dietetition to recommend and monitor the FODMAP diet (DRUG, 2015).

The pharmacotherapy of IBS should be tailored to the main IBS complains: pain, constipation or diarrhea.

Pain

Antispasmodics are drugs with several different mechanisms of action and are used in all forms of IBS patients. They may act as anticholinergics, calcium channel blockers, peripheral μ, k and δ opiate receptors agonists and may intervene with modulation of the release of peptides, including motilin, vasoactive intestinal peptide, gastrin and glucagon. Antispasmodic drugs largely used in IBS are: dicycylomine, otilonium, mebeverine, Peppermint oil, trimebutine.

Tricyclics antidepressants (TCAs) such as: desipramine and amitriptyline and also serotonin reuptake inhibitors (SSRIs) such as paroxetine or sertraline and alosetron, had showed efficacy in IBS patients.

Constipation

Soluble fiber such as psyllium, inuline and isphagula and osmotic laxative such as polyethylene glycol (PEG) and lactulose are used classically in IBS –C form for the improvement of colonic transit. New drugs for IBS-are available now in several countries, unfortunately not in Romania. Prucalopride is a highly selective 5-HT4 agonists, used in Europe as second- or third-line after laxatives failure. Also available for constipation resistant patients are: Lubiprostone which is a selective type 2 chloride-channel (ClC-2) and Linaclotide which acts as a guanylate cyclase-C agonist increasing cyclic guanosine monophosphate production.

Diarrhea

Available in Romania for the treatment of IBS-D are: Loperamide (synthetic peripheral μ-opioid receptor agonist), Diosmectite (Smecta) with absorptive effect and Rifaximin, which is a nonabsorbable antibiotic. These drugs are used as first or second choice drugs depending on gastroenterologist's opinion. Also, most of antispasmodics, tricyclics antidepressants (TCAs) and serotonin reuptake inhibitors (SSRIs) have antidiarrhea effect. Alosetron, a highly selective 5-HT3 antagonist and Eluxadoline which is a mixed μ-receptor agonist/δ-opioid receptor antagonist are not yet available in Romania. An important part of IBS - D patients may in fact have biliary malabsorption. In these patients cholestyramine, colesevelam and colestipol may be useful as therapy but also as diagnostic trial. Unfortunately they are also unavailable in Romania. (Drug, 2015, Adriani et al., 2018).

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II.2. THE EVALUATION OF THE PHENOTYPES OF IRRITABLE BOWEL SYNDROME PATIENTS

II.2.1. INTRODUCTION

Irritable Bowel Syndrome is a heterogeneous condition of unknown cause. It is characterized by abdominal pain and altered bowel habits. There is no known cause and no biological marker of this condition. Present data seems to suggest that there is no unique pathophysiological mechanism which may lead to symptom generation.

Irritable Bowel Syndrome diagnosis is based on symptomatic criteria which was altered and improved in time. It started with the Manning criteria, after with the Kruise criteria and than continued with Rome I, II, III and IV criteria (Tack and Drossman 2017)

The Rome criterias represents nowadays the fundamentum for the correct diagnosis of IBS. These are important research tools that are used to have a uniform methodology for clinical trials or translational research.

However, they are not sufficient to characterize reasonably the large spectrum of IBS patients and to describe accurately different subpopulations. These questionnaire should be widely applicable in different settings, allowing valid comparisons of findings from different centers. This is crucial for studying functional digestive disorders including the IBS using genetic, epigenetic and microbiota methodology. In these studies, inclusion of important number of subjects (patients and normal controls) from multiple and international centers is a necessity.

According to the Rome criteria (including Rome IV), the classification of IBS patients is limited to differences in defecation pattern yielding four different subgroups or phenotypes: IBS with constipation, IBS with diarrhea, mixed IBS and unsub-typed IBS (Longstreth et al., 2006; Tack and Drossman 2017).

However, for epi-/genetic, microbiota and pathophysiological studies, patients should be as homogeneous as possible. They have to be better characterized and factors influencing the phenotype, such as diet, psychological comorbidity and many others should be evaluated in more detail to achieve a homogeneous population. Also, physiological, biological and imagistic investigations should be included in the patient's evaluation, reducing complexity and increasing the chance to identify genes or biomarkers crucial to biological processes underlying the pathophysiology of the disease under study.

This in depth characterization of IBS patients may lead to identification of specific subphenotypes or so-called intermediate phenotypes (quantitative traits) that may represent an important approach to improve the homogeneity of IBS subpopulation. This will permit building large and homogenous .cohorts, allowing statistically solid and reliable data analysis. Ultimately, the data collection should allow the assessment of possible links between symptoms, life style, epi-/genetic abnormalities, dysbiosis, and physiological alterations.

The aim of this review was to discuss the requirements to standardize the process of selecting and phenotyping IBS patient. It is also a tool on organizing the collection and storage of patient information/samples. The proposed approach is the result of a thoughtful and thorough

discussion among experts including myself as part of the European COST Action BM1106 GENIEUR (www.GENIEUR.eu).

II.2.2. STANDARDIZATION OF DATA AND SAMPLE COLLECTION

Standardization of data and sample collection holds many challenges. They are mostly due to differences in the methodology used to collect information or samples or perform the physiological tests. For instance, the procedure in which blood, tissue and stool is sampled for epi-/genetics and microbiota analysis, has clearly to be defined in order to preserve the material and to prevent degradation. Moreover, data should be collected and registered in a standardized case report form, constructed in such a way that information can be entered easily and stored in a uniform format in a database.

Not all centers will have the financial or logistic disponibilities in order to perform all tests or collect the entire data set. However, a minimal set of information and samples must be collected in all patients. Relevant information that cannot be collected at all sites should be included in different 'modules'. Detailed assessment of dietary intake, collection of biopsies for assessment of permeability, immunohistochemistry, molecular biological testing, measurement of visceral sensitivity (barostat), GI transit, or even functional brain imaging are not established in every center, time consuming and expensive and thus will be restricted to centers of expertise.

II.2.3. GENERAL INFORMATIONS ON PATIENTS

General information such as demographics, including date of birth, gender, ethnical background, BMI, education, and profession needs to be collected from all subjects as these factors are known to influence the occurrence of symptoms or their reporting (Lovell and Ford, 2012). Special attention should be paid to ethnicity (Carter et al., 2015). Similarly, family aggregation should be recorded (Waehrens et al., 2015), (Levy et al., 2001), providing valuable information to identify new genetic factors in a family/twin study design. Given the potential role of immune activation in IBS (Ohman and Simren, 2010) the presence of inflammatory bowel diseases (IBD) in family members needs to be checked as well. Similarly, adding celiac disease in the interrogation of family history can be of interest.

Known risk factors for the development of IBS should be carefully inventoried and checked for such as adverse early life events, abuse, stress, and onset after a GI infection are critical. Comorbid associations should be investigated and recorded, in particular atopic conditions. A prior history of abdominal surgery and other comorbid conditions suspected to be linked to IBS, i.e., chronic fatigue syndrome, uro - gynecological symptoms, fibromyalgia, other FGIDs, and psychiatric disorders (Whitehead et al., 2002).

Food is an important factor that may trigger IBS symptoms (Lacy, 2015). It also may influence the composition of the microbiome. In conclusion dietary information is becoming more relevant and have to be recorded. Some subjects may follow dietary restrictions and these factors have to be recorded, also (Bohn et al., 2015). A minimal set of required dietary information should be identified.

Special attention should also be given to the medications taken by the patients. One should

record antibiotics, antidepressants, and also pro-/pre-/ symbiotics, frequently used as self-medication. Recording the latter, as well as recent use of antibiotics, is particularly relevant for studies on the microbiome. The type of delivery, i.e., vaginal *vs* cesarean section, could be of interest due to different bacterial colonization pattern.

Data related to the clinical presentation are also important. The type and severity of symptoms are crucial for phenotyping patients (Drossman et al., 2011) and obviously should be always recorded as detailed as possible using standardized questionnaires. As IBS patients from primary care may have different phenotypes compared to patients from tertiary centers, it is recommended to record if patients are recruited from primary, secondary, or tertiary care or from defined groups (i.e., employees of a company).

To date, phenotyping of IBS patients is largely based on stool pattern. One of the best tools to determine this variable is the Bristol Stool Form Scale (BSFS) (Lewis and Heaton, 1997). Indeed, stool form correlates better with whole-gut and colonic transit than defecation frequency (Saad et al., 2010). The BSFS is recommended by the Rome committees and also validated in several European languages (Chira and Dumitrascu, 2015). Finally, although IBS is a symptom-based diagnosis, a minimal set of diagnostic tests should be included to exclude confounding organic conditions. A blood test excluding anemia and inflammation (C-reactive protein) is therefore mandatory. Moreover, conditions mimicking IBS (especially IBS with diarrhea), like lactose malabsorption and celiac disease should ideally be ruled out. Many gastroenterologists perform a lower digestive endoscopy to exclude organic disease, but in the absence of alarm signs, the decision to perform colonoscopy with biopsies remains at the discretion of the individual practitioner (Dumitrascu, 2011). Functional tests are useful and important for phenotyping, but the availability is limited to specialized centers.

Last but not least, standardized criteria have to be defined to select controls. Healthy controls should be subjects with no GI symptoms and no chronic disorders that may affect research outcomes.

All data recommend to be collected for IBS phenotyping can be retrieved from the following website: www.GENIEUR.eu.

II.2.4. SYMPTOM ASSESSMENT

Evaluation of the digestive symptoms, including its severity is crucial for IBS patient's phenotyping. It is important to carefully characterize the IBS symptoms, but due to the frequent overlap with other FGIDs, thorough assessment of overlapping FGIDs should also be included. The gold standard to obtain a careful clinical phenotyping based on the symptom profile is to use validated questionnaires. It should be emphasized that there may be some language particularities issues with symptom description that should be taken into account. We have to highlight that, at this moment, at this moment, we have a Romanian language version of most of questionnaires and also for the GENIEUR CRF.

Diagnosing FGIDs: Rome IV diagnostic questionnaire for adult FGIDs

Functional GI disorders are defined by diagnostic criteria, together with normal findings on a

lmted number of routine investigations and tests (Drossman, 1999). The GENIEUR CRF for patient's phenotyping was developed based on Rome III criteria. However, it may be used easily updating for the currently Rome IV criteria.

Assessment of IBS symptom pattern & severity

Besides confirming the diagnosis of IBS and other FGIDs, it is also of importance to assess the overall severity of IBS, and the severity and pattern of different IBS symptoms. Among several others, the two most widely used questionnaires are the IBS severity scoring system (IBS-SSS) (Francis et al., 1997), and the Gastrointestinal Symptom Rating Scale (GSRS) (Svedlund et al., 1988), which has also been developed into an IBS-specific version (GSRS-IBS).

- ***IBS severity scoring system***

The IBS-SSS was developed as a simple, easy to use scoring system for IBS to reliably capture effects of treatments and / or other interventions (Francis et al., 1997). It has undergone sufficient validation, and has also been used extensively as an outcome measure in trials assessing different treatment options for IBS (Bohn et al., 2015), (Miller et al., 2015), (Ringstrom et al., 2010). The questionnaire includes five items; abdominal pain intensity, abdominal pain frequency, abdominal distension, dissatisfaction with bowel habits, influence of IBS on life in general ('life interference'); each scored 0–100. Maximum IBS-SSS is 500, with higher scores indicating more severe symptoms. Frequently accepted cut-off levels are used to divide patients into severity groups: <175, mild IBS; 175–300, moderate IBS; >300, severe IBS. In treatment trials a reduction in IBS-SSS total score of 50 is considered clinical improvement.

- ***Gastrointestinal Symptom Rating Scale (GSRS)***

The GSRS has 15 items, each scored using a 7-point Likert scale (1–7), combined into five domains (reflux, indigestion, diarrhea, constipation and abdominal pain) identified through factor analysis (Dimenas et al., 1995). The higher the scores the more severe are the symptoms. A more recent development is the GSRS-IBS, the IBS-specific version of the GSRS (Wiklund et al., 2003). This 13-item questionnaire determines the pattern and severity of IBS-related symptoms during the past week using a similar 7-point Likert scale with descriptive anchors as the original GSRS (ranging from 'no discomfort' to 'very severe discomfort'). The items are divided into five domains: pain, bloating, constipation, diarrhea, and satiety. One advantage with GSRS and GSRS-IBS relative to IBS-SSS for use in large-scale IBS studies is that they also include questions about upper GI symptoms, which is relevant for careful phenotyping. Moreover, with the GSRS and GSRS-IBS it is possible to separately determine the perceived severity of diarrhea and constipation, which is not possible with IBS-SSS, as this questionnaire only asks for dissatisfaction with bowel habits in general.

- ***Bristol Stool Form Scale (BSFS)***

IBS subgrouping is based on stool consistency defined by the widely used BSFS (Longstreth et al., 2006). This is a 7-point scale to describe the stool consistency; 1, separate hard

lumps like nuts; 2, sausage shaped but lumpy; 3, like a sausage or snake but with cracks on its surface; 4, like a sausage or snake, smooth and soft; 5, soft blobs with clear cut edges; 6, fluffy pieces with ragged edges, a mushy stool; 7, watery, no solid pieces. The use of the BSFS in bowel habit diaries has been found to be a useful guide to assess intestinal transit time (O'Donnell et al., 1990), (Heaton and O'Donnell, 1994).

- ***Assessment of functional dyspepsia***

The rationale for including a psychometric instrument to measure dyspepsia symptom severity in an IBS cohort is the frequent comorbidity of functional dyspepsia (FD) in IBS. This is not only the case in healthcare seeking patients (Wang et al., 2008), but also in the general population (Rasmussen et al., 2015). Several validated instruments exist to assess dyspeptic symp- toms, such as the Glasgow Dyspesia Severity Score (el-Omar et al., 1996), the Leeds Dyspepsia Questionnaire (Moayyedi et al., 1998), and the Canadian Dyspepsia score (Veldhuyzen van Zanten et al., 1993). One of the most widely used nowdays dyspepsia instruments, the Nepean Dyspepsia Index (NDI) (Talley et al., 1999), and also Short Form Nepean Dyspepsia Index seems to be particularly useful and largely used.

- ***Assessment of psychological distress and somatic symptom severity*** *('somatization')*

The rationale for including instruments to measure presence and severity of depressive and anxiety disorders, as well as (extra-intestinal) somatic symptom severity ('somatization') lies in the observation that IBS patients have elevated levels of anxiety and depression symptoms, as well as of extra-GI symptoms including comorbidity with anxiety, depressive (Henningsen et al., 2003) and somatoform/ somatic symptom disorders (Whitehead et al., 2002), (Henningsen and Herzog, 2008), (Whitehead et al., 2007). Furthermore, higher levels of anxiety, depressive, and extra-intestinal somatic symptoms have been shown to be associated with higher levels of impairment in IBS and treatment responses are associated with improvements in anxiety, depression, and somatization (Creed et al., 2005).

- ***Patient Health Questionnaire***

The Patient Health Questionnaire (PHQ) modules on depression, anxiety disorders, and somatic symptom severity ('somatization') have excellent psychometric properties, including criterion validity (sensitivity & specificity based on cut-offs, see below), internal consistency, test–retest reliability, and sensitivity to change. They have been translated to and validated in many languages, and have been validated in a wide variety of populations and medical settings, including many patient groups with somatic symptoms (Kroenke et al., 2010).

Other commonly used measures include the Symptom Checklist-90 (SCL-90 ; a 90-item questionnaire including, among others, subscales for the severity of depression, somatization, and different types of anxiety symptoms) as well as the Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983) a 14-item instrument which includes subscales for anxiety and depression severity (seven items each) and has validated cut-points for possible and probable

diagnosis of anxiety or depressive disorders. The PHQ modules are preferred over the HADS because of recent concerns on the factor structure of the latter questionnaire and more specifically on its inability to differentiate between anxiety and depression thereby rendering it more useful as a general measure of psychological distress.

- ***Depression Module (PHQ-9)***

The PHQ-9 consists of nine depressive symptom items based on the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) criteria for major depressive episode (Kroenke et al., 2001); the frequency of each is rated over the prior 2 weeks on a Likert scale ranging from 0 (not at all) to 3 (almost every day). The total score can be used as a continuous measure of depressive symptom severity ranging from 0 to 27 or, alternatively, cut-off points of 5, 10, 15, and 20 can be used representing mild, moderate, moderately severe, and severe levels of depressive symptoms. The presence of depressive disorder can be determined using a DSM-IV based diagnostic algorithm, or an optimal cut-off point ≥10 (with the latter performing better in terms of sensitivity).

- ***Anxiety module*** *(GAD-7)*

The GAD-7 consists of seven anxiety symptoms based on DSM-IV criteria for generalized anxiety disorder (Spitzer et al., 2006), which are scored as in the PHQ-9, and can be summed up to generate a continuous anxiety severity score ranging from 0 to 21, with 5, 10, and 15 representing cut-off points for mild, moderate, and severe levels of anxiety symptoms, respectively. In a manner similar to the PHQ-9, ≥10 represents the optimal cut-off point for 'caseness', in this case for generalized anxiety disorder. However, although it was originally developed as an instrument to detect generalized anxiety disorder, the GAD-7 was also shown to have good sensitivity and specificity (at the same cut-off point of ≥10) as a screening tool for panic, social anxiety, and post-traumatic stress disorder (Kroenke et al., 2010), which are also frequently comorbid with IBS.

- ***Somatic symptom severity*** *('somatization') module (PHQ-15)*

The PHQ-15 consists of 15 somatic symptom items that account for more than 90% of symptoms seen in primary care, and which also constitute the diagnostic criteria for the now abandoned DSM-IV category of somatization disorder (Kroenke et al., 2002). Subjects rate how much they have been bothered by each symptom during the past month on a Likert scale ranging from 0 ('not at all') to 2 ('bothered a lot'). The total (sum) score thus ranges from 0 to 30, with cut-off points of 5, 10, and 15 representing thresholds for mild, moderate, and severe somatic symptom severity, respectively. As the PHQ-15 included 3 GI symptom items ('stomach pain', 'constipation, loose bowels, or diarrhea', and 'nausea, gas, or indigestion'), one of which constitutes a core IBS symptom, it is recommended to omit these three items when calculating the total score if the aim is to distinguish GI symptom severity from extra-intestinal symptom severity, or test relationships between both. This reduced version (PHQ-12) has been used and validated in IBS patients by Spiller *et al*.

- ***Visceral Sensitivity Index***

Contrary to what may be suggested by the name, this instrument measures gastrointestinal symptom-specific anxiety (GSA), which can be defined as 'anxiety related to GI sensations, symptoms or the contexts in which these may occur' (Labus et al., 2004). It covers five dimensions of GI-related cognitions and behaviors; worry, fear, vigilance, sensitivity, and avoidance. The rationale to include this instrument in addition to an instrument measuring anxiety in general, lies in the findings that GSA has been shown to be more strongly associated with IBS symptom severity (Labus et al., 2004).

The Visceral Sensitivity Index is the only validated instrument to measure GSA, with good psychometric properties.

- ***Assessment of disease-specific quality of Life***

Irritable bowel syndrome has a profound impact on quality of life (Monnikes, 2011), and therefore validated disease-specific quality of life questionnaires are often included in large-scale IBS studies. Some of the most widely used instruments are the IBS-QoL (Patrick et al., 1998), the functional digestive disorders quality of life (Chassany et al., 1999), and the irritable bowel syndrome quality of life questionnaire (IBSQOL) (Hahn et al., 1997). All of these have been validated and have been used in clinical trials (Chassany et al., 1999; Drossman et al., 2007; Watson et al., 2001).

II.2.5 PHENOTYPING BASED ON FUNCTIONAL / PHYSIOLOGICAL MEASUREMENTS

In order to determine useful intermediate phenotypes, correlation of patient's symptoms with physiological features, gut microbiota, immune and permeability characteristics seems to be important.

Physiological testing

The GENIEUR consortium developed protocols in order to standardize research activities on IBS physiological testing (visceral sensitivity and permeability studies) and taking into account local and national regulations. The protocols have been designed to be inclusive, in order to allow to non-specialist, non-tertiary centers, to contribute to the cohort (see www.GENIEUR.eu).

- ***Visceral sensitivity testing – rectal barostat***

Mechanical distension of the distal colon can be undertaken to evaluate visceral perception and sensitivity in IBS, with previous reports suggesting that up to 60% of patients have heightened sensitivity to distension compared with healthy controls (Bouin et al., 2002), (Kuiken et al., 2005). Visceral hyper-sensitivity has been proposed as a biomarker in IBS. Distension in the rectum, has become the major site for this study, as it is more accessible and more technical straightforward. Also, there is reported good correlation between centers (Keszthelyi et al., 2012).

Rectal sensitivity can be evaluated by utilizing a distensible polyethylene bag placed in the rectum in conjunction with a barostat, a device that maintains a constant pressure within the aforementioned bag. The barostat can delineate changes in the tone of the rectal wall by measuring alterations in volume and pressure within the bag. Although several distension protocols have been used, rapid rectal distension using the ascending method of limits (AML) and random phasic distension (RPD) protocols, are considered to be the most reproducible within individuals and across study centers (Cremonini et al., 2005). However, AML and RPD protocols take up to 60 min to perform. Sauter *et al.* have recently proposed and validated a Rapid Barostat Bag (RBB) technique, which is as a viable alternative to formal barostat testing in centers where this is not practical (Sauter et al., 2014).

Categorizing patients as hypersensitive, normosensitive, or indeed hyposensitive somewhat depends on the distension protocol and normal reference range used.

- ***Colonic transit study***

Different methods exist to investigate colon transit time. The standard measurement of colonic transit time has been performed with radio-opaque markers or colonic scintigraphy. The traditional approach is to assess the progression time of radio-opaque markers along the large bowel. Colonic scintigraphy can evaluate whole-gut transit (Rao et al., 2011). Recently, wireless motility capsules have also been validated as a technique in measuring colon transit time (Maqbool et al., 2009).

For practical reasons, assessment of colon transit is best based on retention of radioopaque markers seen on abdominal X-ray following their ingestion 3–7 days earlier. These methods have been widely adopted since Hinton *et al.* first described this technique in 1969 (Hinton et al., 1969). They distinguish constipation subgroups such as normal or slow transit constipation, and assess segmental transit times in patients with delayed total colon transit. Retention of ≥5 markers 5 days after ingestion of 24 markers is considered abnormal (Rao et al., 2009), and normal values for other variants of colonic transit time measurements also exists (Tornblom et al., 2012). These tests are simple and inexpensive as well as reliable and reproducible. However it requires good compliance of the patient, exposures patients to radiation, and does not measure the transit of a physiological meal.

- **Genetic/epigenetic analysis**

Irritable bowel syndrome frequently clusters within families, thus suggesting a degree of heritability (Saito et al., 2008; Buonavolonta et al., 2010; Saito et al., 2010). Furthermore, twin studies have demonstrated that the genetic heritability is in the order of 22–57% and the reported concordance rates for IBS differ between monozygotic and dizygotic twins, with 33% concordance in the former and 13% in the latter (Saito, 2011). Hence the collection of data from such ‘IBS families’ and twins may provide invaluable genetic insights into the pathophysiology of IBS. Although several candidate genes have been investigated in IBS (Gazouli et al., 2016) the major weakness of such an approach in IBS studies has been the paucity of replication of findings in independent cohorts and the relatively small sample sizes which in turn results in

limited statistical power to detect, what is almost certainly, a small effect. Therefore, there is a large unmet need for international initiatives collecting information and samples in a standardized manner.

Genome-wide association studies (GWAS) and next generation sequencing represent potentially useful techniques for systematically evaluating genetic factor within IBS, but also may provide novel insights into the pathophysiology of the disorder. Many common diseases represent complex disorders of multifactorial origin and have recently been successfully been dissected on genome level (http://www.genome.gov/GWAStudies/).

To date, the largest population based IBS GWAS study has examined more than 500 patients with an IBS-like phenotype, in comparison to 5000 matched controls from a twin registry in the discovery sample and replicated these ftndings in a further cohort of approximately 3500 IBS patients and controls (Ek et al., 2015). Furthermore, as outlined in detail recently, some of the major flaws in IBS genetics research are attributed to the limited phenotype information. Consequently, more detailed phenotyping of larger case–control cohorts is mandatory before meaningful conclusions can be drawn. Similarly, epigenetic changes as a consequence of environmental stresses/nutrition resulting in DNA methylation and/or differential miRNA profiles may also provide important insights into the pathophysiology and stress related exacerbation of symptoms seen in IBS. A recent, albeit small, study has provided preliminary evidence for differential methylation positions using genome wide technology (Mahurkar et al., 2016) and few miRNA studies generated additional evidence as recently summarized in a recent review). Therefore, to address these methodological deficiencies, an international consortium needs to be established to collect blood samples suitable for genetic and epigenetic analysis, patient information and functional data from large numbers of patients (see www.GENIEUR.eu).

Microbiota analysis

As a consequence of advances in high throughput DNA sequencing over the recent past, quantitation of the human microbiota has become feasible. Given the marked interaction between the gut microbiota and the structure and function of the GI tract, it is not surprising that the microbiota has been the subject of intense research interest within IBS. A number of research groups have used culture-independent techniques to examine the role of the microbiota in different IBS subtypes (Jeffery et al., 2012). Hitherto, the sample sizes of the studied patient cohorts have been relatively small, in addition to a lack of uniformity regarding sampling methods and the collection of phenotypical data between studies, thereby rendering direct comparison achallenge. By standardizing the fecal sample collection (see www.GENIEUR.eu) and the information relevant for microbiota analysis (diet, antibiotic use, psychological trait, and state, etc.), more robust data will be obtained and the interaction with genetic factors of the host can be studied in great detail.

Colonic biopsy sampling

Colonic biopsies can be used to monitor inflammatory events in the intestine as well as changes in neuronal plasticity, neurotransmitter alterations, and intestinal permeability. In

addition, differential gene expression (mRNAs, ncRNAs, miRNAs), epigenetic modification of DNA which may impact expression by switching genes on or off in a long-term manner is of utmost interest to gain more insight in the mechanisms underlying IBS. Only by studying biopsies from large cohorts of well phenotyped and characterized IBS patients, new biomarkers and improved insights into the pathophysiology of IBS can be made. Storage of samples in a tissue bank however requires dedicated personnel and logistics, limiting this approach to specialized centers. Again, only if samples are collected using the same standardized operation procedures (see www.GENIEUR.eu), data from different centers can combined to yield large numbers leading to more robust inferences.

II.2.6. IMPLEMENTATION OF LARGE DATABASES ON IRRITABLE BOWEL SYNDROME PATIENTS

Organizational issues

The development and implementation of a pan - European IBS sample collection and database poses considerable challenges. Local ethical regulations, validation and translation of questionnaires, harmonization, standardization, best practices, standard operation procedures, data protection, and intellectual property rights all have to be taken into account. Professional electronic custom made databases are costly, not only when design and development is concerned, but also when considering ongoing maintenance. Similar arguments are valid for the storage of collected samples.

Local ethics & legal implications

A substantial hurdle in international sample collection and database implementation are the variable standards at national/local medical ethical committees (METCs). There is no European legislation for biobanking. The current EU framework on data protection and medical confidentiality is based on directive 2004/23/EC, which has important implications for human tissues and cells and human application as well as transplants. Material transfer agreements and informed consent have to be adapted individually Electronic documentation is needed while data availability and data protection have to be well balanced.

Translation into different languages

When setting up a large pan-European or international sample collection and database, especially related to questionnaires dealing with patient symptomatology and psychological aspects, language considerations are centrally important. Although papers published in peer-reviewed journals usually report data from questionnaires that are available and mostly validated in English, these questionnaires are usually not translated in other languages in a validated manner. Questionaires have to be validated and adapted taking into consideration local culture, religion, language-specifics and interpretation. In addition, copyright issues of translating the respective questionnaires have to be taken account.

II.2.7. CONCLUSION

Despite the challenges discussed above, implementation of a large European or international biobank and database offers valuable opportunities and is fundamental to address many of the knowledge gaps that exist within the fteld. The necessity of unifying and harmonizing approaches across Europe, and ideally the world, allows greater data compatibility, and larger databases across countries and will improves the quality of multi-centers trials.

II.3. EXAMINING THE RELATIONSHIP BETWEEN DIET AND IRRITABLE BOWEL SYNDROME

II.3.1. INTRODUCTION

Irritable bowel syndrome is a common functional gut disorder with unknown cause present in patients throughout the world. According to Rome IV criteria, the main symptom of IBS is chronic abdominal pain associated with change in the bowel movements (Mearin, F. et al 2016). Based on the stool patterns, according to Rome IV criteria there are four phenotypes of IBS patients: diarrhea-predominant (IBS-D), constipation predominant (IBS-C), mixt form IBS (IBS-M) and patients which cannot be correctly included in these categories (IBS-U) (Mearin, F. et al 2016). Although many patients recognize the impact of specific food in symptom occurrence, still few population-based studies evaluated the importance of diet in IBS and its role remains uncertain and under-studied (Eswaran et al., 2011, Morcos et al., 2009). Many patients report that food may precipitate or aggravate their symptoms, (Rey and Talley, 2009). It is recognized that up to 84% of IBS subjects are reporting food-related symptoms (Rej et al., 2019). The mechanism through which diet may influence the occurrence of irritable bowel syndrome is diverse. Primary mechanisms are osmotic, mechanical, chemical, neuroendocrine and modulation of microbiota. Secondary mechanisms are through fermentation by-products, alteration of intralunimal pH and effects of altered microbiota (Spencer et al., 2014).

The type of diet varies largely due to different factors, the geographical differences being one of the elements which have been recognized to have an influence. Evaluating the importance of diet in IBS symptoms generation may have clear therapeutic implications. The single study conducted on the general population from Romania, using Rome I criteria, revealed IBS prevalence of 14.49% (8.4% man and 17.7% women) with no significant difference between the age groups (Drug et al., 2000). The study was conducted in 1998 in the same area with the present study, on 338 subjects (220 women, 118 men) with mean age 44 (standard deviation 14.8, range 17 to 80 years old) and had a home visiting design.

The aim of the study was to determine the prevalence of IBS in general urban population and to evaluate the type of diet associated with IBS symptoms.

II.3.2. MATERIAL AND METHOD

Population

The study included a sample of 300 subjects (>18 years old) from a population of 18.000 subjects living in the Pacurari urban area, Iasi, Romania. The sample size and demographic characteristics were estimated to be representative for the general population of the geographic area using *Epi Info™ 3.5.2* (CDC) software. For a population survey or descriptive study using random (not cluster) sampling, a population size of 18.000 subjects and an expected frequency of 14 %, and worst acceptable value of 8 %, the sample size would have been 128 - confidence interval (CI) of 95 % - or 219 (CI of 99%). The inclusion criteria were age over 18 years and the residency in this urban area. The selection of subjects was randomized, using a function in *Microsoft Excel™* software, from family doctors patient lists. The family doctors invited the selected subjects by phone for interview and measurement in their offices.

Measures

Two interview-based questionnaires were delivered to all subjects: a Rome III questionnaire (Drossman, 2006, Drossman and Dumitrascu, 2006, Drossman et al., 2006) for diagnosis of IBS and a food-frequency questionnaire (FFQ) for evaluation of eating habits and frequency of food intake for the last six month. General medical history (overweight / obesity, diabetes mellitus, hypertension, cancer, cardiovascular, liver, digestive, endocrine, loco-motor system, skin, respiratory, neuro-psychiatric diseases and sleep disorders) was also included in interview together with objective evaluation of obesity (weight and height were measured by doctors, in their offices).

Age, gender and educational level were studied as demographic factors. Educational level was categorized into three classes: low (no school or elementary school only), medium (high school) and high (college or university). Health-related conditions were investigated: smoking (dichotomized as "current smokers" and "non-smokers"), physical activity (dichotomized as "physically active" if exercise moderate to vigorous at least weekly and "physically inactive" for less), self perceived stress (using a 3 point scale – "high – medium - low level") and general well-being (using a 5 point scale "very good – good - acceptable - poor – very poor condition"). Body mass index (BMI) was also calculated and subjects were grouped into four categories: underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obese (≥30.0 kg/m^2).

A FFQ was designed to reveal habitual intake over a six months period. This questionnaire was based on a FFQ developed for use in adults in Romania, but was modified to include more dietary questions. We asked about the main categories of foods consumed in our region, detailing the foods considered of interest to our study. Consumption frequencies were noted: "never or rarely", "monthly", "once a week", "several times a week", "once a day" and "several times a day". We also investigated individual eating habits (including daily breakfast, number of meals and snacks a day, use of home prepared food, meal with family, eating in a hurry).

Descriptive statistics were performed with *SPSS 17.0*. We have used mean for parametric characteristics and median for non-parametric or ordinal variables. The median was used to characterize the frequency of food consumption in the studied population and subjects were divided into two categories of consumers (less than median frequency and equal or more than median frequency). Spearman's correlation and cross-tabulation analysis (chi square test) was used initially to reveal any association between medical personal history, eating habits, food consumption frequency and other associated conditions. Finally, we used logistic regression (binary, univariate) and calculated odd ratios (ORs) and 95 % confidence interval (95% CI) for significant predictors of IBS derived from initial analysis. A value of $p<0.05$ in both analyses was considered to be statistically relevant.

The study was approved by the Ethics Committee of the University of Medicine and Pharmacy "Grigore T. Popa" Iasi, and a patient informed consent was obtained from all subjects.

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II.3.3. RESULTS

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

II.3.3.1 The prevalence study

The prevalence of IBS was 19.17 % (19.47% for females and 18.75 % for males) (fig. 13). Evaluation of the age distribution indicated increased prevalence of IBS for subjects above the mean age of sample, with a maximum in the decade 60-69 years (37.5%, $p <0.01$) (fig. 14). Educational level of subjects influenced the prevalence of IBS in the general population, but not significantly ($p=0.066$). The trend showed higher prevalence of IBS symptoms toward low educated people (12.5% among high educated people, 23.0% in medium and 30.3 % in low educated people). Profession did not reveal any difference between subjects with and without IBS. A history of digestive diseases was more common in subjects with IBS versus non-IBS subjects (29.7% vs. 7.7%, $p<0.01$). Also, patients with IBS had more common cardio-vascular diseases (64.9% vs. 18.6%, $p<0.01$), including arterial hypertension (75.7 % vs. 31.4%, $p<0.01$). Obesity (59.5% vs. 24.4%, $p<0.01$) and diseases of the loco-motor system (27% vs. 12.2%, $p<0.05$) were more common on IBS subjects. History of other diseases, including psychiatric disorders, was not more common in IBS subjects.

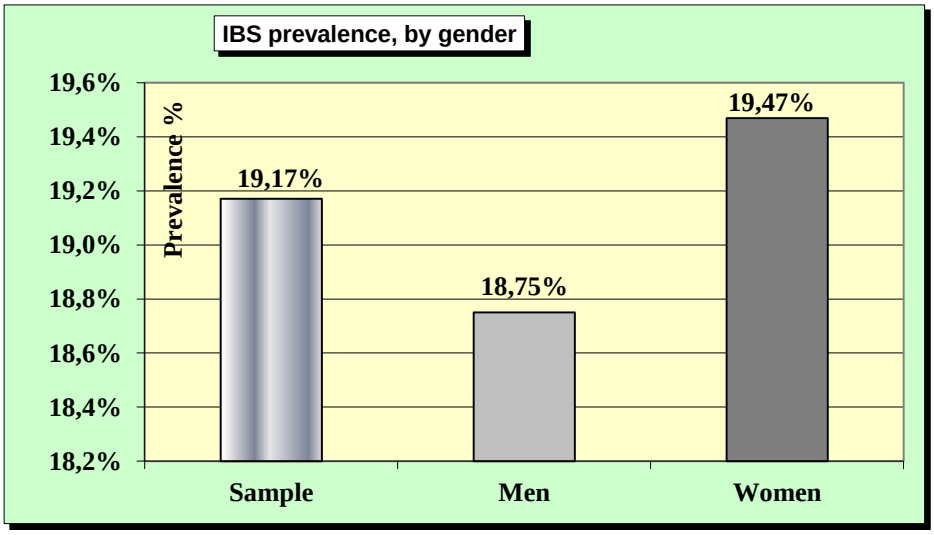

Fig. 13. IBS prevalence, by gender

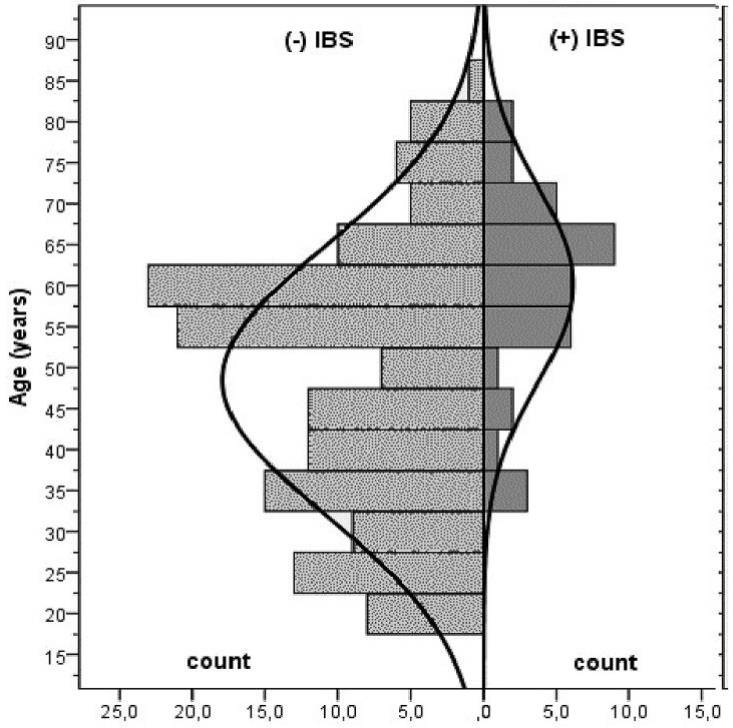

Fig. 14. Age distribution of IBS and non-IBS subjects

II.3.3.2 IBS and health-related behaviors / conditions

Smoking was not associated with IBS: 13.5 % of IBS subjects were smokers, vs. 29.5% non-IBS subjects ($p>0.05$). Alcohol (beer, wine or spirits) was also not associated with IBS symptoms ($p>0.05$). 10.9 % participants used daily alcoholic beverage. However, 86.5% of IBS subjects and 60.2% of non-IBS subjects were physically inactive ($p<0.01$). Perception of stress was not associated with IBS (high level of stress were perceived in 10.8% of IBS patients and in 14.2% of non-IBS patients and medium level of stress in 89.2% respectively 72.3 %, $p>0.05$). IBS vs. non-IBS subjects perceived their well being status to be poor: 13.3% versus 7.6% ($p>0.05$), acceptable (50% vs. 34.7%, $p>0.05$), good (36.7% vs. 40.7%, $p>0.05$). No IBS subjects and 16.9% of non-IBS subjects perceived a very good condition. No subject perceived a very poor condition of well-being. In the sample studied, 49.5% were overweight and 20.8% obese. Presence of obesity was not significantly different in IBS (21.6%) and non-IBS subjects (20.6%) ($p>0.05$).

II.3.3.3 IBS and diet

Median frequency of food consumption in the studied population is presented in figure 15. Data for food consumption frequency in IBS and non IBS subjects are presented in table XVI. Using median as cut-off point, the IBS subjects are eating significantly more frequent the following foods: canned food ($p<0.001$), fruit compotes (canned or not) ($p<0.001$) processed meat ($p<0.01$), beef meat ($p<0.001$), milk ($p<0.05$), pulses (legumes)($p<0.05$), cereals or grain bread /pasta ($p<0.01$), cafeteria products ($p<0.01$), herb teas ($p<0.001$). It was not significantly different consumption for the following type of foods: fish, eggs, fats, vegetables with 5%

carbohydrate (lettuce, spinach, tomatoes, peppers), white bread, sugar and sweets, alcoholic beverages and coffee between IBS and non-IBS subjects (tab. XVII).

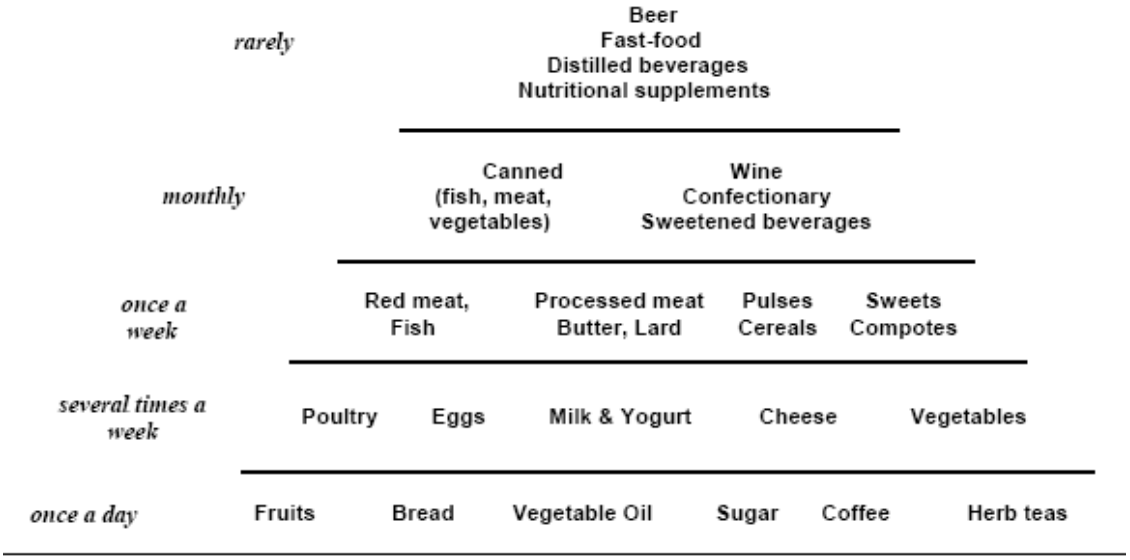

Fig. 15. Median frequency of food consumption

Table XVI. Median frequency of food consumption among IBS and non-IBS subjects

FOOD CONSUMPTION		Non- IBS subjects		IBS Subjects		p-value
		No.	%	No.	%	
Pork	less than once a week	69	44.2%	20	54.1%	0.281
	at least once a week	87	55.8%	17	45.9%	
Beef	less than once a week	69	44.2%	1	2.7%	**<0.001**
	at least once a week	87	55.8%	36	97.3%	
Poultry	once a week or less	28	17.9%	2	5.4%	0.058
	at least several times a week	128	82.1%	35	94.6%	
Processed meat	less than once a week	57	39.7%	4	13.5%	**0.002**
	at least once a week	99	60.3%	33	86.5%	
Fish (fresh)	less than once a week	57	36.5%	9	24.3%	0.159
	at least once a week	99	63.5%	28	75.7%	
Fish (canned)	rarely	79	50.6%	4	10.8%	**<0.001**
	at least monthly	77	49.4%	33	89.2%	
Canned mixed (meat/ fish + vegetables)	rarely	87	55.8%	5	13.5%	**<0.001**
	at least monthly	69	44.2%	32	86.5%	
Eggs	once a week or less	37	23.7%	11	29.7%	0.447
	at least several times a week	119	76.3%	26	70.3%	
Milk	once a week or less	34	21.8%	1	2.7%	**0.013**
	at least several times a week	122	78.2%	36	97.3%	
Cheese	once a week or less	23	14.7%	1	2.7%	0.085
	at least several times a week	133	85.3%	36	97.3%	

FOOD CONSUMPTION		Non- IBS subjects		IBS Subjects		p-value
		No.	%	No.	%	
Butter, Lard	less than once a week	64	41.0%	11	29.7%	0.205
	at least once a week	92	59.0%	26	70.3%	
Vegetable oil	less than once a day	28	17.9%	10	27.0%	0.212
	at least once a day	128	82.1%	27	73.0%	
Potatoes	once a week or less	48	30.8%	6	16.2%	0.076
	at least several times a week	108	69.2%	31	83.8%	
Vegetables with 5% carbohydrates (lettuce, spinach, tomatoes, peppers)	once a week or less	45	28.8%	9	24.3%	0.582
	at least several times a week	111	71.2%	28	75.7%	
Vegetables with 10% carbohydrates (carrots, onions, beets)	once a week or less	27	17.3%	1	2.7%	**0.044**
	at least several times a week	129	82.7%	36	97.3%	
Pulses (beans, peas, soybeans, lentils)	less than once a week	51	32.7%	4	10.8%	**0.014**
	at least once a week	105	67.3%	33	89.2%	
Fruits	less than once a day	33	21.2%	5	13.5%	0.293
	at least once a day	123	78.8%	32	86.5%	
White bread	less than once a day	15	9.6%	5	13.5%	0.484
	at least once a day	141	90.4%	32	86.5%	
Grain bread / pasta	once a week or less	76	48.7%	5	13.5%	**<0.001**
	at least several times a week	80	51.3%	32	86.5%	
Corn flower*	less than once a week	23	14.7%	1	2.7%	0.085
	at least once a week	133	85.3%	36	97.3%	
Cereals	less than once a week	74	47.4%	6	16.2%	**0.001**
	at least once a week	82	52.6%	31	83.8%	
Canned vegetables	rarely	75	48.1%	3	8.1%	**<0.001**
	at least monthly	81	51.9%	34	91.9%	
Sugar	less than once a day	62	39.7%	18	48.6%	0.323
	at least once a day	94	60.3%	19	51.4%	
Sweets	less than once a week	75	48.1%	20	54.1%	0.513
	at least once a week	81	51.9%	17	45.9%	
Confectionary (cakes, cream, ice-cream)	rarely	64	41.0%	4	10.8%	**0.001**
	at least monthly	92	59.0%	33	89.2%	
Compotes	less than once a week	84	53.8%	5	13.5%	**<0.001**
	at least once a week	72	46.2%	32	86.5%	
Alcoholic beverages						
Beer	never /rarely	100	64.1%	25	67.6%	0.692
	at least monthly	56	35.9%	12	32.4%	
Wine	rarely	80	51.3%	13	35.1%	0.077
	at least monthly	76	48.7%	24	64.9%	
Distilled beverages (brandy, vodka, whisky)	never / rarely	115	73.7%	25	67.6%	0.451
	at least monthly	41	26.3%	12	32.4%	

FOOD CONSUMPTION		Non- IBS subjects		IBS Subjects		p-value
		No.	%	No.	%	
Other food/ beverages						
Plain water	rarely	81	51.9%	14	37.8%	0.123
	more/day	75	48.1%	23	62.2%	
Mineral water	rarely	67	42.9%	11	29.7%	0.141
	at least monthly	89	57.1%	26	70.3%	
Carbonated sweetened beverages	rarely	74	47.4%	11	29.7%	0.051
	at least monthly	82	52.6%	26	70.3%	
Coffee	less than once a day	33	21.2%	6	16.2%	0.501
	at least once a day	123	78.8%	31	83.8%	
Herb teas	less than once a day	75	48.1%	6	16.2%	**<0.001**
	at least once a day	81	51.9%	31	83.8%	
Fast-food (hamburger, hot-dog, chips, pretzels)	never / rarely	90	57.7%	15	40.5%	0.060
	at least monthly	66	42.3%	22	59.5%	

*Corn flower were excluded from cereals in a separate question because it is a staple food in Moldova

Table XVII. Significantly univariate associations of IBS with frequency of food consumption

Food categories Frequency of food consumption	**OR (95% CI)**
Canned food	
no, rarely	1
at least monthly	23.74 (3.17-177.7)**
Processed meat	
less than once a week	1
at least once a week	4.75 (1.60-14.09)**
Milk	
once a week or less	1
at least several times a week	10.03 (1.55-418.93)*
Vegetables (10% CH)	
once a week or less	1
at least several times a week	7.53 (1.15-316.93)*
Pulses (legumes)	
less than once a week	1
at least once a week	4.01 (1.31-16.31)**
Grain bread /pasta / cereals	
once a week or less	1
at least several times a week	8.75 (2.03-37.8)**
Confectionary (cakes, cream, ice-cream)	
rarely	1
at least monthly	5.74 (1.89-23.22)**
Compotes	
less than once a week	1
at least once a week	7.47 (2.59-23.11)***
Herb teas	
less than once a day	1
at least once a day	4.78 (1.77-13.59)***

OR: odds ratio; CI: confidence interval; CH: carbohydrates; *p<0.05; **p<0.01; ***p<0.001

II.3.3.4. Eating habits and IBS

Eating in a hurry was more frequent among the IBS patients (41.6 %) than non-IBS subjects (22 %) ($p<0.05$). Other eating habits (daily breakfast, number of meals per day, meals with family or frequent use of home prepared food) were not significantly different (tab. XVIII).

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

EATING HABITS	ANSWERS	Non- IBS		IBS		Significance
		No.	%	No.	%	
Breakfast	Not daily	55	35.3	14	37.8	p>0.05
	Daily	101	64.7	23	62.2	
Number of meals and snacks/day	1-2/day	57	42.2	18	51.4	p>0.05
	3/day	48	35.6	14	40.0	
	More than 3/day	30	22.2	3	8.6	
Home cooked food	One /day or less	65	42.2	16	43.2	p>0.05
	2 /day or more	89	57.8	21	56.8	
Meal with family / day	One /day or less	101	66.0	22	59.5	p>0.05
	2 /day or more	52	34.0	15	40.5	
Eating in a hurry	No	117	78.0	21	58.3	**p<0.05**

II.3.4. DISCUSSION

Worlwide mean prevalence of IBS is considered to be 11.2% (Lovell and Ford, 2012). However, the prevalence may range from 5% to 65% (Kay et al., 1994). In Romania, an IBS prevalence study in general population was conducted in 1998 in the same area and was included in the Lovell study. The study design included household visits and used Rome I criteria. The IBS prevalence was 14.49% (8.4% man and 17.7% women) (Drug et al., 2000). Comparing the two studies we can see a modest, not significantly increase of the prevalence - 19.47 ± 5.65 % (95% CI) for the present study and 14.49 ± 3.79 % (95% CI) for the previous one ($p>0.05$). Although the geographic area was the same, the design was somehow different and this could have influenced the results. The present study had a population sample with higher mean age and the subjects were invited to the doctor's office. It is recognized that inviting the subjects in the doctor's office may influence the selection.

The prevalence of IBS was higher in women (as most studies have found), even did not reach statistical significance (Chang et al., 2006, Spiller, 2004). The prevalence was also increased in elderly people. Age factor may have a contradictory effect. IBS incidence was showed to decrease with age (Hillilä and Färkkilä, 2004) but the prevalence may rise with ageing similar with our study (Grundmann and Yoon, 2010). It also may be an overlooked problem (Agrawal et al., 2009). Female gender and increasing age are associated with higher consultation rates in most studies in both western and developing countries although not all agree (Rey and Talley, 2009).

Education level and profession did not influence the prevalence of IBS. In different populations was found that those with higher educational levels or professional people were more likely to be physically active (Dowler, 2001) and have a healthy diet (Johansson et al.,

1999). However, no association with socioeconomic class or education has been reported (Hillilä and Färkkilä, 2004, Ford et al., 2008). Also, low socioeconomic status in childhood may carry an increased risk of suffering from irritable bowel syndrome in adulthood (Rey and Talley, 2009).

Similar with other data, IBS subjects in our study were more common to have gastrointestinal diseases co-morbidities in their past history, but not psychiatric disorders (Talley et al., 2001). Higher mean age in IBS subjects may explain the increased prevalence of cardiovascular and loco-motor system diseases in the IBS subjects.

Comparable with other studies, smoking and alcohol was not more common in IBS subjects. Most guidelines recommends alcohol reduction for majority of IBS patients even the evidence is vey week and not based on RCT (Rej et al., 2019). The general self-perceived well being of IBS subjects was worse than in non-IBS. In a 10-years longitudinal study, poor quality of life at baseline was a strong predictor for the new onset of IBS (Ford et al., 2008).

Our study is based on a food frequency questionnaire to capture habitual intake over a long period (Mcneill et al., 2009). The FFQ is appropriate for exploring dietary patterns based on frequencies, but has not been validated for estimating total intakes of energy or nutrients (Oellingrath et al., 2011). The FFQ did not include portion sizes, and calculating energy and nutrient intakes was not feasible. However, the reproducibility and validity of major dietary patterns assessed using FFQs have previously been found to be satisfactory for studying diet-disease relation (Hu et al., 1999).

In our study, certain categories of food (canned food, processed meat, milk, high carbohydrates vegetables, pulses, whole cereals, confectionary, compotes or herb teas) were significantly related with IBS. Food may contribute to symptom onset through several mechanisms including food allergy and intolerance. Also, certain food may alter the composition of the luminal milieu, either directly or indirectly through effects on bacterial metabolism. Finally, IBS symptoms may develop following exposure to food-borne pathogens (Morcos et al., 2009).

A cross-sectional study such as the present one, may reveal only association and not causality between the studied elements. A correlation between the studied elements may have several explanations in our case. Frequent consumption of a particular food may positively or negatively influence the presence of disease – for example, canned food, processed meat, milk, or fibre-rich products - 10% carbohydrate vegetables (carrots, onions, beets), pulses (beans, peas, soybeans, lentils), grain cereals/ bread/ pasta or sweet foods - confectionary (cakes, cream, ice-cream), compotes - may affect digestive transit, gas production, gut microbiota and also may cause abdominal discomfort or pain. Most of studies also reveiled that milk and milk products, what products, cabbage, onion, peas/beans and most recently the FODMAPS (fermentable oligo-, di-, monosaccharides, and polyols) may be associated with IBS symptoms. However, the association with canned food was just recently recognized (Rej et al., 2019, El-Salhy et al., 2019).

The presence of IBS may lead, on the other hand to a specific lifestyle or diet, which may possible explain the increased use of herb teas in IBS subjects. The relationship between dietary factors and IBS independent of other potential confounding factors (ex. socioeconomic status)

could not be evaluated without a multivariate modeling. A further study using a larger sample may permit a multivariate analysis and consequently may reduce the confounding factors.

II.3.3.5. CONCLUSIONS

This survey, conducted in general urban population and using Rome III criteria revealed that IBS may be associated with higher consumption of canned food, processed meat, legumes, whole cereals, confectionary, fruit compotes and herb tea. Further studies are needed to explore the mechanisms which may explain the association. Examining, the relationship between diet and microbiota, using new investigational techniques, may also be a step forward for new research. However, investigating and modulating diet may be an important element for the correct management of these patients.

II.4. SMALL INTESTINAL BACTERIAL OVERGROWTH IS ASSOCIATED WITH SYMPTOMS IN IRRITABLE BOWEL SYNDROME. EVIDENCE FROM A MULTICENTRE STUDY IN ROMANIA

II.4.1. INTRODUCTION

Over the past years, there has been a considerable amount of studies suggesting that gut microbiota plays an important role in the occurrence of symptoms and also in the pathogenesis of IBS. Small intestinal bacterial overgrowth (SIBO) is a clinical condition caused by an increase number of bacteria in the small bowel (majority abnormal flora) and causing gastrointestinal symptoms. These bacteria are frequently coliforms, normaly found in the colon and include predominantly Gram-negative aerobic and anaerobic species that ferment carbohydrates and producing gas (Sachdev and Pimentel, 2012).

It may result in intestinal inflammation and malabsorbtion (Lin, 2004). Also, SIBO was associated with irritable bowel syndrome, inflammatory bowel disease (IBD), systemic sclerosis, motility disorders, fatty liver disease, liver cirrhosis, postgrastrectomy syndrome but also with other conditions (Pimentel and Lembo, 2020). The patients with SIBO may present abdominal pain, diarrhea, bloating (Saad and Chey, 2014).

The presence of more than 10^3 colony-forming units/ml (CFU/ml) measured in duodenal/jejunal aspirate is considered abnormal, triggering the diagnosis of SIBO (Vanner, 2008; Rezaie et al., 2017). This direct diagnostic method is considered to be the gold standard which led to the definition of SIBO. However, it is very rarely used in clinical practice. SIBO is causing a pathologic fermentation of nutrients and production in excess of gas. An alternative method for the diagnosis of SIBO is measurement of exhaled hydrogen gas on the breath after ingestion of a fixed quantity of a carbohydrate substrate such as glucose or lactulose (Rezaie et al., 2017).

The hydrogen breath test (HBT) is currently the most used test for the diagnosis of SIBO. Growing evidence on the importance of SIBO in IBS had led to the indication of antibiotic in the treatment of IBS (Lamanna and Orsi, 1984; Pimentel et al., 2003; Lupascu et al., 2005; Lin, 2004, Rezaie et al., 2017). One of these antibiotics indicated in IBS is Rifaximine, a semi synthetic, rifampicin derivate drug with virtually no systemic absorption and a favorable safety profile. Prevalence of SIBO in IBS may vary according to geographic area.

The aim of the study was to evaluate the prevalence of SIBO in IBS vs. no IBS patients in Romania and to assess the effect of rifaximine on symptoms.

II.4.2. MATERIAL AND METHOD

A prospective multicenter study was performed in six medical centers from Romania including two groups: a group of IBS patients and a group of healthy volunteers.

PATIENTS

Inclusion criteria. Patients with IBS were diagnosed according to Rome lll criteria (Drossman, 2006) after the exclusion of any organic disease including a comprehensive diagnostic work-out including colonoscopy, malabsorption testing, hyperthyroidisms and other conditions clinical suggested.

Exclusion criteria. Any organic disorders which may explain the symptoms such as: intestinal tumors, inflammatory bowel disease, celiac disease, diabetes, cirrhosis or medication. Patients with antibiotic therapy, colonoscopy or barium studies in previous 4 week were also excluded.

The subjects answered a questionnaire containing information on baseline demographics, severity of symptoms (Linker scale) personal medical history. An informed consent was signed by all subjects.

Controls. Two groups of controls were created: an age and sex matched group of healthy volunteers and an age and sex matched group of IBS patients, not included in the intervention.

STUDY DESIGN.

All IBS patients referred to the participating centers during 6 months were also referred to glucose hydrogen breath test (GHBT). A total number of 331 IBS patients were included: 105 with diarrhea (IBS-D), 101 with constipation (lBS-C), 90 with unclassified IBS (lBS-U), and 35 with alternation constipation/diarrhea (IBS -M) (fig. l6).

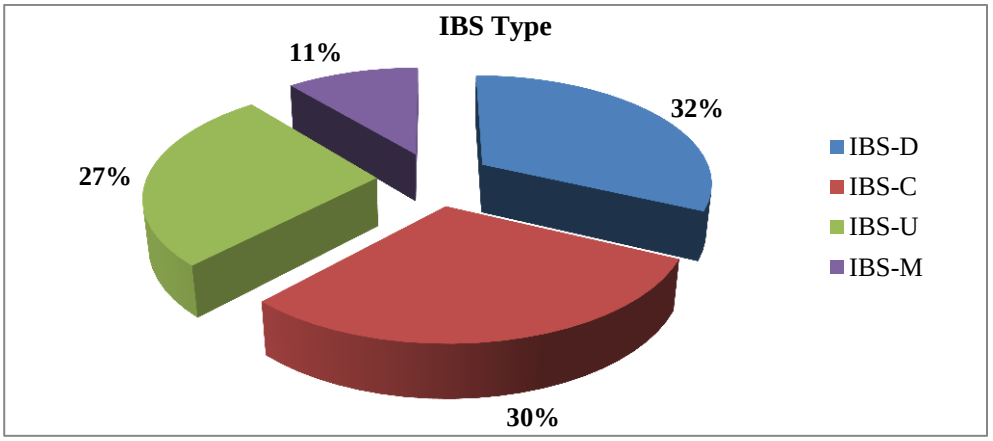

Figure 16. IBS groups repartition according to symptoms

SIBO testing. The fermentable substrate was represented by 50 g of glucose dissolved in 250 ml water. The test was done in fasted patients with no fermentable sugar previous day diet. A baseline of expired air was obtained at the beginning of the study and afterwords, the patients drinked the substrate. The breath samples were collected immediately after drinking the substrate until 120 minutes at 15 minutes interval. The test was considered positive if a clear H 2 peak exceeding 2 0 ppm was recorded before the end of the study (fig. 17).

Patients were not allowed to chew gum, smoke and perform any kind of physical exercise 2 hours before and during the test. Subjects were asked to brush their teeth and disinfect their mouth with chlorhexidine. Vitamins and laxatives were forbidden at least in the 24 hr before the GHBT. A number of 10 subjects were excluded because fasting H_2 was greater than 10 ppm.

Intervention. Patients positive for SIBO have been treated for 7 days with rifaximin 1200 ng/day. Patient's symptoms were assessed before and after the end of the treatment using a 5 points Likert scale for pain, stool, bloating (no improvement 1, little improvement 2, partial improvement 3, almost complete improvement 4, complete improvement 5). One week after treatment the patients were retested for the presence of SIBO.

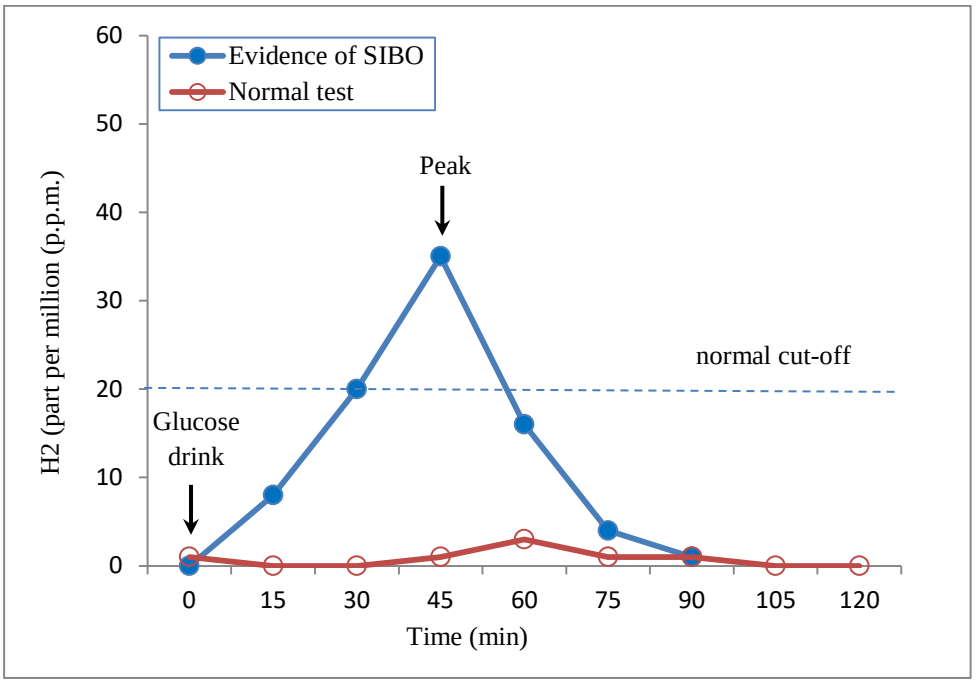

Figure 17. Example of a positive GHBT (adapted from Portincasa P.)

Patients with a negative test stopped the treatment. Those with persistent positive test for SIBO have been retreated with rifaximin for one more week and retested 1 week after the end of treatment. Patients with persistent positive test for SIBO have been reevaluatedfor compliance or for underlying disease. The control group of 105 healthy volunteers was also tested for SIBO and positive subjects were treated with rifaximine. An IBS control group of 20 patients were also included in the study and receive conventional therapy. Response to rifaximine was considered positive if GHBT became negative and IBS symptoms improved from baseline (fig.18).

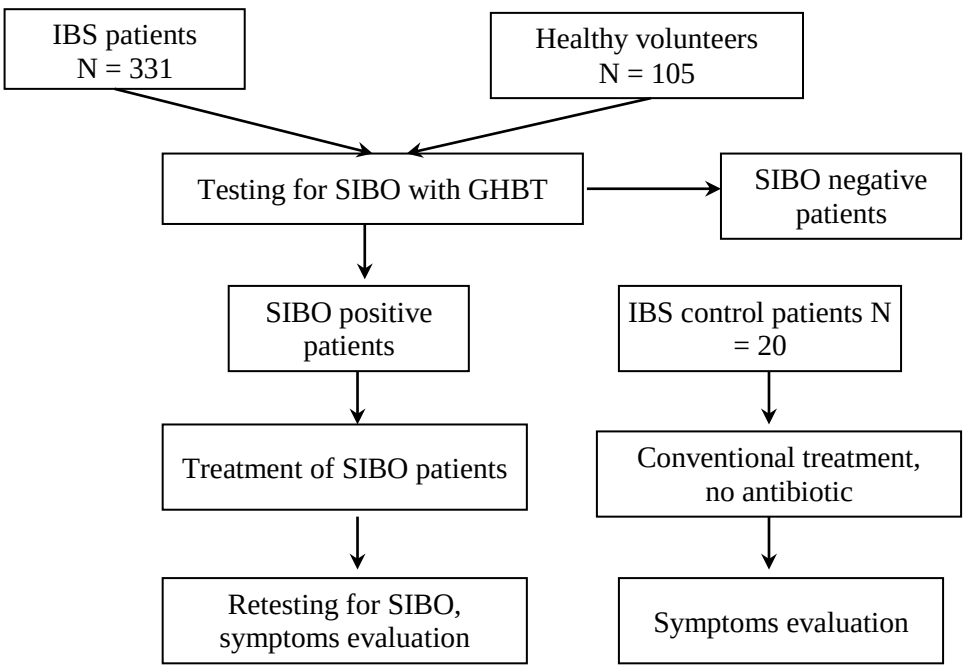

Figure 18. Flow chart of the protocol

II.4.3. RESULTS

Characteristics of the groups

The study included a group of 331 IBS patients, 208 females (62.8%) and 123 males (37.1%), a group of 105 healthy volunteers (HV), 70 females (66.6%) and 35 males (33.3%) and 20 age and sex marched IBS patients (tab. XIX).

Table XIX. Gender and age repartition of patients

	IBS patients	Healthy volunteers	Control patients	P value
Gender repartition	208 females 123 males	70 females 35 males	12 females 8 males	P = 0.83
Mean age	54.5 ± 12.3	59 ± 13	53 ± 12.7	P = 0.64

After testing for SIBO using the GHBT, 105 from 331 IBS patients were positive (31.7%) and also 7 (6.6%) healthy controls (N=105). The calculated OR was 105/331(31.7%) vs.7/105 (6.6%) O R = 6.5, p <0.00011. Demographic, clinical and laboratory parameters of the study subjects have been compared. There was no statistical difference between patients with IBS and controls regarding age and gender (p = ns). Out of 105 positive patients, 48 were part of the IBS-D group (45.7%), 21 of the IBS-C (20%) group, 19 IBS-M (18.1%) and 17 IBS-U(16.2%) (fig. 19).

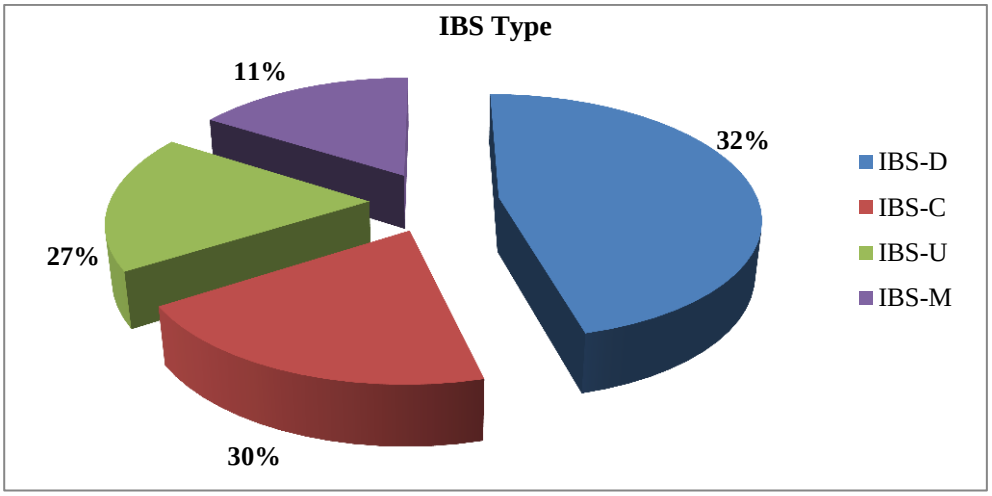

Figure 19. IBS group patients with SIBO

Among patients with SIBO, 29 positive were dropped-out. SIBO was more frequent in the IBS-D patients than non-IBS-D (48/105 vs. 57/226 (O R: 2.5, p = 0.0002) and among the IBS-M group than non I BS-M [19 /35 vs.88/296 (OR: 2.23.p - 0.02)]. Constipation and unspecified symptoms are not predictive for SIBO (OR= 0.45)

A number of 7 subjects out of 105 controls presented with positive GHBT (6.6%). All positive patients for SIBO have been treated with rifaximin 1200m g (400 mgx 3/day) for 7 days. One week after the end of treatment 76 out of 105 SIBO patients and 5 healthy volunteers have

been retested to reassess the efficacy of the treatment (negative GHBT and relief of symptoms). Only 11 patients (14.4%) were still positive for SIBO from the IBS group and none healthy volunteer. All positive patients showed a negative GHBT after another 7 days of antibiotics (fig. 20).

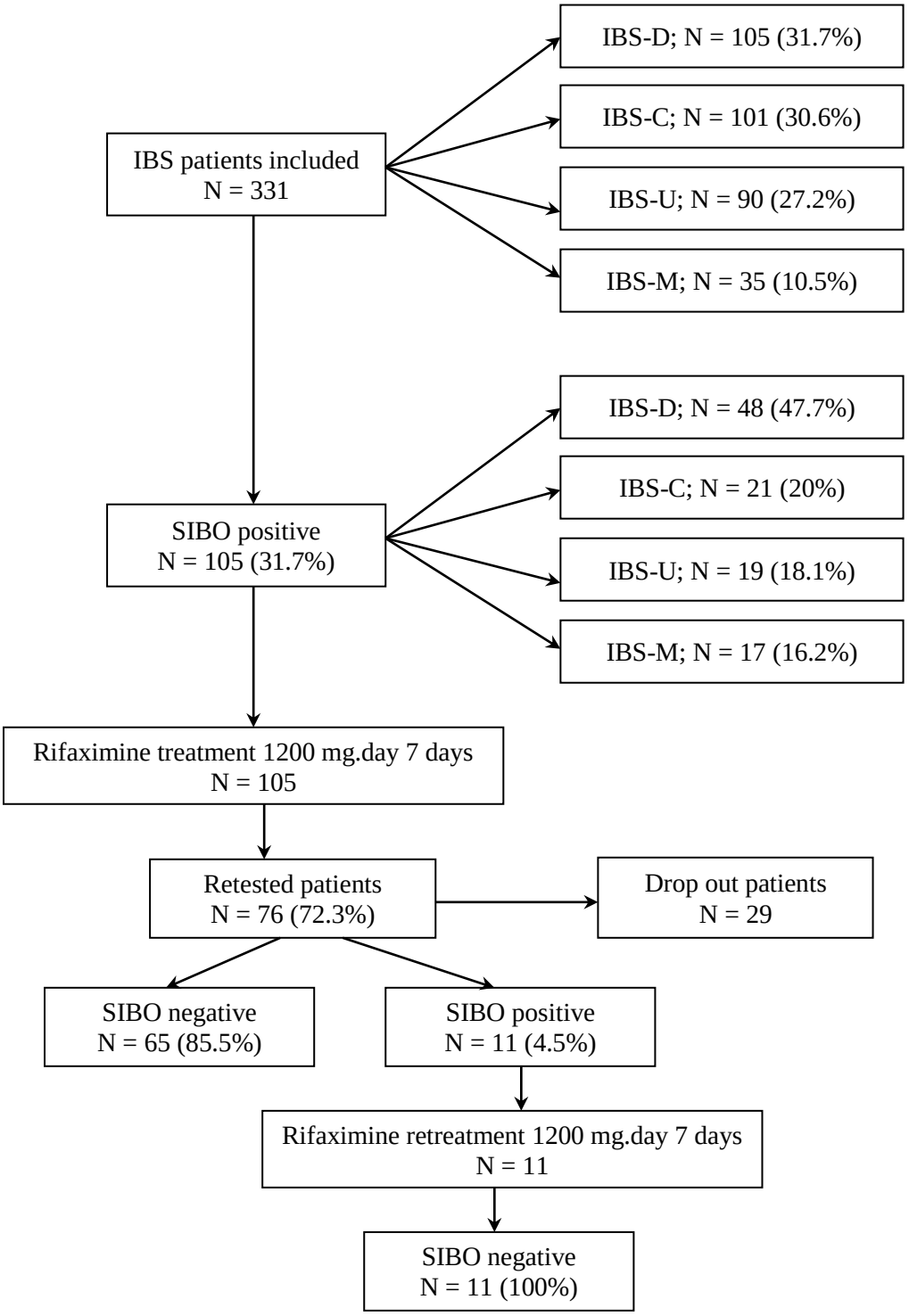

Figure 20. Flow-chart of study

EVOLUTION OF SYMPTOMS

A questionnaire regarding symptoms improvement has been given to each patient from SIBO group after the end of treatment. 49 patients (46.6%) showed a complete response to treatment, 27 patients from IBS-D group (56.2%), 9 IBS-M (52.9%), 7 IBS-U (36.9%) and 6 patients from the IBS-C group (28.5%). 33 patients (31.4%) showed a partial response to treatment when evaluated with the Linker scale, 17 patients from IBS-D (35.4%) showing less number stools with increase consistency. 35.3% from IBS-M, 26.3% from IBS-U and 23.8% from IBS-C also showed less number of stools with increase consistency 23 (22%) patients showed no improvement of symptoms at the end of treatment .

In the IBS control group 7 patients (35%) showed partial improvement, and 5% complete improvement (tab. XX) Overall there was a significant improvement in the rifaximin group and IBS control group (p=0.0005).

Table XX. Repartition of patients according to symptoms improvement

Patients	Gender	Age	Complete response	P-value	Partial response	P-value	No response	P value
IBS-SIBO (105)	60 females 45 males	54.1 ± 12	48 patients (46.6%)	P = 0.0005	33 patients (31.4%)	P = 0.7	23 patients (22%)	P = 0.0005
IBS control (20)	12 females 8 males	53 ± 12.7	1 patient (5%)		7 patients (35%)		12 patients (60%)	

II.4.4. DISCUSSION

Alterations of gastrointestinal motility and visceral hypersensitivity were considered traditionally the main pathogenic mechanisms of IBS. Recently, however, the role of inflammation and alteration of microbiota including SIBO are considered to be important pathogenic mechanism for IBS (Mayer et al., 2014). Symptoms of IBS and SIBO overlap to a large degree (Farrar et al., 1972), (Peralta et al., 2009).

In our study, 31.7% of the IBS patients and 6.6% of normal volunteers have been diagnosed with SIBO. Using LHBT, Pimentel et al (Pimentel et al., 2003) found abnormal breath test in 93/111(84%) patients with IBS and SIBO has been proposed as etiologic factor for IBS. Another study performed on 65 IBS patients and 102 controls found positive glucose breath test in 31% and 4% respectively (Lupascu et al., 2005). Almost the same results (35% positive GHBT) were obtained in the study of Reddymasu (Reddymasu et al., 2010). On children also, LHBT was abnormal in 66% of cases (Scarpellini et al., 2013).

Contradictory data showing low prevalence of SIBO in IBS patients also exists.

In a study performed in India that included 225 consecutive patients with IBS according to Rome II criteria and 100 controls SIBO was found in 11.1% and l% respectively (Rana et al., 2008). Breath test are considered to have low sensitivity and specificity for SIBO and many consider direct bacterial assessment of intestinal aspirate a better method (Simren and Stotzer, 2006; Lin, 2004). According to a systematic review by Khoshini et al. the sensitivity of breath test using lactulose as substrate has ranged from 31% to 68% and specificity from 44% to 100%.

The sensitivity of glucose breath testing has varied from 20% to 93% and specificity from 30% to 86% when compared with cultures of aspirates from the small bowel (Khoshini et al., 2008).

It is recognized that SIBO is more prevalent in IBS patients. The prevalence of SIBO among patients with IBS ranged between 4% and 78%, and that among controls, between 1% and 40% (Ghoshal et al., 2017). In a meta-analysis published by Ford (Ford et al., 2009) of the 12 studies including 1,921 patients with IBS, the pooled prevalence for positive LHBT was 54% (95% confidence interval [CI], 32% to 76%) and for GHBT 31% (95% CI, 14% to 50%), respectively. The odds ratio (OR) for any test showing positive SIBO result among patients with IBS as compared to controls was 3.45 to 4.7.69. In another meta-analysis on 11 studies, breath testing was found to be abnormal among patients with IBS than controls (OR, 4.46; 95% CI, 1.69 to 11.80) (Ghoshal et al., 2017). Clinical studies with direct assessment of SIBO through aspirate detected 4-12% positive results in IBS patients (Husebye, 2005).

In our study we have found a large proportion of patients with SIBO in the IBS-D group (45.7%) similar with data reported in the literature. In a study performed on 204 IBS Rome II patients 46% were positive, majority with IBS-D (73%) and only 13% in IBS-C. and 14 % IBS-M (Posserud et al., 2007). Due to the fact that data suggest the importance of SIBO in IBS (especially in IBS-D), testing for SIBO is currently recommended for IBS patients (Pimentel and Lembo, 2020).

Our study showed that 7 days rifaximin treatment is efficient for treating IBS SIBO positive patients in 85.5% of cases with significantly improvement of symptoms. Other studies showed comparable results, with 50% (Majewski and McCallum, 2007) and 64% improvement after rifaximine treatment. Meta-analysis of five studies demonstrated that rifaximin was significantly associated with improvement of global IBS symptoms compared with placebo [42.2% *vs* 32.4%, respectively; OR, 1.6 (95% CI, 1.2– 2.0); $p < 0.001$]; (Menees et al., 2012). Data from four studies showed rifaximin was significantly associated with improvement in bloating *versus* placebo 10–14 days after treatment [41.6% *versus* 31.7%; OR, 1.6 (95% CI, 1.2–2.0); $p < 0.001$].75 The number needed to treat for rifaximin has been reported as 8 ($n = 7$ studies) to 11 ($n = 4$–6 studies) (Chey et al., 2020).

The advantage of this treatment was also the rapid and persistent positive effect with also positive negative effect of developing resistant organisms (Meyrat et al., 2012).

II.4.5. CONCLUSIONS

In our study, a significant proportion (31.7%) of patients with IBS were found positive for SIBO, majority being in the IBS- D group (47.7%). This is suggesting that SIBO may be an important factor in the occourance of IBS symptoms in our patients.

Rifaximine normalized 85.5% GHBT tests in IBS patients, with significant or partial improvement in 46.6% and 31.4% of patients.

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II.5. THE RELATIONSHIP OF TRUST BETWEEN PATIENT AND DOCTOR IN IRRITABLE BOWEL SYNDROME: A QUALITATIVE STUDY

II.5.1. INTRODUCTION

Mutual trust in doctor-patient relationship plays an important role in correct and efficient management of patients (Jin et al., 2008, Abel and Efird, 2013, Nguyen et al., 2008). This is so, because mutual trust is at the core of doctor-patient relationship, which in turn, is the vehicle for improving and maintained patients' health. The significance of patients' trust in their doctors is well recognized and it is associated with patients' willingness to seek care, to disclose symptoms and to be compliant with therapeutic recommendations (Holman and Lorig, 2004, Oprea et al., 2010, Rogers and Braunack-Mayer, 2009). Although doctors' trust in their patients is less discussed and studied in the literature, (Rogers and Braunack-Mayer, 2009, Rogers, 2002) it plays a significant role in chronic care, because in chronic care doctors have to transfer significant responsibilities associated with self-care. This is so, because patients are under medical observation only once in several months and, they have to be engaged in self-care for most of the time by making health care decisions according to the changing conditions of their illnesses and their lives (Bodenheimer et al., 2002, Bodenheimer et al., 2002). In addition, a willingness to trust patients is a significant ethical step in recognizing the patient as a person with diligence rather than merely a passive recipient of care (Rogers and Braunack-Mayer, 2009).

Our study was focus on identifying the pathways through which mutual trust in doctor-patient relationship in the context of irritable bowel syndrome is built and maintained. We choose to carry out a qualitative study because explaining pathways and generating theory is a particular strength of qualitative research (Denzin and Lincoln, 1998, Given and Gale, 2008). Irritable bowel syndrome has been used as a case example for mutual trust in the context of chronic diseases. Irritable bowel patients are often confronted with anxieties about possible diagnosis. For instance, most of irritable bowel patients have a sudden relief feeling when cancer diagnosis is excluded through colonoscopy (Jilcott Pitts et al., 2013). However, some uncertainties may persist in the presence of a functional or unclear diagnosis (Mikocka-Walus et al., 2012). On the other hand, there are indications that anxiety distorts the reception of information the patient needs before colonoscopy (Rollbusch et al., 2014, Bessissow et al., 2013).

II.5.2. MATERIAL AND METHOD

Sampling and recruitment

Fifteen irritable-bowel patients have been recruited through recommendation from hospital-based gastroenterology practices as well as general practitioners' offices. All these places were located in the Iași area (Romania). Maximum variability of the sample has been targeted. The average age was 50 years and 6 months. The youngest participant was 21 and the oldest participant was 74 years old. Six of the participants were males and nine were females. Six participants had graduate studies, one had postgraduate studies and eight had undergraduate studies.

Data collection

Data were collected between 22nd of January 2015 and 5th of August 2015 via semi-structured interviews, which were audio-recorded and latter written. An interview guide developed by the research team was used and gradually improved to maintain a degree of consistency between interviews but also help reaching the saturation point. Interviews were conducted face-to-face, after informed consent explained and signed.

Data analysis

Data analysis began following the first three interviews. A constant comparative method has been used, in order to find out repetitive patterns (Glaser, 1965). These patterns attempted conceptual comparisons while at least one feature was constant (e.g. same patient in interaction with different doctors, same specialty approached by different patient with irritable bowel, different patient with same type of long-lasting relationship with their GP etc.)

Written versions were imported in QSR NVIVO 9. After an initial analysis that considered smaller units, a node structure was created. It contained nodes grouped in order to discover patterns of how mutual trust is built and maintained. One of these three larger nodes was context which has been considered either temporally (e.g. long-lasting relationship with the doctor), emotionally (e.g. fearful uncertainty about a potential disease), informational (e.g. well-informed patient).

Acknowledgement

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II.5.3. RESULTS

The successive results in the context of the relationship make both patient and doctors trust each other

One of the most frequent patterns we managed to identify emphasized the importance of results in the medical care, for both patient and doctor. Results meant for patient the proof of being worthy of trust. On the other hand, for the doctor, good results in medical care meant a patient who complied with recommendations and followed treatments. It also dealt with truth, as a component of doctor-patient relationship: patients did tell the truth about how they managed their own treatment:

S.T. (male, 48 years old, graduate studies)

Investigator*: Something else? What is important in order for the patient to trust his doctor?*

Patient*: To look for results... If one had good results with one doctor, you keep going to the same one. One doesn't go to see someone else because one has heard that the other doctor might be better than the current one […] when you had results, it's good to keep seeing the same doctor.*

[…]

Patient*: […] the doctor sees if you follow your treatment, if you come when called […]. I mean, patient's behavior makes the doctor trust him/her. I mean, if the doctor sees that there is no feedback from the patient, what can he do? She has her hands tied. And then, it's logic she won't trust the patient.*

I.M. (male, 72 years old, undergraduate studies)

Investigator*: How did you realize Miss X is competent… that she knows what she is doing?*

Patient: I was well after the treatment she gave me.

Both doctor and patient ask pertinent questions, which, in turn, are interpreted as competence from both sides, increasing trust

Another pattern of building mutual trust is by the quality and quantity of questions and answers from either doctor or patient. Patient interprets the quantity of questions the doctors asks as a sign the doctor cares and is really interested to find the correct diagnosis and treatment. The doctor seems to interpret the questions asked by the patient and his answers as a constructive involvement in the therapeutic process and a competence to supply valid information:

D.A. (27, female, graduate studies)

Investigator: […] how did trust appear, in your case with this doctor?

Patient: I would say during the consultation […] the fact that she asked me many questions…

O.V. (24, female, graduate studies)

Patient: *There is a certain aura of a person whom you trust. She was smiling, calm, and immediately asked me: what happened? Please, tell me! She waited; she was patient and asked me, indeed, a lot of questions. She put no diagnosis before asking me a lot of questions.*

Investigator: *What does it mean she asks you a lot of questions ?*

Patient: *It means she is interested and she will make a precise diagnosis. It won't be something general or vague.*

Patient gets a sense of psychological awareness about his/her anxieties which, in turn, instills trust

Another pattern of communication we identified among the patients we interviewed is related to anxiety and exaggeration. Patient is worried about possible unidentified diagnosis or courses of treatment. The doctor supports the patient, by gently showing him/her that the reactions might be over exaggerated. Subsequently, patient becomes aware of his/her psychological processes, and this, in turn, makes him/her trust doctor even more. This happens especially in the context of a long-lasting relationship.

TM (57, female, graduate studies)

Patient: *She knows how to temperate me, if you want to see yet another doctor she says: "Let's see, let's think about it a bit more…"* [….]

Patient: *… I kept doing investigations with many doctors. But my GP told me, in the end:"Mrs. X, let's stop. You went to see enough doctors, and I am sticking with my initial opinion that it's about anxiety. It leads you to an end which is not necessarily beneficial to you: to keep asking for opinions and it's not the case. These are useless expenses and efforts. Let's stop a little and see…" She probably realized the state I was in, and this was normal.*

Anxieties are soothed once investigations are carried out and reasons for fear are excluded

Another pattern of interaction we identified, deals with how uncertainty and anxiety is soothed once investigations are carried out and reasons for fears are excluded. In the context of uncertainty about health problems, patient is worried he/she might have something worse. The investigations have the power to exclude a possible bad diagnosis (like cancer). Therefore, patient becomes suddenly soothed and reinforced in his/her feeling of trust towards the doctor.

D.D. (41, male, postgraduate studies)

Patient: *I'm the type of person that doesn't connect much with doctors. But afterwards, when something happens that might get out of control […] and I feel it's something that might be serious….*

Investigator: *What?*

Patient: *Incurable diseases, especially. I am honestly thinking about cancer. This could hit me unexpectedly […].*

Patient: *It meant he somehow gained my trust […]. It appeared like a serious investigation… They did, how do they call that? …an echography. […] Following the echography he said there is nothing to be seen, and I should just need to run some blood tests to confirm everything is OK.*

Patient gets to be a witness to the imaging diagnosis procedure and this instills trust, despite lack of medical information

A way of building trust that we could identify from what our patients told us came from the fact that they could be a witness of the colonoscopy. This soothed their anxieties, on one hand, but also instilled trust through "seeing with my own eyes" idea. Apparently, for some patients, it was important that they had this visual "proof" of the exclusion of a worse possible diagnosis.

D.A. (62 years old, male, undergraduate studies)

Investigator : *What made you consider her nice ?*

Patient : *The way she talked… I mean… she spoke to me nicely, consulted me, seated me nicely in there… «Look for yourself on the monitor »*

Tudor: *And what did this mean to you? …the fact that she said: «Look for yourself on the monitor » […] What did you think about it?*

Patient: *Well, a satisfaction because… Anyway… I had no idea if there was something wrong but… I could see the interior of the big bowel, how they call it...*

M.M. (21 years old, female, undergraduate student)

Patient: *[…] I think I lean towards colonoscopy. Because without something concrete… without me seeing if there is something or not*

Investigator: *I see. Some people told me about colonoscopy, and about the fact that the doctor allowed them to see for themselves on the monitor.. […] What did it mean to you, the fact that you could look on the monitor?*

Patient: It gave me more trust.

II.5.4. DISCUSSION

In this qualitative study we focused on identifying the pathways through which trust in doctor-patient relationship in the context of irritable bowel syndrome is built. We identified several themes which bear resemblance to themes taken out from narratives in other studies dealing with trust. Therefore, we are going to present similarities and differences between our findings and the current literature on the subject.

Outcomes in doctor-patient relationship

One of the themes we identified relates to the importance of results in the medical care, for both patient and doctor. We illustrated that, for some of our patients, results meant for patient the proof of being worthy of trust. Patients' perception of good level of competence meant fulfillment of the treatment expectations that the patients had. This seemed to instill trust in the doctor and the facility and also to maintain it, as shown by previous studies (Gopichandran and Chetlapalli, 2013). Equally, we understood the theme we identified as an interaction in which the successive positive results in the context of a longer relationship made both patient and doctors trust each other and maintain the trust based relationship. This result bears some resemblance to what is mentioned in the current literature concerning loyalty with respect to returning to the same doctor, whatever the illness (Gopichandran and Chetlapalli, 2013) or asking for the second opinion (Hillen et al., 2012).

We identified a theme concerning the results in the context of a longer relationship. This theme has similarities and differences with what is mentioned in literature long-term trust (Hillen et al., 2012) or secure trust (Tarrant et al., 2010). Our finding is similar with previous conclusions that trust is maintained if patients perceive that the care they received has been effective (Tarrant et al., 2010). With respect with dissimilarities with these previous studies, it's worth mentioning what some of them deal with cancer patients. In them, caring behaviors and showing interest in the patient are considered most important for building trust, not the theme of repeated positive treatment results that we extracted from our data. Cancer patients also build

trust due to perceived threat of cancer, which is different from that IBS patients experience (Hillen et al., 2012).

Our findings bear also resemblance to the mechanism previously identified with respect to patient honesty and being believed by the doctor. In previous studies, providers want to trust that their patients are disclosing information important for managing their health. On the other hand, patients hope providers will believe what they say about their health and concerns (Ratanawongsa, et al.,2011). In short, our findings seem to confirm the idea that both honesty and competence are key features that contribute to the maintenance of trust in longterm relationships.

Patient-centered communication

In our interviews, we identified a pathway of trust building by the rich exchange of questions and answers from either doctor or patient. This pathway deals with an interaction between the two parties. It was made apparent that patients interpret the quantity of questions the doctor asks as a sign the doctor is really interested to find the correct diagnosis and treatment. The caring attitude the doctor has, together with the perceived competence in information gathering, seemed crucial in patients' narratives of trust. On the other hand, the doctor seems to understand the questions asked by the patient and his answers as a constructive involvement in the therapeutic process and a competence to supply valid information. Patient's competence is relevant for doctor's trust in them. Elements of this interaction are apparent in previous research, although they are not presented together, as an interaction per se, but separately. Some studies indeed mentioned listening to the patient, and addressing all questions as determinants of trust in health care providers (Gopichandran and Chetlapalli, 2013). Our results are also similar to what has been found in research regarding cervical cancer screening with, patient-centered communication as one of the best represented themes. The physician being attentive, taking time with the patient, and focusing on the patient was perceived as important (McAlearney, et al., 2012) while not taking the necessary time to explain answer questions is presented as illustrating lack of patient-centered communication (McAlearney, et al., 2012).

Previous literature showed that not providing an explanation to the patient was an important theme of distrust (Tytgat et al., 2003; Hillen, et al., 2012). This can be somehow connected to our results, although the quality and quantity of questions asked by both doctors and patients is a different theme than the repeated question-asking and being answered. Our study puts an emphasis on interaction as a key finding in understanding the pathway through which trust is built and maintained.

Witnessing diagnostic procedure

In our study, we found a theme related to witnessing the diagnosis procedure. Apparently, this soothed patient's anxieties, on one hand, but also instilled trust through "seeing with one's own eyes" certitude. Even if it doesn't superpose completely, the theme we found might be similar to how patients valued laboratory tests as a competent medical maneuver despite not knowing particularly what tests are performed (Gopichandran & Chetlapalli, 2013; O'Donnell, *et*

al., 2008). Our findings also showed that even if patients didn't have medical knowledge to interpret the colonoscopy, witnessing the procedure instilled trust. Apparently, it's possible that both pathways are true: on one hand, colonoscopy is regarded as a competent diagnosis maneuver. On the other hand, patient gets to see by himself/ herself that there is nothing dangerous inside his/her body. This result is interesting in the context of actual trends concerning sedation use in colonoscopy (Tetzlaff, 2016). Even though witnessing the procedure itself might soon be considered unfeasible, presenting the recorded procedure to the patient might prove important with respect to the preservation of the trust-related benefit.

Reassurance as reinforcement

One theme that emerged in our study is how uncertainty and anxiety is soothed once investigations are carried out and reasons for fears are excluded. In the context of uncertainty about health proble ms, patient is worried about receiving bad news. The investigations have the power to exclude a possible bad diagnosis or prognosis. Therefore, patient becomes suddenly soothed and reinforced in his/ her feeling of trust towards the doctor. Our finding is somehow connected with other ways of reassuring the patient. By explaining ways in which patient's disease is not as bad as it might have been, patients gets reassured and a feeling of trust (Wright, et al., 2004).

Carelessness

Concerning distrust, the narratives of our patients mentioned carelessness as a pathway of building distrust in the relationship. In our study, this theme is illustrated by a patient with uncertainties and anxieties about her condition who notices minor and attention-related mistakes the doctor makes. This fact casts doubt if the doctor can be relied upon. This can complete the apparently contradictory findings of previous studies about trust in health care where patients are found, on one hand, as willing to overlook the pitfalls in a well-established relationship. The willingness to tolerate mistakes may be understood as a consequence of trust; it is also an indicator of the level of trust (Gopichandran & Chetlapalli, 2013). On the other hand, previous studies showed that inappropriate or missed diagnoses are a distinct theme of distrust (McAlearney, et al., 2012). There is a big resemblance between the theme of perceived mistakes we identified in our study, and what patients understood as mistakes in the aforementioned research, even if the stake is different in the case of IBS and in the case of cancer. As a corollary, in our own study, doctor's unrecognized mistake is understood by the patient in the context of a relationship which is not, yet, built on trust. Therefore, the pitfall participates in the building of distrust towards the doctor.

The panic label

In our study we showed examples where the "panicky" or "scared" label is applied by the doctor. The patient starts questioning if he/she is taken seriously and loses trust. It makes patient doubt that the doctor will do all the things necessary to arrive at a correct diagnosis. We found no examples in literature dealing with this specific interaction. However, studies showed that missed

diagnoses are mentioned as a distinct theme of distrust, and this illustrates the direction. The theme of doctors not taking the time to explain or answer questions is presented as a distinct feature of distrust in the healthcare provider (McAlearney, et al., 2012). This might come in contrast with how patients seem to perceive medical thorough investigations: doing laboratory tests to make the correct diagnosis is perceived as a competent medical maneuver (Gopichandran & Chetlapalli, 2013).

Insight

An original finding of our study deals with another pattern of communication we identified among the patients we interviewed. This script of doctor-patient interaction is related to anxiety and overreacting with respect to medical checkups. In this scenario, the patient is worried about possible unidentified diagnosisor courses of treatment. The doctor supports the patient, by showing him/her that the reactions might be over exaggerated. Subsequently, patient becomes aware of his/her psychological processes, and this, in turn, makes him/her trust doctor even more. This happens especially in the context of a long-lasting relationship. We consider this theme to be a distinct theme compared with the reassurance theme. It does not deal with the simple reinforcement the patient gets when, finally, his worries are soothed by the exclusion of serious illnesses. This theme pertains with how patient perceives that the doctor helped him/her become aware of his/her own emotional processes when coping with anxiety through extensive check-ups and verifications. We consider this theme to be the psychotherapeutic equivalent of insight (Richfield, 1954), when patient becomes aware and considers valid an interpretation of his own emotional processes, and, subsequently, is empowered in relationship with them.

Confidentiality

Another important result of our study is the lack of emphasis on confidentiality. Almost all our patients did not emphasize confidentiality as an important feature of their trust in the doctor. They didn't mention situations where confidentiality is kept or transgressed, despite specific question targeting this topic. This totally supports the findings of previous studies, where patients judged confidentiality as an unimportant consideration or determinant of trust (Hillen, *et al.*, 2012) but is inconsistent with studies that mentioned lack of respect for patient privacy and confidentiality as a key point of patient health-care provider communication expressed by patients (Farahani, et al., 2011).

In this study, we focused on identifying the pathways through which trust in doctor-patient relationship in the context of irritable bowel syndrome is built. The empirical literature dealt so far with disparate features of trust, not considering interactions and the mutual influence between the patient's trust in the doctor and the doctor's trust in the patient. The originality of our endeavor resides in the focus we had on interactions, identifying sequences of interactions or scripts in which there are revealed the mechanisms of how mutual trust is built and maintained in the context of the relationship between the doctor and the IBS patient. Among themes that are reconsidered from an interaction point of view, we identified other themes which were not previously approached, like insight or the labeling of patient as panicky.

The present study has limits that derive from the particulars of its methodology and research question. Building up and adapting interview guides proved a difficult task when trying to catch-up the particulars of doctor-patient interaction. Sometimes, this was not possible without additional questions that might be considered leading when taken out of the context. In what the number of participants is concerned, the fifteen patients we interviewed didn't allow the exploration of all particular or smaller themes that emerged. Despite looking for maximum variability, some patients provided more illustrative examples than others, possibly related with their degree of language mastery and expression. It is also worth mentioning that the objective of the study proved difficult to connect with present literature, as previous studies focused on disparate features of trust and not mechanisms or scripts. This further complicated the process of data gathering. Among the limits of the study, we can also point out that the qualitative endeavor doesn't allow for generalizations usual in quantitative studies.

Other studies can further explore the dynamics of mutual trust by targeting specific interactional scripts or sequences (e.g.: *What did you think? And how did he react?).* In parallel, quantitative studies can use the data presented here in order to build quantitative questionnaires with items derived from the specific patterns we described. This will allow for new factors to be identified by Exploratory Factor Analysis, together with the discovery of culturally-appropriated psychometrical constructs.

II.5.5. CONCLUSIONS

In this qualitative study we investigated the pathways through which mutual trust in doctor-patient relationship in the context of irritable bowel syndrome is built. Irritable bowel syndrome has been used as an example for mutual trust in the context of chronic diseases thanks to the particulars of uncertainty and anxiety feelings these patients have. Several themes emerged from our analysis, which dealt with interactions leading to one-side or mutual trust. Patients talked about the successive medical outcomes in the context of long-lasting relationships, patient-centered communication, the insight obtained by the patient with the help of his/her doctor, the value of witnessing the diagnostic procedure and the function of reassurance of doctor-patient interaction. Pitfalls in doctor-patient mutual trust dealt with perceived mistakes and the "panicky" label the doctors used. Most of our results connect with similar outcomes mentioned in the empirical literature, but bring an extra approach based on interaction and mutual influence. Our study brings information on mechanisms for how mutual trust is built and maintained: particular interactions of perception, thought and behavior. These interactions are described and discussed. Our study completes the understanding of how mutual trust is built in doctor-patient interaction, bringing more input into future qualitative and quantitative investigations.

II.6. THE INTERNET AND THE RELATIONSHIP BETWEEN PATIENTS AND GASTROENTEROLOGISTS. A MULTI-CENTER STUDY

II.6.1. INTRODUCTION

The fast-paced evolution of the Internet has changed many aspects of our life both at individual and population levels. Also, in recent years, the Internet has been gaining ground as primary source of medical information (Ziebland and Wyke, 2012). It is used by an ever-increasing number of patients. Romania is a country with many internet users: 58% of general population (http://www.internetlivestats.com/) and more than 84 % of youth (http://www.statista.com/statistics/348061/daily-internet-usage-age-group-romania/). When considering the countries with the highest Internet connection speed, Romania ranks in top 10 worldwide (https://en.wikipedia.org/wiki/List_of_countries_by_Internet_connection_speeds). In Romania, in 2016, four out of the top ten Internet inquiries included in the category "what is" were health related (https://www.google.com/trends/). These important changes may have an impact on the doctor-patient relationship. Understanding how the Internet affects the patients is of interest for physicians and could require from the medical side, adaptive changes in the approach of the patients. Current data show an increasing tendency for search of medical information before taking medical advice. A study published in 2006 revealed that more than 70% of the Internet users from European countries searched the Internet for health purposes (Andreassen, 2007).

The use of the Internet by the general population is on the rise, spanning from 50% - 60% in Eastern Europe, to nearly 90% in the Northern and Western Europe (http://www.internetlivestats.com/). Patients are usually searching for differential diagnoses, treatment options, side effects of medication, or are looking for data about their own physicians. Nearly 40% of the population visits physicians rating sites (Kadry et al., 2011). This information may have impact on their perceptions on the physician's reputation and practice (Belle et al., 2013). Motivated by the needs of acknowledgment, of uncertainty reduction and of perspective, patients may search for health information online in order to achieve the goals of preparing for meeting a doctor, complementing it, validating it, and/or challenging its outcome (Caiata-Zufferey et al., 2010). Others may seek emotional support (Ziebland and Wyke, 2012).

A considerable rise in the role of peer-to-peer support using online forums was noticed (Willis, 2014). These forums allow patients with similar diseases to advise each other and share medical information. Many would argue that sharing experiences with peers can positively affect one's health (Ziebland and Wyke, 2012). The insufficient information provided by health professionals (verbal or written) may explain the patients' interest in Internet health information (Ziebland and Wyke, 2012, Van De Belt et al., 2014).

The aim of the study was to evaluate the impact of the Internet on gastroenterologist – patient relationship with focus on functional digestive disorders. Our study might be especially important for medical practitioners to explain the ongoing change in their patients' mentality due to the constantly increasing use of online medical information, and also to improve patients' education and counseling.

II.6.2. MATERIAL AND METHOD

Protocol

A prospective multi-center study was conducted, including a representative sample selected from five major health-providing regional centers distributed throughout Romania.

Subjects

We included adult patients, aged 18-80 years, on their first visit to any of the Gastroenterology clinics participating in this study, in consecutive order. The asymptomatic patients or those returning for follow up were excluded.

Questionnaires

We designed a structured questionnaire, validated on a pilot group, which had two parts, one to be filled by patients and the other by physicians. We collected demographic data in addition to other information, such as having Internet access, if they performed an online search for medical data, if their search helped them to find out a potential diagnosis, if the Internet influenced them to choose a medical specialty, if the patients previously used a medication as a result of their search. We also investigated if the patient had prior a general practitioner (GP) appointment, if they followed the GP's treatment or if they got a prior medical advice from a specialist doctor for the same problem. They were also asked to quantify the extent to which the Internet had influenced them in this process (”Low influence”,”Relative influence” or”High influence”).

The second part was designed to be filled by physicians, stating both the initial and the final diagnoses. The doctors scored subjectively, in one item question, the collaboration with the patient – understanding and compliance with recommendation (scored from 1 to10, with 1 being the worst and 10 being the best collaboration grade). The examining doctor also positively diagnosed the presence of Gastro-Esophageal Reflux Disease (GERD) according to Montreal definition, the dyspeptic syndrome/functional dyspepsia or the irritable bowel syndrome (using Rome III criteria).

Statistics

We analyzed the data using PASW Statistics version 17.0 for descriptive statistics, Pearson's correlation between characteristics of the sample and logistic regression analysis (multivariable, for independent variables like demographic characteristics, previous visit to GP or online search for medical information and collaboration score as outcome variable), considering $p<0.05$ as statistically significant.

Ethics

The study protocol was approved by the local ethics committee. All the participants in this study provided informed written consent prior to study enrollment.

Acknowledgement

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II.6.3 RESULTS

Description of responders.

The sample consisted of 485 subjects from five regional centers. The demographic characteristics of the sample are presented in table XXI.

Mean age was 50.4 ± 15.7 years. Patients had different educational background, most of them being high school graduates or having university degrees. Out of these patients, 307 (64.8%) reported that they were Internet users.

Table XXI. Demographic characteristics of study sample

	No. of subjects	**Valid Percent**
Regional center		
Craiova	114	23.5%
Bucharest	103	21.2%
Brasov	76	15.7%
Cluj	100	20.6%
Iasi	92	19.0%
Gender		
Women	242	49.9%
Men	243	50.1%
Education level		
Analphabet	2	0.5%
Primary school	23	5.7%
General school	63	15.6%
Professional school	25	6.2%
High school	148	36.5%
Higher education	144	35.6%

Internet use.

52.9 % of the patients performed online search for medical information related to their symptoms prior to getting medical advice - 75.2 % of the people who have internet connection and only 4.9 % of those without internet connection (who used internet in public spaces) ($p<0.001$). When considering Internet search for medical information before getting medical advice, we found no significant difference between women and men (55.0% versus 50.8%, $p>0.05$), but a strong, inverse correlation with age ($r = -0.367$; $p<0.001$).

Most of the patients stated that they used the Internet to identify the most appropriate medical specialist for their condition (tab. XXII).

Table XXII. Internet influence on medical behavior of the subjects

Type of internet influence	**Count**	**Percentage of internet searchers**
Choosing the right specialty	162	63.03%
Helped me think that I have a specific disease	94	36.56%
Followed a treatment	37	14.39%

Also, they admitted that the Internet helped them to better understand their medical condition and the recommendations offered by the physician – 75.7 % of subjects considered that Internet has a relative or high influence on the understanding of medical explanations.

Internet use and medical visits.

The majority of the patients visited the GP before getting medical advice from a specialist doctor (87.8%). Out of these patients, 39.1% did not follow the treatment recommended by the GP. Only 7.3% declared that they practiced self-medication based on Internet recommendations, 58.2% of the patients admitted that they had visited at least one specialist doctor for the same condition before getting current medical advice.

Internet access was negatively correlated with previous visits to GP (85.3% versus 92.2%, $p = 0.041$).

Patients who had searched for their symptoms online were less likely to follow the treatment prescribed by the GP (53.6% versus 67.5%, $p = 0.004$, OR = 0.369, CI95% = 0.236 - 0.577), yet had better collaboration scores ($r = 0.17$, $p = 0.003$).

50.3% of the patients were diagnosed with functional diseases (fig. 21).

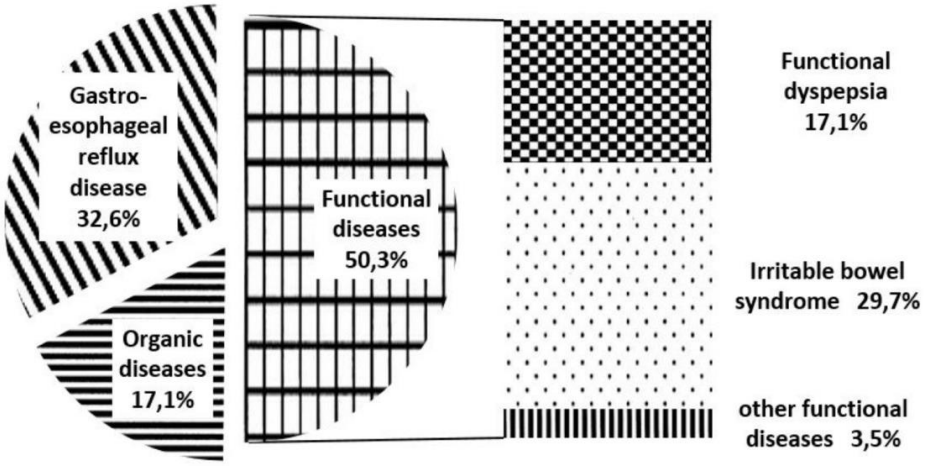

Figure 21. Final diagnosis in specialist doctor medical advice

Although patients with functional diseases searched the Internet in higher numbers as compared to patients who did receive a diagnosis for an organic disease (62% versus 49%, $p = 0.022$), the former were not more frequently influenced by the information provided by the Internet and their online search had no influence on their doctor-patient collaboration score ($p>0.05$).

The education level was positively correlated with the collaboration scores ($r = 0.26$, $p<0.001$), and patients with higher education were more likely to search for information on the Internet before meeting a specialist doctor ($r = 0.37$, $p<0.001$).

Advanced age was negatively correlated with both education level and collaboration score (-0.34, and -0.20, $p<0.001$, Pearson 2 - tailed correlations).

Using a multivariable regression analysis, the best collaboration score (10 points) was inversely correlated with age (OR = 0.79, CI95% = 0.65-0.96, $p = 0.017$) and positively

correlated with higher education level (OR = 3.15, CI95% = 1.17 - 8.43, p = 0.023), previous visit to GP (OR = 3.05, CI95% = 1.14 - 8.15, p = 0.026) and online search for medical information (OR = 2.38, CI95% = 1.01 - 5.65, p = 0.049).

II.6.4. DISCUSSION

We are contemplating today an unbelievable development of the internet with more than 4,525,000,000 users in the world, or more than 50% of the world population. (http://www.internetlivestats.com/).

The relationship between medical doctor and patient is very important in gastroenterology, in both chronic organic and functional conditions (Tanaka et al., 2011). More and more patients (60 %) use internet for personal information related to health issues and to their own concerns (Teriaky et al., 2015, Angelucci et al., 2009). As the information on internet is not critically checked and can be provided by non-professionals or even charlatans, the opinions of the patients may be misleading. Patients' beliefs may therefore differ from the medical evidence (Levy et al., 2014, Reimer and Bytzer, 2007). Few data were published concerning the extent to which the Internet influences the doctor-patient relationship. There is no doubt that Internet is a major communication instrument of our era, and it is estimated that almost half of the world's population is connected to the Internet (http://www.internetlivestats.com/).

This study showed that more than half of the GI outpatients seeks online health information before visiting a doctor, with no gender differences, as other studies found in other countries (Bidmon and Terlutter, 2015). However, a strong correlation with younger age, similar to most studies was also revealed in our study (Beck et al., 2014).

Functional patients have hypervigilance (Posserud et al., 2009). Therefore, they pay more attention to own symptoms compared with chronic organic sufferers. This behavior is supposed to explain the more frequent access of internet compared to other patients.

In a recent study, patients using the Internet were more likely to self-diagnose their condition (87% versus 46% of non-users of Internet), and this self-diagnosis can lead to anxiety until a medical expert confirms or disapproves it (Teriaky et al., 2015).

In our study, we noticed a negative influence of Internet on patient-GP relationship and a tendency to use the information obtained through online search as an alternative to visiting the GP. This may happen not only in the specialization of Gastroenterology, but also in other Medicine disciplines.

Romanian medical system allows the patient to be examined directly by the specialist doctor (especially in private clinics), without prior GP referral. The current tendency of bypassing the role of the GP is alarming and it will have serious consequences resulting in overcrowding the health system unless no measures are taken. This study could form the basis of further work looking at perhaps the need to implement this rule in Romania whereby referral was compulsory before a specialist is seen (as it's done in most other EU and Australasian countries) (Stainkey et al., 2010, Loudon, 2008). For this, we must to estimate the cost/efficiency aspects, if it is harmful or it is useful and proven to save time and resources.

Another alarming sign is the increasing number of patients not following the treatment prescribed by the GP before getting advice from a specialist doctor. We believe that there is an

urgent need to develop strategies regarding the use of the Internet in the family medicine practice. When taking in consideration that almost 65% of our patients are connected to the Internet and 75% of them already use the Internet for health purposes, the use of an online national health platform would make possible for patients to check for their symptoms, watch instructive videos on various diseases, check for urgent health messages and request a video interview with their family doctor when appropriate.

The Internet can be a useful instrument in educating patients. In Japan, endoscopy live demonstrations is one of the most effective and attractive methods used for educating patients with pancreatic-biliary disorders and with other gastrointestinal diseases (Shimizu et al., 2016). In USA, the Internet is being used increasingly as a source of information for prescriptions, and clinicians guide their patients to specific websites (Nguyen et al., 2014). Although more than a half of the patients visited the recommended website, an email reminder increased the frequency of site visits and improved the patients' compliance (Ritterband et al., 2005, Aung et al., 2015).

Also, Internet-based cognitive behavior therapy (ICBT) has shown promising results in treating digestive disorders, including irritable bowel syndrome (IBS) and was found to be more cost-effective than the waiting list, with an 87% chance of leading to both reduced social costs and clinical effectiveness (Ljotsson et al., 2011). Although few studies explored the outcomes of Internet-based e-Health technology in Gastroenterology, a systematic review showed that this promising instrument can be used to enhance and promote gastrointestinal disease management and mental health (Knowles and Mikocka-Walus, 2014). However, the internet online psychological interventions studies for mental and physical health outcome in IBS and IBD were included in a systematic review and meta-analysis published in 2018. Data showed insufficient evidence to demonstrate the effectiveness of cognitive behavioral therapy delivered on-line for these conditions (Hanlon I. et al., 2018). Recent study published in 2019 showed that, in a multicenter randomized trial, web-delivered cognitive-behavioural therapy was more efficient the usual IBS treatment (Everitt HA. Et al., 2019).

The Internet is a useful tool in prevention campaigns for spreading health information, especially those targeting young adults, who consider the Internet a valid source of advice on health matters and trust online information (Beck et al., 2014).

II.6.5. CONCLUSIONS

The Internet is a modern and popular source of health information. Although having access to Internet negatively correlates with visiting the GP, the Internet search tends to improve the doctor-patient relationship. Being younger, more educated and having access to the Internet significantly correlates with obtaining a higher collaboration score given by the physician at the end of the meeting. The trend of Internet usage as health source is unstoppable, thus it is logical for health providers to be an online presence, read what their patients are exposed to and recommend good sites to get informed from. Doctors should accommodate themselves to the new role of the Internet by being non-judgmental and by trying to promote good information while protecting their patients against misleading ones.

SECTION II.
FUTURE EVOLUTION AND DEVELOPMENT PLANS

The thesis of habilitation is a good opportunity to look into the past, evaluating the personal achievements and should also be a good moment to plan for the future. As a clinician, it is not always easy to find the balance between a high quality clinical work, scientific challenges and academic activities. The magnitude of these activities may vary in time, having different importance in different lifetime moments. They are influenced by the evolution of the academic and clinical duties, but also by personal preferences and assets.

The academic activity after completion of habilitation thesis implies, in consequence, duties of conducting PhD thesis and continuous involved in research and postdoctoral training. However, cutting edge clinical work should also be a target for someone who choosed to be specialist in gastroenterology.

In this section I shall present the future perspectives for development in scientific, academic and professional activities, focusing on my scientific future plans. However, one should take into account that planning in science should be very much open to the science development reality which is offering us as new opportunities also as tools and targets.

II. 1. PERSPECTIVES IN SCIENTIFIC ACTIVITY

Good quality clinical and translational research in any field, of course also in neurogastroenterology and digestive endoscopy needs up-to-date technology, adapted to the research aim. Available technology represents a starting point which needs to be updated.

The Institute of Gastroenterology and Hepatology represents a clinical unit of Emergency Clinical Hospital “St Spiridon” Iasi. It includes a Digestive Endoscopy Unit and has a facility that allows performance of functional digestive tests, imaging techniques including abdominal ultrasonography and radiology. High-tech imaging such as Computer Tomography, Magnetic Resonance Imaging, and Scintigraphy are available in the hospital. Also, good clinical lab is available within the hospital.

Several clinical and translational research infrastructures are available within the University of Medicine and Pharmacy “Grigore T Popa” Departments (such as immunology, pathology, biochemistry, microbiology, and genetics). This includes also the “Advanced Research and Development Center for Experimental Medicine (CEMEX). Good institutional relationship is allowing us to access also, other research infrastructure available in the nearby Clinical Hospitals.

AVAILABLE RESEARCH INFRASTRUCTURE IS REPREZENTED BY:

- SANDHIL pH impedance unit (available partially due to University grant and private funds from the Romanian Society of Neurogastroenterology);

- WAGNER Analyzer Unit for respiratory testing of H Pylori infection, intestinal malabsorption and digestive transit time;
- Standard digestive manometry unit – Synectics – actually nonfunctional; needs urgent acquisition of new high resolution manometry equipment;
- 2 SYNECTICS pH metry boxs and BILITEC unit;
- OLYMPUS and PENTAX Standard HR digestive endoscopy units.

It is evident that the present infrastructure is totally insufficient for performance in clinical and translational research in neurogastroenterology and digestive endoscopy and that urgent investments in infrastructure is a necessity. The existence of high resolution manometry for esophageal and anorectal studies and also cutting edge endoscopy devices together with modern radiology is a must for quality research in neurogastroenterology.

Improving research infrastructure could be done by applying to all possible sources of financing. Applying for European structural grants, available European and national resources for research funding is a key element for developing research.

II.1.1. RESEARCH IN THE AREA OF EXTRADIGESTIVE GERD

IMPROVING DIAGNOSTIC OF EXTRADIGESTIVE GERD - PEPSINE – MARKER FOR EXTRADIGESTIVE GERD

Pepsin is proteolytic enzyme secreted as pepsinogen produced exclusively by the stomach and activated in acidic environments (Saritas Yuksel and Vaezi, 2012). A pepsin test called: Peptest is a non-invasive, quick and inexpensive tool for the diagnosis of LPR. It uses 2 monoclonal anti-bodies to human pepsin to detect the presence of pepsin in saliva. Its results can be obtained in 5-15 minutes. Due to its benefits and ease of application, a positive salivary pepsin test in a patient suspected of having LPR can be a cost effective, accurate and alternative diagnostic method for GERD. Yuksel et al. (Saritas Yuksel et al., 2012) found in their study a sensitivity and specificity of 87%, a positive predictive value of 85% and a negative predictive value of 68% for the pepsin test in the diagnosis of LPR. In another cross-sectional study, *in vitro* pepsin detection tests were compared to the 24-hour double probe pH monitoring results for patients with suspected LPR (Ocak et al., 2015). The authors found a sensitivity and specificity of the pepsin detection test of 33% and 100%, respectively. Recent study on pepsine and GERD showed that patients with NERD had higher average pepsin concentrations compared with Functional Heartburn patients. A weak correlation was determined between intercellular spaces as marker of GERD and salivary pepsin among patients with NERD ($r = 0.669$, $P = 0.035$) (Li et al., 2017).

A meta-analysis including 5 studies was published recently (Guo et al., 2018) showed a sensitivity of 0.60 (95% CI 0.41–0.76, and specificity 0.71 of (95% CI 0.51–0.86) for pepsine in saliva as diagnostic marker for Gastroesophageal Reflux Disease suggesting that Pepsine test is not yet ready for high accuracy diagnostic or GERD. However it may have a role in a personalized diagnostic algorithm.

STUDY I

Aim to establish if adding salivary pepsin **concentration measurement** to classic diagnostic algorithm including digestive endoscopy and pH-impedance measurement will help to determine causative relationship between GERD and reflux laryngitis.

Study design

Study will include patients with reflux laryngitis based on the ENT surgeon diagnosis. Included patients will may have GERD symptoms but also patients without typical GERD symptoms will be included.

All patients will be examined by upper GI endoscopy and 24-hour ambulatory multichannel impedance-pH (MII-pH) monitoring after at least 1 week of pharmacological washout and also salivary pepsin concentrations will be determined by enzyme-linked immunosorbent assay before bedtime and after awakening.

STUDY II

Aim to establish if **adding** salivary pepsin concentration measurement determination to classic diagnostic algorithm including digestive endoscopy and pH-impedance measurement will help to determine causative relationship between GERD and CHRONIC COUTH AND BRONCHIAL ASTHMA.

Study design

Study will include patients with chronic couth of unknown diagnosis and patients with bronchial asthma of unknown cause. Included patients will may have GERD symptoms but also patients without typical GERD symptoms will be included.

All patients will have upper GI endoscopy and 24-hour ambulatory multichannel impedance-pH (MII-pH) monitoring after at least 1 week of pharmacological washout and also salivary pepsin concentrations will be determined by enzyme-linked immunosorbent assay before bedtime and after awakening.

SURVEILLANCE IN PATIENTS WITH ESOPHAGEAL ATRESIA/TRACHEO-ESOPHAGEAL FISTULA – A MODEL OF EXTRADIGESTIVE GERD

In pediatric patients, different surgical techniques for esophageal replacement have been widely used in long-gap esophageal atresia (LGEA) over the past few decades with different outcomes. However, a gastroesophageal reflux disease is a common condition of these patients especially in subjects with gastric tube reconstruction (Liu et al., 2017). Early surveillance of these patients is a pediatric surgeon task. However, long term correct management and surveillance have to be done by an experienced gastroenterologist. This should be done taking into consideration the higher risk of Barrett esophagus, esophageal cancer but also of restrictive and obstructive lung disease that can affect up to 78% of patients (Mirra et al., 2017). Including these patients in data base may help in better understanding the pathophysiology of extradigestive GERD but also to have a better management of these patients.

THE RELATIONSHIP BETWEEN HEART RATE VARIABILITY AND ESOPHAGEAL IMPEDANCE-PH MONITORING PARAMETERS IN PATIENTS WITH GASTROESOPHAGEAL REFLUX DISEASE: ATRIAL FIBRILLATION RISK PREDICTION

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice with a high morbi-mortality; its incidence has risen in countries with rapidly aging populations, with 120 000–215 000 newly diagnosed patients per year (Kirchhof et al., 2016). One in four middle-aged adults in Europe and the US will develop AF. By 2030, 14–17 million AF patients are anticipated in the European Union (Vakil et al., 2006). Gastroesophageal reflux disease (GERD) is one of the most frequent benign disorders of the upper gastrointestinal tract (Vakil et al., 2006b). The range of GERD prevalence estimates in Europe has broadened slightly, ranging from 8.8% to 25.9% (El-Serag et al., 2014). Heart rate variability (HRV) is a noninvasive tool that has been successfully used to estimate modulation of autonomic tone. HRV is known to decrease when sympathetic activity predominates, whereas it increases when parasympathetic activity predominates (Pumprla et al., 2002). Different methods are available for the analysis of HRV (Pumprla et al., 2002). The most widely-used methods are those in time and frequency domains. There are few prospective data about autonomic (sympatho-vagal) imbalance and arrhythmias risk in patients with GERD (Pumprla et al., 2002; Dobrek et al., 2004; Dobrek et al., 2005; Milovanovic et al., 2015).

Understanding the association between gastroesophageal reflux disease (GERD) and non-valvular atrial fibrillation (AF) is extremely important in the global multimodality treatment strategies to improve outcomes in patients with AF. Similar autonomic innervation of the esophagus and left atrium, sympatho-vagal imbalance and inflammation from esophageal acid exposure seem to explain the association between non-valvular AF with GERD. It remains unclear what degree of esophageal acid exposure may induce sympatho-vagal imbalance and trigger AF.

The aim of the study will be to assess prospectively sympatho-vagal imbalance through heart rate variability (HRV) parameters (in time and frequency domain) by 24 hour electrocardiogram (ECG) Holter monitoring in patients with/without GERD, off and on acid-suppression therapy with proton pomp inhibitors.

The patients will be included based on both clinical symptoms and upper gastrointestinal endoscopy by a joint team consisting of gastroenterologist and cardiologist. Simultaneous with 24 hour ECG Holter, esophageal impedance-pH monitoring (for % and time acid and bolus exposure) will be performed. 24 hour ECG Holter monitoring will be repeated after 8 weeks on acid-suppression therapy with proton pomp inhibitors. In addition left atrium structural remodeling parameters by transthoracic echocardiography and the presence of hiatal hernia and esophagitis by upper gastrointestinal endoscopy will be noted. The correlation between HRV and impedance-pH monitoring parameters during esophageal acid exposure depending on degree of severity will be assessed. The variation of HRV parameters off and on acid-suppression therapy with proton pomp inhibitors in relation with left atrium structural remodeling parameters will be analyzed; these parameters this will be compared also in patients with and without GERD. A

prediction model of AF risk in patients with GERD using HRV, impedance-pH monitoring and echocardiography parameters will be developed.

GASTROESOPHAGEAL REFLUX DISEASE AND ESOPHAGITIS AFTER ATRIAL FIBRILLATION RADIOFREQUENCY ABLATION

Due to the close positioning of the esophagus and the atria, patients undergoing AF ablation seem to have an increasing risk to develop esophageal wall injury and GERD (Nolker and Sinha, 2010), (Martinek et al., 2009), (Nolker et al., 2009), (Schmidt et al., 2008), (Kim et al., 2017). These patients could develop esophageal injury by direct thermal injury ranging from erythema and esophagitis, necrosis and ulcer, to a very severe complication, atrio-esophageal fistula.

The **aim** of the study will be to valuate prospectively the presence of symptomatic GERD and esophagitis in naïve patients who had AF radiofrequency ablation. More clearly, we aimed to study prospectively the presence of symptomatic GERD and esophagitis at 3 months after radiofrequency catheter ablation of paroxysmal AF.

II.1.2. RESEARCH IN THE AREA OF NOVEL ENDOSCOPICAL METHODS

NOVEL ENDOSCOPY METHODS FOR THE DETECTION OF GASTRIC CANCER AND IMPROVEMENT OF FUNCTIONAL DYSPEPSIA DIAGNOSIS

Diagnosis of functional dyspepsia is still a difficult task in terms of enlarging or limiting the number of investigations. A previous systematic review and meta-analysis has shown that more than 70% of people will be labeled as having functional dyspepsia (FD) following upper GI endoscopy, with <10% having peptic ulcer disease and <1% having upper GI cancer (Ford, 2010). The current recommendation is to use endoscopy in cases of alarm symptoms and also aged patients (>45-65 years depend on population). However, the alarm symptoms perform poorly for predicting endoscopic findings, with limited predictive value for upper GI malignancy (Vakil et al., 2006). Also there is no clear age limit based on scientific data for endoscopy in uninvestigated dyspepsia in Romania.

A large multicenter study using the new endoscopic technology would **aim** for better characterized and diagnose functional dyspepsia and gastric cancer.

Terahertz radiation (1 THz corresponds to 10^{12} Hz, 33.3 cm^{-1}, 4.14 meV, with a wavelength of 300 μm) is located between the high frequency microwave region and long-wavelength far-infrared region of the electromagnetic spectrum. The region is classically defined as 0.1–10 THz. (Danciu et al., 2019)

A growing body of evidence indicates that THz could represent a useful instrument for identifying carcinomas at an early stage, starting from the fact that the malignant tumors have a greater content of water that strongly absorbs the THz radiation. Additionally, THz imaging could be used for assessing if an excised tissue has tumor-infiltrated margins or not (Brun et al., 2010)

Currently, in vivo THz detection is limited only to the examination of superficial tissues or tumors close to the epithelial layer (i.e., breast). However, significant efforts have been made to extend THz's applications to the diagnosis of more profound tissues and organs. In 2009, Ji et al. developed a miniaturized fiber-coupled THz endoscope structure that generates and detects THz waves using an optical fiber linked with a laser, close to the reflective surface of an organ through the excitation of the detector and generator. Some years later, an innovative THz prototype with single-channel detection based on flexible metal-coated THz waveguides and a polarization specific exposure method was integrated into a commercial optical endoscope and demonstrated its value, which is successfully differentiated between normal and colonic cancer tissues (Doradla et al., 2014)**.** Preliminary studies have been done on oral, esophageal, gastric, colonic, hepatic and pancreatic cancers.

A next step in this field would be to establish the role of terahertz spectroscopy and imaging as a rapid test for malignancy during standard endoscopy with biopsy.

II.1.3. RESEARCH IN THE AREA OF IRRITABLE BOWEL SYNDROME

BIOLOGICAL MARKER FOR IRRITABLE BOWEL SYNDROME - IRITABLE BOWEL SYNDROME AND GENES

Irritable Bowel Syndrome is a gastrointestinal disorder with no specific biological marker for diagnosis. It is characterized by abdominal pain, altered bowel habit, often bloating and/or abdominal distension. In 2016 new Rome IV diagnostic criteria was launched. The ethiopathology of IBS is originating from the combination of genetic polygenic and environmental/nutritional factors. Genetic studies have focused on the relationship between IBS clinical phenotype and the genotype but also on endo-phenotype-genotype association studies. The importance of studying genomics in IBS was highlighted also be the existence of GENIEUR network (Genes in Irritable Bowel Syndrome in Europe) which was initially a COST financed project, presently being supported by the European Society of Neurogastroenterology. I am actively participating in this network at the beginning as a COST MC member, presently as an ESNM board member. I succeed to involve in this GENIEUR network several colleagues from the University of Medicine and Pharmacy "Grigore T. Popa" Iasi, with complementary background: genetitians, microbiologists, epidemiologists and also gastroenterologists. One of the most important personal input on GENIEUR was my work on the better phenotypic characterization of IBS patients (Boeckxstaens et al., 2016).

The research **aim** will be to analyze, using molecular technique a possible genetic susceptibility for the disease in the IBS patients. During the project we shall analyze the associations of genetic variations of SNP (rs25531 in *SLC6A4*; rs1062613 in *HTR3A*, rs62625044 in *HTR3E*, rs4263839 in *TNFSF15*) and indels (5HTTLPR) in the target population. Also, we shall make genotype-phenotype correlations, molecular characterization of subgroups of patients by combination of two genetic polymorphisms and correlation with co morbid

psychiatric symptoms in IBS.

The proposed research will have a case-control study design. Patients will be selected from IBS patients referred by the family doctors or other specialties to the Institute of Gastroenterology and Hepatology Iasi. From each subject we shall collect data, using a questionnaire and blood sample for DNA extraction. The study questionnaire will be based on the translated and validated GENIEUR recommendation both for the IBS and the control group, using the Rome IV criteria.

The *novelty* of the study will be that we shall use new Rome IV criteria for IBS diagnosis and also new genetic molecular targets, never used before in IBS patients in Romania. Also, will contribute to creation of an European data and genetic bank on IBS patients, a follow-up purpose of COST Action BM1106 GENIEUR. In this way, our study will contribute to better understanding of IBS etiopathogenesis.

RELATIOSHIP BETWEEN DIET AND FUNCTIONAL DIGESTIVE DISORDERS

Present data is showing convincing evidence that vegetarians have lower rates of coronary heart disease, (low LDL, hypertension, diabetes mellitus) overall cancer rates (colon cancer) (Fraser, 2009). The role of diet in physiopathology and treatment of IBS is still under-evaluated. A predominantly vegetable diet is commonly associated with bloating and flatulence. It is also known that vegetarianism may affect **gut microbiota**, intestinal peristalsis and inflammation (Zimmer et al., 2012; David et al., 2014).

The **aim** of the study will be to evaluate the prevalence of IBS among vegetarians.

Vegetarians will be recruited from: vegetarian and healthy food websites, the Vegetarian Society, from friends and relatives of participants. Questionnaire will be based on: Rome IV criteria, GENIEUR CRF and food frequency questionnaire centered on a validated European Prospective Investigation of Cancer (EPIC) protocol). Socio-demographic factors and general medical history will also be included in interview together with objective evaluation of overweight.

IMPROVING MANAGEMENT IN IRRITABLE BOWEL SYNDROME –

- *IMFLAMATORY BOWEL DISEASE / IRRITABLE BOWEL SYNDROME A SOCIAL MODEL OF CHRONIC DISEASE*

How two different but somehow related disease are effecting the social life of the patients but also the social environment will be studied by a multidisciplinary team including a social worker (Doru Botezat PhD) and gastroenterologist (Vasile Drug MD PhD).

The study is presently underway and we are including patients with inflammatory disease and patients with IBS. The social worker is using a composite methodology including 4 sources of data: free interviews with patients, media, social observations in medical environment, and interviews with gastroenterologists.

- *STUDY ON THE EVALUATION OF THE IMPORTANCE OF TRUST IN DOCTOR AND/OR IN INTERNET IN IRRITABLE BOWEL SYNDROME*

A patient's trust in their doctor is essential for his/her compliance to treatment (Kerse et al., 2004). However, many patients with Irritable Bowel Syndrome (IBS) prefer to look up over the Internet for information in order to diagnose and treat themselves (Tangri and Chande, 2011). In order to further study this issue, a standardized measure of trust might prove useful. Several attempts to use quantitative measures of trust have been made (Leisen and Hyman, 2001), (Dugan et al., 2005). We haven't identified in literature a specific tool to measure for IBS patient's trust in their doctor, or a quantitative tool to measure the patient's reliance on Internet information.

AIM and Research Objectives

Our first objective will be to develop scales measuring the IBS patients' trust in their doctor as well as their reliance on the information provided by the Internet.

The second objective will be to test the hypothesis of a significant difference between those who look up for information over the Internet and those who don't search the internet with respect to the measure of trust in their gastroenterologist.

Research Subjects

A multicenter study including relatively small number of subjects (N=80) with IBS respecting the Roma-IV criteria will be recruited via public hospitals and private gastroenterology practices around the country. Socio-demographic information will be gathered as well. The questionnaires will be anonymous. All patients will be asked if they consulted the Internet for information about IBS. The questionnaires will include a specific section for doctors. They will be asked to confirm Roma-IV criteria, to specify patient's provenance (public hospital or private practice) and to state the type of IBS (diarrhea, constipation or mixed).

Instruments

The study subjects will first complete a questionnaire of trust in their gastroenterologist. The questionnaire will be elaborated based on items already existent in current literature for other medical specialties (Kao et al., 1998; Leisen and Hyman, 2001; Hall et al., 2002; Moseley et al.: 2006; Hamelin et al., 2012; Okere et al., 2014; Aloba et al., 2014). We also include several items that target specific information gathered in previous qualitative research. For the second measure, targeting patient's reliance on Internet information, a similar approach is used. Only those patients who report having used Internet for IBS information will complete the second questionnaire. Two questionnaires will be put in place, and their reliability will be tested.

II.2. PERSPECTIVES IN ACADEMIC AND PROFESSIONAL ACTIVITY

The academic activity should integrate the research and professional activity through teaching. In order to be efficient, teaching should be based on the novel achievements on science using up-to-date methodology.

Future didactic activities with Ph D, postdoctoral students and young doctors

Presenting new and up-to-date scientific data in clinical medicine is essential for PhD, postdoctoral students and young doctors. These new data have to be presented objectively, based on evidence based methology. Furthermore, it is my intention to promote knowledge of "evidence based medicine" in the field of gastroenterology and also on medical semiology. It is my intention to promote a "Journal Club" that could be a good opportunity for pH D students, young doctors and fellows to understand better the importance of the new scientific data but also to improve their skills for doing interesting presentations. Interdisciplinary meetings together with colleagues with other specialties to better understand the scientific challenges are also important and it is in my intention have them promoted.

Promoting problems solving learning methodology may be a solution in our times when we have an inflation of scientific information. Learning to access and to use information is important for the medical profession in our time. It is my intention to promote the entire panoply of methods available for learning.

Evaluation of students is an important part of the education process. Implementing a list of clinical maneuvers that medical students should perform in order to succeed the exam should be considered essential for better educated students. I am intending to participate to expand the MCQs, including more clinical scenarios.

I am willing to promote exchange of PhD students and post-doctoral fellows but also of young doctors with the important International Academic, especially from Europe and also from other Universities from Romania.

Stimulating clinical research and involving also undergraduates and young doctors in these activities is also part of the academic training that I am willing to continue.

The activities of professional medical societies are important also for medical education imposing a level of competence. Organizing continuous medical educations courses under the auspice of Romanian Society of Gastroenterology, Romanian Society of Digestive Endoscopy but also of Romanian Society of Neurogastroenterology is an intrinsic part of academic activities that I intend to fulfill.

Gastroenterology is a very active specialty with multiple novelties occurring in the daily practice. In regard of prosessional activity, my main objectives will include acquiring new skills and competences, to create functional teams for better patient management. Participating in the colon cancer screening program as expert and helping to implement a colon cancer screening program in our region is an important professional task also with academic and scientific opportunities.

***IN CONCLUSION**, the scientific activity in our field should answer the questions and fulfill the needs that our clinical work is raising. Increasing the quality of life and also the lifespan are two daily problems in medicine. Neurogastroenterology health problems are very common in the general population with clear negative impact in the quality of life. In this way intensive clinical research is a must in the field.*

Clinical research, and including my research activity should be strategically planned and my thesis is highlighting this aspect, but also has to be open for the new developments that may arrive in the field.

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