Journal of
Clinical Medicine

Gastrointestinal Tract Disorders

Edited by
Marilena Durazzo

Printed Edition of the Special Issue Published in
Journal of Clinical Medicine

www.mdpi.com/journal/medicines

MDPI

Gastrointestinal Tract Disorders

Gastrointestinal Tract Disorders

Editor

Marilena Durazzo

MDPI • Basel • Beijing • Wuhan • Barcelona • Belgrade • Manchester • Tokyo • Cluj • Tianjin

Editor
Marilena Durazzo
University of Turin
Italy

Editorial Office
MDPI
St. Alban-Anlage 66
4052 Basel, Switzerland

This is a reprint of articles from the Special Issue published online in the open access journal *Journal of Clinical Medicine* (ISSN 2077-0383) (available at: https://www.mdpi.com/journal/jcm/special_issues/gastrointestinal_tract).

For citation purposes, cite each article independently as indicated on the article page online and as indicated below:

LastName, A.A.; LastName, B.B.; LastName, C.C. Article Title. *Journal Name* **Year**, *Volume Number*, Page Range.

ISBN 978-3-0365-3715-3 (Hbk)
ISBN 978-3-0365-3716-0 (PDF)

Contents

About the Editor

Marilena Durazzo is presently the Head of Internal Medicine 3 Unit, Molinette Hospital of Turin, and a confirmed Associate Professor of Internal Medicine, University of Turin. She is also the Vice-Director of the General Hospital Department. She graduated in medicine in 1978, after which she then specialized in gastroenterology (1982). During 90s she was a visiting scientist in Internal Medicine, University of Hannover, Germany. Prof. Durazzo is the author of more than 140 publications in scientific international journals with impact factors, about 20 book chapters and a monograph. Nowadays, her main fields of scientific and research interest include chronic viral hepatitis, nonalcoholic steatohepatitis, metabolic syndrome and gastroenterological problems in the elderly.

Journal of
Clinical Medicine

MDPI

Editorial

Trends in the Comprehension and Management of Gastrointestinal Tract Disorders

Marilena Durazzo [1,*], Arianna Ferro [1], Sharmila Fagoonee [2] and Rinaldo Pellicano [3]

[1] Department of Medical Sciences, University of Turin, C.so A.M. Dogliotti 14, 10126 Turin, Italy; direzione.scienzemediche@unito.it
[2] Institute for Biostructure and Bioimaging, National Research Council, Molecular Biotechnology Centre, 10126 Turin, Italy; sharmila.fagoonee@unito.it
[3] Unit of Gastroenterology, Città della Salute e della Scienza Hospital, C.so Bramante 88, 10126 Turin, Italy; rinaldo_pellican@hotmail.com
* Correspondence: marilena.durazzo@unito.it; Tel.: +39-0110918473

During the last decade, relevant advances have been made in the knowledge of the pathogenetic mechanisms of gastrointestinal (GI) tract disorders. This has led to a better management of these morbidities that, regarding the healthcare required for a longer lifespan, represent a significant burden for all national health systems around the world [1]. In fact, aging increases the risk for chronic and neoplastic diseases as well as the worsening of already existent disturbances [2].

The epidemiological changes that have occurred lately have been influenced by the improved management of some health conditions, as well as exogenous factors. For instance, after the introduction of new drugs and new strategies for the cure of *Helicobacter pylori* infection—particularly, the single-capsule bismuth quadruple therapy [3] and the use of tailored approaches [4]—a drastic reduction in terms of the incidence and prevalence of this infection as well as other related morbidities (peptic ulcer disease) is expected [5]. On the other hand, the increasing prevalence of a more "Westernized" lifestyle (including dietary changes and a decrease in physical activity) has been associated with a diet rich in fat and protein, and a rise in the incidence of gastroesophageal reflux disease (GERD), which affects esophageal and extra-esophageal systems [6]. In parallel, this change in lifestyle has been associated with an increased incidence of metabolic-associated fatty liver disease (MAFLD) [7], which in the clinical setting, reflects in a shift from a higher prevalence of viral liver diseases to a higher prevalence of dysmetabolic liver diseases [8], as well as a significant increase in the incidence of malignancies as colorectal cancer (CRC). Within this negative context, the increased incidence of disorders caused by inappropriate alcohol consumption [9], associated with both hepatic and extra-hepatic alcohol-related disorders play a major role [10]. Since modifiable factors can be corrected after the appropriate education and psychological support, carrying out this task has become a priority.

Inflammatory bowel diseases (IBD) represent another prime example that highlights therapeutic improvements of GI tract disorders. The introduction, in the clinical setting, of biologic drugs has allowed the management of IBD patients to become optimized for steroid-refractory or steroid-resistant diseases [11], improving not only the mucosal state (with a mucosal remission) but also the clinical consequences of this inflammation [12]. These advances have allow us to focus on the endpoint beyond the clinical and endoscopic parameters, including the patient's quality of life [13].

Increasingly often, the interdisciplinary aspect of GI pathologies is the object of studies that aim to optimize the management of patients with complex diseases, a prime example being the management of GI conditions in diabetic patients. Nevertheless, despite the intense efforts made from basic research [14] to the clinical setting [15], diabetic gastroparesis remains a challenge for clinicians. The involvement of the hepato-pancreato-biliary tract in the context of autoimmune manifestations is another key example of this [16]. While some

Citation: Durazzo, M.; Ferro, A.; Fagoonee, S.; Pellicano, R. Trends in the Comprehension and Management of Gastrointestinal Tract Disorders. *J. Clin. Med.* **2022**, *11*, 1730. https://doi.org/10.3390/jcm11061730

Received: 18 March 2022
Accepted: 18 March 2022
Published: 21 March 2022

diseases, such as autoimmune hepatitis and primary biliary cholangitis, are well-known, others, such as autoimmune pancreatitis, represent a challenge for clinicians in several fields (gastroenterologists, experts in endoscopic or radiologic imaging, and immunologists). In the presence of these conditions, a multidisciplinary approach is essential to determine both the appropriate diagnosis and optimal treatment for managing patients, whilst avoiding undervaluation, overmedicalization and unnecessary costs.

The emergence of microbiota–microbiome investigations into the spotlight has opened a door to several research possibilities and could, in theory, help to offer therapeutic interventions for a broad series of GI diseases. These interventions could range from benign, non-inflammatory [17] or inflammatory types [18] to malignant diseases [19].

Finally, the current pandemic, caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has drastically impacted human society, causing not only diseases of the respiratory tract [20] but also of the digestive tract [21]. Furthermore, some GI conditions, such as bleeding and the increased the risk of death among patients with coronavirus disease-19 (COVID-19), were discovered [22]. Patients affected by COVID-19 also experienced an increase in GI symptoms, but this was not associated with hospitalization or mortality rates [23]. This could be due to the fact that a relevant part of these manifestations were likely to have had an anxiety-induced functional basis. This has also been associated with GI disturbances reported by medical students during the COVID-19 lockdown periods [24].

Author Contributions: Conceptualization, M.D. and R.P.; writing—original draft preparation, A.F. and R.P.; writing—review and editing, S.F.; supervision, M.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We apologize to all the investigators whose important works have not been cited due to space restrictions.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Peery, A.F.; Crockett, S.D.; Murphy, C.C.; Jensen, E.T.; Kim, H.P.; Egberg, M.D.; Lund, J.L.; Moon, A.M.; Pate, V.; Barnes, E.L.; et al. Burden and Cost of Gastrointestinal, Liver, and Pancreatic Diseases in the United States: Update 2021. *Gastroenterology* **2022**, *162*, 621–644. [CrossRef]
2. Durazzo, M.; Campion, D.; Fagoonee, S.; Pellicano, R. Gastrointestinal tract disorders in the elderly. *Minerva Med.* **2017**, *108*, 575–591. [CrossRef]
3. Nyssen, O.P.; Perez-Aisa, A.; Castro-Fernandez, M.; Pellicano, R.; Huguet, J.M.; Rodrigo, L.; Ortuñ, J.; Gomez-Rodriguez, B.J.; Pinto, R.M.; Areia, M.; et al. European Registry on Helicobacter pylori management: Single-capsule bismuth quadruple therapy is effective in real-world clinical practice. *United Eur. Gastroenterol. J.* **2021**, *9*, 38–46. [CrossRef] [PubMed]
4. Choe, A.R.; Shim, K.N.; Park, Y.; Song, E.M.; Tae, C.H.; Jung, S. Cost-Effectiveness, Efficacy, and Safety Analysis of Tailored Therapy in Patients with Helicobacter pylori Infection. *J. Clin. Med.* **2021**, *10*, 2619. [CrossRef]
5. Mladenova, I. Clinical Relevance of *Helicobacter pylori* Infection. *J. Clin. Med.* **2021**, *10*, 3473. [CrossRef] [PubMed]
6. Durazzo, M.; Lupi, G.; Cicerchia, F.; Ferro, A.; Barutta, F.; Beccuti, G.; Gruden, G.; Pellicano, R. Extra-Esophageal Presentation of Gastroesophageal Reflux Disease: 2020 Update. *J. Clin. Med.* **2020**, *9*, 2559. [CrossRef] [PubMed]
7. Eslam, M.; Sanyal, A.J.; George, J.; on behalf of the International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* **2020**, *158*, 1999–2014.e1991. [CrossRef]
8. Saracco, G.M.; Evangelista, A.; Fagoonee, S.; Ciccone, G.; Bugianesi, E.; Caviglia, G.P.; Abate, M.L.; Rizzetto, M.; Pellicano, R.; Smedile, A. Etiology of chronic liver diseases in the Northwest of Italy, 1998 through 2014. *World J. Gastroenterol.* **2016**, *22*, 8187–8193. [CrossRef]
9. Testino, G.; Fagoonee, S.; Caputo, F.; Pellicano, R. The early identification of alcohol use disorders and liver injury: Proposal for a diagnostic algorithm. *Panminerva Med.* **2021**, *63*, 361–367. [CrossRef]

10. Rosa, G.M.; Scagliola, R.; Zoppoli, G.; Perna, V.; Buscaglia, A.; Berri, A.; DELLA Bona, R.; Porto, I.; Pellicano, R.; Testino, G. May standard basal echocardiogram allow to obtain predictors of asymptomatic cardiac dysfunction in alcoholics? *Minerva Med.* **2022**, *112*, 786–791. [CrossRef]
11. Bertani, L.; Caviglia, G.P.; Antonioli, L.; Pellicano, R.; Fagoonee, S.; Astegiano, M.; Saracco, G.M.; Bugianesi, E.; Blandizzi, C.; Costa, F.; et al. Serum Interleukin-6 and -8 as Predictors of Response to Vedolizumab in Inflammatory Bowel Diseases. *J. Clin. Med.* **2020**, *9*, 1323. [CrossRef]
12. Scarozza, P.; De Cristofaro, E.; Scucchi, L.; Rocchetti, I.; Marafini, I.; Neri, B.; Salvatori, S.; Biancone, L.; Calabrese, E.; Monteleone, G. Effect of Vedolizumab on Anemia of Chronic Disease in Patients with Inflammatory Bowel Diseases. *J. Clin. Med.* **2020**, *9*, 2126. [CrossRef] [PubMed]
13. Sochal, M.; Małecka-Panas, E.; Gabryelska, A.; Talar-Wojnarowska, R.; Szmyd, B.; Krzywdzińska, M.; Białasiewicz, P. Determinants of sleep quality in inflammatory bowel diseases. *J. Clin. Med.* **2020**, *9*, 2921. [CrossRef] [PubMed]
14. Ha, C.; Kim, H.; Cha, R.; Lee, J.; Lee, S.; Ryu, J.H.; Lee, O.J. Effect of DA-9701 on the Gastrointestinal Motility in the Streptozotocin-Induced Diabetic Mice. *J. Clin. Med.* **2021**, *10*, 5282. [CrossRef] [PubMed]
15. Bonetto, S.; Gruden, G.; Beccuti, G.; Ferro, A.; Saracco, G.; Pellicano, R. Management of Dyspepsia and Gastroparesis in Patients with Diabetes. A Clinical Point of View in the Year 2021. *J. Clin. Med.* **2021**, *10*, 1313. [CrossRef] [PubMed]
16. Kunovsky, L.; Dite, P.; Jabandziev, P.; Kala, Z.; Vaculova, J.; Andrasina, T.; Hrunka, M.; Bojkova, M.; Trna, J. Autoimmune Diseases of Digestive Organs—A Multidisciplinary Challenge: A Focus on Hepatopancreatobiliary Manifestation. *J. Clin. Med.* **2021**, *10*, 5796. [CrossRef]
17. Caviglia, G.P.; Tucci, A.; Pellicano, R.; Fagoonee, S.; Rosso, C.; Abate, M.L.; Olivero, A.; Armandi, A.; Vanni, E.; Saracco, G.M.; et al. Clinical Response and Changes of Cytokines and Zonulin Levels in Patients with Diarrhoea-Predominant Irritable Bowel Syndrome Treated with *Bifidobacterium Longum* ES1 for 8 or 12 Weeks: A Preliminary Report. *J. Clin. Med.* **2020**, *9*, 2353. [CrossRef]
18. Larussa, T.; Abenavoli, L.; Fabiano, G.; Mancuso, M.A.; Polimeni, N.; Dumitrascu, D.L.; Luzza, F. Gut microbiota in in-flammatory bowel disease: A target for therapy not to be missed. *Minerva Gastroenterol.* **2021**, *67*, 357–368.
19. Nouri, R.; Hasani, A.; Asgharzadeh, M.; Sefidan, F.Y.; Hemmati, F.; Rezaee, M.A. Roles of gut microbiota in colorectal carcinogenesis providing a perspective for early diagnosis and treatment. *Curr. Pharm. Biotechnol.* **2022**. *online ahead of print*. [CrossRef]
20. Koenigkam-Santos, M.; Wada, D.T.; Benatti, M.N.; Siyuan, L.; Batah, S.S.; Cetlin, A.A.; de Menezes, M.B.; Fabro, A.T. SARS-Cov-2 pneumonia phe-notyping on imaging exams of patients submitted to minimally invasive autopsy. *Ann. Transl. Med.* **2022**, *10*, 140. [CrossRef]
21. Burkett, A.E.; Sher, S.B.; Patel, C.R.; Ildin-Eltoum, I.; Dhall, D.; Margaroli, C.; Peter, S.; Lee, G.; Bajpai, P.; Benson, P.V.; et al. Gastrointestinal Manifestations of COVID-19 Infection: Clinicopathologic Findings in Intestinal Resections Performed at Single Institution. *Front. Med.* **2022**, *9*, 811546. [CrossRef] [PubMed]
22. Zuin, M.; Rigatelli, G.; Fogato, L.; Zuliani, G.; Roncon, L. Higher risk of death in COVID-19 patients complicated by gastrointestinal bleeding events: A meta-analysis. *Minerva Gastroenterol.* **2021**, *67*, 408–410.
23. Wang, Y.; Li, Y.; Zhang, Y.; Liu, Y.; Liu, Y. Are gastrointestinal symptoms associated with higher risk of Mortality in COVID-19 patients? A systematic review and meta-analysis. *BMC Gastroenterol.* **2022**, *22*, 106. [CrossRef] [PubMed]
24. Abenavoli, L.; Cinaglia, P.; Lombardo, G.; Boffoli, E.; Scida, M.; Procopio, A.; Larussa, T.; Boccuto, L.; Zanza, C.; Longhitano, Y.; et al. Anxiety and Gastrointestinal Symptoms Related to COVID-19 during Italian Lockdown. *J. Clin. Med.* **2021**, *10*, 1221. [CrossRef] [PubMed]

Journal of
Clinical Medicine

MDPI

Review

Extra-Esophageal Presentation of Gastroesophageal Reflux Disease: 2020 Update

Marilena Durazzo [1,2,*], Giulia Lupi [1,2], Francesca Cicerchia [1,2], Arianna Ferro [1,2], Federica Barutta [1,2], Guglielmo Beccuti [1,2], Gabriella Gruden [1,2] and Rinaldo Pellicano [3]

1 Department of Medical Sciences, University of Turin, C.so A.M. Dogliotti 14, 10126 Turin, Italy; giulia.lupi@unito.it (G.L.); fracice7@gmail.com (F.C.); arianna.ferro@unito.it (A.F.); federica.barutta@unito.it (F.B.); guglielmo.beccuti@unito.it (G.B.); gabriella.gruden@unito.it (G.G.)
2 3th Internal Medicine Unit, Città della Salute e della Scienza Hospital, C.so Bramante 88, 10126 Turin, Italy
3 Unit of Gastroenterology, Città della Salute e della Scienza Hospital, C.so Bramante 88, 10126 Turin, Italy; rinaldo_pellican@hotmail.com
* Correspondence: marilena.durazzo@unito.it; Tel.: +39-0-116-708-4783

Received: 8 July 2020; Accepted: 5 August 2020; Published: 7 August 2020

Abstract: Gastroesophageal reflux disease (GERD) is defined by the presence of symptoms induced by the reflux of the stomach contents into the esophagus. Although clinical manifestations of GERD typically involve the esophagus, extra-esophageal manifestations are widespread and less known. In this review, we discuss extra-esophageal manifestations of GERD, focusing on clinical presentations, diagnosis, and treatment. Common extra-esophageal manifestations of GERD include chronic cough, asthma, laryngitis, dental erosions, and gingivitis. Extra-esophageal involvement can be present also when classic GERD symptoms are absent, making the diagnosis more challenging. Although available clinical studies are heterogeneous and frequently of low quality, a trial with proton pump inhibitors can be suggested as a first-line diagnostic strategy in case of suspected extra-esophageal manifestations of GERD.

Keywords: gastroesophageal reflux; cough; asthma; laryngo-pharyngeal reflux; chest pain; tooth erosions

1. Introduction

Gastroesophageal reflux disease (GERD) is a common gastrointestinal (GI) condition with a worldwide diffusion and high prevalence in Western countries. The 2006 Montreal consensus defined GERD as a condition that develops when the reflux of the stomach contents causes troublesome symptoms and/or complications into the esophagus [1]. Tissue damage related to GERD range from esophagitis to Barrett's esophagus and esophageal adenocarcinoma; troublesome symptoms attributable to reflux can be esophageal (heartburn, regurgitation) or extra-esophageal (EE) [2–5]. GERD can be further classified by the presence of erosions on endoscopic examination (Erosive Reflux Disease [ERD] and Nonerosive Reflux Disease [NERD]) [5].

GERD-related EE manifestations are frequent and represent a diagnostic and therapeutic challenge, being able to involve lungs, upper airways, and mouth, presenting with asthma, laryngitis, chronic cough, dental erosions, and non-cardiac chest pain (Figure 1).

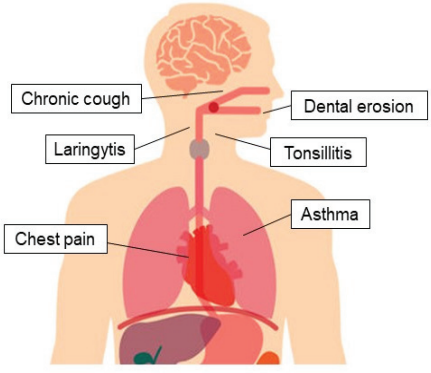


Figure 1. Extra-esophageal presentation of gastroesophageal reflux disease.

It has been estimated that one-third of patients with GERD may have atypical or EE symptoms [6]: non-cardiac chest pain is the most common complaint (23.1%), followed by pulmonary manifestations (bronchitis—14.0%, asthma—9.3%) and head and neck symptoms (hoarseness—14.8%, globous sensation—7.0%) [7]. In a prospective European study, the prevalence of EE symptoms was 32.8% in patients complaining of heartburn, with a higher proportion in those with ERD (34.9%) than in those with NERD (30.5%) [6]. Chest pain (14.5%), chronic cough (13%), laryngeal disorders (10.4%), and asthma (4.8%) were the commonest disorders associated with GERD [6].

The prevalence of EE disorders in patients not complaining of typical symptoms of GERD is hard to define, due to the increased difficulty of establishing the correct diagnosis. It has been estimated that between 20% and 60% of patients with GERD have head and neck symptoms without any considerable heartburn. Thus, the diagnosis of GERD-related EE manifestations requires a strong collaboration between specialists to exclude alternative causes [8].

Physiologically, the competence of esophageal sphincters (lower and upper) protect the esophageal and laryngeal mucosa from acid refluxate, while the esophago-glottic closure reflex protects the airway. Peristaltic waves perform mechanical clearance by promoting the progression of the bolus through the esophagus: primary peristalsis is a voluntary process that occurs concurrently with swallowing; thus, it is typical of daytime, while secondary peristalsis is involuntary and predominates during the night. Saliva produced during meals neutralizes acids with its content of bicarbonate and plays a chemical clearance during primary peristalsis [9]. When a reflux event happens, esophageal peristalsis pushes the refluxate back in the stomach, while swallowed saliva neutralize acid [9].

The degree and the duration of acid exposure are responsible for the severity of esophageal mucosal injury and GERD-related symptoms, depending from the incompetence of protective mechanisms. Impairment of the esophageal sphincters is the main predisposing condition: upper esophageal sphincter (UES) insufficiency can be diagnosed by esophageal manometry or pH monitoring. Factors associated with EE are the same as those of GERD, either endogenous, as gastric acidity, pepsin, bile, and pancreatic enzymes, or exogenous such as smoke, alcohol, drugs, and hypertonic solutions [10].

Two main mechanisms have been proposed to explain GERD-related EE manifestations: direct damage induced by the aspiration of gastric materials, and indirect damage, which is vagus nerve mediated.

In the hypothesis of a direct stimulus, cough, laryngitis, or asthma exacerbation appear consequently to a tracheal or bronchial aspirate that stimulates the pharynx and larynx. An intact lower esophageal sphincter (LES) and UES protect from gastroesophago-pharyngeal reflux, while high basal UES pressure and the esophago-glottic closure reflex prevent pharyngeal and laryngeal contact with refluxate [10]. The hypothesis of an indirect mechanism is based on the common embryonic origin and vagus innervation of the esophagus and the bronchial tree, considering cough, bronchial

spasm, and cardiac-type chest pain induced by the stimulation of the vagal reflex arc from the distal esophageal reflux [10].

A response to the empiric proton pump inhibitor (PPI) therapy (PPI test) would ideally confirm the diagnosis; however, in a meta-analysis, response to PPIs had only sensitivity of 78% and specificity of 54% in the diagnosis of GERD [11]. GERD-related EE manifestations are less responsive to standard therapy with PPIs [12]. Ambulatory 24-h esophageal pH monitoring is indicated in the evaluation of patients' refractory to a PPI test and when the diagnosis of GERD is uncertain. This diagnostic test is the only capable of assessing the association between refluxates and reflux symptoms, being particularly useful in detecting GERD-related EE manifestations [5].

Upper gastrointestinal endoscopy is recommended when alarm signs are present (e.g., anemia, undesired loss of weight), in cases of no response to PPI treatment (no decrease of GERD symptoms after short PPI treatment, recurrence of EE symptoms besides 3 months of PPI treatment), dysphagia, suspicious of other causes of heartburn (e.g., eosinophilic esophagitis), long-lasting EE symptoms, the presence of GERD complications, the presence of Barrett esophagus, and fundoplication (before and after).

2. Pulmonary Manifestations

An association between GERD and respiratory symptoms has been suggested by several epidemiological studies [13], although a causative association has not been demonstrated yet. Here, we discuss the most frequently reported pulmonary manifestations of GERD: chronic cough, asthma, and aspiration pneumonia.

2.1. Chronic Cough

Cough is defined as chronic when it persists over 8 weeks; cough of a much longer duration is defined as chronic refractory cough [14]. Common causes of chronic cough are side effects due to commonly used drugs (especially angiotensin converting enzyme [ACE] inhibitors), tracheo-broncomalacia, chronic obstructive pulmonary disease (COPD), bronchiectasis, asthma, obstructive sleep apnea, rhinosinusitis, and GERD [9,10,13]. In non-smoking patients with normal chest X-rays who are not taking ACE inhibitors, chronic cough is determined in 86% of cases by asthma, postnasal drip syndrome (PNDS), and GERD, although often multiple causes co-exist in a single patient [10].

Several studies suggested a significant relationship between chronic cough and GERD, with prevalence rates of 10% to 56%, which is mainly due to referral bias to centers with specialized interest [6]. In a large prospective European study, the PROGERD study, chronic cough could be attributed to GERD in 13% of patients [6]. In a recent systematic review, Irwin et al. identified GERD as the cause of 85% of chronic cough worldwide, especially in Western countries [15]. In Japan, GERD is described as a rare cause of chronic cough, accounting only for the 7.7% of all causes. The lower prevalence of obesity and the less common Western diet are the main factors associated with the rarity of GERD in this country [16].

When GERD causes cough, GI symptoms can be absent up to 75% of the time, making the diagnosis more challenging [3]. Furthermore, cough and GERD are common diseases and often co-exist, but the association does not imply a causative relationship in all cases: Eastburn et al. showed an occurrence by chance in 25% of cases [17]. Temporal association between reflux episodes and cough could help address correctly chronic cough to reflux, although a diagnostic gold standard is lacking [10].

2.1.1. Pathogenesis

The two main theories proposed to explain GERD-related cough are the reflex theory, considering cough consequent to a vagal-mediated esophageal–tracheobronchial reflex induced by reflux, and reflux theory, suggesting a micro aspiration of refluxed gastric material in the tracheobronchial tract as the cause of cough [16,18].

2.1.2. Diagnosis

GERD-induced cough is usually dry, and it is often exacerbated by postural changes, food intake, and phonation. Chronic cough is quite often the only manifestation of GERD [16]. In patients complaining of chronic cough, it is firstly necessary to exclude pulmonary diseases by performing a radiologic investigation, such as chest x-ray or pulmonary computed tomography (CT). Few cases will require a bronchoscopy also for diagnostic or therapeutic reasons.

When GERD causes cough by irritating the larynx, laryngoscopy can demonstrate signs consistent with "reflux laryngitis" (posterior laryngitis with red arytenoids and piled-up inter-arytenoid mucosa). At bronchoscopy, abnormalities consistent with aspiration can be detected, such as subglottic stenosis, hemorrhagic tracheo-bronchitis, and erythema of subsegmental bronchi. Evidence of inflammation and edema of the larynx and lower airways should not be automatically addressed to GERD because these findings can be associated to other causes of cough or to cough itself. If imaging and endoscopy are normal, it can be assumed that GERD causes cough by stimulating an esophageal–bronchial reflex [3]. In the gastric refluxate, there are multiple potential mediators of cough other than acid, so several mechanisms can be proposed [10].

Patients with laryngeal or pulmonary manifestations of GERD usually are firstly visited by pulmonology and otolaryngology specialists, and only upon a second presentation are they generally admitted by gastroenterologists. In such typical situations, upper gastrointestinal endoscopy is rather often ordered.

A normal esophagogastroduodenoscopy (EGD) is a common finding in patients with GERD-induced cough; only a few have esophagitis or Barrett's epithelium. Hence, a normal EGD does not rule out the presence of GERD or its involvement in pulmonary abnormalities. Therefore, upper endoscopy should not be performed to diagnose GERD-related asthma, chronic cough, or laryngitis. Furthermore, the diagnosis of esophagitis does not confirm the relationship between GERD and potential EE manifestations [8].

While 24-h esophageal pH monitoring can detect only acid reflux episodes, impedance-pH monitoring can also detect non-acid reflux [19]. During impedance-pH monitoring, reflux episodes are detected considering characteristic impedance changes (e.g., progressing variations in intraluminal impedance), while pH data are used to distinguish acid from non-acid refluxes. The temporal association between reflux events detected at the 24-h reflux monitoring and symptoms is defined by symptom index (SI) and symptom-association probability (SAP) [19,20].

Esophageal manometry and the pH monitoring off-PPI can be recommended in patients with cough unresponsive to treatment and who are considered for surgical options [2].

Recently, Burton et al. have suggested the use of scintigraphy with Tc-99m to identify alterations in the esophageal motility and lung aspiration of refluxate [4].

Given the low availability of pH monitoring, its invasiveness, and the common association between chronic cough and GERD, it is frequent to diagnose GERD-related cough with an empiric trial of PPIs. Up to 79% of patients with cough secondary to GERD experienced a resolution of symptoms after PPI therapy, thus confirming the diagnosis [10]. However, American Gastroenterological Association (AGA) Guidelines recommend 24-h pH monitoring before starting a PPI trial in patients with suspected GERD-related EE manifestations and an absence of typical esophageal findings [5].

2.1.3. Treatment

Although there is poor evidence to support this approach, PPIs are the commonest treatment used in the suspect of GERD-induced chronic cough. Several studies have shown an improvement of chronic cough with this treatment; however, a recent randomized controlled trial (RCT) did not show differences between PPIs and placebo [21–23]. A possible explanation can be found in the small sample size included and in the type of quality of life (QoL) questionnaires used to address the usefulness of the treatment [23].

A Cochrane systematic review reported insufficient evidence to conclude for PPI efficacy in treating cough associated with GERD, although some beneficial effect was seen in a sub-analysis [24]. Chronic cough had a high response rate to placebo, and this fact interferes with statistical results in clinical studies. Clinicians prescribing PPI drugs should consider their potential side effects, and maintaining treatment should be planned only when demonstrated useful [24].

Chang et al., in a meta-analysis of RCTs comparing PPI drugs versus placebo, evidenced the efficacy of treatment in patients with GERD-associated cough in a subgroup analysis. In the pooled analysis, there was no effect on the main outcomes, although all studies favored PPIs. The number needed to treat (NNT) to achieve cough resolution was 5. The authors evidenced a smaller effect of treatment on cough compared to the results of non-controlled trials, which was probably related to the placebo effect, which is as high as 85%. A limit of this meta-analysis is the lack of data from RCTs including patients with chronic cough without GERD symptoms. Furthermore, in the included studies, there were no consistent data on the efficacy of dietary changes or surgical treatment [21].

In 2006, the American College of Chest Physician (ACCP) Guidelines on Reflux-Cough Syndrome have been published. These guidelines recommend behavioral changes such as weight loss in patients who are overweight, sleeping with head elevated, and meal avoidance three hours before bedtime. PPI treatment is recommended in patients with symptoms of heartburn and regurgitation; in those with cough but no gastroesophageal symptoms, PPIs should not be prescribed alone, although can be considered in association with lifestyle modifications. In the latter case, prescribing PPIs without behavioral changes are not likely to resolve symptoms [25].

While GI symptoms usually resolve after 4–8 weeks of treatment, the literature suggests that improvement in cough may take up to 3 months. Generally, a positive response to PPIs is evident within a few weeks, being the strongest indicator for disease resolution. It is crucial to reassess shortly the patient response to avoid the prolonged use of useless therapies [10].

Some experts recommend twice daily initial dosing of PPI drugs in patients with chronic cough, although several studies suggested the non-superiority of the twice daily regimen versus the once daily regimen [10]. In resistant cases, the addition of a histamine H2 receptor antagonist (H2-blockers) and/or baclofen may be helpful [22].

Anti-reflux surgery (as Niessen's fundoplication) may have a role in medical resistant reflux-associated chronic cough when there is not a major motility disorder (absent peristalsis, achalasia, distal esophageal spasm, hypercontractility) [2].

2.2. Asthma

Asthma is defined by the American Thoracic Society (ATS) as "a condition with a history of discrete attacks of wheezing, coughing or dyspnea and increase in forced expiratory volume in one-second (FEV1) of 20% from baseline after bronchodilator administration or decrease in FEV1 of 20% after methacholine bronchoprovocation" [26].

Gastroesophageal reflux has been proposed as a trigger for asthma, also when clinically silent, and an effective treatment of reflux could improve asthma control [27].

A significant association between asthma and GERD has been shown in epidemiological studies: up to 50% of patients with asthma have associated GERD [6]. However, the prevalence of asthma in patients with GERD is still uncertain: study reports from 30% to 90%, compared to an average of 24% in controls [9]. A large European prospective study (PROGERD) showed that 4.8% of GERD patients may have asthma [6], while a higher prevalence (24–29%) of silent GERD can be found in difficult-to-control asthmatic cases [28]. Broers et al. reported that the average percentage of GERD prevalence in asthmatic patients was 46.54%, based on symptoms alone and 52.70% based on pH-monitoring and endoscopy, whereas in control groups, the prevalence of GERD was 23.59% based on symptoms evaluation [9].

Although a temporal association between asthma and GERD exists, gastroesophageal reflux does not always trigger asthma [29]. According to Avidan et al., half of all coughs and wheezes in asthmatics are associated with esophageal acid reflux, and at 24-h pH monitoring, it is documented that the reflux

episodes lead to cough [18]. However, while an occasional episode of cough can rarely bring to reflux, it is more common that the reflux episode that leads to cough [18].

Similarly, to the challenges encountered in the case of chronic cough, the diagnosis of GERD-related asthma is difficult: upper endoscopy, pH impedance, and the PPI test also when positive, do not always demonstrate the association between the diseases. Silent reflux and night reflux are highly prevalent in patients with asthma and respiratory symptoms: during sleep, the usual protective responses are lacking, increasing the damage provoked by the refluxates [30].

An unresolved question is if asthma worsens GERD or GERD exacerbates asthma. In asthmatic patients, many factors can contribute to GERD worsening: cough and increased respiratory effort, lung hyperinflation, with diaphragm contraction and increased pressure gradient across the LES. Asthma medications such as theophylline, β-agonists, and corticosteroids may promote reflux. On the contrary, GERD as the underlying cause of asthma should be suspected in patients with adult onset of asthma, no family history, no allergic component, a low response to traditional asthma medications, symptoms onset preceded by heartburn and regurgitation, or with postprandial worsening [10,28].

2.2.1. Pathogenesis

Asthma and chronic cough share the two main theories of association with GERD. In the reflux theory, the micro aspiration of gastric reflux determines a direct damage to pulmonary parenchyma, causing symptoms such as cough and wheezing, and inducing histologic damage, possibly leading to acute lung injury and acute respiratory distress syndrome. In the reflex theory, refluxates stimulate the vagal nerve, leading to bronchoconstriction [10].

Bronchial hyper-responsiveness is typical of asthma and is defined by an abnormal bronchoconstriction induced by various agents. Esophageal reflux exacerbates asthma by inducing bronchial hyper-responsiveness to the micro aspiration of refluxate, esophageal-triggered vagal reflexes, and esophageal-triggered neuroinflammation through the release of cytokines as tachykinins [27].

2.2.2. Treatment

Lifestyle changes, such as elevation of the head of the bed, smoking cessation, and dietary changes (reduction of fat, chocolate, alcohol, citrus, tomato, coffee, and tea intake, avoidance of large meals and of eating three hours before bedtime) are recommended to improve reflux control and could help obtain improved bronchial symptoms, although there are no RCTs to confirm this hypothesis.

Although PPIs demonstrated superiority over H2-blockers to cure esophagitis, the efficacy of the former in treating GERD-related asthma is still matter of debate: some studies reported an improvement of symptoms and lung function with reflux treatment, while others did not demonstrate this effect [10,31,32]. In a Cochrane systematic review of randomized, placebo-controlled trials conducted in asthma patients, six studies investigated the effect of PPIs and five investigated that of H2-blockers. The authors found no clear effect on lung function, airway responsiveness, or asthma symptoms [29]. Although most of the included trials reported at least one significant outcome, there was no consistency in the results: FEV1 increase [33,34], reduction of β-agonist use [34–36], significant improvement in asthma symptoms [34,36,37], and improvement of nocturnal asthma [35,38,39] after treatment with PPIs were reported by two or three studies each. Interestingly, only one trial evaluated the effect of behavioral changes and one evaluated the outcome of surgical approach. No study reported hospitalizations or emergency room visits resulting from asthma [29]. A meta-analysis, summarizing PPI treatment in asthma patients, concluded that there was a small but significant improvement in morning PEF (Peak Expiratory Flow) rate after PPI therapy, although it was highly probable that this amelioration had minimal clinical significance; no overall improvement in lung function and asthma symptom scores was revealed. This meta-analysis included studies comparing asthmatic patients with and without diagnosis of GERD: both groups showed small but statistically significant improvements in the morning PEF rate with PPI therapy, although a larger benefit was seen in those with GERD.

Differences in treatment length or cumulative PPI dosage were not associated with a better morning PEF rate outcome [31].

Controversial results on the effect of PPIs in asthmatic patients arise from differing methodologies, small sample sizes, and an absence of placebo group of published studies. Currently, there is no evidence to recommend PPIs in all asthmatic patients, while patients with nocturnal asthma or nocturnal reflux might have some beneficial effects [32].

The actual recommendations in patients with GERD-related asthma (with or without concomitant esophageal symptoms) consist of an initial empiric trial of once or twice daily PPIs for 2–3 months. In patients responsive to therapy, PPIs should be tapered to the minimal dose necessary to control symptoms. In those unresponsive, testing for reflux by pH testing or impedance–pH monitoring can rule out pathological reflux [10].

In some study, anti-reflux surgery showed some beneficial effect on GERD-related asthma: disease control scores dropped, and the consumption of asthma medication decreased. However, consistent evidence encouraging the routinely use of this approach is lacking, and further investigation should be performed [29].

3. Laryngitis

Laryngo-pharyngeal reflux (LPR) is defined by the 2002 Position Statement of the American Academy of Otolaryngology-Head and Neck Surgery as a disorder of retrograde flow of gastric contents into the larynx and hypopharynx [40]. It is a common GERD-related EE manifestation: up to 10–15% of all visits to otolaryngology offices are prompted by manifestations of LPR [20].

GERD can cause a variety of laryngeal symptoms, such as hoarseness, sore or burning throat, pain with swallowing, sensation of a lump in the throat, cough, repetitive throat clearing, excessive phlegm, difficulty swallowing, and voice fatigue. These complaints are non-specific of GERD and LPR, and they can be also caused by allergens, smoke, and various irritant agents [10]. In a large case-control study, patients with esophagitis or esophageal strictures had higher odds ratios (OR) for pharyngitis (OR: 1.60), aphonia (OR: 1.81), and chronic laryngitis (OR: 2.01) compared with controls [12]. Many patients diagnosed with laryngeal reflux do not suffer from the classic symptoms of GERD [19]: heartburn is absent in more than half of the patients with LPR [40]. In the PROGERD study, the prevalence of laryngeal disorders was 10.4%, and it was associated with older age, longer GERD duration, and obesity. Interestingly, smokers had laryngeal disorders less often than non-smokers, which was probably due to a desensitized laryngeal mucosa [6].

Laryngeal manifestations of GERD can be explained by a direct damage induced by the acid–peptic contact in the larynx via esophago-pharyngeal reflux (micro-aspiration theory), or by an indirect acidification of the distal esophagus through vagally mediated reflexes (esophageal–bronchial reflex theory). Both mechanisms lead to chronic throat clearing and coughing, inducing mucosal damage and typical signs and symptoms [10].

Laryngeal mucosa is more susceptible to injury than esophageal mucosa: acid refluxate, contents of acid and pepsin, and biliary reflux cause inflammatory and precancerous laryngeal lesions. Nevertheless, the absence of saliva clearance leads to more serious damage compared to the esophagus [10].

3.1. Diagnosis

Laryngoscopic findings of reflux-mediated disease are erythema, edema, lymphoid hyperplasia of the posterior larynx, ulcerations, subglottic or posterior glottic stenosis, vocal cord polyps, granuloma, leucoplakia, and cancer [10,41]. Although frequent in reflux laryngitis, most of them are non-specific. Edema and erythema, which are often used to define reflux-induced laryngitis, lack specificity and are highly operator-dependent parameters [10]: in fact, signs of laryngeal irritation are present in over 80% of healthy controls [5]. Allergy, smoking, and voice abuse are common causes of laryngeal irritation and induce the same alteration of LPR.

The use of ambulatory pH monitoring to diagnose LPR is debatable. Hypopharyngeal and proximal esophageal pH monitoring have sensitivities of 40% and 55%, respectively [10,42]. Although pH-monitoring detects reflux in only 40% of patients showing symptoms of laryngeal dysfunction, impedance monitoring can detect the presence also of weakly acid and alkaline reflux, gas, or liquid refluxate possibly causing laryngeal dysfunction [10,41].

A promising non-invasive test to diagnose reflux, although still controversial in its clinical applications, is the salivary detection of pepsin [43,44]. Pepsin is a proteolytic enzyme secreted in the gastric fundus as pepsinogen and activated in the acidic environment: its identification in non-gastric sites can detect the presence of significant reflux. Methods to measure pepsin levels are still not standardized, with heterogeneous accuracy. Using the Western blot technique for sputum and salivary pepsin samples in patients with EE reflux, Kim et al. reported a sensitivity and specificity of 89% and 68%, respectively, based on the pH monitoring results [45]. A monoclonal antibodies assay has shown in a recent, prospective, blinded study positive and negative predictive values of 87% and 78%, respectively [46].

3.2. Treatment

Hanson et al. reported a great response rate to the medical and non-medical treatment of LPR: half of the patients responded to behavioral changes, while 54% of those who failed this approach responded to H2-blockers [47]. PPI therapy is the standard treatment in patients with chronic throat symptoms if GERD is suspected as the underlying cause, although a single-dose PPI treatment has not demonstrated superiority compared to placebo in treating LPR [48]. An empirical trial of double-dose PPIs is recommended as first-line therapy in patients with suspected LPR to aggressively suppress the hypopharyngeal acid reflux [48]. A 2016 meta-analysis of 13 RCTs on patients with LPR showed an improvement in reflux symptoms (measured with the reflux symptoms index [RSI]) with twice-daily treatment for 3–6 months, although a difference in the response rate and effect on the laryngeal mucosa was not observed between PPIs and the placebo [49]. On the other hand, a recent meta-analysis of controlled studies including patients with LPR demonstrated no benefit of PPI therapy [50]. This negative finding can be partially explained by the difficulty of identifying patients with LPR, due to the absence of a specific diagnostic tool. The diagnosis of GERD-related laryngitis is presumed in the presence of symptoms such as throat clearing, cough, globous, and signs as laryngeal edema and erythema, although these are non-specific for reflux. Patients unresponsive to PPI therapy can have either a non-reflux related disease or a functional component. The lack of effect of PPIs in clinical trials can be also explained by the high placebo response rates of approximately 40%.

Empirical PPI therapy for a period of one or two months is a reasonable initial approach in patients without warning symptoms and with a high suspicion of reflux-related laryngeal disease. If symptoms improve, therapy might be prolonged up to 6 months to allow the healing of laryngeal tissue, after which the dose should be tapered to minimal acid suppression, resulting in continued response. In patients unresponsive to PPIs, impedance or pH monitoring can be used to rule out reflux as the cause of laryngeal complaint.

Ren et al. considered a combination of PPIs and prokinetics effective in improving QoL, although it had no significant effect on the symptoms or endoscopic responses of GERD-related EE [51].

Among non-pharmacological treatments of LPR, diet modification appeared to be effective: patients following a low-fat, high-protein, and alkaline diet had higher rates of symptom resolution [52]. However, a recent systematic review concludes that there is insufficient evidence to recommend diet modifications for LPR [53].

4. Oral Cavity

Saliva is main defense mechanism from acid exposure present in the oral cavity: the quality and amount of saliva provide protection through acid clearance and neutralization [54]. The amount of saliva produced varies throughout the day, depending on circadian rhythms and stimulation from food:

a salivary flow rate of 0.2 mL/min (milliliters per minute) is the lower limit of normal unstimulated whole saliva output, while 0.7 mL/min is the lower limit of stimulated salivary flow [55]. Saliva functions involve the removal of pathogenic bacteria that can destroy tissues and cause dental caries in conditions of poor oral hygiene. The presence of lysozyme, lactoferrin, thiocyanate ions, and antibodies make the saliva an excellent antibacterial, while its neutral pH protects the inorganic material of the teeth.

Salivary flow volume and swallowing function are significantly reduced in patients with GERD [56]. The reduction of saliva amount leads to oral dryness, sometimes evolving to xerostomia [57]. Gengivitis, defined as the inflammation of the periodontal soft tissue, is a possible consequence of saliva reduction. The coexistence of bruxism can exacerbate periodontal disease [56].

4.1. Dental Erosion

Dental erosion (DE) is an irreversible loss of dental hard tissue by a chemical process that does not involve bacteria, and it is a known major oral symptom caused by acid reflux in patients with GERD, according to the Montreal Definition and Classification [1]. The median prevalence of DE in patients with GERD range widely, from 5% to 47.5% [54,58], with higher severity compared to healthy subjects [59].

DE are caused by a combination of extrinsic factors, such as demineralizing acidic foods, acidic beverages, and medications, and intrinsic causes of tooth erosion, such as recurrent vomiting or regurgitation of gastric contents [54]. Hydroxyapatite crystals, the main component of dental enamel, are damaged if exposed to a pH lower than 5.5. Gastric refluxate has often a pH lower than 2.0, being able to erode dental tissues, depending on the duration and the number of reflux episodes and the function of protective factors such as saliva [60]. Although both DE and dental caries determine the loss of mineral component of the teeth, the former occurs in plaque-free surfaces, while dental caries depend on the exposure to weak acids from cariogenic plaque [54]. While DE can be caused by acid reflux, dental caries do not appear to be related to GERD [56]. A defensive role of acid reflux has been suggested in preventing the formation of dental caries by inhibiting bacterial growth in the mouth [59]. Under normal circumstances, saliva withdraw acid and buffer the remaining [58]: in GERD patients, swallowing function and salivary flow volume are significantly decreased, suggesting a role in the pathogenesis of DE [58]. Direct contact with acid is considered the main mechanism of injury: the acid reflux lowers the pH of the oral cavity, leading to dissolution of the inorganic material of the teeth (hydroxyapatite crystals in the enamel), and consequently to DE, with an irreversible loss of dental substance. DE predisposes the teeth to friction (flattening of the occlusal surface) and abrasion (wear of the tooth substance), which can lead to tooth loss, aesthetic deterioration, and a change in facial appearance [61].

DE is classified taking into account the number and degree of severity of erosion: grade 0 (absence of erosion), grade 1 (loss of the enamel-like cream colored appearance), grade 2 (loss of the enamel surface features: smooth dull appearance, without dentin exposure), grade 3 (involvement of enamel and dentin), and grade 4 (severe structural involvement with destruction of the tooth) [59]. DE caused by GERD can involve any surfaces of the teeth, although it is more often encountered on the labial (buccal), occlusal, and lingual surfaces: reflux acid attacks first the palatal surfaces of the upper teeth, and later, if the condition continues, other teeth may be affected. The palatal surfaces of upper teeth are highly susceptible to erosion being the first encountered by gastric reflux; they are relatively far from major salivary glands, and the tongue keeps the contact of the refluxate against them [58]. The lower lingual surfaces are less affected, which is likely because there is plenty of saliva coming from the submandibular glands [58].

In children with GERD, primary teeth are affected more than permanent ones, being less mineralized and thinner; therefore, they are are more prone to acid erosion [62].

Given the high prevalence of DE in GERD patients, collaboration between dentists and gastroenterologists should be promoted. Subjects with unexplainable DE should be referred to the gastroenterology to investigate the presence of undiagnosed GERD [59].

4.2. Oral Soft Tissue Disorders

Oral soft tissue can be damaged by GERD, too [56]. Association with GERD has been proposed for tonsillitis, mucosal atrophy, erythema of the soft palate and uvula, glossitis, epithelial atrophy, xerostomia, and dysgeusia [63]. Common oral cavity complaints in GERD patients are oral dryness, acid and bitter taste, halitosis, itching and burning, and pharyngeal discomfort [56].

GERD can induce oral mucosa damage, although mucosal changes are not pathognomonic of GERD: oral candidiasis, Sjögren syndrome, drug-related xerostomia, poor oral hygiene, dietary changes, and smoking-induced oral lesions present with similar patterns [57]. Palatal regions are typically damaged by GERD [60].

Although mucosal lesions have been found in patients with reflux disease, the literature does not evidence differences between GERD patients and healthy controls in periodontal lesions [59]. Given their non-specificity, the oral soft tissue disorders are not considered a GERD-related EE manifestation in the 2006 Montreal consensus [1].

4.3. Diagnosis

An early diagnosis and suppression of acid reflux through lifestyle changes and medication have been reported to prevent damage to the soft and hard tissues of the oral cavity [62].

This diagnosis is generally made by inspecting the oral cavity by a dentistry or dental hygienist. Assessment of the oropharynx and larynx for signs of GERD may help the clinician to establish a diagnosis and subsequent treatment of patients. Since DE is the predominant oral manifestation of GERD, dental examination plays an important role in the early diagnosis of GERD in otherwise asymptomatic patients [62]. Association with typical or atypical reflux symptoms should support the suspicion of underlying GERD.

Due to the low sensitivity of diagnostic tests such as endoscopy and pH monitoring, and the low specificity of laryngoscopy, response to acid-suppressive therapy is now considered the first diagnostic step in patients suspected of having GERD-related oral symptoms [64].

4.4. Treatment

In patients with DE, preventive and therapeutic strategies are important. Recommended strategies to stop the progression of this condition include taking antacids immediately after heartburn or after the sensation of acid reflux in the oropharynx, rinsing the mouth with neutral pH mouthwash or neutral sodium fluoride, avoiding brushing teeth immediately after reflux episodes, applying fluoride gel immediately after reflux, avoiding xerostomic medications, lubricating oral cavity with saliva substitute, or stimulating salivary flow with sugar-free chewing gum [65]. Dietary changes are recommended, too, such as avoiding highly processed acidic foods that are rich in fats and added sugars (sour candies, spicy, salty snacks, carbonated beverages, energy and sport drinks), while minimally processed and fresh acidic foods (fresh fruit, tomatoes, and savory vegetables) can be included in mixed meals [65]. Behavioral modifications include stopping smoking and good oral hygiene.

Current guidelines suggest empirical therapy with PPIs twice daily in patients with suspected GERD-related oral manifestations. There are currently no studies on the effect of anti-reflux surgical therapy on GERD-related DE. In patients with LPR who do not respond to appropriate PPI therapy, studies suggest that surgical fundoplication does not lead to a further improvement of laryngeal outcomes or throat symptoms. Therefore, surgical fundoplication is not recommended in this context, while it may be considered as a second-line therapy in patients responsive to PPI but relapsing to suspension [66].

5. Chest Pain

GERD-related chest pain is the most frequent atypical manifestation of GERD [6,7]. Although the Montreal Classification considers non-cardiac chest pain as an esophageal syndrome, we discuss it separately from the commonest symptoms of typical GERD, such as heartburn and regurgitation, given its similarity in diagnosis and treatment with EE manifestation [19,67].

GERD-related chest pain is defined as recurrent episodes of substernal pain radiating to the back, neck, jaw, or arms, which can last from minutes to hours and is due to pathological esophageal acid exposure [68].

When chest pain does not have a cardiological origin, it is defined as non-cardiac chest pain (NCCP). NCCP includes heterogeneous causes of various severity: musculoskeletal, pulmonary (pneumonia, pulmonary embolism, lung cancer, sarcoidosis, pneumothorax and pneumomediastinum, pleural effusions), vascular (aortic disorders, pulmonary hypertension), drug-related, psychological, and GI disorders (Table 1). Of these, the most frequent etiology of NCCP is GERD [68]. Focusing on epidemiology, NCCP affects both sexes equally, although females tend to consult healthcare providers more often than males. With older age, cardiac chest pain is more common, with a subsequent decrease in the prevalence of NCCP. Chest pain is a common presentation to emergency departments [69], although only 25% of individuals who experience this symptom present to a hospital [70].

Table 1. Non-Cardiac Chest Pain Etiologies. GERD: gastroesophageal reflux disease.

Etiological Site	Specific Disorder
Muscoloskeletal	Costochondritis Fibromyalgia
Esophageal	GERD Esophageal motor disorders (achalasia, hypercontractile esophagus and distal esophageal spasm) Esophageal cancer Functional chest pain Eosinophilic esophagitis
Gastrointestinal	Gastritis Pancreatitis Cholecystitis
Pulmonary	Pneumonia Pulmonary embolism Lung cancer Sarcoidosis Pneumothorax Pleural effusion
Vascular	Aortitis Aortic dissection
Miscellaneous	Herpes zoster Sickle cell crisis Psychological disorders

Beyond GERD (30–60% of cases), other esophageal causes of NCCP are esophageal dysmotility (15–30%) and esophageal hypersensitivity [68,69,71], alone or in combination.

The mechanism by which gastroesophageal reflux causes NCCP remains poorly understood. It is still unclear why esophageal exposure to gastric content in some patients causes heartburn and in others, it causes chest pain. In addition, the same patient can sometimes experience chest pain and heartburn on other occasions [68].

GERD-related chest pain is induced by abnormal exposure of the esophageal mucosa to stomach acid content. Under the physiopathological aspect, chest pain could be triggered by the stimulation of acid-sensitive chemoreceptors, mechanoreceptors, or thermoreceptors of the esophageal mucosa.

Esophageal NCCP may be alleviated by an assumption of high-dose anti-secretory drugs, although in some cases, it can benefit from nitrate treatment, complicating the differential diagnosis with angina pectoris [66]. Esophageal chest pain is often related to meals, although it can be precipitated by emotions and exercise, being harder to distinguish from cardiac chest pain [72]. Risk factors for coronary artery disease (CAD), such as smoking, obesity and diabetes, are also risk factors for esophageal abnormality and GERD, complicating the diagnostic differential [72]. CAD and GERD can also coexist, and their prevalence increases with advanced age. Hence, signs and symptoms of the latter should not be considered mutually exclusive of CAD [69]. Epidemiological data have shown that 50% of patients with coronary disease have suffered from one or more symptoms typical of GERD [69]. On the contrary, one-third to one-half of patients presenting with severe chest pain have no evidence of CAD after invasive examination [68].

Functional chest pain should undergo differential diagnosis with GERD-related chest pain. It has been defined by the ROME IV classification as a retrosternal chest pain or discomfort, without esophageal symptoms and without evidence of GERD, esophageal motor disorders, or eosinophilic esophagitis (EoE) [73] as the cause of symptoms that have occurred for the past 3 months with a frequency of at least once a week [74]. Suspected mechanisms include abnormal mechano-physical properties of the esophagus, esophageal hypersensitivity, autonomic dysregulation, and altered central processing of esophageal stimuli [74,75].

5.1. Diagnosis

When a patient complaints of chest pain, it is necessary firstly to exclude the cardiac origin of pain, using highly available tests such as electrocardiogram, echocardiography, troponin dosage, and, considering the pretest probability, more specific exams as single photon emission computed tomography (SPECT), stress echocardiography, and coronary computed tomography. Coronary angiography remains the gold standard, but it is an invasive test, and its use is limited to highly suspicious coronary ischemic pain, especially in people over 40 years old [68]. Once serious cardiac conditions have been excluded, it is crucial to rule out life-threatening conditions other than ischemic heart disease, such as pulmonary embolism, aortic dissection, and pneumothorax (Table 2).

The upper digestive tract, the biliary tree, the thoracic wall, or the pulmonary system should be further investigated in the diagnostic work-up after life-threatening conditions have been ruled out.

In the suspect of GERD-related NCCP, a PPI trial could be used by primary care physicians as the initial diagnostic tool after the exclusion of non-esophageal causes: rabeprazole 20 mg twice daily for two weeks has shown a sensitivity of 81% and specificity of 62% in diagnosing GERD-related NCCP [76]. In a systematic review of the diagnostic accuracy of the PPI test in these patients, sensitivity and specificity were 0.89 and 0.88, respectively [77]. The recommended duration is at least two weeks of treatment, and any PPI can be used, although a high dose is recommended: from 40 to 80 mg daily for omeprazole, 30–90 mg for lansoprazole, and 40 mg for rabeprazole [78]. The PPI test is defined positive if a reduction of 50–75% of symptoms burden is recorded [79].

Endoscopic pathological findings are less frequent in patients with GERD-related chest pain compared to those with typical symptoms of GERD. In fact, hiatal hernia, erosive esophagitis, and Barrett's esophagus was found in 28.6%, 19.4% and 4.4% of subjects complaining of NCCP, respectively, compared to 44.8%, 27.8%, and 9.1% of patients with typical GERD symptoms [80]. The ASGE guideline recommended EGD in patients with symptoms suggestive of complicated GERD or alarm symptoms, for follow-up of patients with severe esophagitis to rule out underlying Barrett's esophagus and to screen for Barrett's esophagus in patients with multiple risk factors [81]. When NCCP diagnosis is uncertain, it is recommended to perform upper endoscopy to diagnose other conditions apart from GERD as eosinophilic esophagitis.

The 24-h pH monitoring permits revealing reflux events by identifying pH reductions, with abnormal findings in 40–50% of the cases [71]. The AGA suggest using together with esophageal pH recording a symptom reflux association scheme to accurately diagnose when the chest pain symptom is due to gastroesophageal reflux [71]. The impact of pH-impedance measurement is relevant in patients who do not have esophagitis and do not respond to anti-secretory therapy. In fact, some patients experience chest pain triggered by non-acid reflux, which is identifiable by impedance measurement but not pH monitoring [71].

Esophageal manometry can be helpful in distinguishing GERD from esophageal motor disorders as achalasia and distal esophageal spasm [68].

5.2. Differential Diagnosis

GERD-related chest pain should be distinguished from NCCP induced by esophageal motility, visceral hypersensitivity, and disorders of gut–brain interaction such as functional esophageal chest pain, reflux hypersensitivity, and functional heartburn [74,82].

Esophageal motility disorders present with an increase of amplitude and duration of esophageal contractions, generating pain. Manometry can measure these contractions, identifying pressure changes along the entire esophageal tract. Various motility abnormalities are associated with chest pain: hypertensive LES, non-specific esophageal motor disorder, hypercontractile esophagus, distal esophageal spasm, and achalasia [69]. A temporal correlation between sustained contractions of the esophageal longitudinal muscle and esophageal chest pain has been demonstrated [83,84].

Visceral hypersensitivity is the mechanism proposed to explain esophageal NCCP in cases with normal pH measurement. In these patients, a non-pathological reflux (based on characteristics or duration) triggers painful symptoms, such as heartburn or chest pain. Visceral hypersensitivity increases the perception of stimuli due to neuronal hyperexcitability as peripheral sensitization of esophageal sensory afferents and modulation of afferent neural function at the spinal dorsal root or the central nervous system [71]. Esophageal sensitivity has been studied by instilling hydrochloric acid into the distal esophagus in subjects affected by NCCP and healthy volunteers: all patients with NCCP had a reproduction of their pain during instillation. In addition, after acid exposure, the pain threshold dropped further and for longer in NCCP patients than in healthy subjects, identifying the development of secondary allodynia (harmless visceral stimulus hypersensitivity in normal tissue close to the lesion), although its mechanism remains unclear [69,85]. Hypersensitivity to visceral and somatic pain may also be caused by central sensitization.

In several GI disorders, such as irritable bowel syndrome, an increase of mucosal mast cells (MMCs) has been shown to be associated with symptom generation. Furthermore, esophageal MMC count can be associated to visceral hypersensitivity and esophageal dysmotility [86].

Disorders of Gut–Brain Interaction (DGBI) have been extensively discussed in the Rome IV classification of functional disorders: they are defined as a group of disorders classified by GI symptoms related to any combination of motility disturbances, visceral hypersensitivity, altered mucosal and immune function, gut microbiota, and/or central nervous system processing [74]. Functional esophageal chest pain, functional heartburn, and reflux hypersensitivity are the main esophageal phenotypes of DGBI, and these are characterized by the presence of chronic symptoms attributed to the esophagus without evidence of structural, inflammatory, motor, or metabolic disorders [74]. Criteria must be fulfilled for the past 3 months with symptom onset at least 6 months before diagnosis with a frequency of at least twice a week [82]. In the suspect of GERD-related NCCP, patients should firstly undergo a high-dose PPIs trial; if there is no response, endoscopy with esophageal biopsies should be performed to rule out EoE. Afterwards, pH monitoring and esophageal manometry should therefore be performed to exclude NERD or esophageal dysmotility. Once all these investigations are negative, the symptom can be considered an expression of a functional disorder [87].

Functional chest pain accounts for more than one-third of the patients diagnosed with esophageal related NCCP; esophageal hypersensitivity, with the painful perception from normal stimuli, is the

mechanism proposed to explain this condition. Treatment goals include symptoms control and improvement in quality of life, using neuromodulators (as tricyclic anti-depressants, selective serotonin reuptake inhibitors), alternative and complementary medicine, and psychological intervention [88].

Patients with functional heartburn do not respond to PPI trial, have a normal acid exposure and negative symptom–reflux association, while those with reflux hypersensitivity present with normal acid exposure and positive symptom–reflux association [87].

Given the presence of symptoms unrelated to reflux episodes, functional heartburn is primarily treated with neuromodulators, but psychological intervention and complementary and alternative medicine may also play a role; anti-reflux surgery should be avoided [89].

Patients with reflux hypersensitivity have clinical symptoms during reflux episodes with normal esophageal acid exposure; the mainstay of treatment is esophageal neuromodulators, while surgical anti-reflux management can be used in selected cases [90]. Drugs and behavioral modifications to reduce reflux events are always recommended [90].

It should be highlighted that functional heartburn and reflux hypersensitivity can overlap with GERD [87].

5.3. Natural Course

Patients with NCCP have good outcome and higher life expectancy than those with cardiac pain. Thus, NCCP does not change the prognosis of patients with GERD [69].

Although the life expectancy of GERD patients with NCCP is not affected, QoL is often impaired by this complaint: most patients report an impairment of functional status, chronic use of drugs (PPIs, cardiac, and psychiatric), repeated hospital admissions, and multiple cardiac and non-cardiac investigations [69]. As a result, the economic impact of NCCP on the healthcare system is higher than it should be. In addition to the cost of multiple clinical and emergency room visits, hospital admissions, and prescribed drugs, indirect costs, such as loss of working days and patient QoL, should be considered [69,91].

Once it is confirmed that the esophagus is the source of pain, patients are less likely to feel disabled and reduce the request of medical evaluation. When GERD is identified as the cause of pain, anti-reflux therapy is started, generally with good outcome.

NCCP is associated with psychological diseases, such as panic disorder, anxiety, and depression, which can cause chest pain independently from GERD or enhance reflux perception [71,92]. Of all the GERD-related EE manifestations, chest pain is the most associated to psychometric abnormalities. NCCP patients with psychological disorders show lower QoL, more frequent chest pain, and lower treatment satisfaction than NCCP patients without psychological co-morbidity [92].

Table 2. Life-threatening conditions of chest pain.

Etiological Site	Life-Threatening Condition
Cardiac	STEMI (ST elevation myocardial infarction)
	Cardiac tamponade
	Cardiac wall rupture
Vascular	Aortic dissection
Pulmonary	Pulmonary embolism
	Pneumothorax
	Pneumomediastinum

5.4. Treatment

The pharmacological treatment of GERD-related chest pain is complex and still under investigation: the cornerstone is represented by PPIs and H2-blockers, with the former considered the main first-line therapy. Patients with diagnosed GERD (endoscopic findings and/or abnormal pH test) improve symptoms in 78–92% of cases with anti-reflux treatment. In contrast, response to PPI treatment in

NCCP patients without objective evidence of GERD range between 10% and 14% [93]. Furthermore, the duration of PPI therapy with has yet to be clarified, although a 2–3 month course is generally recommended [68]. On the other hand, a lack of response to PPI trial of 2 weeks should lead to the discontinuation of PPI treatment [91]. In a recent systematic review and meta-analysis, PPI treatment in GERD-related NCCP was more effective than placebo, while results in NCCP patients without GERD were inconsistent [91]. In an uncontrolled trial, 2 weeks of high-dose rabeprazole (40 mg) resulted in symptom improvement in 81% of NCCP patients with GERD, which was statistically significant when compared with non-GERD-related NCCP patients [76]. Today, a full course of treatment with double-dose PPI, over a period of 2 months, is still considered the best initial therapeutic approach for GERD-related NCCP [71].

Laparoscopic Nissen fundoplication is a surgery technique that restores the anti-reflux barrier by reinforcing EGJ basal pressures, repairing hiatal hernias, and it enhances the peristaltic function of the esophagus. Both complete and partial surgical fundoplication have been performed in patients with GERD-related NCCP: 81–96% of those with correlation of symptoms to reflux events had an improvement of symptoms after surgery compared to those without correlation [71]. Surgical fundoplication has been shown to be more effective in patients with typical GERD symptoms associated to NCCP, and in those who responded to PPI therapy, compared to those with atypical manifestations of the disease and low response to PPIs [71]. This effective procedure has some side effects: Esophagogastric junction is significantly altered after surgery, leading to more frequent motility disorders, bolus pressurization, and post-operative dysphagia. Post-operative dysphagia can affect up to 90% of post-fundoplication patients with various severity (graded in four-point Likert-like scale). Laparoscopic Nissen fundoplication is currently the "gold standard" technique for the surgical treatment of GERD, but it is indicated when an optimal dose of PPIs does not control the disease or medical long-term therapy cannot be taken [87,94].

When chest pain is due to esophageal mucosa hypersensitivity, recommended treatment includes visceral pain modulators such as tricyclic antidepressants (TCA), trazodone, adenosine antagonists, serotonin–norepinephrine reuptake inhibitors (SNRI), and selective serotonin reuptake inhibitors (SSRI). Although trials evaluating pain modulators are small and often not placebo controlled, these medications remain the mainstay of esophageal hypersensitivity. Of them, venlafaxine and sertraline have showed the most encouraging data for pain modulation in NCCP patients [68,71,92].

Given the association between NCCP and psychological disorder, cognitive behavior therapy (CBT) has been investigated as a possible intervention. Demiryoguran et al. found that in patients who underwent CBT, there was a significant reduction in the number of days with chest pain, severity of symptoms, psychological distress, reduced activity due to pain, and depressed mood compared to controls. However, further investigations are required before suggesting CBT routinely to treat NCCP [92]. CBT should be also considered in patients with elevated levels of hypervigilance and anxiety.

6. Conclusions

The diagnosis of GERD-related EE manifestation is not simple and often of exclusion. EGD plays a marginal role, being more useful if alarm symptoms are present. The 24-h esophageal pH monitoring is of relevance in the diagnostic work-up of EE manifestations. This test allows diagnosing acid reflux events in the esophagus, and when using pH impedance monitoring, refluxates of both acidic and non-acidic material into the esophagus can be identified as well. A PPI test is often used as the first diagnostic step. In atypical cases, diagnostic tools such as laryngoscopy and bronchoscopy may be useful to detect abnormalities associated with reflux damage.

Table 3 shows schematically shown the main diagnostic tools mentioned above.

Table 3. Diagnostic tools for extra-esophageal (EE) GERD. EGD: esophagogastroduodenoscopy, PPIs: proton pump inhibitors.

Diagnostic Tool	Recommendation
EGD	Recommended if alarm symptoms (weight loss, age > 50, anemia)
24-h esophageal pH monitoring	Recommended for chronic cough, asthma, laryngitis, oral cavity injury, non-cardiac chest pain, aspiration pneumonia
pH impedance monitoring	Recommended for asthma, laryngitis
PPIs trial	Recommended for chronic cough, asthma, laryngitis aspiration pneumonia, oral cavity injury
Laryngoscopy	Recommended for laryngitis
Bronchoscopy	Recommended for chronic cough

Lifestyle modifications, such as elevation of the head of the bed, weight reduction, smoking cessation, and dietary changes (reduction of fat, chocolate, alcohol, citrus, tomato, coffee and tea intake, avoidance of large meals and of eating three hours before bedtime) are always recommended, both in typical GERD and in its related EE manifestations. Pharmacological therapy is used in all forms of GERD. This is especially effective in patients with evidence of acid reflux to pH monitoring. H2 blockers are not superior to PPIs but can be used as a valid alternative. In some difficult-to-treat cases, the association between PPI an H2 blockers can be tried. The anti-reflux surgery can be used in cases of NNCP or chronic cough associated with evidence of acid reflux to pH monitoring in patients responsive but dependent from PPI therapy. In NCCP patients, due to esophageal hypersensitivity, visceral pain modulators should also be considered.

Author Contributions: M.D. and R.P. designed the review and critically revised the work; G.L. and F.C. performed the research; G.L., F.C., F.B., G.B. and A.F. wrote the paper; G.G. revised the text. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: We apologize to all the investigators whose important works have not been cited due to space restrictions.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Vakil, N.; Van Zanten, S.V.; Kahrilas, P.; Dent, J.; Jones, R.; Global Consensus Group. The Montreal definition and classification of gastroesophageal reflux disease: A global evidence-based consensus. *Am. J. Gastroenterol.* **2006**, *101*, 1900–1920. [CrossRef]
2. Kahrilas, P.J.; Altman, K.W.; Chang, A.B.; Field, S.K.; Harding, S.M.; Lane, A.P.; Lim, K.; McGarvey, L.; Smith, J.; Irwin, R.S.; et al. Chronic cough due to gastroesophageal reflux in adults: CHEST guideline and expert panel report. *Chest* **2016**, *150*, 1341–1360. [CrossRef]
3. Irwin, R.S.; Madison, J.M. Diagnosis and treatment of chronic cough due to gastro-esophageal reflux disease and postnasal drip syndrome. *Pulm. Pharmacol. Ther.* **2002**, *15*, 261–266. [CrossRef]
4. Burton, L.; Falk, G.L.; Parsons, S.; Cusi, M.; Van Der Wall, H. Benchmarking of a Simple Scintigraphic Test for Gastro-oesophageal Reflux Disease That Assesses Oesophageal Disease and Its Pulmonary Complications. *Mol. Imaging Radionucl. Ther.* **2018**, *27*, 113. [CrossRef]
5. Katz, P.O.; Gerson, L.B.; Vela, M.F. Guidelines for the diagnosis and management of gastroesophageal reflux disease. *Am. J. Gastroenterol.* **2013**, *108*, 308–328. [CrossRef]
6. Jaspersen, D.; Kulig, M.; Labenz, J.; Leodolter, A.; Lind, T.; Meyer-Sabellek, W.; Vieth, M.; Willich, S.N.; Lindner, D.; Stolte, M.; et al. Prevalence of extra-oesophageal manifestations in gastro-oesophageal reflux disease: An analysis based on the ProGERD Study. *Aliment Pharmacol. Ther.* **2003**, *17*, 1515–1520. [CrossRef] [PubMed]

7. Locke, G.R., 3rd; Talley, N.J.; Fett, S.L.; Zinsmeister, A.R.; Melton, L.J., 3rd. Prevalence and clinical spectrum of gastroesophageal reflux: A population-based study in Olmsted County, Minnesota. *Gastroenterology* **1997**, *112*, 1448–1456. [CrossRef]
8. Cesario, S.; Scida, S.; Miraglia, C.; Barchi, A.; Nouvenne, A.; Leandro, G.; Meschi, T.; De Angelis, G.L.; Di Mario, F. Diagnosis of GERD in typical and atypical manifestations. *Acta Biomed.* **2018**, *89*, 33–39. [CrossRef]
9. Broers, C.; Tack, J.; Pauwels, A. Review article: Gastro-esophageal reflux disease in asthma and chronic obstructive pulmonary disease. *Aliment. Pharmacol. Ther.* **2018**, *47*, 176–191. [CrossRef] [PubMed]
10. Ates, F.; Vaezi, M.F. Approach to the patient with presumed extraoesophageal GERD. *Best Pract. Res. Clin. Gastroenterol.* **2013**, *27*, 415–431. [CrossRef]
11. Numans, M.E.; Lau, J.; de Wit, N.J.; Bonis, P.A. Short-term treatment with proton-pump inhibitors as a test for gastroesophageal reflux disease: A meta-analysis of diagnostic test characteristics. *Ann. Intern. Med.* **2004**, *140*, 518–527. [CrossRef] [PubMed]
12. Ghisa, M.; Della Coletta, M.; Barbuscio, I.; Marabotto, E.; Barberio, B.; Frazzoni, M.; De Bortoli, N.; Zentilin, P.; Tolone, S.; Ottonello, A.; et al. Updates in the field of non-esophageal gastroesophageal reflux disorder. *Expert Rev. Gastroenterol. Hepatol.* **2019**, *13*, 827–838. [CrossRef] [PubMed]
13. Havemann, B.D.; Henderson, C.A.; El-Serag, H.B. The association between gastro-esophageal reflux disease and asthma: A systematic review. *Gut* **2007**, *56*, 1654–1664. [CrossRef] [PubMed]
14. Good, J.T., Jr.; Rollins, D.R.; Kolakowski, C.A.; Stevens, A.D.; Denson, J.L.; Martin, R.J. New insights in the diagnosis of chronic refractory cough. *Respir. Med.* **2018**, *141*, 103–110. [CrossRef]
15. Irwin, R.S.; French, C.L.; Chang, A.B.; Altman, K.W.; CHEST Expert Cough Panel. Classification of cough as a symptom in adults and management algorithms: CHEST guideline and expert panel report. *Chest* **2018**, *153*, 196–209. [CrossRef]
16. Niimi, A. Cough associated with gastro-oesophageal reflux disease (GORD): Japanese experience. *Pulm. Pharmacol. Ther.* **2017**, *47*, 59–65. [CrossRef]
17. Eastburn, M.M.; Katelaris, P.H.; Chang, A.B. Defining the relationship between gastroesophageal reflux and cough: Probabilities, possibilities and limitations. *Cough* **2007**, *3*, 4. [CrossRef]
18. Avidan, B.; Sonnenberg, A.; Schnell, T.G.; Sontag, S.J. Temporal associations between coughing or wheezing and acid reflux in asthmatics. *Gut* **2001**, *49*, 767–772. [CrossRef]
19. Sifrim, D.; Castell, D.; Dent, J.; Kahrilas, P.J. Gastro-oesophageal reflux monitoring: Review and consensus report on detection and definitions of acid, non-acid, and gas reflux. *Gut* **2004**, *53*, 1024–1031. [CrossRef]
20. Wiener, G.J.; Richter, J.E.; Copper, J.B.; Wu, W.C.; Castell, D.O. The symptom index: A clinically important parameter of ambulatory 24-hour esophageal pH monitoring. *Am. J. Gastroenterol.* **1988**, *83*, 358–361.
21. Chang, A.B.; Lasserson, T.J.; Kiljander, T.O.; Connor, F.L.; Gaffney, J.T.; Garske, L.A. Systematic review and meta-analysis of randomised controlled trials of gastro-oesophageal reflux interventions for chronic cough associated with gastro-oesophageal reflux. *Bmj* **2006**, *332*, 11–17. [CrossRef] [PubMed]
22. Michaudet, C.; Malaty, J. Chronic Cough: Evaluation and Management. *Am. Fam. Physician* **2017**, *96*, 575–580. [PubMed]
23. Shaheen, N.J.; Crockett, S.D.; Bright, S.D.; Madanick, R.D.; Buckmire, R.; Couch, M. Randomised clinical trial: High-dose acid suppression for chronic cough—A double-blind, placebo-controlled study. *Aliment. Pharmacol. Ther.* **2011**, *33*, 225–234. [CrossRef]
24. Chang, A.B.; Lasserson, T.J.; Gaffney, J.; Connor, F.L.; Garske, L.A. Gastro-oesophageal reflux treatment for prolonged non-specific cough in children and adults. *Cochrane Database Syst. Rev.* **2006**, *4*, CD004823. [CrossRef]
25. Irwin, R.S. Chronic cough due to gastroesophageal reflux disease: ACCP evidence-based clinical practice guidelines. *Chest* **2006**, *129*, 80S–94S. [CrossRef] [PubMed]
26. Reddel, H.K.; Taylor, D.R.; Bateman, E.D.; Boulet, L.P.; Boushey, H.A.; Busse, W.W.; Casale, T.B.; Chanez, P.; Enright, P.L.; Gibson, P.G.; et al. An official American Thoracic Society/European Respiratory Society statement: Asthma control and exacerbations: Standardizing endpoints for clinical asthma trials and clinical practice. *Am. J. Respir. Crit. Care Med.* **2009**, *180*, 59–99. [CrossRef]
27. Stein, M.R. Possible mechanisms of influence of esophageal acid on airway hyperresponsiveness. *Am. J. Med.* **2003**, *115*, 55S–59S. [CrossRef]

28. Irwin, R.S.; Curley, F.J.; French, C.L. Difficult-to-control asthma: Contributing factors and outcome of a systematic management protocol. *Chest* **1993**, *103*, 1662–1669. [CrossRef]
29. Gibson, P.G.; Henry, R.L.; Coughlan, J.J.L. Gastro-oesophageal reflux treatment for asthma in adults and children. *Cochrane Database Syst. Rev.* **2003**, *2*, CD001496. [CrossRef] [PubMed]
30. Gislason, T.; Janson, C.; Vermeire, P.; Plaschke, P.; Bjoïrnsson, E.; Gislason, D.; Boman, G. Respiratory symptoms and nocturnal gastroesophageal reflux: A population-based study of young adults in three European countries. *Chest* **2002**, *121*, 158–163. [CrossRef]
31. Chan, W.W.; Chiou, E.; Obstein, K.L.; Tignor, A.S.; Whitlock, T.L. The efficacy of proton pump inhibitors for the treatment of asthma in adults: A meta-analysis. *Arch. Intern. Med.* **2011**, *171*, 620–629. [CrossRef] [PubMed]
32. Harding, S.M.; Richter, J.E.; Guzzo, M.R.; Schan, C.A.; Alexander, R.W.; Bradley, L.A. Asthma and gastroesophageal reflux: Acid suppressive therapy improves asthma outcome. *Am. J. Med.* **1996**, *100*, 395–405. [CrossRef]
33. Meier, J.H.; McNally, P.R.; Punja, M.; Freeman, S.R.; Sudduth, R.H.; Stocker, N.; Perry, M.; Spaulding, H.S. Does omeprazole (Prilosec) improve respiratory function in asthmatics with gastroesophageal reflux? *Dig. Dis. Sci.* **1994**, *39*, 2127–2133. [CrossRef] [PubMed]
34. Larrain, A.; Carrasco, E.; Galleguillos, F.; Sepulveda, R.; Pope, C.E., II. Medical and surgical treatment of non-allergic asthma associated with gastroesophageal reflux. *Chest* **1991**, *99*, 1330–1335. [CrossRef]
35. Ekstrom, T.; Lindgren, B.R.; Tibbling, L. Effects of ranitidine treatment on patients with asthma and a history of gastroesophageal reflux: A double blind crossover study. *Thorax* **1989**, *44*, 12–23. [CrossRef] [PubMed]
36. Kjellen, G.; Tibbling, L.; Wranne, B. Effect of conservative treatment of oesophageal dysfunction on bronchial asthma. *Eur. J. Respir. Dis.* **1981**, *62*, 190–197.
37. Levin, T.R.; Sperling, R.M.; McQuaid, K.R. Omeprazole improves peak expiratory flow rate and quality of life in asthmatics with gastroesophageal reflux. *Am. J. Gastroenterol.* **1998**, *93*, 1060–1063. [CrossRef]
38. Goodall, R.J.; Earis, J.E.; Cooper, D.N.; Bernstein, A.; Temple, J.G. Relationship between asthma and gastro-oesophageal reflux. *Thorax* **1981**, *36*, 116–121. [CrossRef]
39. Kiljander, T.O.; Salomaa, E.R.M.; Hietanen, E.K.; Terho, E.O. Gastroesophageal reflux in asthmatics. A double-blind, placebo-controlled crossover study with omeprazole. *Chest* **1999**, *116*, 1257–1264. [CrossRef]
40. Koufman, J.A.; Aviv, J.E.; Casiano, R.R.; Shaw, G.Y. Laryngopharyngeal reflux: Position statement of the committee on speech, voice, and swallowing disorders of the American Academy of Otolaryngology-Head and Neck Surgery. *Otolaryngol. Head Neck Surg.* **2002**, *127*, 32–35. [CrossRef]
41. Zelenik, K.; Kajzrlikova, I.M.; Vitek, P.; Urban, O.; Hanousek, M.; Kominek, P. There is no correlation between signs of reflux laryngitis and reflux oesophagitis in patients with gastro-oesophageal reflux disease symptoms. *Acta Otorhinolaryngol. Ital.* **2017**, *37*, 401–405. [CrossRef] [PubMed]
42. Vaezi, M.F.; Schroeder, P.L.; Richter, J.E. Reproducibility of proximal probe pH parameters in 24-hour ambulatory esophageal pH monitoring. *Am. J. Gastroenterol.* **1997**, *92*, 825–829. [PubMed]
43. Race, C.; Chowdry, J.; Russell, J.M.; Corfe, B.M.; Riley, S.A. Studies of salivary pepsin in patients with gastro-oesophageal reflux disease. *Aliment. Pharmacol. Ther.* **2019**, *49*, 1173–1180. [CrossRef] [PubMed]
44. Woodland, P.; Singendonk, M.M.; Ooi, J.; Nikaki, K.; Wong, T.; Lee, C.; Glasinovic, E.; Koning, R.; Lutter, R.; Benninga, M.A.; et al. Measurement of salivary pepsin to detect gastroesophageal reflux disease is not ready for clinical application. *Clin. Gastroenterol. Hepatol.* **2019**, *17*, 563–565. [CrossRef]
45. Kim, T.H.; Lee, K.J.; Yeo, M.; Kim, D.K.; Cho, S.W. Pepsin detection in the sputum/saliva for the diagnosis of gastroesophageal reflux disease in patients with clinically suspected atypical gastroesophageal reflux disease symptoms. *Digestion* **2008**, *77*, 201–206. [CrossRef]
46. Saritas Yuksel, E.; Hong, S.K.; Strugala, V.; Slaughter, J.C.; Goutte, M.; Garrett, C.G.; Dettmar, P.W.; Vaezi, M.F. Rapid salivary pepsin test: Blinded assessment of test performance in gastroesophageal reflux disease. *Laryngoscope* **2012**, *122*, 1312–1316. [CrossRef]
47. Hanson, D.G.; Kamel, P.L.; Kahrilas, P.J. Outcomes of antireflux therapy for the treatment of chronic laryngitis. *Annu. Otol. Rhinol. Laryngol.* **1995**, *104*, 550–555. [CrossRef]
48. Wo, J.M.; Koopman, J.; Harrell, S.P.; Parker, K.; Winstead, W.; Lentsch, E. Double-blind, placebocontrolled trial with single-dose pantoprazole for laryngopharyngeal reflux. *Am. J. Gastroenterol.* **2006**, *101*, 1972–1978. [CrossRef]

49. Wei, C. A meta-analysis for the role of proton pump inhibitor therapy in patients with laryngopharyngeal reflux. *Eur. Arch. Otorhinolaryngol.* **2016**, *273*, 3795–3801. [CrossRef]
50. Qadeer, M.A.; Phillips, C.O.; Lopez, A.R.; Steward, D.L.; Noordzij, J.P.; Wo, J.M.; Suurna, M.; Havas, T.; Howden, C.W.; Vaezi, M.F. Proton pump inhibitor therapy for suspected GERD-related chronic laryngitis: A meta-analysis of randomized controlled trials. *Am. J. Gastroenterol.* **2006**, *101*, 2646–2654. [CrossRef]
51. Ren, L.H.; Chen, W.X.; Qian, L.J.; Li, S.; Gu, M.; Shi, R.H. Addition of prokinetics to PPI therapy in gastroesophageal reflux disease: A meta-analysis. *World J. Gastroenterol.* **2014**, *20*, 2412–2419. [CrossRef] [PubMed]
52. Koufman, J.A. Low-acid diet for recalcitrant laryngopharyngeal reflux: Therapeutic benefits and their implications. *Ann. Otol. Rhinol. Laryngol.* **2011**, *120*, 281–287. [CrossRef]
53. Min, C.; Park, B.; Sim, S.; Choi, H.G. Dietary modification for laryngopharyngeal reflux: Systematic review. *J. Laryngol. Otol.* **2019**, *133*, 80–86. [CrossRef] [PubMed]
54. Filipi, K.; Halackova, Z.; Filipi, V. Oral health status, salivary factors and microbial analysis in patients with active gastro-oesophageal reflux disease. *Int. Dent. J.* **2011**, *61*, 231–237. [CrossRef] [PubMed]
55. Ghezzi, E.M.; Lange, L.A.; Ship, J.A. Determination of variation of stimulated salivary flow rates. *J. Dent. Res.* **2000**, *79*, 1874–1878. [CrossRef] [PubMed]
56. Watanabe, M.; Nakatani, E.; Yoshikawa, H.; Kanno, T.; Nariai, Y.; Yoshino, A.; Vieth, M.; Kinoshita, Y.; Sekine, J. Oral soft tissue disorders are associated with gastroesophageal reflux disease: Retrospective study. *BMC Gastroenterol.* **2017**, *17*, 92. [CrossRef]
57. Campisi, G.; Lo Russo, L.; Di Liberto, C.; Di Nicola, F.; Butera, D.; Vigneri, S.; Compilato, D.; Lo Muzio, L.; Di Fede, O. Saliva variations in gastro-oesophageal reflux disease. *J. Dent.* **2008**, *36*, 268–271. [CrossRef]
58. Yoshikawa, H.; Furuta, K.; Ueno, M.; Egawa, M.; Yoshino, A.; Kondo, S.; Nariai, Y.; Ishibashi, H.; Kinoshita, Y.; Sekine, J. Oral symptoms including dental erosion in gastroesophageal reflux disease are associated with decreased salivary flow volume and swallowing function. *J. Gastroenterol.* **2012**, *47*, 412–420. [CrossRef] [PubMed]
59. Muñoz, J.V.; Herreros, B.; Sanchiz, V.; Amoros, C.; Hernandez, V.; Pascual, I.; Mora, F.; Minguez, M.; Bagan, J.V.; Benages, A. Dental and periodontal lesions in patients with gastro-oesophageal reflux disease. *Dig. Liver Dis.* **2003**, *35*, 461–467. [CrossRef]
60. Ranjitkar, S.; Smales, R.J.; Kaidonis, J.A. Oral manifestations of gastroesophageal reflux disease. *J. Gastroenterol. Hepatol.* **2012**, *27*, 21–27. [CrossRef]
61. Farrokhi, F.; Vaezi, M.F. Extra-esophageal manifestations of gastroesophageal reflux. *Oral Dis.* **2007**, *13*, 349–359. [CrossRef]
62. Kothimbakkam, S.S.K.; Jayanthi, M.; Nilaya, R.V.; Poornima, V.; Deebiga, K. Oral manifestations of gastroesophageal reflux disease in children: A preliminary observational study. *J. Indian Soc. Pedod. Prev. Dent.* **2018**, *36*, 125–129. [CrossRef]
63. Romano, C.; Cardile, S. Gastroesophageal reflux disease and oral manifestations. *Ital. J. Pediatr.* **2014**, *40*, A73. [CrossRef]
64. Charbel, S.; Khandwala, F.; Vaezi, M.F. The role of esophageal pH monitoring in symptomatic patients on PPI therapy. *Am. J. Gastroenterol.* **2005**, *100*, 283–289. [CrossRef]
65. Marshall, T.A. Dietary assessment and counseling for dental erosion. *J. Am. Dent. Assoc.* **2018**, *149*, 148–152. [CrossRef] [PubMed]
66. Klaus, A.; Swain, J.M.; Hinder, R.A. Laparoscopic antireflux surgery for supraesophageal complications of gastroesophageal reflux disease. *Am. J. Med.* **2001**, *111*, 202S–206S. [CrossRef]
67. Saritas Yuksel, E.; Vaezi, M. Extraesophageal manifestations of gastroesophageal reflux disease: Cough, asthma, laryngitis, chest pain. *Swiss Med. Wkly.* **2012**, *142*, w13544. [CrossRef] [PubMed]
68. Durazzo, M.; Gargiulo, G.; Pellicano, R. Non-cardiac chest pain: A 2018 update. *Minerva Cardioangiol.* **2018**, *66*, 770–783. [CrossRef]
69. Fass, R.; Achem, S.R. Noncardiac chest pain: Epidemiology, natural course and pathogenesis. *J. Neurogastroenterol. Motil.* **2011**, *17*, 110–123. [CrossRef]
70. Ballarino, P.; Cervellin, G.; Trucchi, C.; Altomonte, F.; Bertini, A.; Bonfanti, L.; Bressan, M.A.; Carpinteri, G.; Noto, P.; Gavelli, F.; et al. An Italian registry of chest pain patients in the emergency department: Clinical predictors of acute coronary syndrome. *Minerva Med.* **2020**, *111*, 120–132. [CrossRef]

71. Yamasaki, T.; Fass, R. Noncardiac chest pain: Diagnosis and management. *Curr. Opin. Gastroenterol.* **2017**, *33*, 293–300. [CrossRef] [PubMed]
72. Heatley, M.; Rose, K.; Weston, C. The heart and the oesophagus: Intimate relations. *Postgrad. Med. J.* **2005**, *81*, 515–518. [CrossRef]
73. Mari, A.; Abu Baker, F.; Mahamid, M.; Khoury, T.; Sbeit, W.; Pellicano, R. Eosinophilic esophagitis: Pitfalls and controversies in diagnosis and management. *Minerva Med.* **2020**, *111*, 9–17. [CrossRef] [PubMed]
74. Drossman, D.A. Functional gastrointestinal disorders: History, pathophysiology, clinical features, and Rome IV. *Gastroenterology* **2016**, *150*, 1262–1279. [CrossRef]
75. Aziz, Q.; Fass, R.; Gyawali, C.P.; Miwa, H.; Pandolfino, J.E.; Zerbib, F. Functional esophageal disorders. *Gastroenterology* **2016**, *150*, 1368–1379. [CrossRef]
76. Kim, J.H.; Sinn, D.H.; Son, H.J.; Kim, J.J.; Rhee, J.C.; Rhee, P.L. Comparison of one-week and two-week empirical trial with a high-dose rabeprazole in noncardiac chest pain patients. *J. Gastroenterol. Hepatol.* **2009**, *24*, 1504–1509. [CrossRef] [PubMed]
77. Wertli, M.M.; Ruchti, K.B.; Steurer, J.; Held, U. Diagnostic indicators of noncardiovascular chest pain: A systematic review and meta-analysis. *BMC Med.* **2013**, *11*, 239. [CrossRef] [PubMed]
78. Dickman, R.; Emmons, S.; Cui, H.; Sewell, J.; Hernández, D.; Esquivel, R.F.; Fass, R. The effect of a therapeutic trial of high-dose rabeprazole on symptom response of patients with non-cardiac chest pain: A randomized, double-blind, placebo-controlled, crossover trial. *Aliment. Pharmacol. Ther.* **2005**, *22*, 547–555. [CrossRef]
79. Fass, R. Empirical trials in treatment of gastroesophageal reflux disease. *Dig. Dis.* **2000**, *18*, 20–26. [CrossRef]
80. Dickman, R.; Mattek, N.; Holub, J.; Peters, D.; Fass, R. Prevalence of upper gastrointestinal tract findings in patients with noncardiac chest pain versus those with gastroesophageal reflux disease (GERD) related symptoms: Results from a national endoscopic database. *Am. J. Gastroenterol.* **2007**, *102*, 1173–1179. [CrossRef]
81. ASGE Standards of Practice Committee; Muthusamy, V.R.; Lightdale, J.R.; Acosta, R.D.; Chandrasekhara, V.; Chathadi, K.V.; Eloubeidi, M.A.; Fanelli, R.D.; Fonkalsrud, L.; Faulx, A.L.; et al. The role of endoscopy in the management of GERD. *Gastrointest. Endosc.* **2015**, *81*, 1305–1310. [CrossRef] [PubMed]
82. Schmulson, M. How to use Rome IV criteria in the evaluation of esophageal disorders. *Curr. Opin. Gastroenterol.* **2018**, *34*, 258–265. [CrossRef] [PubMed]
83. Balaban, D.H.; Yamamoto, Y.; Liu, J.; Pehlivanov, N.; Wisniewski, R.; DeSilvey, D.; Mittal, R.K. Sustained esophageal contraction: A marker of esophageal chest pain identified by intraluminal ultrasonography. *Gastroenterology* **1999**, *116*, 29–37. [CrossRef]
84. Pehlivanov, N.; Liu, J.; Mittal, R.K. Sustained esophageal contraction: A motor correlate of heartburn symptom. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2001**, *281*, G743–G751. [CrossRef] [PubMed]
85. Sarkar, S.; Thompson, D.G.; Woolf, C.J.; Hobson, A.R.; Millane, T.; Aziz, Q. Patients with chest pain and occult gastroesophageal reflux demonstrate visceral pain hypersensitivity which may be partially responsive to acid suppression. *Am. J. Gastroenterol.* **2004**, *99*, 1998–2006. [CrossRef]
86. Park, S.W.; Lee, H.; Lee, H.J.; Chung, H.; Park, J.C.; Shin, S.K.; Lee, S.K.; Lee, Y.C. Esophageal mucosal mast cell infiltration and changes in segmental smooth muscle contraction in noncardiac chest pain. *Dis. Esophagus* **2015**, *28*, 512–519. [CrossRef]
87. Marjoux, S.; Roman, S.; Juget-Pietu, F.; Robert, M.; Poncet, G.; Boulez, J.; Mion, F. Impaired postoperative EGJ relaxation as a determinant of post laparoscopic fundoplication dysphagia: A study with high-resolution manometry before and after surgery. *Surg. Endosc.* **2012**, *26*, 3642–3649. [CrossRef]
88. Shaker, R.; Babaei, A.; Naini, S.R. Prevention of esophagopharyngeal reflux by augmenting the upper esophageal sphincter pressure barrier. *Laryngoscope* **2014**, *124*, 2268–2274. [CrossRef]
89. Yamasaki, T.; O'Neil, J.; Fass, R. Update on Functional Heartburn. *Gastroenterol. Hepatol.* **2017**, *13*, 725–734.
90. Yamasaki, T.; Fass, R. Reflux hypersensitivity: A new functional esophageal disorder. *J. Neurogastroenterol. Motil.* **2017**, *23*, 495–503. [CrossRef]
91. Burgstaller, J.M.; Jenni, B.F.; Steurer, J.; Held, U.; Wertli, M.M. Treatment efficacy for noncardiovascular chest pain: A systematic review and meta-analysis. *PLoS ONE* **2014**, *9*, e104722. [CrossRef] [PubMed]
92. Demiryoguran, N.S.; Karcioglu, O.; Topacoglu, H.; Kiyan, S.; Ozbay, D.; Onur, E.; Korkmaz, T.; Demir, O.F. Anxiety disorder in patients with non-specific chest pain in the emergency setting. *Emerg. Med. J.* **2006**, *23*, 99–102. [CrossRef] [PubMed]

93. Kushnir, V.M.; Sayuk, G.S.; Gyawali, C.P. Abnormal GERD parameters on ambulatory pH monitoring predict therapeutic success in noncardiac chest pain. *Am. J. Gastroenterol.* **2010**, *105*, 1032–1038. [CrossRef] [PubMed]
94. Rerych, K.; Kurek, J.; Klimacka-Nawrot, E.; Błońska-Fajfrowska, B.; Stadnicki, A. High-resolution manometry in patients with gastroesophageal reflux disease before and after fundoplication. *J. Neurogastroenterol. Motil.* **2017**, *23*, 55. [CrossRef]

Journal of
Clinical Medicine

MDPI

Review

Diagnosing Constipation Spectrum Disorders in a Primary Care Setting

Joel Heidelbaugh [1], Nicole Martinez de Andino [2], David Pineles [3] and David M. Poppers [3,4,*]

[1] Department of Family Medicine, University of Michigan, Ann Arbor, MI 48109, USA; jheidel@med.umich.edu
[2] Division of Gastroenterology/Hepatology, Department of Surgery, Medical College of Georgia, Augusta University, Augusta, GA 30912, USA; nmartinezdeandino@augusta.edu
[3] Division of Gastroenterology, Department of Medicine, NYU Langone Health, New York, NY 10022, USA; David.Pineles@nyulangone.org
[4] Center for Advanced Therapeutics and Innovation, NYU Langone Health, New York, NY 10022, USA
* Correspondence: David.Poppers@nyulangone.org; Tel.: +1-617-835-7677

Abstract: Understanding pathophysiological causes of constipation is worthwhile in directing therapy and improving symptoms. This review aims to identify and fill gaps in the understanding of the pathophysiology of constipation, understand its prevalence, review diagnostic tools available to primary care physicians (PCPs), and highlight patients' expectations for the management of this common spectrum of disorders. Literature searches conducted via PubMed included terms related to constipation, diagnosis, and patient perceptions. Case studies were developed to highlight the differences between patients who may be appropriately managed in the primary care setting and those requiring specialty consultation. Myriad pathophysiological factors may contribute to constipation, including stool consistency, altered intestinal motility, gut microbiome, anorectal abnormalities, as well as behavioral and psychological factors. Common diagnoses of "primary constipation" include slow-transit constipation, defecation disorders, irritable bowel syndrome with constipation, and chronic idiopathic constipation. A detailed medical history should be conducted to exclude alarm features and PCPs should be familiar with pathophysiological factors that cause constipation, available diagnostic tools, alarm signs, and the various classification criteria for constipation subtypes in order to diagnose and treat patients accordingly. PCPs should understand when a referral to a gastroenterologist, anorectal specialist, pelvic floor physical therapist, and/or mental health specialist is appropriate.

Keywords: chronic idiopathic constipation; constipation; irritable bowel syndrome; pathophysiology; primary care

Citation: Heidelbaugh, J.; Martinez de Andino, N.; Pineles, D.; Poppers, D.M. Diagnosing Constipation Spectrum Disorders in a Primary Care Setting. *J. Clin. Med.* **2021**, *10*, 1092. https://doi.org/10.3390/jcm10051092

Academic Editor: Marilena Durazzo

Received: 17 February 2021
Accepted: 2 March 2021
Published: 5 March 2021

1. Introduction

Chronic constipation affects up to 14% of people during their lifetime [1]. Therefore, primary care physicians (PCPs) need to be familiar with constipation subtypes and their diagnosis. PCPs are equipped to manage constipation and should be confident in determining the cause of their patient's constipation [2] to ensure that appropriate treatment options are considered and recommended. Finally, PCPs should be aware when subspecialty referral is advised, such as when worrisome features ("red-flags") are present.

Chronic constipation is a gastrointestinal disorder that is characterized by lumpy or hard stools, infrequent bowel movements, abdominal cramping, bloating, excessive straining, and/or the sensation of incomplete defecation or "evacuation" [3]. The Rome IV criteria provide a straight-forward guide for diagnosing chronic constipation [4]. A key tool that can be used to aid in diagnosis of constipation is the Bristol Stool Form Scale (BSFS; Figure 1), which categorizes stool form on a graded 7-point scale ranging from separate hard lumps that are difficult to evacuate (BSFS type 1) to mushy, watery stools (BSFS type 7) [5]. Amongst others, the criteria for constipation include BSFS type 1 or

type 2 stools in over 25% of bowel movements and less than 25% of BSFS type 6 or type 7 stools [4]. Patients may also provide self-diagnoses based on their complaints (i.e., hard stools or straining) or by the use of laxatives to relieve symptoms, and it is worthwhile to note that there are discrepancies in the perception of constipation between primary physicians and the patients whom they treat [6].

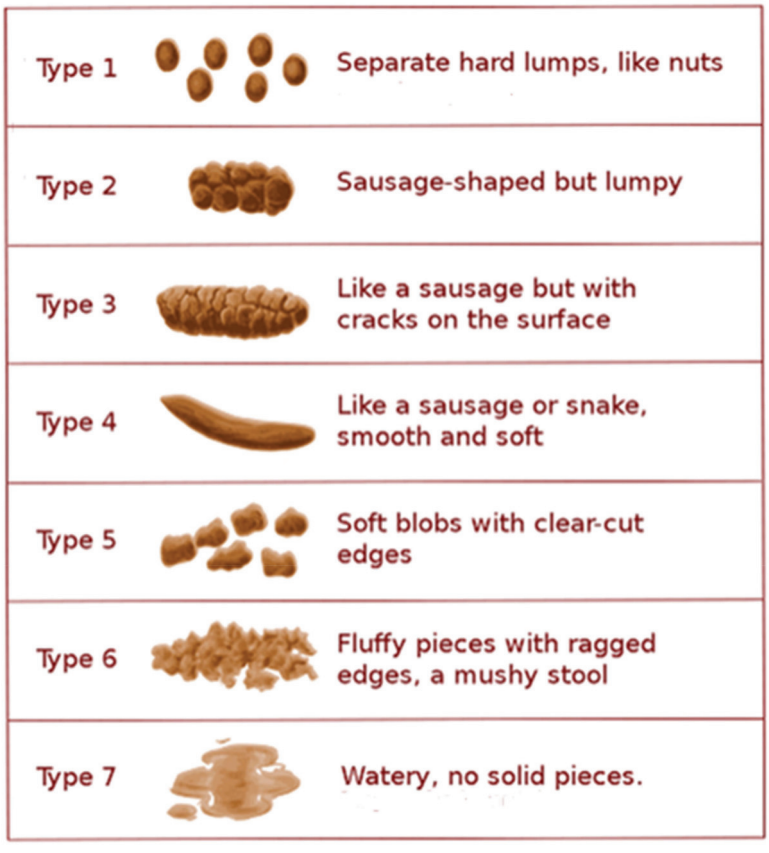


Figure 1. Bristol Stool Form Scale. Copyright 2000 © by Rome Foundation. All Rights Reserved.

Possible causes of constipation are myriad and include insufficient liquid and/or fiber intake, abnormalities in colonic motility, reduced exercise, and physical disorders (e.g., neuromuscular disorders) [7]. Although insufficient liquid intake or limited exercise alone may not be the sole contributing factor in causing constipation, there is evidence suggesting that improved liquid intake and physical activity can improve constipation symptoms in certain patients [8]. Chronic constipation may or may not have an identifiable cause; causes may be primary (related to intrinsic gastrointestinal structure and function) or secondary (related to systemic disease or medication) [1,9]. An identifiable cause of constipation is unknown in the largest subset of chronic constipation sufferers [10]. Primary constipation can be caused by functional colonic abnormalities or by defects in the process of defecation itself [9]. Primary constipation may be present in patients with inflammatory bowel disease (IBD), which includes Crohn's disease (CD), ulcerative colitis (UC) [11], and indeterminant colitis. IBD is more classically associated with diarrhea and bloody stools; however, IBD represents a spectrum in which altered motility and obstruction may occur. Secondary constipation often has an identifiable cause related to medication use or to other underlying

disease processes [1,9]. Secondary constipation may be associated with medication side effects, bowel obstruction (including secondary to adhesions, malignancy, benign strictures, or extrinsic bowel compression from other organs or abnormal lesions within the peritoneal cavity), metabolic disorders (e.g., hypothyroidism, hypercalcemia), neurological disorders (e.g., Parkinson's disease, multiple sclerosis), other systemic disorders (e.g., scleroderma, amyloidosis), as well as psychological disorders (e.g., depression, eating disorders) [9].

2. Patient's Perspective of Constipation Spectrum Disorders

A patient's quality of life (QoL) is understandably negatively correlated with the severity of constipation [1,3,12]. While constipation is defined by characteristics of a patient's bowel habits, the patients underlying concerns may be unrelated (or only partially related) to their bowel movements [1]. The symptom that patients perceive as the most severe is normally the one that they feel is the most bothersome; this may include pain, bloating, abdominal discomfort, or some combination thereof [12]. Patients often report an increasing severity of their symptoms over time (i.e., the longer they have symptoms, the more severe and bothersome they perceive them to be) [12]. Patients often utilize over the counter (OTC) therapies prior to discussing their symptoms with their PCPs. They are often dissatisfied with the results of traditional first-line therapies (such as fiber and over-the-counter laxatives), potentially because these focus on symptomatic management rather than addressing the underlying *causes* of their chronic constipation [12]. It is important to discuss previous treatments with patients in detail because isolated fibers (supplements) may have varying effects on constipation; both psyllium and coarse wheat bran improved symptoms, while finely ground wheat bran may have an unwanted stool-hardening effect [13]. In addition, fiber and/or laxatives do not benefit patients with certain types of chronic constipation, such as functional defecation disorders (DDs), and in fact may exacerbate these issues in a subset of these patients [12,14].

Many patients with constipation have become accepting of the physical and QoL limitations of their symptoms [3] and may not be aware of available effective treatments. Constipation may be associated with depression, anxiety, and other psychosocial issues [12]. It is therefore recommended that PCPs explain to patients how chronic constipation may impact their QoL. In certain cases, in which significant impact upon QoL is noted, early involvement of mental health professionals may be beneficial.

In the absence of alarm symptoms, the most important role of the PCP is to consider how to best manage the expectations of patients with chronic constipation by discussing, among other issues, what tests might need to be performed to confirm (or exclude) diagnoses, the available treatment options and their likelihood of success, potential treatment-related adverse events (such as diarrhea), and options to consider if initial lines of therapy are unsuccessful or do not yield adequate relief. In many cases, empiric therapy may be recommended without diagnostic investigation; however, some limited testing may be clinically warranted depending on the individual patient (e.g., complete blood count, thyroid-stimulating hormone, complete metabolic panel, age-appropriate colorectal cancer screening) [4].

3. Diagnosing Constipation in a Primary Care Setting

The diagnosis and clinical presentation of constipation may also be influenced by patient factors. The prevalence of constipation can vary depending on gender, age, race, and socioeconomic status [15,16]. Women experience constipation at a rate 2.2-fold higher than that in men [17]. While a younger patient population may report increased constipation symptom severity [18], the prevalence of constipation increases with age and is much higher in patients aged $\geq$ 65 years [15,17]. Older patients with higher rates of polypharmacy may also be at risk for drug–drug interactions, thereby complicating treatment efficacy and safety [19,20]. Race and socioeconomic status may also be factors in the risk of developing constipation, with a higher rate of constipation reported in non-white versus white patients and in those of lower socioeconomic status [16,21].

There exist only a few widely used, validated, and standardized tools for the classification of constipation. The BSFS categorizes stool forms ranging from liquid stools to stools that are hard and lumpy in consistency (Figure 1) [5]. By asking patients to indicate their stool form on the BSFS chart, insight can be gained as to the nature of the patient's stool and whether they are consistent with constipation. Rome IV provides criteria to aid in the diagnosis and subclassification of functional GI disorders, including CIC (i.e., functional constipation) and IBS-C (Table 1). According to Rome IV, a constipation diagnosis should be made following a clinical history, physical examination, minimal laboratory tests, and, when clinically appropriate, a colonoscopy or other diagnostic test (such as age-appropriate colorectal cancer screening) [4]. Although currently the Rome criteria may not be widely used by PCPs, it a useful tool that, when utilized in the primary care setting, would help standardize constipation diagnosis and drive appropriate treatment decisions.

Table 1. Comparison between symptoms of IBS-C and CIC.

Condition	Rome IV Criteria for Diagnosis	Other Considerations
IBS-C	• Recurrent abdominal pain (≥1 per week • Change in stool frequency • Change in form of stool • ≥ 25% of bowels movements are BSFS type 1 or 2 • < 25% of bowel movements are BSFS type 6 or 7 [4,10,22]	• Patients must present with abdominal pain for a diagnosis of IBS [2] • Abdominal bloating is often present, though not required for diagnosis [2] • Abdominal pain or discomfort may be relieved with defecation [23,24]
CIC	• Patients should meet ≥2 of the following in the last 3 months [4,10] • < 3 bowel movements/week • Straining for > 25% of bowel movements • Lumpy or hard stools (BSFS type 1 or 2) for > 25% of bowel movements • Sensation of incomplete defecation in > 25% of bowel movements • Sensation of anorectal obstruction/blockage in > 25% of bowel movements • Manual maneuvers to facilitate > 25% of bowel movements • Patients do not meet the Rome IV criteria for IBS-C	• Patients who have some similar symptoms to IBS-C but who do not meet IBS-C criteria are diagnosed with CIC [4,10] • CIC is commonly determined by the frequency of bowel movements [3] • Patients may experience bloating, abdominal pain, or discomfort, but these are not considered as main symptoms for CIC [4,9,10]

BSFS, Bristol Stool Form Scale; CIC, chronic idiopathic constipation; IBS-C, irritable bowel syndrome with constipation.

Internists and other primary physicians should enquire if, and how, a patient may have already attempted to manage their constipation. On average, patients used three OTC products before consulting a health care professional [3]. The failure or inadequacy of previous therapies, and behavioral, dietary, and lifestyle modifications may provide insight into a potential diagnosis [1] as well as the next diagnostic and therapeutic steps that may be considered, potentially minimizing unnecessary delays in treatment escalation or specialist referral. Common OTC therapies for constipation include supplemental fiber, stool softeners, probiotics, prebiotics, and nonprescription laxatives, which are relatively cost-effective compared to prescription treatment [25]. However, only 40% of patients report satisfaction with OTC laxatives [3].

PCPs must capture a detailed clinical history to exclude alarm symptoms (which may indicate a more serious health problem) and evaluate for common comorbidities that may be driving secondary causes of constipation [4]. These alarm symptoms that can present with constipation include unintentional weight loss, iron-deficiency anemia, hematochezia (rectal bleeding/bloody bowel movements), new onset of symptoms at

age 50 or older, and/or severe, persistent, and treatment-refractory constipation [2,26]. Patients with the above symptoms (or other "red flag" symptoms) and those with a family history of colorectal cancer, IBD, or celiac disease should also be considered for expedited specialist referral [1,2]. When taking a patient's history, secondary constipation should be considered [9]. PCPs should be aware if their patient is taking certain medications that can cause secondary constipation [1] and if they have a history of neurologic, endocrine, and metabolic disorders, which may be associated with constipation (Table 2). Opioids alone or in combination with other medications may contribute to chronic constipation [1]. Several therapies specifically target opioid-induced constipation [27], and should be considered as part of the management strategy when opioid-induced constipation is diagnosed. Colorectal cancer screening should be pursued if the patient is not up to date with recommendations [4].

Table 2. Causes of secondary constipation [1].

Secondary Constipation Subtype	Possible Underlying Causes
Medications	Iron supplements, calcium supplements, antidepressants, antihypertensive drugs, opioids, antihistamines, anticholinergics, etc.
Neurological, endocrine, and metabolic	Autonomic neuropathy, Parkinson's disease, multiple sclerosis, spina bifida, spinal cord injuries, diabetes, hypothyroidism, hypercalcemia, pregnancy
Bowel obstruction	Obstructing colonic cancer, luminal stenosis, abdominal adhesions, etc.

Based on the individual patients' history, their risk factors, and the clinician's degree of suspicion with regard to the cause of their constipation, PCPs should determine whether excluding other etiologies by objective testing, imaging, etc., is necessary. However, in the absence of alarm symptoms, if there are comorbidities potentially contributing to constipation, primary physicians can typically manage by empiric therapy and monitoring of outcomes.

Primary constipation is often a complex condition that can be challenging for primary physicians (and indeed subspecialists) to manage [22]. If constipation and its associated symptoms are severe, not improving with conservation and first-line therapies, or are of unclear etiology, the patient should likewise be considered for specialist referral (Figure 2) [2].

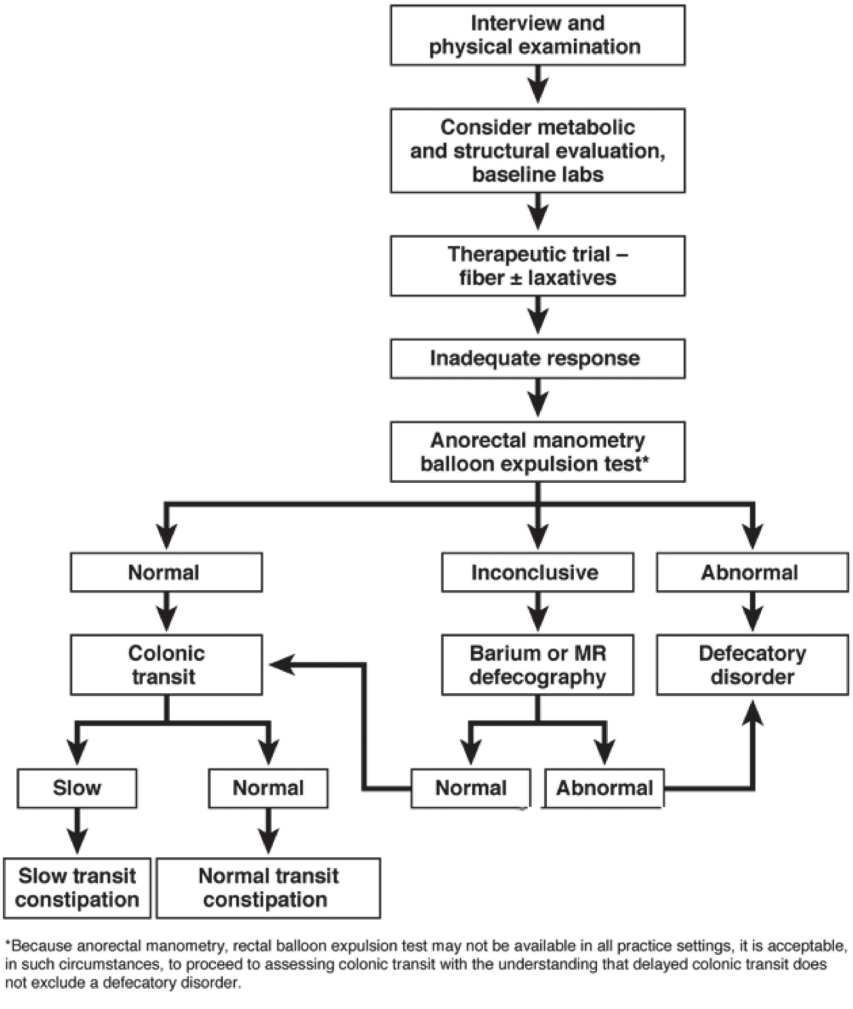


Figure 2. Diagnosing chronic constipation, Reprinted from *Gastroenterology*, Vol. 144, Bharucha, A.E., Dorn, S.D., Lembo, A., Pressman, A., American Gastroenterological Association Medical Position Statement on Constipation, Pages 211–217, 2013, with permission from Elsevier [28]. * As anorectal manometry/rectal balloon expulsion test may not be available in all practice settings, it is acceptable in such circumstances to proceed to assessing colonic transit with the understanding that delayed colonic transit does not exclude a defecatory disorder. MR, magnetic resonance.

As part of a detailed physical exam, abdominal and digital rectal examination (with consideration of in-office anoscopic evaluation in selected patients) should be performed at the time of discussion of the patient's presenting symptoms to help begin to elucidate the cause of chronic constipation [29]. Explaining the purpose and nature of the digital rectal examination to patients with chronic constipation is recommended, as well as the relevance of anorectal anatomy as it relates to defecation and stool evacuation. This topic should be broached sensitively, particularly in select patient populations. A digital rectal exam should be performed in the presence of chronic constipation to assess for rectal tone, puborectalis muscle function, inappropriate anal contraction during defecation, abnormal perineal descent during defecatory effort, and the suggestion of potential dyssynergic defecation; related postural and respiratory function issues may also be assessed during a physical

exam [30]. Palpable abnormalities during a digital rectal exam may require further testing or examination, including imaging studies, manometric or other studies, and possibly consultation by other medical colleagues, such as gastroenterologists, specialists in anorectal disorders (including colorectal surgeons), and pelvic floor–trained physical therapists. PCPs should know how to perform a proper digital rectal examination and assess tone and perianal decent as it is often a revealing element to the clinical evaluation [29]. References are available to guide performing and interpreting digital rectal examinations [31].

3.1. Primary Constipation Diagnoses

Irritable bowel syndrome with constipation (IBS-C) is diagnosed using the Rome IV criteria, in which abdominal pain is required for a diagnosis [4]. Abdominal bloating is a very common feature of IBS but is not required for this diagnosis (Table 1) [2]. IBS-C is complex and can involve multiple mechanisms [2,22]. Specifically, IBS-C may be associated with alterations in motor and secretory functions of the gut [32,33], gut microbiota ("the gut microbiome"), visceral hypersensitivity or hyperalgesia, mucosal dysfunction, and immune dysfunction [22,34]. When the intestinal mucosal barrier is impaired, bacteria may be able to traverse this barrier, which can cause gastrointestinal pain and exacerbate baseline psychological disorders, such as anxiety and depression [22]. IBS is the most commonly diagnosed gastrointestinal tract disorder that is associated with psychological factors [22], although it is important to note that not all psychological conditions lead to the development of IBS. Stress can affect the brain–gut connection (a connection between the central nervous system and enteric/gut-based nervous system), resulting in abnormalities in colonic motility, such as prolonged colonic transit [22]. Impaired serotonin release has been observed in patients with IBS-C [22,35].

Chronic idiopathic constipation is the most commonly diagnosed subtype of chronic constipation [9,10] and can be divided into normal-transit constipation, slow-transit constipation (STC), and DDs [4]. CIC is diagnosed when there are no identifiable physiological or biochemical etiologies of the symptom complex [10]. According to the Rome IV criteria, CIC is diagnosed when a patient presents with chronic constipation but does not meet all the criteria for IBS-C; the main difference is that pain is not a predominant symptom or may not be present in CIC [4,10,36]. Abdominal bloating, when present, can be a challenging symptom of CIC [37]. Patients with CIC may respond well to increased dietary fiber and other conservative measures, although these measures may potentially exacerbate abdominal bloating. These patients may also require more intensive or targeted pharmacologic therapies [9]. Slow-transit constipation is characterized by infrequent bowel movements (typically fewer than once per week), decreased defecatory urge, and bloating or abdominal discomfort [9]. Patients with STC have a prolonged colonic transit time [9]. STC is thought to be caused by a neuromuscular disorder of the colon [9]. For example, patients may have a decreased number of interstitial cells of Cajal (ICCs), which help regulate contractions in the gastrointestinal tract [9]. Colonic transit time may be assessed using a variety of techniques, including radiopaque markers, wireless motility capsules, or scintigraphy [1,38].

Defecation disorders are heterogeneous in nature. Some may be characterized by excessive straining [9]; however, symptoms have a limited utility in determining a DD diagnosis [39]. Patients tend to spend large amounts of time on the toilet each day [9]. In addition, increased pelvic floor tone in patients with DD may increase their risk of hemorrhoids and anal fissure [9]. Underlying structural or mechanical abnormalities may be present [9]. Laxatives are often ineffective and patients may have difficulty evacuating liquid stools [9]. Assessing for dyssynergia includes evaluating a patient for paradoxical increases in anal contraction or decreases in resting anal sphincter pressure or inadequate propulsive forces [9]. A digital rectal exam can assess for abnormal anal contraction while straining, in which case further specialist testing may be needed to confirm a specific defecatory disorder [2]. Gastroenterologists with a focus on anorectal disorders may perform additional diagnostic tests, including anorectal manometry, balloon expulsion testing, and

magnetic resonance (MR) defecography with qualified radiology colleagues, along with pelvic floor physical therapy and biofeedback in concert with those appropriately trained and experienced in this area (Figure 2) [9].

3.2. Overlap Between IBS-C and CIC

There is often significant overlap between IBS-C and CIC. As such, both conditions are often considered to exist on a spectrum of functional constipation disorders rather than as distinct entities, often making it challenging to distinguish in the primary care as well as specialty clinical setting (Table 1) [1,2,4,23,24,36]. Per Rome IV criteria, abdominal pain is the discriminating factor of IBS-C as compared with CIC [2,36]. Pain may be a marker of disease severity, but not necessarily a distinguishing factor in all cases per se [1,2]. Indeed, at times, an individual's symptoms may fluctuate between those more consistent with CIC or IBS-C [2,23].

4. Pathophysiology

Many pathophysiological factors can cause constipation, including abnormalities in colonic absorption, colonic motility, as well as behavioral and psychological factors [1]. Water content, and thereby stool consistency, may correlate with colonic transit time [1,9,22]. Secretion of water into the intestinal lumen is essential for normal stool consistency. The longer stools take to pass through the colon, the more water is absorbed by the colon, thereby increasing the firm consistency of stool [9]. This process may contribute to issues such as the passage of small hard stools (BSFS type 1), or large hard stools (BSFS type 2), both of which may be more difficult for a patient to evacuate (Figure 1) [9]. Water and solute secretion into the intestinal lumen are essential for lubrication and influence stool consistency [10]. Fluid secretion into the gastrointestinal tract is in part regulated by guanylate cyclase-C (GC-C) [10]. Patients with constipation may have impaired or decreased expression of GC-C compared with an unaffected cohort [40]. In contrast, increased GC-C expression may lead to diarrhea [10]. Accordingly, the GC-C receptor is a pharmacologic target for medications, such as plecanatide (currently only approved for use in the US) and linaclotide, that activate this system and thereby enhance fluid secretion into the gut (Figure 3) [24,41]. Although otherwise associated with few adverse events in adults, these agents are associated with an increase in diarrhea likely related to the mechanism of action [24,41]. Different rates of diarrhea between the two may be attributable to pH-independence and affinity to the GC-C receptor [34].

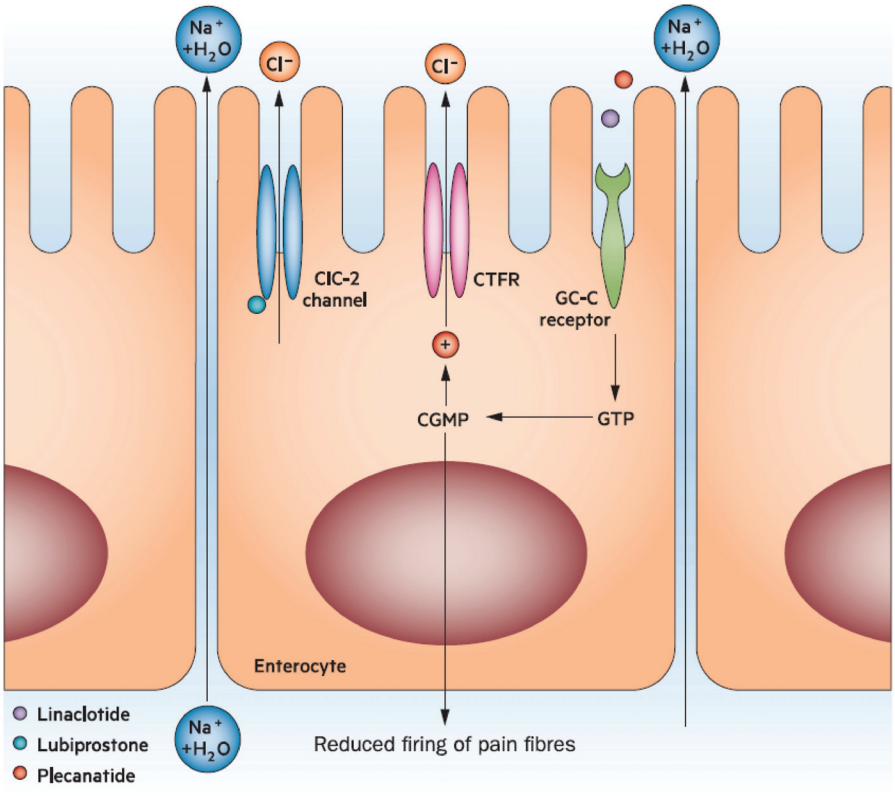


Figure 3. Mechanism of action of common therapies used to manage chronic constipation. Reprinted by permission from Springer Nature Custom Service Center GmbH: Nature Reviews Gastroenterology & Hepatology Agents that act luminally to treat diarrhea and constipation. Menees, S., Saad, R., Chey, W.D., 2012 [42]. Chloride channel activation in the treatment of constipation. ClC-2, type-2 chloride channel; CFTR, cystic fibrosis transmembrane regulator; GC-C, guanylate cyclase-C; GTP, guanosine triphosphate; cGMP, cyclic guanosine monophosphate.

Gut motility and contractility play an important role in stool transit. Small non-peristaltic contractions aid in gut absorption of water [9]. Fiber supplements have been shown to cause mechanical stimulation of the gut mucosa, causing softer stools and faster colonic transit [43]. Larger peristaltic contractions (also called high-amplitude propagated contractions [HAPCs]) help propel stools along the colonic lumen [9]. A decrease in frequency of these larger contractions may be one pathophysiological mechanism involved in constipation [9].

As noted, behavioral and psychological factors can cause or contribute to constipation. Constipation may start in childhood, when most cases are considered idiopathic [44]. Withholding of stools after a difficult bowel movement, a common etiology of functional constipation in the pediatric population, leads to water absorption from the fecal mass, increasing evacuation difficulty, rectal distension, loss of rectal sensation, and eventually loss of the normal defecatory urge [44]. After children with constipation enter adulthood, one-fourth of them may continue to experience symptoms [45]. Psychological factors, such as anxiety and stress, may contribute to constipation by increasing skeletal muscle tension, which can lead to dyssynergic defecation [46].

Alterations in the gut microbiota may also affect gut motility [22]. Various gases are produced by gut microorganisms, including hydrogen sulfide, carbon dioxide, and

methane [22]. The increased production of hydrogen and carbon dioxide from bacterial fermentation of oligosaccharides may cause symptoms of bloating [22]. The increased production of methane in the gut is thought to slow gut motility, contributing to constipation in some patients [22]. Patients with constipation have decreased levels of Bifidobacteria, Lactobacillus, Clostridium leptum, and Faecalibacterium prausnitzii and increased levels of Bacteroides spp and Enterobacteriaceae [47].

Functional and physiological abnormalities of the anorectum can additionally be involved in contributing to constipation [9,46]. Dyssynergia, likely the most common DD, is a spectrum of dysfunctional or disordered contractions and relaxations in muscles involved in defecation. Dyssynergia may present with pathologic habitual behaviors, such as avoidance of defecation (often due to a painful anal fissure), along with various other comorbidities, including back injury, brain–gut dysfunction, eating disorders, and a history of sexual or physical abuse [9]. Less common causes of DD include mechanical factors, such as rectal intussusception, prolapse, rectocele, and abnormal perineal descent [9]. Injuries to pelvic floor muscles during childbirth are attributable to higher rates of constipation in women [7]. When diagnosed in the primary care setting, gynecological referral may be warranted. Guidelines for managing constipation due to defecatory disorders favor pelvic floor retraining by biofeedback as opposed to laxatives [28].

Bile acids may have laxative effects and, as a result, impaired bile acid synthesis can also contribute to constipation [22]. Bile acids inhibit apical Cl^-/OH^- exchange, increase permeability of the intestinal mucosa, activate intracellular secretory mechanisms, and are beneficial to propulsive colonic contractions, thereby improving colonic motility and evacuation [22]. In a study of IBS, the constipation-predominant group had the lowest bile acid values and significantly decreased percentages of two of the most potent secretory bile acids [48].

Medications may affect water regulation in the gut or gut motility. Medications that can cause constipation are myriad, including antidepressants—notably tricyclic antidepressants—that exert profound anticholinergic effects as well as affect serotonin levels [1,9,49]. Serotonin is known to be involved in the regulation of gastrointestinal motility [22]. Antihypertensive drugs, such as certain calcium channel antagonists, may inhibit smooth muscle contraction in the intestinal tract [1,9]. As a result, medications in this class may contribute to increasing colonic transit time. Analgesics, especially opiates, contribute to constipation [1,9]. Opioid receptors are located throughout the gut [50], but constipation is largely caused by delayed transit in the colon and increased colonic fluid absorption resulting in harder, firmer stools [51]. Oral iron supplements are also classically associated with constipation [1,9].

Certain systemic diseases are associated with constipation. Neurological disorders that may induce constipation include autonomic neuropathy, Parkinson's disease, multiple sclerosis, and certain spinal cord injuries [1,9]. Neurological causes of constipation are complex, as they can include neural dysfunction and systemic factors, such as impaired mobility [9]. If the neural connection between the brain and the gut is affected, this may alter bowel function. Endocrine or metabolic disorders may contribute as well, including diabetes, hypothyroidism, and hypercalcemia [1]. These conditions can impact gut function and motility. As a case in point, constipation may affect up to 60% of patients with diabetes [52] (a common disease), helping to explain the frequency of such complaints in the general patient population. Although hypothyroidism may cause constipation, this is not a common condition among patients presenting with constipation [53]. Mechanical bowel obstruction of any etiology can also cause constipation; however, obstructions of acute onset may present differently than those of gradual or subtle onsets [1]. These conditions should always be among the first diagnoses to be excluded prior to continuing the patient's workup and further evaluation.

Pathophysiological factors often overlap and interact in cases of chronic constipation [1]. Researchers seeking to identify an integrated explanatory model for IBS identified three main components that may be associated with constipation: alterations in the pe-

ripheral regulation of gut function (sensory and secretory mechanisms), psychological distress, and brain-gut signaling (visceral hypersensitivity) [54]. As discussed above, sensory and secretory mechanisms of constipation can be impacted by diet (e.g., liquid intake, fiber), the gut microbiome, anorectal abnormalities, and bile acid composition. Depression and anxiety as well as somatization and psychotic disorders were significantly higher ($p < 0.05$) in patients with constipation compared with controls; these types of psychological stressors positively correlated with constipation symptoms (e.g., straining, sensation of anal blockage) [55]. There is evidence suggesting differences in brain-gut signaling in patients with constipation, and such patients often have a higher threshold to sense the urge to evacuate [54].

5. Case Studies

5.1. Case Study 1

A 40-year-old woman reports an average of 2 spontaneous bowel movements each week for the past 6 months. She describes her bowel movements as rarely resulting in a sensation of complete evacuation. She has been researching her symptoms on the internet and has tried to increase her exercise and has purchased fiber supplements from her supermarket. After several weeks she had not observed any improvements in her constipation.

She consulted her local pharmacist, who encouraged her to use a different brand of fiber supplement, but this also did not improve her symptoms. As a result, the pharmacist suggested she try a laxative. The first recommended laxative also failed to relieve the symptoms, so the pharmacist recommended an alternative laxative. During this time, she felt her symptoms were beginning to worsen and she started experiencing abdominal pain and bloating. Her stools also moved from being lumpy and sausage-like (BSFS type 2) to hard, separate lumps (BSFS type 1).

She is now seeking guidance from her PCP to address her abdominal pain, which is her primary symptomatic concern. She has not been diagnosed with depression or anxiety but feels that her constipation and abdominal pain are negatively affecting her QoL. She is otherwise healthy and has not been prescribed any medication that is typically associated with secondary constipation.

As this patient does not have alarm symptoms and secondary constipation is unlikely, her PCP made the clinical diagnosis of IBS-C and continued managing her care. A review of prior treatments determined inadequate trials of OTC laxatives. The patient had previously tried MiraLAX (a polyethylene glycol-based osmotic laxative), and experienced modest but only intermittent relief of constipation and abdominal discomfort. Her PCP began an appropriate treatment course that included use of lubiprostone, which was associated with initial improvement, but was discontinued due to diarrhea and intolerable nausea despite instructions on the optimal manner in which to use the medication. In consultation with fellow internists and a gastroenterology colleague, the patient's primary physician started her on plecanatide 3 mg daily, which began to have an effect over the next several days, with the patient reporting marked improvement in both abdominal discomfort and constipation within 6 days of treatment. After 2 weeks, the patient is nearly symptom-free, and now experiences well-formed bowel movements up to 3 to 4 times weekly.

5.2. Case Study 2

A 53-year-old woman reports an average of one complete spontaneous bowel movement each week for the past several years, which has been stable in nature. She describes most of her bowel movements as incomplete evacuations. She also notes abdominal discomfort and pain, which typically precede defecation and are generally relieved by passage of a bowel movement. Of note, there are no alarm signs/symptoms or so-called "red flags", including the absence of rectal bleeding and unintentional weight loss, and there is no consistent or concerning change in stool size/caliber. She has not undergone prior colonoscopy or other colorectal cancer–screening modalities.

Increased liquid intake, dietary and supplemental fiber (such as psyllium), and aerobic exercise have been of only modest benefit. The use of various OTC stool softeners and laxatives, along with enemas, has provided intermittent but generally short-lived improvement in her symptoms. Her stools have consistently ranged from BSFS type 1–3, without a consistent pattern.

She now seeks advice from her PCP to address her abdominal pain and constipation, her two main symptomatic issues. There are no known alarm signs or symptoms, as noted above. She has no other organic pathology that is likely to contribute, including the absence of other underlying comorbid conditions. She does not use any medications (prescription or OTC) typically associated with secondary constipation/alterations in GI tract motility. However, with worsening constipation and no history of prior colonoscopy, the patient is referred to a gastroenterologist for colorectal cancer screening.

5.3. Case Study 3

A 23-year-old man presents to his PCP with 6 months of altered bowel habits. He reports 2 to 3 bowel movements per week associated with significant straining and incomplete evacuation. He underwent abdominal surgery the previous year for an episode of acute appendicitis with an uncomplicated laparoscopic appendectomy. Since then, he reports intermittent use of tramadol 50 mg every other day. He has no abdominal pain and no "red flag" or alarm symptoms (with the absence of hematochezia, unintentional weight lost, or change in stool caliber). He also reports a longstanding history of major depressive disorder and has been using paroxetine 20 mg daily for 3 years. He currently reports feeling well overall, with a recent Patient Health Questionnaire-9 (PHQ-9) score of 3. He has been taking a daily OTC senna-based laxative without effect.

Abdominal examination is non-tender and non-distended with old, well-healed surgical scars present. Rectal examination is notable for normal rectal tone, no masses, and hard stool in the rectal vault. All blood tests are within the normal range, including a complete blood count, metabolic panel, and thyroid-stimulating hormone (TSH) level.

In consultation with a pain management specialist, the patient is able to be weaned off the tramadol and started on non-opiate analgesia with good effect. In consultation with the patient's psychiatrist, the patient was switched to a tricyclic antidepressant. The PCP then recommended the patient initiate MiraLAX 17 g daily with change in bowel habits to 2 bowel movements (BSFS type 3–4) every day.

5.4. Case Study 4

A 32-year-old female has had 2 vaginal deliveries, one requiring forceps, and both resulting in second-degree vaginal tears. She reports an average of 2 to 3 bowel movements weekly (BSFS type 1–2) since the birth of her second child approximately 1 year ago. She complains of straining, requiring that she spend 15–20 min on the toilet in order to pass a complete bowel movement. She requires digital maneuvers to initiate and complete a bowel movement.

She has tried supplemental fiber in the past, but this was discontinued due to increased bloating and gas. She was managing reasonably well with the occasional use of OTC laxative preparations, but recently, due to rectal pain associated with her bowel movements, the laxatives caused excessive discomfort with the increasing frequency with which she now needs to use the toilet. She is now seeking evaluation by her PCP.

Her PCP performed a digital rectal exam, which revealed suspected DD, and checked her TSH and hemoglobin (Hgb); both were within normal range. The patient was referred for gastroenterology subspecialty consultation and formal anorectal evaluation, which confirmed diagnosis of DD. Pelvic floor exercise and biofeedback were prescribed, ultimately improving the patient's symptoms.

6. Summary

The causes of constipation are multifactorial and complex; however, constipation can be diagnosed and managed in the primary care setting. It is important for primary care providers (including internists, gynecologists, physician assistants, and nurse practitioners) to be familiar with the different subtypes of chronic constipation in order to diagnose and hence treat each patient accordingly. PCPs should also be familiar with alarm signs and symptoms that should trigger referral (or expedited referral) to a gastroenterologist or other appropriate specialist as needed.

Author Contributions: J.H., N.M.d.A., D.P. and D.M.P. contributed to conceptualization and design; all authors contributed in reviewing the literature, drafting and critically revising and editing. All authors have read and agreed to the published version of the manuscript.

Funding: Medical writing and editorial support for this manuscript was funded by Salix Pharmaceuticals, Inc. (Bridgewater, NJ, USA) in accordance with Good Publication Practice guidelines.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: The authors would like to thank Blair Hesp, Julie O'Grady and Nicole Coolbaugh of The Medicine Group, LLC (New Hope, PA, USA), who provided medical writing and editorial assistance, which was funded by Salix Pharmaceuticals, Inc. (Bridgewater, NJ, USA) in accordance with Good Publication Practice guidelines.

Conflicts of Interest: J.H. has nothing to disclose. N.M.d.A. has participated in an advisory board for Salix Pharmaceuticals Inc. D.P. is a consultant for Salix Pharmaceuticals Inc. D.M.P. is a consultant/educator for Olympus and a consultant for Salix Pharmaceuticals Inc., Ambu, and Applied Medical.

References

1. Basilisco, G.; Coletta, M. Chronic constipation: A critical review. *Dig. Liver Dis.* **2013**, *45*, 886–893. [CrossRef] [PubMed]
2. Cash, B.D. Understanding and managing IBS and CIC in the primary care setting. *Gastroenterol. Hepatol.* **2018**, *14*, 3–15.
3. Harris, L.A.; Horn, J.; Kissous-Hunt, M.; Magnus, L.; Quigley, E.M.M. The better understanding and recognition of the disconnects, experiences, and needs of patients with chronic idiopathic constipation (BURDEN-CIC) study: Results of an online questionnaire. *Adv. Ther.* **2017**, *34*, 2661–2673. [CrossRef]
4. Lacy, B.E.; Mearin, F.; Chang, L.; Chey, W.D.; Lembo, A.J.; Simren, M.; Spiller, R. Bowel disorders. *Gastroenterology* **2016**, *150*, 1393–1407.e5. [CrossRef] [PubMed]
5. Lewis, S.J.; Heaton, K.W. Stool form scale as a useful guide to intestinal transit time. *Scand. J. Gastroenterol.* **1997**, *32*, 920–924. [CrossRef] [PubMed]
6. Dimidi, E.; Cox, C.; Grant, R.; Scott, S.M.; Whelan, K. Perceptions of constipation among the general public and people with constipation differ strikingly from those of general and specialist doctors and the ROME IV criteria. *Am. J. Gastroenterol.* **2019**, *114*, 1116–1129. [CrossRef] [PubMed]
7. Serra, J.; Pohl, D.; Azpiroz, F.; Chiarioni, G.; Ducrotté, P.; Gourcerol, G.; Hungin, A.P.S.; Layer, P.; Mendive, J.; Pfeifer, J.; et al. European society of neurogastroenterology and motility guidelines on functional constipation in adults. *Neurogastroenterol. Motil.* **2019**, *32*, e13762. [CrossRef]
8. Ayaz, S.; Hisar, F. The efficacy of education programme for preventing constipation in women. *Int. J. Nurs. Pract.* **2014**, *20*, 275–282. [CrossRef] [PubMed]
9. Andrews, C.N.; Storr, M. The pathophysiology of chronic constipation. *Can. J. Gastroenterol.* **2011**, *25* (Suppl. B), 16b–21b. [CrossRef] [PubMed]
10. Islam, B.N.; Sharman, S.K.; Browning, D.D. Clinical utility of plecanatide in the treatment of chronic idiopathic constipation. *Int. J. Gen. Med.* **2018**, *11*, 323–330. [CrossRef] [PubMed]
11. Barros, L.L.; Farias, A.Q.; Rezaie, A. Gastrointestinal motility and absorptive disorders in patients with inflammatory bowel diseases: Prevalence, diagnosis and treatment. *World J. Gastroenterol.* **2019**, *25*, 4414–4426. [CrossRef]
12. Johanson, J.F.; Kralstein, J. Chronic constipation: A survey of the patient perspective. *Aliment. Pharmacol. Ther.* **2007**, *25*, 599–608. [CrossRef]
13. McRorie, J.W.; Fahey, G.C.; Gibb, R.D.; Chey, W.D. Laxative effects of wheat bran and psyllium: Resolving enduring misconceptions about fiber in treatment guidelines for chronic idiopathic constipation. *J. Am. Assoc. Nurse Pract.* **2020**, *32*, 15–23. [CrossRef]

14. Eswaran, S.; Muir, J.; Chey, W.D. Fiber and functional gastrointestinal disorders. *Am. J. Gastroenterol.* **2013**, *108*, 718–727. [CrossRef] [PubMed]
15. Mugie, S.M.; Benninga, M.A.; Di Lorenzo, C. Epidemiology of constipation in children and adults: A systematic review. *Best Pract. Res. Clin. Gastroenterol.* **2011**, *25*, 3–18. [CrossRef] [PubMed]
16. Kang, J.Y. Systematic review: The influence of geography and ethnicity in irritable bowel syndrome. *Aliment. Pharmacol. Ther.* **2005**, *21*, 663–676. [CrossRef] [PubMed]
17. Higgins, P.D.; Johanson, J.F. Epidemiology of constipation in North America: A systematic review. *Am. J. Gastroenterol.* **2004**, *99*, 750–759. [CrossRef]
18. Nojkov, B.; Baker, J.; Lee, A.; Eswaran, S.L.; Menees, S.B.; Saad, R.J.; Chey, W.D. Impact of age and gender to severity of constipation-related symptoms and quality of life indices in patients with chronic idiopathic constipation. *Gastroenterology* **2017**, *152*, S514–S515. [CrossRef]
19. Morin, L.; Johnell, K.; Laroche, M.-L.; Fastbom, J.; Wastesson, J.W. The epidemiology of polypharmacy in older adults: Register-based prospective cohort study. *Clin. Epidemiol.* **2018**, *10*, 289–298. [CrossRef] [PubMed]
20. Slater, N.; White, S.; Venables, R.; Frisher, M. Factors associated with polypharmacy in primary care: A cross-sectional analysis of data from The English Longitudinal Study of Ageing (ELSA). *BMJ Open* **2018**, *8*, e020270. [CrossRef]
21. Suares, N.C.; Ford, A.C. Prevalence of, and risk factors for, chronic idiopathic constipation in the community: Systematic review and meta-analysis. *Am. J. Gastroenterol.* **2011**, *106*, 1582–1591. [CrossRef] [PubMed]
22. Niewinna, K.; Zielińska, A.; Fichna, J. Recent advances in the pharmacological management of constipation predominant irritable bowel syndrome. *Expert Opin. Pharmacother.* **2020**, *21*, 73–84. [CrossRef] [PubMed]
23. Heidelbaugh, J.J.; Stelwagon, M.; Miller, S.A.; Shea, E.P.; Chey, W.D. The spectrum of constipation-predominant irritable bowel syndrome and chronic idiopathic constipation: US survey assessing symptoms, care seeking, and disease burden. *Am. J. Gastroenterol.* **2015**, *110*, 580–587. [CrossRef]
24. Moayyedi, P.; Mearin, F.; Azpiroz, F.; Andresen, V.; Barbara, G.; Corsetti, M.; Emmanuel, A.; Hungin, A.P.S.; Layer, P.; Stanghellini, V.; et al. Irritable bowel syndrome diagnosis and management: A simplified algorithm for clinical practice. *United Eur. Gastroenterol. J.* **2017**, *5*, 773–788. [CrossRef] [PubMed]
25. Bharucha, A.E.; Wald, A. Chronic constipation. *Mayo Clin. Proc.* **2019**, *94*, 2340–2357. [CrossRef]
26. American College of Gastroenterology Chronic Constipation Task Force. An evidence-based approach to the management of chronic constipation in north america. *Am. J. Gastroenterol.* **2005**, *100*, S1–S4. [CrossRef]
27. Viscusi, E.R. Clinical overview and considerations for the management of opioid-induced constipation in patients with chronic noncancer pain. *Clin. J. Pain* **2019**, *35*, 174–188. [CrossRef]
28. Bharucha, A.E.; Dorn, S.D.; Lembo, A.; Pressman, A. American gastroenterological association medical position statement on constipation. *Gastroenterology* **2013**, *144*, 211–217. [CrossRef] [PubMed]
29. Rao, S.S.; Camilleri, M. Approach to the patient with constipation. In *Yamada's Textbook of Gastroenterology*; Podolsky, D.K., Camilleri, M., Fitz, J.G., Kalloo, A.N., Shanahan, F., Wang, T.C., Eds.; Willey: Hoboken, NJ, USA, 2015; pp. 757–780.
30. Brusciano, L.; Limongelli, P.; Del Genio, G.; Rossetti, G.; Sansone, S.; Healey, A.; Maffettone, V.; Napolitano, V.; Pizza, F.; Tolone, S. Clinical and instrumental parameters in patients with constipation and incontinence: Their potential implications in the functional aspects of these disorders. *Int. J. Color. Dis.* **2009**, *24*, 961–967. [CrossRef] [PubMed]
31. Talley, N.J. How to do and interpret a rectal examination in gastroenterology. *Am. J. Gastroenterol.* **2008**, *103*, 820–822. [CrossRef]
32. Camilleri, M.; Ford, A.C. Irritable bowel syndrome: Pathophysiology and current therapeutic approaches. *Handb. Exp. Pharmacol.* **2016**, *239*, 75–113. [CrossRef]
33. Rao, S.S.; Xiang, X.; Yan, Y.; Rattanakovit, K.; Patcharatrakul, T.; Parr, R.; Ayyala, D.; Sharma, A. Randomised clinical trial: Linaclotide vs placebo-a study of bi-directional gut and brain axis. *Aliment. Pharmacol. Ther.* **2020**, *51*, 1332–1341. [CrossRef]
34. Waldman, S.A.; Camilleri, M. Guanylate cyclase-C as a therapeutic target in gastrointestinal disorders. *Gut* **2018**, *67*, 1543–1552. [CrossRef]
35. Atkinson, W.; Lockhart, S.; Whorwell, P.J.; Keevil, B.; Houghton, L.A. Altered 5-Hydroxytryptamine signaling in patients with constipation- and diarrhea-predominant irritable bowel syndrome. *Gastroenterology* **2006**, *130*, 34–43. [CrossRef]
36. Shah, E.D.; Almario, C.V.; Spiegel, B.M.R.; Chey, W.D. Lower and upper gastrointestinal symptoms differ between individuals with irritable bowel syndrome with constipation or chronic idiopathic constipation. *J. Neurogastroenterol. Motil.* **2018**, *24*, 299–306. [CrossRef] [PubMed]
37. Emmanuel, A.; Quigley, E.M.M.; Simrén, M.; Feng, Y.; Müller-Lissner, S.; Urbain, D.; Tack, J.; Bredenoord, A.J.; Sabaté, J.; Yiannakou, Y.; et al. Factors affecting satisfaction with treatment in European women with chronic constipation: An internet survey. *United Eur. Gastroenterol. J.* **2013**, *1*, 375–384. [CrossRef]
38. Rao, S.S.; Camilleri, M.; Hasler, W.L.; Maurer, A.H.; Parkman, H.P.; Saad, R.; Scott, M.S.; Simren, M.; Soffer, E.; Szarka, L. Evaluation of gastrointestinal transit in clinical practice: Position paper of the American and european neurogastroenterology and motility societies. *Neurogastroenterol. Motil.* **2010**, *23*, 8–23. [CrossRef]
39. Ratuapli, S.K.; Bharucha, A.E.; Noelting, J.; Harvey, D.M.; Zinsmeister, A.R. Phenotypic identification and classification of functional defecatory disorders using high-resolution anorectal manometry. *Gastroenterology* **2013**, *144*, 314–322.e2. [CrossRef]
40. Waldman, S.A.; Tenenbaum, R.; Foehl, H.C.; Winkle, P.; Griffin, P. Blunted evoked prouroguanylin endocrine secretion in chronic constipation. *Clin. Transl. Gastroenterol.* **2019**, *10*, e00016. [CrossRef]

41. Rao, S.S. Plecanatide: A new guanylate cyclase agonist for the treatment of chronic idiopathic constipation. *Ther. Adv. Gastroenterol.* **2018**, *11*, 1756284818777945. [CrossRef] [PubMed]
42. Menees, S.; Saad, R.; Chey, W.D. Agents that act luminally to treat diarrhoea and constipation. *Nat. Rev. Gastroenterol. Hepatol.* **2012**, *9*, 661–674. [CrossRef]
43. McRorie, J.W. Evidence-based approach to fiber supplements and clinically meaningful health benefits, Part 2. *Nutr. Today* **2015**, *50*, 90–97. [CrossRef] [PubMed]
44. Mugie, S.M.; Di Lorenzo, C.; Benninga, M.A. Constipation in childhood. *Nat. Rev. Gastroenterol. Hepatol.* **2011**, *8*, 502–511. [CrossRef] [PubMed]
45. Bongers, M.E.; Van Wijk, M.P.; Reitsma, J.B.; Benninga, M.A. Long-term prognosis for childhood constipation: Clinical outcomes in adulthood. *Pediatrics* **2010**, *126*, e156–e162. [CrossRef] [PubMed]
46. Rao, S.S.; Bharucha, A.E.; Chiarioni, G.; Felt-Bersma, R.; Knowles, C.; Malcolm, A.; Wald, A. Anorectal disorders. *Gastroenterology* **2016**, *150*, 1430–1442.e4. [CrossRef] [PubMed]
47. Zhao, Y.; Yu, Y.-B. Intestinal microbiota and chronic constipation. *SpringerPlus* **2016**, *5*, 1130. [CrossRef]
48. Shin, A.; Camilleri, M.; Vijayvargiya, P.; Busciglio, I.; Burton, D.; Ryks, M.; Rhoten, D.; Lueke, A.; Saenger, A.; Girtman, A.; et al. Bowel functions, fecal unconjugated primary and secondary bile acids, and colonic transit in patients with irritable bowel syndrome. *Clin. Gastroenterol. Hepatol.* **2013**, *11*, 1270–1275.e1. [CrossRef] [PubMed]
49. Siah, K.T.; Wong, R.K.; Whitehead, W.E. Chronic constipation and constipation-predominant IBS: Separate and distinct disorders or a spectrum of disease? *Gastroenterol. Hepatol.* **2016**, *12*, 171–178.
50. Farmer, A.D.; Drewes, A.M.; Chiarioni, G.; de Giorgio, R.; O'Brien, T.; Morlion, B.; Tack, J. Pathophysiology and management of opioid-induced constipation: European expert consensus statement. *United Eur. Gastroenterol. J.* **2019**, *7*, 7–20. [CrossRef]
51. Crockett, S.D.; Greer, K.B.; Heidelbaugh, J.J.; Falck-Ytter, Y.; Hanson, B.J.; Sultan, S. American gastroenterological association institute guideline on the medical management of opioid-induced constipation. *Gastroenterology* **2019**, *156*, 218–226. [CrossRef] [PubMed]
52. Maisey, A. A practical approach to gastrointestinal complications of diabetes. *Diabetes Ther.* **2016**, *7*, 379–386. [CrossRef]
53. Muller-Lissner, S.A.; Kamm, M.A.; Scarpignato, C.; Wald, A. Myths and misconceptions about chronic constipation. *Am. J. Gastroenterol.* **2005**, *100*, 232–242. [CrossRef] [PubMed]
54. Hungin, A.P.; Becher, A.; Cayley, B.; Heidelbaugh, J.J.; Muris, J.W.M.; Rubin, G.; Seifert, B.; Russell, A.; De Wit, N.J. Irritable bowel syndrome: An integrated explanatory model for clinical practice. *Neurogastroenterol. Motil.* **2015**, *27*, 750–763. [CrossRef] [PubMed]
55. Rao, S.S.; Seaton, K.; Miller, M.J.; Schulze, K.; Brown, C.K.; Paulson, J.; Zimmerman, B. Psychological profiles and quality of life differ between patients with dyssynergia and those with slow transit constipation. *J. Psychosom. Res.* **2007**, *63*, 441–449. [CrossRef] [PubMed]

Journal of
Clinical Medicine

MDPI

Review

Management of Dyspepsia and Gastroparesis in Patients with Diabetes. A Clinical Point of View in the Year 2021

Silvia Bonetto [1,*], Gabriella Gruden [2], Guglielmo Beccuti [2], Arianna Ferro [2], Giorgio Maria Saracco [1,2] and Rinaldo Pellicano [1]

[1] Unit of Gastroenterology, Molinette Hospital, 10126 Turin, Italy; giorgiomaria.saracco@unito.it (G.M.S.); rinaldo_pellican@hotmail.com (R.P.)
[2] Department of Medical Sciences, University of Turin, 10126 Turin, Italy; gabriella.gruden@unito.it (G.G.); guglielmo.beccuti@unito.it (G.B.); arianna.ferro@unito.it (A.F.)
* Correspondence: silvia.bonetto@edu.unito.it

Abstract: Diabetes mellitus is a widespread disease, and represents an important public health burden worldwide. Together with cardiovascular, renal and neurological complications, many patients with diabetes present with gastrointestinal symptoms, which configure the so-called diabetic enteropathy. In this review, we will focus on upper gastrointestinal symptoms in patients with diabetes, with particular attention to dyspepsia and diabetic gastroparesis (DG). These two clinical entities share similar pathogenetic mechanisms, which include autonomic neuropathy, alterations in enteric nervous system and histological abnormalities, such as interstitial cells of Cajal depletion. Moreover, the differential diagnosis may be challenging because of overlapping clinical features. Delayed gastric emptying should be documented to differentiate between DG and dyspepsia and it can be assessed through radioactive or non-radioactive methods. The clinical management of dyspepsia includes a wide range of different approaches, above all *Helicobacter pylori* test and treat. As regards DG treatment, a central role is played by dietary modification and glucose control and the first-line pharmacological therapy is represented by the use of prokinetics. A minority of patients with DG refractory to medical treatment may require more invasive therapeutic approaches, including supplemental nutrition, gastric electric stimulation, pyloromyotomy and gastrectomy.

Keywords: dyspepsia; diabetes; gastroparesis

Citation: Bonetto, S.; Gruden, G.; Beccuti, G.; Ferro, A.; Saracco, G.M.; Pellicano, R. Management of Dyspepsia and Gastroparesis in Patients with Diabetes. A Clinical Point of View in the Year 2021. *J. Clin. Med.* **2021**, *10*, 1313. https://doi.org/10.3390/jcm10061313

Academic Editor: Marilena Durazzo

Received: 18 February 2021
Accepted: 20 March 2021
Published: 23 March 2021

1. Introduction

Diabetes mellitus (DM) is a widespread disease. According to the last estimate from the International Diabetes Federation (ID) it affects 463 million people worldwide with increasing prevalence [1]. DM represents an important public health burden, mainly because of its cardiovascular, renal and neurological complications. In addition, many patients with diabetes present with upper gastrointestinal (GI) symptoms and motility alterations. Among the latter, delayed gastric emptying (GE) affects up to 50% of patients with both type 1 and type 2 DM manifesting with dyspepsia, gastroparesis or, for a proportion of patients, remaining asymptomatic [2]. As dyspepsia and diabetic gastroparesis (DG) share similar pathogenetic mechanisms and clinical features, the differential diagnosis may be challenging. Recently, some authors suggested that functional dyspepsia (FD) and DG could be different expressions of the same spectrum of gastric neuromuscular disorders, with common histopathological alterations and comparable clinical manifestations and prognosis [3].

In this review, we will discuss an update of dyspepsia and gastroparesis in patients with diabetes, focusing on pathophysiology, clinical presentation and management of these manifestations.

2. Dyspepsia: Definition and Clinical Classification

The term dyspepsia includes a set of symptoms with epigastric localization, which can be episodic or persistent, with variable intensity and severity. In the clinical setting, it is often difficult to characterize these symptoms and to distinguish dyspepsia from other GI disorders such as gastroesophageal reflux disease (GERD) [4]. The American College of Gastroenterology (ACG) and Canadian Association of Gastroenterology (CAG) clinical guidelines give a useful definition of dyspepsia as predominant epigastric pain which lasts at least one month and is associated with any other upper GI symptom such as epigastric fullness, nausea, vomiting or heartburn [5].

For the appropriate clinical management, it is important to distinguish organic dyspepsia from FD. The former includes patients in whom clinical evaluation, laboratory tests, endoscopy or radiologic studies can identify a pathologic process which is the cause of dyspeptic symptoms, while FD includes all cases of dyspepsia without evidence of an organic cause [6]. The exclusion of organic causes requires endoscopy and, where needed, radiologic investigations, such as ultrasound or computed tomography, along with Helicobacter pylori (H. pylori) testing and treating and re-evaluation of symptoms after its eradication [7].

Functional dyspepsia can be classified on the basis of prevalent symptoms in postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS) [8]. These two entities, however, have blurred boundaries as they frequently overlap and they share similar therapeutic strategies. Moreover, due to their common motility alterations, PDS is more likely to overlap with gastroparesis.

Although different definitions of FD have been previously proposed, the most recent update is represented by Rome IV criteria, shown in Table 1 [9].

Table 1. Rome IV diagnostic criteria of functional dyspepsia modified from [9].

Functional Dyspepsia:
1. One or more of the following: (a) postprandial fullness (b) early satiation (c) epigastric pain (d) epigastric burning AND 2. Exclusion of structural disease which can explain symptoms a. Must fulfill criteria for PDS (Postprandial Distress Syndrome) and/or EPS (Epigastric Pain Syndrome). b. Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis.
Postprandial Distress Syndrome (PDS):
1. One or both of the following for at least 3 days per week and severe enough to impact on usual activities: (a) postprandial fullness (b) early satiation 2. No evidence of organic, systemic, or metabolic disease which can explain symptoms. Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis. Supportive remarks: • Possible co-existence of postprandial epigastric pain or burning, epigastric bloating, excessive belching. • In case of vomiting, other disorders should be considered • Possible association with heartburn • Symptoms relieved by evacuation of feces or gas should not be ascribed to dyspepsia
Epigastric Pain Syndrome(EPS):
1. One or both of the following for at least 1 day per week and severe enough to impact on usual activities: (a) epigastric pain (b) epigastric burning 2. No evidence of organic, systemic, or metabolic disease which can explain symptoms Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis Supportive remarks: • Pain may be induced or relieved by ingestion of a meal or may occur while fasting • Possible coexistence of postprandial epigastric bloating, belching, and nausea • In case of persistent vomiting, other disorders should be considered • Possible association with heartburn • Pain cannot be defined as biliary pain • Symptoms relieved by evacuation of feces or gas should not be ascribed to dyspepsia

H. pylori-associated dyspepsia represents a distinct form of dyspepsia [10]. If dyspepsia resolves six months after bacterial eradication it can be attributed to *H. pylori* infection [11,12] otherwise the disorder is deemed FD [7].

2.1. Organic Dyspepsia

The most common cause of organic dyspepsia is peptic ulcer disease, which is often associated with either *H. pylori* infection or chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) [6]. *H. pylori* is a Gram-negative, micro-aerophilic bacterium, usually acquired during childhood, whose natural habitat is the luminal surface of the gastric epithelium [13]. Since the human stomach is an unfriendly place for microbial survival, *H. pylori* has developed a repertoire of acid resistance mechanisms which allow the microorganism to overcome the mucous layer. In particular, via the enzyme urease, the bacterium creates a cloud of acid neutralizing chemicals around it, offering protection from the acid [14]. *H. pylori* infection is accepted as the most important cause of gastritis and PUD in humans. Moreover, it is recognized as a risk factor for gastric cancer [15] along with potential involvement in the pathogenesis of several extra-gastric manifestations, ranging from hematological diseases (such as idiopathic thrombocytopenic purpura, iron deficiency anemia), to neurological diseases (for example, Parkinson's disease and other forms of neurodegeneration and dementia) [16–19].

Gastric or esophageal cancers are less frequent causes of dyspepsia. However, malignancy has an important impact on prognosis and clinical management of affected patients and should be ruled out in those aged 60 or over or with other risk factors [5]. Neoplastic risk is increased in patients with *H. pylori* infection, family history of gastric malignancy, previous gastric surgery, immigrants from endemic areas, smokers, patients with high alcohol consumption or with a long history of heartburn [6]. Moreover, the risk of gastric cancer is nearly doubled in males [5]. Pancreatic diseases, such as acute and chronic pancreatitis, can present with dyspepsia too. Pancreatic pain, however, is often more severe than epigastric pain related to dyspepsia, moreover chronic pancreatitis is usually associated with weight loss and other symptoms due to pancreatic insufficiency [6]. Other GI diseases associated with dyspepsia include gallstones, superior mesenteric artery syndrome, eosinophilic esophagitis, amyloidosis and lymphomas. The diseases that should be considered in the differential diagnosis of dyspepsia are summarized in Table 2.

Table 2. Differential diagnoses of dyspepsia.

• ESOPHAGEAL DISEASES	• Gastroesophageal reflux disease • Eosinophilic esophagitis • Achalasia • Esophageal cancer
• GASTRIC DISEASES	• Peptic ulcer • Erosive and non-erosive gastritis • Helicobacter Pylori-related dyspepsia • Gastroparesis • Gastric cancer
• DUODENAL DISEASES	• Duodenal ulcer • Duodenal cancer
• PANCREATIC DISEASES	• Acute pancreatitis • Chronic pancreatitis • Pancreatic cancer
• HEPATOBILIARY DISEASES	• Biliary lithiasis • Cholangitis • Cholangiocarcinoma
• VASCULAR DISEASES	• Superior mesenteric artery syndrome
• SYSTEMIC DISEASES	• Lymphoma • Amyloidosis • Connective tissue diseases (e.g., scleroderma)

2.2. Functional Dyspepsia

The etiology of FD remains unclear. It is considered a multifactorial disease, related to genetic, environmental, and socio-cultural factors [10,20]. The pathogenesis of FD involves different mechanisms, such as delayed GE [21], gastric accommodation impairment [22], hypersensitivity to gastric distention, altered chemosensitivity and altered duodenal sensitivity to acids and lipids [6,23]. Impaired intestinal permeability is involved in FD pathogenesis too and it is related to mucosal inflammation [23].

Intestinal physiological functions are modulated by GI endocrine mediators, such as ghrelin, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), motilin and glucose-dependent insulinotropic peptide (GIP). These molecules influence mucosal immune system, permeability and enteric nervous system (ENS) through endocrine and paracrine mechanisms and might contribute to development of dyspepsia, even if more studies are needed for a better understanding of their role in FD [10].

An emerging factor in FD development is the role of microbial imbalance of GI tract. The term human microbiota is referred to the rich microbial community, consisting of more than 10^{14} microorganisms, that colonizes the GI tract [24]. The perturbation of this ecosystem as well as of mucosal integrity allows bacterial translocation and plays a key role in the development of GI and systemic diseases [25–27]. Molecules produced by microbiota components can modify intestinal motility, and, at the same time, motility influences microbiota composition. Moreover, bacterial mediators can act like neurotransmitters, thus interacting with ENS. Gut microbiota and its mediators also influence intestinal permeability [28]. They can modify the composition of the mucus layer and tight junctions through modulation of genetic expression [10]. Moreover, alteration of the mucosal immune system, inflammatory response and modification of gut microbiota after an acute gastroenteritis can predispose to development of FD [12]. A previous GI infection is a risk factor for irritable bowel syndrome (IBS) and FD development in about 10% of patients [29]. The role of *H. pylori* in the development of FD is complex and controversial. Whilst the bacterium induces alterations in gastric acid secretion, gut hormones production and motility [10], studies have reported conflicting results regarding changes to post-prandial gastric motility in *H. pylori*-infected patients [11].

As in other functional GI disorders, a central role in FD is played by the interaction between the GI tract and central nervous system. The main site of this two-way effect is amygdala, which is involved in emotions and pain and in satiety and fullness perception [10]. The brain-gut axis is structurally constituted by direct connections between the central nervous system and myenteric plexus. It is through this pathway that emotion can influence GE, intestinal motility, mucosal secretion and barrier function. Conversely, mental function can be influenced by GI motility, visceral inflammation and injury [8]. As a result of this interaction, mood disorders and psycho-social factors have a demonstrated relationship with both FD development and reduction in quality of life of these patients [23]. Therefore, FD and other functional GI disorders can be defined as the result of the interaction of biopsychosocial factors and gut physiology through the brain-gut axis.

3. Dyspepsia in Diabetic Patients

Dyspeptic symptoms are a frequent finding in patients with diabetes and they are part of the so-called diabetic enteropathy (DE), which includes the GI manifestations of DM [30]. Autonomic neuropathy has an important pathogenetic role in DE, together with interstitial cells of Cajal depletion and reduced expression of neuronal nitric oxide syntethase [30]. These alterations lead to abnormal GI motility, causing symptoms such as dyspepsia, nausea, vomiting, constipation and fecal incontinence.

Despite the high incidence of dyspepsia in patients with diabetes, the current literature offers limited data about the clinical features and the appropriate management of dyspepsia in this population. In case of a patient with DM presenting with upper GI symptoms, organic disease and medication side effects should be excluded: GLP-1 analogues, for example, can cause nausea and vomiting [30]. Moreover, DG should be excluded through

GE measurement, as discussed below [2,31]. When organic dyspepsia, medication side effects and DG are excluded, the clinical management is analogous to that of non-diabetic patients, except for a more important therapeutic role of prokinetics in patients with DM.

4. *H. pylori* Infection in Patients with Diabetes

Many studies have analyzed the prevalence of *H. pylori* infection in symptomatic and asymptomatic patients with DM [32–34]. Hyperglycemia has been suggested as a predisposing factor for *H. pylori* colonization [32]. A recent case–control serological study demonstrated a significantly higher prevalence of *H. pylori* infection in patients with DM, who had positive antibody titers in 50.7% of cases, compared to 38.2% of controls [33]. Moreover, *H. pylori* positive patients showed higher incidence of GI symptoms, including bloating, distention, vomiting, abdominal pain, constipation and diarrhea, as well as systemic manifestations such as hypertension, muscular symptoms and chronic bronchitis, which is potentially attributable to *H. pylori* contribution to inducing systemic inflammation [33].

Among patients with DM, *H. pylori* infection has been shown to be higher in patients with gastroparesis, and bacterial eradication reduced symptoms such as upper abdominal pain and distention, early satiety and anorexia [34], thus suggesting a pathogenetic role of *H. pylori* in DG and reaffirming the therapeutic role of its eradication.

5. Diabetic Gastroparesis

DG is characterized by upper GI symptoms, such as epigastric distress, nausea, vomiting, early satiety or bloating, which occur in DM in the absence of organic obstruction. Epidemiologic studies about DG show heterogeneous data: in a study, among type 1 and type 2 diabetic patients with GI symptoms, the incidence of documented gastroparesis was 60% [35], while a more recent community-based study showed a ten-year cumulative incidence of gastroparesis of 5.2% in type 1 diabetes versus 1% in type 2 diabetes [36].

Whilst GE is often delayed in gastroparesis, the entity of motility alteration has a poor correlation with the severity of symptoms [37].

Glycemic control plays a key role in DG as it influences GE [2,38]. Severe acute hyperglycemia, in fact, has shown to delay GE in both healthy subjects and patients within type 1 DM, while its effects in type 2 diabetes are not clear [38,39]. Moreover, a prospective, observational, follow-up study showed that baseline levels of glycated hemoglobin (HbA1c) and duration of DM at baseline were independently associated with delayed GE, thus supporting a relationship between long-term hyperglycemic exposure and GE [40]. A subsequent cross-sectional study involving 147 type 2 diabetics confirmed the correlation of DG with blood glucose levels, HbA1c and duration of diabetes [41]. Currently, there are limited data on the long-term impact of improving glycemic control on patients with GE [38,42].

One of the main pathogenetic mechanisms of DG is autonomic neuropathy, characterized by loss of cells in motor and sensory sympathetic ganglia and structural changes of vagal nerve fibers, such as demyelination and axonal degeneration. These alterations often are multifocal, suggesting an ischemic injury [2]. Alterations in ENS and gut wall contribute to development of DG too and are a result of different processes, including apoptosis, oxidative stress, advanced glycation end products, and neuroimmune mechanisms [2]. Histological findings in both diabetic and non-diabetic gastroparetic patients showed loss of interstitial cells of Cajal (ICCs) and ganglion cells, fibrosis of the pylorus and lymphocytic infiltration around myenteric plexus [37]. Notably, gastroparesis and FD show the same histopathologichal changes, such as reduction of ICCs and anti-inflammatory C206+ macrophages, as demonstrated by histologic examination of full-thickness stomach biopsies [3]. These findings suggest a common pathophysiology and a possible target for new therapies, focused on the pathogenic mechanism of these diseases instead of mere symptom relief.

Comorbid abdominal pain with gastroparesis, has been related to visceral hypersensitivity, however, this symptom may be partly unrelated to gastric sensorimotor dysfunction. In a study of 32 patients with gastroparesis, 20 with comorbid DM, more than 60% had positive Carnett's sign, which indicates somatic rather than visceral pain, and about half of them were hypervigilant to pain. Furthermore, more than one-third of these patients met criteria for neuropathic pain [43].

As in FD and other functional disorders [44], DG is associated with depression. In comparison with general population, diabetic patients have a higher prevalence of depression [45], which is often severe and has shown to play a role in expression of GI sensorimotor dysfunctions [2]. On the other side, DG has a negative impact on patients' quality of life, with increased anxiety and depression [2].

In patients with DM and upper GI symptoms, gastroparesis can be diagnosed by the presence of delayed GE without gastric outlet obstruction [2]. The gold standard to define and quantify delayed GE is scintigraphy [46], during the test a solid radiolabeled meal is administered to the patient and a series of scintigraphic images is acquired: delayed GE is diagnosed if more than 60% of the meal is retained at 2 h or more than 10% of the meal is retained at 4 h [47]. GE scintigraphy, however, can be expensive and exposes patients to radioactivity. Moreover, GE is delayed by hyperglycemia, therefore, blood glucose levels should be controlled at the moment of the test. Ideally, glycemia should be lower than 200 mg/dL, if it is higher than 275 mg/dL the test cannot be performed or, in alternative, insulin should be administered to lower blood glucose levels [47].

An alternative, non-radioactive method for delayed GE documentation is the 13C-octanoic acid breath test [48], which has shown a strong correlation with GE scintigraphy in diabetic populations [46,49]. Hence, ^{13}C-octanoic acid breath test represents a suitable alternative to investigate delayed GE in patients with DM in clinical practice.

Although many patients with DM have abnormal GE, few develop overt clinical symptoms, furthermore, part of symptomatic diabetics has little or no delay in GE. Differential diagnosis between gastroparesis and FD may be challenging, however, a delayed GE, the presence of vomiting and a lack of response to prokinetics are more suggestive of DG rather than of FD [10].

6. Clinical Management of Dyspepsia

The ACG/CAG clinical guidelines [5] provide indications on the diagnostic work-up which should be performed in patients with dyspeptic symptoms in addition to pharmacological therapies. According to guidelines, patients under the age of 60 should not undergo endoscopy to exclude malignancy, while, as previously mentioned, upper GI neoplasia should be excluded in elderly and in subjects with neoplastic risk factors [5]. The ACG/CAG clinical guidelines do not recommend the routine use of motility studies, which should only be performed in case of FD when gastroparesis is strongly suspected, as in patients with predominant symptoms of nausea and vomiting, who do not respond to empiric therapy. As discussed above, gastroparesis is diagnosed by documentation of delayed GE, investigated through GE scintigraphy or ^{13}C-octanoic acid breath test, after exclusion of mechanical obstruction through radiologic or endoscopic examination [5].

Patients under the age of 60 should have a non-invasive test for *H. pylori* infection and they should be subsequently treated if the test is positive, while they should receive an empirical treatment with proton pump inhibitors (PPIs) if they are *H. pylori* negative or they are still symptomatic after bacterial eradication [5]. Even in the absence of gastric acid secretion abnormalities, PPIs showed to be effective in relieving FD symptoms and their efficacy was not related to concomitant GERD or *H. pylori* positivity [50].

Patients with dyspepsia not responding to PPIs and *H. pylori* eradication, can be offered a prokinetic therapy, despite the limited effectiveness data only available in non-diabetic dyspeptic patients [5]. However, only in dyspepsia related to DE, prokinetics have shown efficacy in improving gastric motility and reducing symptoms [30]. Prokinetics include serotonin-4 receptor agonists such as cisapride, mosapride and tandospirone citrate, which

can be effective in relieving abdominal pain [51] and dopamine-2 receptor antagonists, like metoclopramide, which have shown efficacy similar to cisapride in improving GE and a better control of nausea, vomiting and early satiety [52]. However, metoclopramide is associated with important side effects, including hyperprolactinemia, closely related to gynecomastia and galactorrhea, and extrapyramidal symptoms, such as drug-induced parkinsonism and tardive dyskinesia [2].

Acotiamide is a prokinetic, currently approved for use in Japan and India for FD. It inhibits pre-sinaptic acetil-cholinesterase and antagonized presynaptic M1 and M2 receptors and it seems to relieve PDS symptoms, with a good tolerability [53,54].

An alternative to prokinetic drugs is represented by neuromodulators. In fact, triciclic antidepressant therapy (TCAs), such as amitriptyline, showed to relieve abdominal pain and improve the quality of life in patients with dyspepsia [55,56]. Data on serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs) are controversial as some studies showed an efficacy similar to TCAs [57], while other studies did not demonstrate any efficacy in symptom relief [53]. In the clinical setting the decision between prokinetics and TCAs should be made on a case-by-case basis [5].

As previously discussed, microbiota is receiving increasing attention in the context of FD and some authors have studied a potential effect of therapies targeting on gut microbiota, such as rifaximin [58] or supplementation with Lactobacillus strains [59], which act restoring the physiological microbiota. However, data about the indication to treat dyspeptic patients with probiotics remain scarce.

Finally, patients with FD not responding to drug therapy should be offered psychological therapies. Considering the role of psychological factors in the development of FD, in fact, these treatments may provide a significant symptom relief [10]. The quality of evidence about this approach is very low and the available studies are heterogeneous and do not suggest a specific psychological intervention [5].

Some authors have also proposed complementary and alternative treatments, such as herbal supplements, acupuncture and hypnosis [10], however, the available data are limited and more studies are needed to assess the efficacy of these therapies.

The above discussed therapeutic options for FD are summarized in Figure 1. Together with these pharmacological and non-pharmacological approaches, remains crucial the therapeutic role of lifestyle modifications, such as weight loss in obese patients, cessation of smoking, diet variations, NSAIDs avoidance [10]. These interventions represent the first step in FD treatment and should be associated with any other therapy.

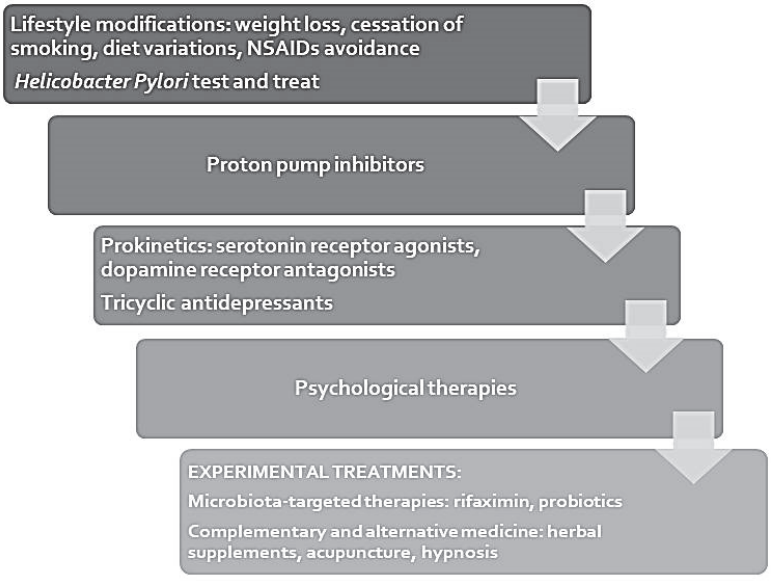


Figure 1. Treatment algorithm for functional dyspepsia.

7. Clinical Management of Gastroparesis

In regard to DG, a stepwise therapeutic approach is also recommended. There is a scarcity of data on appropriate dietary intervention with much of the data extrapolated from other conditions. Typically, dietary advice commences with a low-fat, low-fiber diet but may require liquid meals, enteral or parenteral nutritional support. Avoidance of drugs which delay GE, such as GLP-1 analogues and opioids is recommended [2]. As above described, higher glycemic levels are associated with delayed GE: an accurate glycemic control is therefore essential in the clinical management of DG. However, data on long-term improvement in terms of glycemic control are limited [38,42].

Prokinetic drugs, including metoclopramide and erythromycin represent the first-line therapy. Metoclopramide proved to significantly reduce symptoms of DG through both central antagonism on dopamine receptors and peripheral cholinergic effect [2], but its use is limited by the previously described side effects. Domperidone is a peripheral dopamine receptor antagonist with prokinetic effect, which showed to improve symptoms, with a positive effect sustained over time [2]. As domperidone does not cross the blood–brain barrier, the risk of hyperprolactinemia and extrapyramidal symptoms is significantly lower in comparison with metoclopramide. On the other side, it should be administered with caution in patients with impaired liver function or at increased risk of cardiac events (such as prolonged QT interval) and its co-administration with QT-prolonging drugs is contraindicated [2]. As regards erythromycin, early studies showed a significant reduction in the total symptom score after acute intravenous and chronic oral administration [60], however further investigations demonstrated that its long-term efficacy is often limited by development of tachyphylaxis [60,61]. Moreover, erythromycin is associated with potentially severe adverse events, such as QT prolongation and ventricular arrhythmia [60,62].

Prucalopride is a serotonin receptor agonist which is mainly used in the treatment of constipation. Recently, a randomized placebo-controlled study analyzed its efficacy in thirty-four patients with DG: prucalopride significantly reduced GE time and improved symptoms, evaluated through Gastroparesis Cardinal Symptom Index [63]. These data are promising, but still need to be confirmed in larger sample studies.

Additionally, antiemetics are useful for symptom control in patients with DG. Among them, aprepitant, a neurokinin-1 inhibitor, showed to increase gastric accommodation and reduce nausea and vomiting in DE, even if it had no effect on GE [62].

Agonists of ghrelin and 5-hydroxytryptophan receptors, which are still experimental, are giving promising results [2,37]. Relamorelin is a ghrelin agonist administered by subcutaneous injection, which showed to reduce symptoms and increase GE half-time in phase 2 trials [2,64]. Its main side effect was glycemic control impairment, with more frequent hyperglycemia episodes and higher HbA1c levels [2,64].

As above mentioned, a better comprehension of pathogenic mechanisms of DG could lead to new effective therapies. One of these possible research targets is represented by micro-RNAs. MiR-10b-5p regulates development and function of ICCs and pancreatic β cells through the KLF11-KIT pathway, in murine models, knockout of mir-10b in KIT+ cells led to DM and gastroparesis [65]. In these mice, injection of miR-10b-5p mimic or Klf11 small interfering RNAs were effective in improving glucose homeostasis and gastric motility [65], thus suggesting a potential therapeutic role of micro-RNAs.

As concerns the possible role of alternative medicine in DG, cannabinoids use has been suggested because of their positive effects on chemotherapy-induced nausea and vomiting. However, there are no studies investigating the use of synthetic or herbal cannabinoids in the symptomatic treatment of dyspepsia or gastroparesis [66]. Acupuncture, instead, showed promising results in improving gastric emptying in both murine models and human studies [66]. Its effects seem to be mediated by vagal activity, but other mechanisms could be involved [66]. In murine models, in fact, electroacupuncture was associated with reduced apoptosis and increased proliferation of ICCs [67].

In refractory DG, more invasive therapeutic approaches should be considered and evaluated on a case-by-case basis. These treatments include supplemental nutrition, preferably enteral, administered through a feeding jejunostomy, gastric electric stimulation, pyloromyotomy, and sleeve or total gastrectomy [2,38].

Hospital admission should be considered in case of gastroparesis associated to refractory vomiting, dehydration, electrolyte abnormalities and malnutrition [68]. Clinical management of hospitalized patients requires pharmacological control of symptoms, intravenous hydration, electrolyte correction, glucose control and nutritional support. Enteral nutrition should be preferred, even if in case of severe DG gastric feeding is often not tolerated, thus a nasoduodenal or nasojejunal tube placement can be necessary [68]. Although enteral nutrition should be the first choice, short-term parenteral nutrition may be needed for selected patients, when nasoenteric tube placement or feeding is not tolerated or is contraindicated [68].

Figure 2 shows a therapeutic algorithm for DG, which includes the above discussed treatment options.

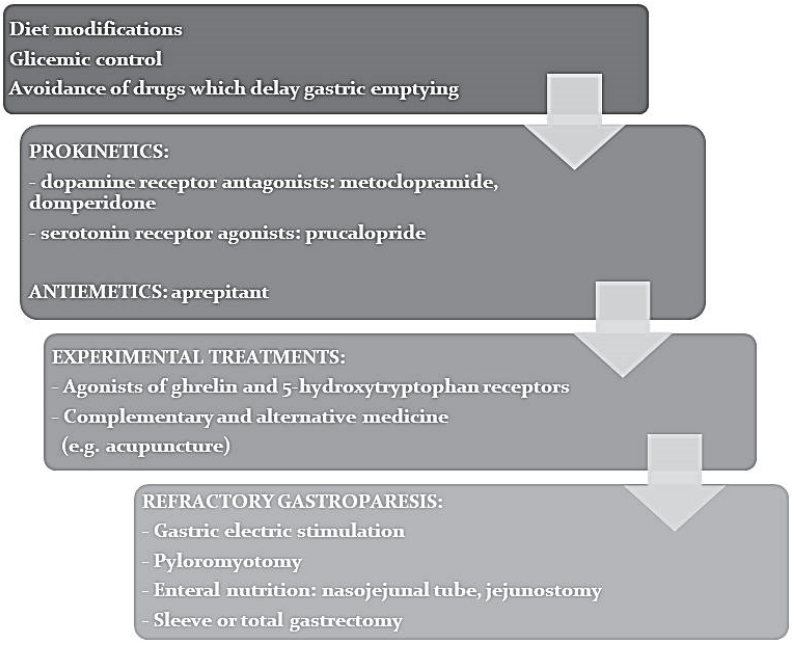


Figure 2. Treatment algorithm for diabetic gastroparesis.

8. Conclusions

FD and gastroparesis are characterized by a complex pathogenesis whose mechanisms remain unclear. This is even more true for patients with diabetes who often suffer from these disturbances. As a consequence, their management should be based initially on international guidelines and tailored to their individual needs. Well-designed studies are needed in this field.

Author Contributions: S.B. and R.P. designed the review, performed the research and wrote the paper; G.G., G.B., A.F. and G.M.S. critically revised the work. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: We thank Ingrid J. Hickman (Princess Alexandra Hospital, Brisbane, Queensland, Australia) for her comments on the manuscript and for her precious language revision.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. International Diabetes Federation. *IDF Diabetes Atlas*, 9th ed.; International Diabetes Federation: Brussels, Belgium, 2019.
2. Bharucha, A.E.; Kudva, Y.C.; Prichard, D.O. Diabetic gastroparesis. *Endocr. Rev.* **2019**, *40*, 1318–1352. [CrossRef] [PubMed]
3. Pasricha, P.J.; Grover, M.; Yates, K.P.; Abell, T.L.; Bernard, C.E.; Koch, K.L.; McCallum, R.W.; Sarosiek, I.; Kuo, B.; Bulat, R.; et al. Functional dyspepsia and gastroparesis in tertiary care are interchangeable syndromes with common clinical and pathological features. *Gastroenterology* **2021**. [CrossRef]
4. Durazzo, M.; Lupi, G.; Cicerchia, F.; Ferro, A.; Barutta, F.; Beccuti, G.; Gruden, G.; Pellicano, R. Extra-esophageal presentation of gastroesophageal reflux disease: 2020 Update. *J. Clin. Med.* **2020**, *9*, 2559. [CrossRef] [PubMed]
5. Moayyedi, P.; Lacy, B.E.; Andrews, C.N.; Enns, R.A.; Howden, C.W.; Vakil, N. ACG and CAG Clinical Guideline: Management of Dyspepsia. *Am. J. Gastroenterol.* **2017**, *112*, 988–1013. [CrossRef] [PubMed]

6. Oustamanolakis, P.; Tack, J. Dyspepsia: Organic versus functional. *J. Clin. Gastroenterol.* **2012**, *46*, 175–190. [CrossRef]
7. Sugano, K.; Tack, J.; Kuipers, E.J.; Graham, D.Y.; El-Omar, E.M.; Miura, S.; Haruma, K.; Asaka, M.; Uemura, N.; Malfertheiner, P. Faculty members of Kyoto Global Consensus Conference. Kyoto global consensus report on Helicobacter pylori gastritis. *Gut* **2015**, *64*, 1353–1367. [CrossRef]
8. Drossman, D.A. Functional gastrointestinal disorders: History, pathophysiology, clinical features and Rome IV. *Gastroenterology* **2016**, *150*, 1262–1279. [CrossRef]
9. Stanghellini, V.; Chan, F.K.; Hasler, W.L.; Malagelada, J.R.; Suzuki, H.; Tack, J.; Talley, N.J. Gastroduodenal disorders. *Gastroenterology* **2016**, *150*, 1380–1392. [CrossRef]
10. Mosso, E.; Bonetto, S.; Longobardi, G.; Abenavoli, L.; Ribaldone, D.G.; Saracco, G.M.; Pellicano, R. Management of functional dyspepsia in the year 2020. A clinical point of view. *Minerva Gastroenterol. Dietol.* **2020**, *66*, 331–342.
11. Koletzko, L.; Macke, L.; Schulz, C.; Malfertheiner, P. Helicobacter pylori eradication in dyspepsia: New evidence for symptomatic benefit. *Best Pract. Res. Clin. Gastr.* **2019**, *40–41*, 101637. [CrossRef]
12. Walker, M.M.; Potter, M.D.; Talley, N.J. Tangible pathologies in functional dyspepsia. *Best Pract. Res. Clin. Gastroent.* **2019**, *40*, 101650. [CrossRef]
13. Fagoonee, S.; Pellicano, R. Helicobacter pylori: Molecular basis for colonization and survival in gastric environment and resistance to antibiotics. A short review. *Infect. Dis.* **2019**, *51*, 399–408. [CrossRef]
14. Marinoni, C.; Ribaldone, D.G.; Rosso, C.; Astegiano, M.; Caviglia, G.P. Diagnosis of Helicobacter pylori infection: A look into molecular aspects of urea breath test. *Minerva Biotecnol.* **2019**, *31*, 100–107. [CrossRef]
15. Pellicano, R.; Ribaldone, D.G.; Fagoonee, S.; Astegiano, M.; Saracco, G.M.; Mégraud, F. A 2016 panorama of Helicobacter pylori infection: Key messages for clinicians. *Panminerva Med.* **2016**, *58*, 304–317. [PubMed]
16. Buzás, G.M. Helicobacter pylori and non-alcoholic fatty liver disease. *Minerva Gastroenterol. Dietol.* **2020**, *66*, 267–279. [CrossRef] [PubMed]
17. Yang, F.; Xu, Y.L.; Zhu, R.F. Helicobacter pylori infection and the risk of colorectal carcinoma: A systematic review and meta-analysis. *Minerva Med.* **2019**, *110*, 464–470. [CrossRef]
18. Valenzano, M.; Bisio, A.; Grassi, G. Helicobacter pylori and diabetes mellitus: A controversial relationship. *Minerva Endocrinol.* **2019**, *44*, 301–309. [CrossRef]
19. Santambrogio, E.; Orsucci, L. Helicobacter pylori and hematological disorders. *Minerva Gastroenterol. Dietol.* **2019**, *65*, 204–213. [CrossRef]
20. Chuah, K.H.; Mahadeva, S. Cultural factors influencing functional gastrointestinal disorders in the East. *J. Neurogastroenterol. Motil.* **2018**, *24*, 536–543. [CrossRef]
21. Stanghellini, V.; Tosetti, C.; Paternico, A.; Barbara, G.; Morselli-Labate, A.M.; Monetti, N.; Marengo, M.; Corinaldesi, R. Risk indicators of delayed gastric emptying of solids in patients with functional dyspepsia. *Gastroenterology* **1996**, *110*, 1036–1042. [CrossRef]
22. Tack, J.; Van den Houte, K.; Carbone, F. The unfulfilled promise of prokinetics for functional dyspepsia/post-prandial distress syndrome. *Am. J. Gastroenterol.* **2019**, *114*, 204–206. [CrossRef] [PubMed]
23. Miwa, H.; Oshima, T.; Tomita, T.; Fukui, H.; Kondo, T.; Yamasaki, T.; Watari, J. Recent understanding of the pathophysiology of functional dyspepsia: Role of the duodenum as the pathogenic center. *J. Gastroenterol.* **2019**, *54*, 305–311. [CrossRef] [PubMed]
24. Pellicano, R. Human microbiota and inflammatory bowel diseases: New pieces of an intricate puzzle. *Minerva Gastroenterol. Dietol.* **2019**, *65*, 171–172. [CrossRef]
25. Korotkyi, O.; Vovk, A.; Galenova, T.; Vovk, T.; Dvorschenko, K.; Luzza, F.; Abenavoli, L.; Kobyliak, N.; Falalyeyeva, T. Effect of probiotic on serum cytokines and matrix metalloproteinases profiles during monoiodoacetate-induced osteoarthritis in rats. *Minerva Biotecnol.* **2019**, *31*, 68–73. [CrossRef]
26. Korotkyi, O.; Dvorshchenko, K.; Falalyeyeva, T.; Sulaieva, O.; Kobyliak, N.; Abenavoli, L.; Fagoonee, S.; Pellicano, R.; Ostapchenko, L. Combined effects of probiotic and chondroprotector during osteoarthritis in rats. *Panminerva Med.* **2020**, *62*, 93–101. [CrossRef] [PubMed]
27. Pasini, E.; Corsetti, G.; Assanelli, D.; Testa, C.; Romano, C.; Dioguardi, F.S.; Aquilani, R. Effects of chronic exercise on gut microbiota and intestinal barrier in human with type 2 diabetes. *Minerva Med.* **2019**, *110*, 3–11. [CrossRef]
28. Caviglia, G.P.; Rosso, C.; Ribaldone, D.G.; Dughera, F.; Fagoonee, S.; Astegiano, M.; Pellicano, R. Physiopathology of intestinal barrier and the role of zonulin. *Minerva Biotecnol.* **2019**, *31*, 83–92. [CrossRef]
29. Adriani, A.; Ribaldone, D.G.; Astegiano, M.; Durazzo, M.; Saracco, G.M.; Pellicano, R. Irritable bowel syndrome: The clinical approach. *Panminerva Med.* **2018**, *60*, 213–222. [CrossRef]
30. Gotfried, J.; Priest, S.; Schey, R. Diabetes and the Small Intestine. *Curr. Treat Options Gastroenterol.* **2017**, *15*, 490–507. [CrossRef]
31. Marathe, C.S.; Rayner, C.K.; Wu, T.; Jones, K.L.; Horowitz, M. Gastrointestinal Disorders in Diabetes. In *Endotext [Internet]*; Feingold, K.R., Anawalt, B., Boyce, A., Chrousos, G., de Herder, W.W., Dungan, K., Grossman, A., Hershman, J.M., Hofland, J., Kaltsas, G., et al., Eds.; MDText.com, Inc.: South Dartmouth, MA, USA, 2000.
32. Devrajani, B.R.; Shah, S.Z.; Soomro, A.A.; Devrajani, T. Type 2 diabetes mellitus: A risk factor for Helicobacter pylori infection: A hospital based case-control study. *Int. J. Diabetes Dev. Ctries.* **2010**, *30*, 22–26. [CrossRef]
33. Bener, A.; Ağan, A.F.; Al-Hamaq, A.O.A.A.; Barisik, C.C.; Öztürk, M.; Ömer, A. Prevalence of Helicobacter pylori infection among Type 2 Diabetes Mellitus. *Adv. Biomed. Res.* **2020**, *9*, 27. [CrossRef] [PubMed]

34. Huang, J. Analysis of the Relationship between Helicobacter pylori infection and diabetic gastroparesis. *Chin. Med. J.* **2017**, *130*, 2680–2685. [CrossRef] [PubMed]
35. Feldman, M.; Schiller, L.R. Disorders of gastrointestinal motility associated with diabetes mellitus. *Ann. Intern. Med.* **1983**, *98*, 378–384. [CrossRef]
36. Choung, R.S.; Locke, G.R., III; Schleck, C.D.; Zinsmeister, A.R.; Melton, L.J., III; Talley, N.J. Risk of gastroparesis in subjects with type 1 and 2 diabetes in the general population. *Am. J. Gastroenterol.* **2012**, *107*, 82–88. [CrossRef] [PubMed]
37. Tack, J.; van den Houte, K.; Carbone, F. Gastroduodenal motility disorders. *Curr. Opin. Gastroenterol.* **2018**, *34*, 428–435. [CrossRef]
38. Halland, M.; Bharucha, A.E. Relationship between control of glycemia and gastric emptying disturbances in diabetes mellitus. *Clin. Gastroenterol. Hepatol.* **2016**, *14*, 929–936. [CrossRef]
39. MacGregor, I.L.; Gueller, R.; Watts, H.D.; Meyer, J.H. The effect of acute hyperglycemia on gastric emptying in man. *Gastroenterology* **1976**, *70*, 190–196. [CrossRef]
40. Bharucha, A.E.; Batey-Schaefer, B.; Cleary, P.A.; Murray, J.A.; Cowie, C.; Lorenzi, G.; Driscoll, M.; Harth, J.; Larkin, M.; Christofi, M.; et al. Diabetes Control and Complications Trial–Epidemiology of Diabetes Interventions and Complications Research Group. Delayed gastric emptying is associated with early and long-term hyperglycemia in type 1 diabetes mellitus. *Gastroenterology* **2015**, *149*, 330–339. [CrossRef]
41. Alnasser, S.A.; Algeffari, M.A. Prevalence and risk factors of gastroparesis-related symptoms among patients with type 2 diabetes. *Int. J. Health Sci.* **2016**, *10*, 397–404.
42. Laway, B.A.; Malik, T.S.; Khan, S.H.; Rather, T.A. Prevalence of abnormal gastric emptying in asymptomatic women with newly detected diabetes and its reversibility after glycemic control-a prospective case control study. *J. Diabetes Complicat.* **2013**, *27*, 78–81. [CrossRef]
43. Jehangir, A.; Abdallah, R.T.; Parkman, H.P. Characterizing abdominal pain in patients with gastroparesis into neuropathic and nociceptive components. *J. Clin. Gastroenterol.* **2019**, *53*, 427–433. [CrossRef] [PubMed]
44. Durazzo, M.; Gargiulo, G.; Pellicano, R. Non-cardiac chest pain: A 2018 update. *Minerva Cardioangiol.* **2018**, *66*, 770–783. [CrossRef] [PubMed]
45. Roy, T.; Lloyd, C.E. Epidemiology of depression and diabetes: A systematic review. *J. Affect Disord.* **2012**, *142*, S8–S21. [CrossRef]
46. Zahn, A.; Langhans, C.D.; Hoffner, S.; Haberkorn, U.; Rating, D.; Haass, M.; Enck, P.; Stremmel, W.; Rühl, A. Measurement of gastric emptying by 13C-octanoic acid breath test versus scintigraphy in diabetics. *Z. Gastroenterol.* **2003**, *41*, 383–390.
47. Farrell, M.B. Gastric Emptying Scintigraphy. *J. Nucl. Med. Technol.* **2019**, *47*, 111–119. [CrossRef] [PubMed]
48. Ghoos, Y.F.; Maes, B.D.; Geypens, B.J.; Mys, G.; Hiele, M.I.; Rutgeerts, P.J.; Vantrappen, G. Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. *Gastroenterology* **1993**, *104*, 1640–1647. [CrossRef]
49. Ziegler, D.; Schadewaldt, P.; Pour Mirza, A.; Piolot, R.; Schommartz, B.; Reinhardt, M.; Vosberg, H.; Brösicke, H.; Gries, F.A. [13C]octanoic acid breath test for non-invasive assessment of gastric emptying in diabetic patients: Validation and relationship to gastric symptoms and cardiovascular autonomic function. *Diabetologia* **1996**, *39*, 823–830. [CrossRef]
50. Pinto-Sanchez, M.I.; Yuan, Y.; Hassan, A.; Bercik, P.; Moayyedi, P. Proton pump inhibitors and functional dyspepsia. *Cochrane Database Syst. Rev.* **2017**, *11*, CD01119.
51. Jin, M.; Mo, Y.; Ye, K.; Chen, M.; Liu, Y.; He, C. Efficacy of serotonin receptor agonists in the treatment of functional dyspepsia: A meta-analysis. *Arch Med. Sci.* **2019**, *15*, 23–32. [CrossRef] [PubMed]
52. Mansi, C.; Borro, P.; Giacomini, M.; Biagini, R.; Mele, M.R.; Pandolfo, N.; Savarino, V. Comparative effects of levosulpiride and cisapride on gastric emptying and symptoms in patients with functional dyspepsia and gastroparesis. *Aliment Pharmacol. Ther.* **2000**, *14*, 561–569. [CrossRef]
53. Tack, J.; Masuy, I.; Van Den Houte, K.; Wauters, L.; Schol, J.; Vanuytsel, T.; Vandenberghe, A.; Carbone, F. Drugs under development for the treatment of functional dyspepsia and related disorders. *Expert Opin. Investig. Drugs* **2019**, *28*, 871–889. [CrossRef]
54. Matsueda, K.; Hongo, M.; Tack, J.; Saito, Y.; Kato, H. A placebo-controlled trial of acotiamide for meal-related symptoms of functional dyspepsia. *Gut* **2012**, *61*, 821–828. [CrossRef]
55. Talley, N.J.; Locke, G.R.; Saito, Y.A.; Almazar, A.E.; Bouras, E.P.; Howden, C.W.; Lacy, B.E.; DiBaise, J.K.; Prather, C.M.; Abraham, B.P.; et al. Effect of amitriptyline and escitalopram on functional dyspepsia: A multicenter, randomized controlled study. *Gastroenterology* **2015**, *149*, 340–349.e2. [CrossRef]
56. Lacy, B.E.; Saito, Y.A.; Camilleri, M.; Bouras, E.; DiBaise, J.K.; Herrick, L.M.; Szarka, L.A.; Tilkes, K.; Zinsmeister, A.R.; Talley, N.J. Effects of antidepressants on gastric function in patients with functional dyspepsia. *Am. J. Gastroenterol.* **2018**, *113*, 216–224. [CrossRef] [PubMed]
57. Tomita, T.; Oshima, T.; Miwa, H. New approaches to diagnosis and treatment of functional dyspepsia. *Curr. Gastroenterol. Rep.* **2018**, *20*, 55. [CrossRef]
58. Tan, V.P.; Liu, K.S.; Lam, F.Y.; Hung, I.F.N.; Yuen, M.F.; Leung, W.K. Randomised clinical trial: Rifaximin versus placebo for the treatment of functional dyspepsia. *Aliment Pharmacol Ther.* **2017**, *45*, 767–776. [CrossRef] [PubMed]
59. Igarashi, M.; Nakae, H.; Matsuoka, T.; Takahashi, S.; Hisada, T.; Tomita, J.; Koga, Y. Alteration in the gastric microbiota and its restoration by probiotics in patients with functional dyspepsia. *BMJ Open Gastro* **2017**, *3*, e000144. [CrossRef] [PubMed]
60. Asha, M.Z.; Khalil, S.F.H. Pharmacological Approaches to Diabetic Gastroparesis: A systematic review of randomised clinical trials. *Sultan Qaboos Univ. Med. J.* **2019**, *19*, e291–e304. [CrossRef] [PubMed]

61. Dhir, R.; Richter, J.E. Erythromycin in the short- and long-term control of dyspepsia symptoms in patients with gastroparesis. *J. Clin. Gastroenterol.* **2004**, *38*, 237–242. [CrossRef]
62. Grover, M.; Farrugia, G.; Stanghellini, V. Gastroparesis: A turning point in understanding and treatment. *Gut* **2019**, *68*, 2238–2250. [CrossRef]
63. Carbone, F.; Van den Houte, K.; Clevers, E.; Andrews, C.N.; Papathanasopoulos, A.; Holvoet, L.; Van Oudenhove, L.; Caenepeel, P.; Arts, J.; Vanuytsel, T.; et al. Prucalopride in Gastroparesis: A Randomized Placebo-Controlled Crossover Study. *Am. J. Gastroenterol.* **2019**, *114*, 1265–1274. [CrossRef]
64. Camilleri, M.; McCallum, R.W.; Tack, J.; Spence, S.C.; Gottesdiener, K.; Fiedorek, F.T. Efficacy and Safety of Relamorelin in Diabetics With Symptoms of Gastroparesis: A Randomized, Placebo-Controlled Study. *Gastroenterology* **2017**, *153*, 1240–1250.e2. [CrossRef] [PubMed]
65. Singh, R.; Ha, S.E.; Wei, L.; Jin, B.; Zogg, H.; Poudrier, S.M.; Jorgensen, B.G.; Park, C.; Ronkon, C.F.; Bartlett, A.; et al. miR-10b-5p Rescues Diabetes and Gastrointestinal Dysmotility. *Gastroenterology* **2021**. [CrossRef] [PubMed]
66. Lee, L.A.; Chen, J.; Yin, J. Complementary and alternative medicine for gastroparesis. *Gastroenterol. Clin. N. Am.* **2015**, *44*, 137–150. [CrossRef] [PubMed]
67. Chen, Y.; Xu, J.J.; Liu, S.; Hou, X.H. Electroacupuncture at ST36 ameliorates gastric emptying and rescues networks of interstitial cells of Cajal in the stomach of diabetic rats. *PLoS ONE* **2013**, *8*, e83904. [CrossRef]
68. Rangan, V.; Ukleja, A. Gastroparesis in the Hospital Setting. *Nutr. Clin. Pract.* **2021**, *36*, 50–66. [CrossRef]

Journal of
Clinical Medicine

MDPI

Review

Clinical Relevance of *Helicobacter pylori* Infection

Irena Mladenova

Medical Faculty, Department of Hygiene, Epidemiology, Microbiology, Parasitology and Infectious Diseases, Trakia University, 6000 Stara Zagora, Bulgaria; imladenova@yahoo.com; Tel.: +359-897-324472

Abstract: *Helicobacter pylori (H. pylori)* is a Gram-negative helical, microaerophilic bacterium which colonizes the antrum and body of the stomach, surviving in its harsh environment through mechanisms of acid resistance and colonization factors. It infects approximately 50% of the world population. Although the prevalence of this infection varies from country to country, as well as between different ethnic, social or age groups, it is estimated that about 50% of the human population only carries this microorganism. While *H. pylori* has been found to play a major etiological and pathogenic role in chronic gastritis, peptic ulcer disease and gastric cancer, its importance for many types of extragastric disease needs to be further investigated. The choice of tests to diagnose *H. pylori* infection, defined as invasive or non-invasive, depends on the clinical indication as to whether to perform upper gastrointestinal endoscopy. Focusing on bacterial eradication, the treatment should be decided locally based on the use of antibiotics and documented antibiotic resistance. The author provides an overview of the current state of knowledge about the clinical aspects of *H. pylori* infection, especially its diagnostic and therapeutic management.

Keywords: *Helicobacter pylori*; chronic gastritis; peptic ulcer disease; gastric cancer; MALT-lymphoma; therapy; vaccines

Citation: Mladenova, I. Clinical Relevance of *Helicobacter pylori* Infection. *J. Clin. Med.* **2021**, *10*, 3473. https://doi.org/10.3390/jcm10163473

Academic Editor: Marilena Durazzo

Received: 14 July 2021
Accepted: 4 August 2021
Published: 6 August 2021

1. Introduction

The identification of *Helicobacter pylori (H. pylori)* by researchers Warren and Marshall in 1982 revolutionized the concept of gastric inhospitality and the consideration of peptic ulcer as a noninfectious disease. This microorganism is a Gram negative helical, microaerophilic bacteria which colonizes the antrum and body of the stomach, surviving in its harsh environment through mechanisms of acid resistance and colonization factors [1]. Through the enzyme urease, the microorganism creates a cloud of acid neutralizing substances around itself, offering protection from the acid [2]. Since *H. pylori* infects more than 50% of the world's population, it represents one of the most common infections in humans, usually acquired in the preschool period, with a risk of acquisition declining after 5 years of age and influenced by poorer living conditions during childhood [3].

Although the prevalence of this infection is different in all countries and also between ethnic, social, or age groups, much higher rates of *H. pylori* infection have been reported in developing countries than in developed countries. Nevertheless, it is important to highlight that only a minority of infected people develops health issues and life-threatening diseases [3].

In this paper, the author provides an overview of the current state of knowledge about the clinical aspects of *H. pylori* infection, especially its diagnostic and therapeutic management.

2. Clinical Impact of *H. pylori* Infection

2.1. Gastroduodenal Diseases

2.1.1. Gastritis and Peptic Ulcer

H. pylori is a major etiological and pathogenic factor for chronic gastritis, peptic ulcer (PUD) and gastric cancer [3]. In a recent review, a total of 55 randomized controlled trials and long-term treatments of peptic ulcer disease were included. The authors concluded

that one or two-week eradication treatment of H. pylori infection is a very effective therapy for *H. pylori*-positive patients with duodenal ulcer compared to ulcer healing drugs alone or without therapy [4]. The Kyoto Global Consensus Meeting has been convened to reach a global consensus on the classification of chronic gastritis and duodenitis, to differentiate *H. pylori*-induced dyspepsia from functional dyspepsia, to adequately diagnose gastritis, and to help clinicians decide when, whom and how to treat. An anonymous electronic consensus-building system has been adopted using the Delphi method. In order to better organize the definition of gastritis and duodenitis based on the etiology, the experts recommended a new classification of these conditions. In particular, an innovative diagnostic algorithm of *H. pylori*-associated dyspepsia has been proposed [5].

Classifications of chronic gastritis have been offered over time. Currently, pathologists mainly follow the recommendations of the revised Sydney System. OLGA (Operative Link for Gastritis Assessment) and OLGIM (Operative Link for Gastritis Intestinal Metaplasia) assessments reported by pathologists are used by clinicians to differentiate patients with chronic gastritis for special monitoring [6]. Those who have chronic gastritis without *H. pylori* infection should be considered to have a disease caused by a previous *H. pylori* infection [7].

To understand the diseases associated with *H. pylori.* it is important to study the virulence factors. Lately, factors have been reported for the colonization HopQ, SabA, BabA, OipA, and necessary factors necessary which are a sign of a pathogenicity island, such as vacuolating cytotoxin A (VacA), cytotoxin-associated gene antigen (CagA), and the outer membrane vesicles [8,9]. Several studies have evaluated the prevalence of *H. pylori* CagA and VacA genotypes, assessing the relationship with the type of damage caused by gastroduodenal mucosa. In patients with PUD, the genotype's vacA s1 cagA-positive strain has a close relationship. The vacAs1 subtype has been detected in all patients. Hence, VacA s1 is an important marker of virulence and patients harboring these strains are more likely to develop ulcers. VacA s1 could serve as the only best marker for the virulence of *H. pylori* [10].

2.1.2. Dyspepsia

The prevalence of functional dyspepsia in the general population is 10–20%. It is a functional disorder of the gastrointestinal tract. Typical dyspeptic symptoms are nausea, epigastric pain, and a feeling of fullness. Proton pump inhibitors (PPIs) and *H. pylori* eradication are the most commonly used treatment. Impaired quality of life in patients with functional dyspepsia suggests the need for definitive diagnosis, followed by symptomatic treatment and preventive management of relapse [11,12].

Functional dyspepsia is often associated with *H. pylori* infection. A patient who has an endoscopy without a pathology is defined as having functional dyspepsia and *H. pylori* therapy must be offered in case of infection [13]. Patients with dyspepsia and *H. pylori* infection may have clinical features that distinguish them from those with functional dyspepsia but without infection. In some studies, the authors evaluated the existence of clinical differences between uninfected individuals with functional dyspepsia and those with *H. pylori* infection and dyspepsia.

The prevalence of *H. pylori* in 578 dyspeptic patients without significant lesions detected by endoscopy was 58% (divided into two groups, i.e., positive or negative for *H. pylori*). Cases of dyspepsia and *H. pylori* infection have been associated with obesity, blood pressure, diabetes, and metabolic syndrome, but finally the paired analysis negated all the differences. Thus, dyspeptic patients positive for *H. pylori* have the same clinical features as uninfected ones [14].

Many randomized trials have investigated the effects of *H. pylori* eradication in patients with functional dyspepsia. The pooled estimates were measured by the fixed or random effect model from 25 randomized controlled trials in 5555 patients with functional dyspepsia. The pooled risk ratio (RR) was 1.23 (95% confidence interval (CI): 1.12–1.36). *H. pylori* eradication induced improvement in symptoms during more than 1 year follow-up (RR = 1.24, $p < 0.0001$) but not with follow-up time less than 1 year (RR = 1.26, $p = 0.27$).

Seven studies did not show a benefit from *H. pylori* eradication on quality of life. In six studies, eradication therapy reduced the incidence of PUD. Ten studies reported that patients who received eradication therapy had higher probability of experiencing histological regression of chronic gastritis than untreated ones [15].

Thus, the eradication of *H. pylori* in patients with functional dyspepsia has to be individual evaluated [16].

2.1.3. Gastric Cancer

H. pylori infection is considered to be the biggest risk factor for stomach cancer, a disease that takes hundreds of thousands of lives a year. This bacterium was classified as a group I carcinogen in 1994 by the World Health Organization (WHO) [3].

Approximately 75% of the global burden of gastric cancer and 5.5% of malignancies worldwide are due to inflammation and injury caused by *H. pylori*. It has been shown that *H. pylori* eradication therapy reduced the incidence of gastric cancer in high-risk areas. In Japan the treatment against *H. pylori* infection is effective to prevent stomach cancer (Lin et al.) [17]. However, the extent of benefit of the same approach in people living in areas with different prevalence of gastric cancer remains unclear. A meta-analysis included randomized controlled trials to assay the effects of eradication therapy on the risk of developing gastric cancer. People with *H. pylori* eradication had a lower incidence of gastric cancer than those without eradication therapy. The baseline incidence of gastric cancer altered the benefit of *H. pylori* eradication. Eradication provided significant benefit to both asymptomatic infected patients and to ones followed by endoscopic resection for gastric cancer. Therefore, there was a link between the elimination of *H. pylori* infection and the reduced incidence of the stomach cancer. The benefits of eradication varied depending on the incidence of gastric cancer but were associated to the baseline risk [18].

As stomach cancer is one of the leading cause of cancer-related deaths worldwide, the approach to preventing this malignancy is a very important public health issue. Gastric cancer is associated with an inflammatory tumor with multistage and multifactorial carcinogenesis. The process includes a series of steps with development of metaplastic epithelium, dysplasia and gastric cancer. *H. pylori* infection is critical for the development of the disease and studies have consistently shown that bacterial eradication reduces inflammation of the gastric mucosa, stopping the progression of the atrophy, metaplasia and dysplasia and lowering the risk of PUD onset and carcinogenesis development. The screening and eradication of *H. pylori* have recently begun only in high-risk populations. Elimination of gastric cancer requires information for implementing effective *H. pylori* screening and treatment programs, taking into account other health priorities in each particular population [19].

The pathogenesis caused by *H. pylori* is mainly attributed to its virulence factors, including urease, flagella, VacA, and CagA. The last two factors, VacA and CagA, play a key role. Infection with vacA-positive strains of *H. pylori* can lead in the stomach mucosa to vacuolation and apoptosis, while infection with CagA-positive strains can result in severe gastritis and gastric cancer. Studies focused on gastric carcinogenesis divide risk factors into categories such as host responses, genotypes, strain variation, and environmental factors. By assessing the interactions between these factors, we can understand the risk and progression of the disease in people with persistent colonization [20].

The CagA protein is an oncoprotein that can induce malignancies in mammals. On delivery, CagA disrupts multiple host pathways by acting as a scaffold. CagA-induced gastric carcinogenesis progresses through a shock-triggering mechanism in which the prooncogenic actions of CagA are followed by a series of genetic or epigenetic changes composed of cancer-prone cells during long-term infection with CagA-positive *H. pylori* [21]. Identifying high-risk individuals is important for monitoring and preventing stomach cancer. The presence of first-degree relatives diagnosed with gastric cancer is a strong risk factor for gastric cancer. The pathogenic mechanisms are unclear. There is an increased risk of developing stomach cancer among patients having two or more affected first-degree relatives with a family history of *H. pylori* infection. Eradication of *H. pylori* is the most

important strategy to prevent stomach cancer in first-degree relatives of patients with stomach cancer. Early eradication of *H. pylori* can prevent progression to intestinal metaplasia and reduce the possibility of developing gastric carcinogenesis in these individuals [22].

It is recommended to adopt classification systems for stratification of gastric cancer risk and modern endoscopy to improve the image for the diagnosis of gastritis. Eradication therapy against *H. pylori* prior to the development of preno-plastic changes has been recommended to minimize the risk of severe complications from this infection [5]. These changes could be an important factor in identifying high risk individuals for gastric cancer [23]. Patients undergoing endoscopic treatment for gastric cancer are at high risk of developing metachronous gastric cancer. Patients with precancerous lesions who do not reverse after treatment with *H. pylori* are at the 'point of no return' and may be at high risk of developing stomach cancer. An earlier eradication of *H. pylori* should prevent the development of gastric cancer before the onset of precancerous lesions [24,25].

The authors of one study recommended, for previous cases of atrophic gastritis caused by *H. pylori*, endoscopic monitoring every 3 years for high risk patients, including those with endoscopic severe atrophy or intestinal metaplasia [18]. Japan introduced a strategy for *H. pylori* eradication in 2013 to reduce the number of new cases of gastric cancer and deaths caused by this malignancy and, respectively, the medical costs. It was estimated that the number of deaths from stomach cancer could be reduced to 30,000 per year by 2020, but the annual number of deaths in 2017 remained above 45,000. The effect of the strategy may not appear until 2023. The risk of gastric cancer is likely to increase in some populations due to the widespread use of PPIs and dysbiosis in the stomach mucosa. In one study a combined therapy with PPIs and aspirin has been proposed after the eradication of *H. pylori* [26]. To reduce the incidence of stomach cancer health promotion has to be included, including adequate physical activity, low alcohol intake, dietary nutrition, and quitting smoking [27,28].

In prospective studies conducted by immunoblot assay (compared to those using ELISA-based methods), the worldwide attributed fraction for *H. pylori* in non-cardia gastric cancer has increased to 89.0% (6.2% of all cancers). In this way, the role of *H. pylori* as a main cause of gastric cancer has been enhanced [29]. The incidence and case fatality rate from stomach cancer are higher in developing countries than in developed countries. The prognosis of gastric cancer is much poorer because of its diagnostic delay. Approximately 2% of *H. pylori*-positive individuals develop gastric cancer [30].

2.1.4. Gastric Mucosa-Associated Lymphoid Tissue (MALT) Lymphoma

The low-grade B-cell lymphoma, called the mucosal associated lymphoid tissue (MALT) lymphoma, is formed in the stomach in response to antigenic stimulation, primarily associated with *H. pylori* infection. There is a strong relationship between *H. pylori* infection and low-grade MALT lymphoma. Furthermore, *H. pylori* eradication in patients with low-grade MALT lymphoma leads to tumor regression [31]. *H. pylori* strains of gastric MALT lymphoma appear to be less virulent than those associated with PUD or gastric carcinoma. A specific antigenic profile of Lewis has been identified in these strains and may represent an alternative mechanism to avoid the immune response of the macro-organism, thus allowing a continuous antigenic stimulation of the lymphocytes in the tissue [32].

In France, in a population-based study, the clinical characteristics and survival of patients with MALT lymphoma were analyzed. Among 460 confirmed patients only 44 showed early transformation into diffuse large B-cell lymphoma and were thought to have initially missed high-grade lymphoma. *H. pylori* was detected in 57% of the cases. Eradication was obtained in 76% of patients and complete remission occurred in 70%. The overall 5-year survival was 79% [33]. The diagnosis of MALT lymphoma has to be posed by endoscopic biopsy confirmed by an experienced pathologist. There are many variable endoscopic pictures such as erosion, lesion, atrophy, and ulcer, so many biopsies are needed to make an accurate diagnosis. Eradication therapy is the basis of treatment in all patients, at all stages of the disease. If remission does not occur after eradication

therapy, radiation therapy or chemotherapy should be given. In the case of advanced disease, immunotherapy with an anti-CD20 monoclonal antibody may be used [34].

3. Extra-Gastroduodenal Diseases

Many studies have shown that *H. pylori* infection can influence the onset of several extra-gastroduodenal diseases. The role of this bacteria in idiopathic thrombocytopenic purpura and iron deficiency anemia is currently well documented. Emerging data suggest that it may also contribute to insulin resistance, diabetes mellitus, non-alcoholic liver disease, and metabolic syndrome. In addition, it may increase the risk of coronary artery disease and neurodegenerative disease [35,36].

Several meta-analyses have been conducted on the potential association between cardiovascular disease and *H. pylori* infection. Meta-analytical approaches with fixed and random effects have been performed. The findings suggest a possible link between *H. pylori* infection and the risk of myocardial infarction [37,38]. Another field of investigation regards special populations, such as patients with chronic kidney disease, who present gastric mucosal injuries and dyspepsia more often than the general population. These diseases have a multifactorial pathogenesis and *H. pylori* infection could play a limited role in their development [39].

4. Diagnostic Methods

4.1. Initial Diagnosis

When endoscopy is required, the current diagnostic invasive approaches are biopsy and histology, immunohistochemistry, urease detection, culture assay, and polymerase chain reaction (PCR). The implementation of sequencing technologies is subject to the recommended guidelines for the management of *H. pylori* infection. The determination of the gold standard among all methods remains controversial, especially for epidemiological studies. Because of the declining sensitivity of invasive tests, non-invasive tests, including serology, stool antigen test and urea breath test, have been largely used for detecting *H. pylori.* Urea breath test and stool antigen test, among the non-invasive tests, are the best methods to detect the active infection. The sensitivity of serology tests is high but the specificity is relatively low. The guidelines show that no test can be considered the gold standard for diagnosing H. pylori and there are advantages and disadvantages of all methods [40–43].

4.2. Confirmation of Eradication

Confirmation of *H. pylori* eradication is always recommended. Stool antigen test and urea breath test can be used to confirm eradication when endoscopy is not required and have to be accomplished at least 4 weeks after the end of the therapy [43–45]. Since it is known that PPIs exert transient negative effects on *H. pylori* viability, morphology, and urease test, cessation of these drugs at least 14 days before testing for eradication could help avoid false-negative results (Maastricht V) [46].

5. Treatment of *H. pylori* Infection

5.1. First-Line Treatment

Considering the choosing of the regimen for *H. pylori* eradication, previous exposure to antibiotics should be accounted. The triple clarithromycin-based therapy must be confined to patients without prior exposure to macrolides living in areas with a low resistance to clarithromycin. At present, bismuth quadruple therapy or concomitant non-bismuth quadruple therapy (PPI, amoxicillin, clarithromycin and nitroimidazole) should be the preferred regimen. This has been shown to be most effective in overcoming antibiotic resistance. After the failure of the first-line therapy, the rescue regimen should avoid antibiotics that have been used before. If the patient has received first-line treatment containing clarithromycin, the preferred treatments are bismuth salts schemes or levofloxacin. Treatment with levofloxacin, which is known as a second-line therapy after treatment

with clarithromycin, should also be recommended after failure of quadriceps containing bismuth salts [47,48].

Recent key topics include studies to evaluate the effectiveness of bismuth quadruple therapies. Now there is strong evidence that it is the best first-line therapy in most countries. In fact, antibiotic resistance has been comprehensively studied and a drastic increase in resistance to clarithromycin and levofloxacin has been noted [49]. The utility of vonoprazan (a competing potassium acid blocker) instead of PPI therapy has also been considered, especially in resistant and difficult-to-treat groups. However, presently this drug is used only in Japan. The diversity of the intestinal microbiota is altered soon after the eradication of *H. pylori* with triple therapy and has been restored after 2 months [50].

In 2017, the WHO identified as a high priority for research the resistant *H. pylori* strains to clarithromycin. Resistance to metronidazole and fluoroquinolones has also increased worldwide [51]. The international consensuses for *H. pylori* eradication recommended quadruple therapy with bismuth or non-bismuth for 2 weeks as a first-line treatment in areas with a high resistance to clarithromycin and/or metronidazole [52]. These schemes provide good levels of eradication. A new approach is needed to reduce antimicrobials and to protect against resistance in case of dual therapy. A good option is vonoprazan and amoxicillin. This could be a breakthrough in the era of increasing antibiotic resistance. This scheme can provide an acceptable degree of eradication, reduces antimicrobial resistance because of the use of single antibiotic and includes an inhibitory effect of vonoprazan on the acid secretion of the stomach. Assessed in a first period only in Japan recently, its efficacy has also been reported in Australia. In a single-center study, conducted in the period January 2017–September 2019, treatment with vonoprazan-containing antibiotic therapy was capable of achieving 100% efficacy in patients treated for the first time and even 91% efficacy in patients with previous eradication failure (Gunaratne et al. [53]).

If the treatment of *H. pylori* fails, new approaches are needed. A meta-analysis aimed to study the role of symbiotics in eradication therapy. A random effects model has been applied to the pooling analysis due to the heterogeneity of the studies. The results have shown that the symbiotic may improve eradication with RR: 1.28. Frequent adverse effects from the antibiotic therapy have been significantly diminished by adding a symbiotic to standard antibiotic treatment. The meta-analysis has suggested that symbiotics might improve the eradication rate of *H. pylori* infection, and reduce the adverse effects [54].

5.2. Second-Line Treatment

The failure of the first-line therapy for *H. pylori* infection requires a second-line therapy which is challenged due to potential microbiological resistance to the antibiotics included initially [55]. There is no "golden" standard in rescue eradication therapy after failure of first-line treatment. The advice of the Maastricht V/Florence Consensus Report is in favor of quadruple bismuth therapy or triple/quadruple fluoroquinolone–amoxicillin therapy as a second-line therapy [46]. Meta-analyses proved that the eradication results of quadruple bismuth therapy and levofloxacin–amoxicillin therapy are almost equal, while the first has more adverse effects than the second. The rate of eradication of triple and quadruple levofloxacin-based therapies is suboptimal.

In case of fluoroquinolone resistance, the triple or quadruple levofloxacin–amoxicillin therapy has a lower efficacy of eradication. A 10-day therapy consisting of PPI, bismuth, tetracycline and levofloxacin was recently developed, which achieved a significantly higher degree of eradication compared to triple therapy with PPI–levofloxacin–amoxicillin (98% vs. 69%) in patients after failure of standard therapies [55,56].

As a second-line treatment, the tetracycline–levofloxacin, bismuth-based or levofloxacin–amoxicillin quadruple therapies could be administered for *H. pylori* eradication. Recent data suggest that 10-days tetracycline–levofloxacin therapy is an effective scheme and a candidate for rescue treatment after failure of eradication by all first-line schemes for *H. pylori* infection. A document comparing the recommendations in the guidelines of expert groups in Europe, Canada and the United States has been published. The guidelines recommend

bismuth quadruple therapy for first-line treatment, replacing triple clarithromycin-based therapy. In case of unsuccessful treatment, because of the resistance to antibiotics or another drugs, they must be avoided in eradication therapy. Second-line therapies have to be quadruple bismuth therapy; triple levofloxacin therapy, a triple scheme based on rifabutin or amoxicillin in high doses plus PPI due to the suspicion of resistance, can be used for subsequent treatment [57,58].

5.3. Third-Line (and Further) Treatment

After two failed therapies, susceptibility-guided treatments have been administered as a third-line strategy. This could be a rescue treatment. Nevertheless, evidence in favor of this therapy is insufficient and the cure rate is moderate [58]. The efficacy of the third-line therapies for *H. pylori* is suboptimal, even after a bacterial culture. Resistance to many antibiotics is the main factor for treatment failure. The effectiveness and safety of 2-weeks eradication using high doses of amoxicillin, metronidazole and esomeprazole in patients with two previous failures of therapy has been assessed. Triple therapy with esomeprazole 40 mg twice daily, amoxicillin 1 g three times daily and metronidazole 500 mg three times daily for 14 days has been implemented as third-line therapy after first therapy, including clarithromycin, and a second-line treatment, including quinolone [59].

The microbiological resistance against antibiotics is increasing worldwide and the rate of failure of *H. pylori* first and second eradication lines is increasing. The role of cultural assay in testing of antibiotic susceptibility is very important to avoid the use of ineffective therapy. There are many causes of eradication failure, including poor compliance of patient with the eradication scheme, smoking, or factors related to treatment such as doses and length of therapy. Treatment could be modified specifically for the respective patient. Individuals at high risk of developing gastric cancer may receive definitive benefits after third or fourth line therapy [60].

Since there is the possibility that *H. pylori* eradication fails, in this case the indication to continue PPI treatment should be considered in patients at high risk for PUD complications, such as at increasing age and those who need a long term treatment with gastrolesive drugs such NSAID and for patients taking anticoagulation (Kanno and Moayyedi) [61].

5.4. Adding an Adjuvant Treatment

Probiotics have inhibitory effect on *H. pylori* and have been used as adjunctive therapy in *H. pylori* eradication. Probiotics have improved eradication rate of *H. pylori* and side effects of antibiotic treatment. Treatment outcomes are conflicting due to species, doses, and length of administration. Additional studies on the safety of adjuvant probiotics in eradication therapy of *H. pylori* are needed [62–64].

In one randomized placebo-controlled study the efficacy of probiotics as an adjuvant to eradication therapy of *H. pylori* has been evaluated. A total of 159 patients receiving sequential eradication therapy against *H. pylori* were included. The degree of elimination, patient compliance, and side effects of eradication therapy were recorded in each treatment group. Adjuvant application of a probiotic in 14 days sequential therapy for *H. pylori* has been associated with a higher rate of *H. pylori* eradication, lower rates of discontinuation associated with diarrhea, less frequent side effects and higher adherence to treatment [65].

6. Relapse and Reinfection

Recurrence can occur either through relapse or through reinfection. To determine relapse or reinfection, and to match the treatment and follow-up of patients to the nature of relapses, it is mandatory to study genotype [66]. Compared to reinfection, the relapse time window is usually shorter, followed by a recurrence of *H. pylori*-related diseases. Reinfection after an effective eradication therapy is very rare [67]. Several factors are responsible for *H. pylori* reinfection, such as the presence of *H. pylori* positive family members, poor living conditions, and health status. The factors for *H. pylori* relapse need further study [68].

7. Vaccine

Unfortunately, there is not an effective vaccine against *H. pylori*. This could be the best weapon against *H. pylori* and prevention of gastric cancer, respectively. A radical change in therapeutic strategies is needed to guide the final decisions about the management of *H. pylori*. The unique nature of *H. pylori* creates obstructions to the development of an immunogenic vaccine. In developing countries, the most reasonable and logical approach would be to recommend a preventive vaccine against *H. pylori* among children as a mandatory national program to reduce the likelihood of early acquisition of infection. Attempts to produce a prophylactic vaccine will be postponed to the future [69]. The modulating of the host immune responses by *H. pylori* results in increasing regulatory T cell proliferation. It is possible to generate protective immune responses by immunization with various *H. pylori* antigens or their epitopes, in combination with an adjuvant, so far only shown in mouse models. New non-toxic adjuvants have recently been developed, consisting of modified bacterial enterotoxins or nanoparticles, which can not only increase the efficacy of the vaccine but also help vaccines to be applied in clinical practice [70].

Knowledge of the immune mechanisms during *H. pylori* infection due to the host's complex response to the pathogen and the factors that allow bacterial persistence, such as *H. pylori* genetic diversity, are needed for an effective vaccine [71]. It would be a strong tool to prevent gastric cancer. Despite the high prevalence of the infection worldwide and evidence that vaccination can prevent children from acquiring *H. pylori* infection, the development of such a vaccine is not a current priority for major pharmaceutical companies. More investment is needed to step up research into the *H. pylori* vaccine [72,73]. This has to involve immunizing mice with classical and recombinant *H. pylori* antigens in order to develop a vaccine against *H. pylori*. Efficacy is very difficult to prove, usually involving many clinical trials. Promising results have been reported by Ming et al. in 2015 [74].

8. Conclusions

In clinical practice, since a decision must be made with therapeutic intent when the bacteria *H. pylori* is found, treatment should be based on topical antibiotic use and documented data on antibiotic resistance. In countries with a high rate of clarithromycin resistance (>15%), in the first line therapy, either 'concomitant' or bismuth-based quadruple therapies are recommended as empirical treatment if antimicrobial susceptibility testing is not possible. In the second-line therapy, as with empirical treatment, if antimicrobial susceptibility testing is not possible, quadruple therapy which was not used as a first-line treatment is recommended. Triple therapy combining PPI with amoxicillin and levofloxacin is also possible. Drugs such as rifabutin and furazolidone should be reserved for further steps.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Warren, J.R.; Marshall, B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* **1983**, *1*, 1273–1275. [PubMed]
2. Fagoonee, S.; Pellicano, R. *Helicobacter pylori*: Molecular basis for colonization and survival in gastric environment and resistance to antibiotics. A short review. *Infect. Dis.* **2019**, *25*, 1–10. [CrossRef]
3. International Agency for Research on Cancer; World Health Organization. Infection with Helicobacter pylori. In *Schistosomes, Liver Flukes and Helicobacter pylori*; IARC: Lyon, France, 1994; pp. 177–202.
4. Ford, A.C.; Gurusamy, K.S.; Delaney, B.; Forman, D.; Moayyedi, P. Eradication therapy for peptic ulcer disease in Helicobacter pylori-positive people. *Cochrane Database Syst Rev.* **2016**, *4*, CD003840. [CrossRef]
5. Sugano, K.; Tack, J.; Kuipers, E.J.; Graham, D.Y.; El-Omar, E.M.; Miura, S.; Haruma, K.; Asaka, M.; Uemura, N.; Malfertheiner, P. Kyoto global consensus report on *Helicobacter pylori* gastritis. *Gut* **2015**, *64*, 1353–1367. [CrossRef]

6. Bacha, D.; Walha, M.; Slama, S.B.; Romdhane, H.B.; Bouraoui, S.; Bellil, K.; Lahmar, A. Chronic gastritis classifications. *Tunis Med.* **2018**, *96*, 405–410.
7. Kishikawa, H.; Ojiro, K.; Nakamura, K.; Katayama, T.; Arahata, K.; Takarabe, S.; Miura, S.; Kanai, T.; Nishida, J. Previous *Helicobacter pylori* infection–induced atrophic gastritis: A distinct disease entity in an understudied population without a history of eradication. *Helicobacter* **2020**, *25*, e12669. [CrossRef]
8. Smith, S.I.; Jolaiya, T.; Onyekwere, C.; Fowora, M.; Ugiagbe, R.; Agbo, I.; Cookey, C.; Lesi, O.; Ndububa, D.; Adekanle, O.; et al. Prevalence of *Helicobacter pylori* infection among dyspeptic patients with and without type 2 diabetes mellitus in Nigeria. *Minerva Gastroenterol. Dietol.* **2019**, *65*, 36–41. [CrossRef] [PubMed]
9. Ansari, S.; Yamaoka, Y. *Helicobacter pylori* virulence factors exploiting gastric colonization and its pathogenicity. *Toxins* **2019**, *11*, 677. [CrossRef]
10. Jeyamani, L.; Jayarajan, J.; Leelakrishnan, V.; Swaminathan, M. CagA and VacA genes of *Helicobacter pylori* and their clinical relevance. *Indian J. Pathol. Microbiol.* **2018**, *61*, 66–69. [CrossRef]
11. Madisch, A.; Andresen, V.; Enck, P.; Labenz, J.; Frieling, T.; Schemann, M. The diagnosis and treatment of functional dyspepsia. *Dtsch. Arztebl. Int.* **2018**, *115*, 222–232. [CrossRef]
12. Mosso, E.; Bonetto, S.; Longobardi, G.; Abenavoli, L.; Ribaldone, D.G.; Saracco, G.M.; Pellicano, R. Management of functional dyspepsia in the year 2020. A clinical point of view. *Minerva Gastroenterol. Dietol.* **2020**, *66*, 331–342.
13. Moayyedi, P.; Lacy, B.E.; Andrews, C.N.; Enns, R.A.; Howden, C.W.; Vakil, N. Management of Dyspepsia. *Am. J. Gastroenterol.* **2017**, *112*, 988–1013. [CrossRef] [PubMed]
14. Rodríguez-García, J.L.; Carmona-Sánchez, R. Functional dyspepsia and dyspepsia associated with *Helicobacter pylori* infection: Do they have different clinical characteristics? *Rev. Gastroenterol. Mex.* **2016**, *81*, 126–133. [CrossRef] [PubMed]
15. Du, L.J.; Chen, B.R.; Kim, J.J.; Kim, S.; Shen, J.H.; Dai, N. Helicobacter pylori eradication therapy for functional dyspepsia: Systematic review and meta-analysis. *World J. Gastroenterol.* **2016**, *22*, 3486–3495. [CrossRef] [PubMed]
16. Aksu, A.Ü.; Yılmaz, G.; Gürkan, Ö.E.; Sarı, S.; Dalgıç, B. The effect of *Helicobacter pylori* eradication on functional dyspepsia in Turkish children. *Helicobacter* **2018**, *23*, e12497. [CrossRef] [PubMed]
17. Lin, Y.; Kawai, S.; Sasakabe, T.; Nagata, C.; Naito, M.; Tanaka, K.; Sugawara, Y.; Mizoue, T.; Sawada, N.; Matsuo, K.; et al. Effects of Helicobacter pylori eradication on gastric cancer incidence in the Japanese population: A systematic evidence review. *Jpn. J. Clin. Oncol.* **2021**, *51*, 1158–1170. [CrossRef]
18. Lee, Y.C.; Chiang, T.H.; Chou, C.K.; Tu, Y.K.; Liao, W.C.; Wu, M.S.; Graham, D.Y. Association between *Helicobacter pylori* eradication and gastric cancer incidence: A systematic review and meta-analysis. *Gastroenterology* **2016**, *150*, 1113–1124. [CrossRef]
19. Wu, J.Y.; Lee, Y.C.; Graham, D.Y. Eradication of *Helicobacter pylori* to prevent gastric cancer: A critical appraisal. *Expert Rev. Gastroenterol. Hepatol.* **2019**, *13*, 17–24. [CrossRef]
20. Amieva, M.; Peek, R.M., Jr. Pathobiology of *Helicobacter pylori*-induced gastric cancer. *Gastroenterology* **2016**, *150*, 64–78. [CrossRef]
21. Hatakeyama, M. *Helicobacter pylori* CagA and gastric cancer: A paradigm for hit-and-run carcinogenesis. *Cell Host Microbe* **2014**, *15*, 306–316. [CrossRef]
22. Choi, Y.J.; Kim, N. Gastric cancer and family history. *Korean J. Int. Med.* **2016**, *31*, 1042–1053. [CrossRef] [PubMed]
23. Ribaldone, D.G.; Simondi, D.; Petrini, E.; Astegiano, M.; Durazzo, M. Non-invasive biomarkers for gastric cancer diagnosis: Ready for prime time? *Minerva Biotecnol.* **2019**, *31*, 3–10. [CrossRef]
24. Li, L.; Yu, C. *Helicobacter pylori* infection following endoscopic resection of early gastric cancer. *BioMed Res. Int.* **2019**, *16*, 9824964. [CrossRef] [PubMed]
25. Venerito, M.; Vasapolli, R.; Rokkas, T.; Malfertheiner, P. Gastric cancer: Epidemiology, prevention, and therapy. *Helicobacter* **2018**, *23*, e12518. [CrossRef]
26. Uno, Y. Prevention of gastric cancer by *Helicobacter pylori* eradication: A review from Japan. *Cancer Med.* **2019**, *8*, 3992–4000. [CrossRef]
27. Na, H.-K.; Lee, J.Y. Molecular basis of alcohol-related gastric and colon cancer. *Int. J. Mol. Sci.* **2017**, *18*, 1116. [CrossRef] [PubMed]
28. den Hoed, C.M.; Kuipers, E.J. Gastric cancer: How can we reduce the incidence of this disease? *Curr. Gastroenterol. Rep.* **2016**, *18*, 34. [CrossRef]
29. Plummer, M.; Franceschi, S.; Vignat, J.; Forman, D.; de Martel, C. Global burden of gastric cancer attributable to *Helicobacter pylori*. *Int. J. Cancer* **2015**, *136*, 487–490. [CrossRef]
30. Sukri, A.; Hanafiah, A.; Zin, N.M.; Kosai, N.R. Epidemiology and role of *Helicobacter pylori* virulence factors in gastric cancer carcinogenesis. *APMIS* **2020**, *128*, 150–161. [CrossRef]
31. Wotherspoon, A.C. Gastric MALT lymphoma and *Helicobacter pylori*. *Yale J. Biol. Med.* **1996**, *69*, 61–68.
32. Floch, P.; Mégraud, F.; Lehours, P. *Helicobacter pylori* strains and gastric MALT lymphoma. *Toxins* **2017**, *9*, 132. [CrossRef]
33. Matysiak-Budnik, T.; Jamet, P.; Ruskoné-Fourmestraux, A.; de Mascarel, A.; Velten, M.; Maynadié, M.; Woronoff, A.S.; Trétarre, B.; Marrer, E.; Delafosse, P.; et al. Gastric MALT lymphoma in a population-based study in France: Clinical features, treatments and survival. *Aliment. Pharmacol. Ther.* **2019**, *50*, 654–663. [CrossRef]
34. Gong, E.J.; Choi, K.D. Diagnosis and treatment of gastric mucosa-associated lymphoid tissue lymphoma. *Korean J. Gastroenterol.* **2019**, *74*, 304–313. [CrossRef]
35. Pellicano, R.; Ianiro, G.; Fagoonee, S.; Settanni, C.R.; Gasbarrini, A. Extragastric diseases and *Helicobacter pylori*. *Helicobacter* **2020**, *25*, e12741. [CrossRef]

36. Ponzetto, A.; Marchet, A.; Pellicano, R.; Lovera, N.; Chianale, G.; Nobili, M.; Rizzetto, M.; Cerrato, P. Association of *Helicobacter pylori* infection with ischemic stroke of non-cardiac origin: The BAT.MAN project study. *Hepato Gastroenterol.* **2002**, *49*, 631–634.
37. Pellicano, R.; Mladenova, I.; Broutet, N.; Salmi, L.R.; Megraud, F. Is there an association between *Helicobacter pylori* and coronary heart disease? *Eur. J. Epidemiol.* **1999**, *15*, 611–619. [CrossRef] [PubMed]
38. Mladenova, I. *Helicobacter pylori* and cardiovascular disease: Update 2019. *Minerva Cardioangiol.* **2019**, *67*, 425–432. [CrossRef] [PubMed]
39. Zizzi, C.F.; Pellicano, R.; Biancone, L. The relationship between *Helicobacter pylori* and chronic kidney disease: Update 2020. *Minerva Gastroenterol. Dietol.* **2020**, *66*, 343–349.
40. Jones, N.L.; Koletzko, S.; Goodman, K.; Bontems, P.; Cadranel, S.; Casswall, T.; Czinn, S.; Gold, B.D.; Guarner, J.; Elitsur, Y.; et al. Joint ESPGHAN/NASPGHAN. Guidelines for the management of *Helicobacter pylori* in children and adolescents (Update 2016). *J. Pediatr. Gastroenterol. Nutr.* **2017**, *64*, 991–1003. [CrossRef]
41. Peng, C.; Hu, Y.; Ge, Z.M.; Zou, Q.M.; Lyu, N.H. Diagnosis and treatment of *Helicobacter pylori* infections in children and elderly populations. *Chronic Dis. Transl. Med.* **2020**, *5*, 243–251. [CrossRef]
42. Pohl, D.; Keller, P.M.; Bordier, V.; Wagner, K. Review of current diagnostic methods and advances in *Helicobacter pylori* diagnostics in the era of next generation sequencing. *World J. Gastroenterol.* **2019**, *25*, 4629–4660. [CrossRef]
43. Miftahussurur, M.; Yamaoka, Y. Diagnostic methods of *Helicobacter pylori* infection for epidemiological studies: Critical importance of indirect test validation. *BioMed Res. Int.* **2016**, *2016*, 4819423. [CrossRef] [PubMed]
44. Wang, X.M.; Yee, K.C.; Hazeki-Taylor, N.; Li, J.; Fu, H.Y.; Huang, M.L.; Zhang, G.Y. Oral *Helicobacter pylori*, its relationship to successful eradication of gastric *H. pylori* and saliva culture confirmation. *J. Physiol. Pharmacol.* **2014**, *65*, 559–566.
45. Diaconu, S.; Predescu, A.; Moldoveanu, A.; Pop, C.S.; Fierbinţeanu-Braticevici, C. *Helicobacter pylori* infection: Old and new. *J. Med. Life* **2017**, *10*, 112–117.
46. Malfertheiner, P.; Megraud, F.; O'Morain, C.A.; Gisbert, J.P.; Kuipers, E.J.; Axon, A.T.; Bazzoli, F.; Gasbarrini, A.; Atherton, J.; Graham, D.Y.; et al. Management of *Helicobacter pylori* infection—The Maastricht V/Florence Consensus Report. *Gut* **2017**, *66*, 6–30. [CrossRef]
47. Pellicano, R.; Zagari, R.M.; Zhang, S.; Saracco, G.M.; Moss, S.F. Pharmacological considerations and step by step proposal for the treatment of *Helicobacter pylori* infection in the year 2018. *Minerva Gastroenterol. Dietol.* **2018**, *64*, 310–321. [CrossRef]
48. Chey, W.D.; Leontiadis, G.I.; Howden, C.W.; Moss, S.F. ACG Clinical Guideline: Treatment of *Helicobacter pylori* infection. *Am. J. Gastroenterol.* **2017**, *112*, 212–239. [CrossRef]
49. Gong, E.J.; Ahn, J.Y.; Kim, J.M.; Lee, S.M.; Na, H.K.; Lee, J.H.; Jung, K.W.; Choi, K.D.; Kim, D.H.; Song, H.J.; et al. Genotypic and Phenotypic Resistance to Clarithromycin in *Helicobacter pylori* Strains. *J. Clin. Med.* **2020**, *9*, 1930. [CrossRef]
50. O'Connor, A.; Liou, J.M.; Gisbert, J.P. O'Morain, C. Review: Treatment of *Helicobacter pylori* infection 2019. *Helicobacter* **2019**, *24*, e12640. [PubMed]
51. World Health Organization. Available online: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed (accessed on 13 July 2021).
52. Suzuki, S.; Esaki, M.; Kusano, C.; Ikehara, H.; Gotoda, T. Development of *Helicobacter pylori* treatment: How do we manage antimicrobial resistance? *World J. Gastroenterol.* **2019**, *25*, 1907–1912. [CrossRef] [PubMed]
53. Gunaratne, A.W.; Hamblin, H.; Clancy, A.; Magat, A.J.M.C.; Dawson, M.V.M.; Tu, J.; Borody, T.J. Combinations of antibiotics and vonoprazan for the treatment of Helicobacter pylori infections—Exploratory study. *Helicobacter* **2021**, e12830. [CrossRef]
54. Pourmasoumi, M.; Najafgholizadeh, A.; Hadi, A.; Mansour-Ghanaei, F.; Joukar, F. The effect of synbiotics in improving *Helicobacter pylori* eradication: A systematic review and meta-analysis. *Complement. Ther. Med.* **2019**, *43*, 36–43. [CrossRef]
55. Lin, T.F.; Hsu, P.I. Second-line rescue treatment of *Helicobacter pylori* infection: Where are we now? *World J. Gastroenterol.* **2018**, *24*, 4548–4553. [CrossRef] [PubMed]
56. Nguyen, C.T.; Davis, K.A.; Nisly, S.A.; Li, J. Treatment of *Helicobacter pylori* in special patient populations. *Pharmacotherapy* **2019**, *39*, 1012–1022. [CrossRef]
57. Fallone, C.A.; Moss, S.F.; Malfertheiner, P. Reconciliation of recent *Helicobacter pylori* treatment guidelines in a time of increasing resistance to antibiotics. *Gastroenterology* **2019**, *157*, 44–53. [CrossRef] [PubMed]
58. Puig, I.; López-Góngora, S.; Calvet, X.; Villoria, A.; Baylina, M.; Sanchez-Delgado, J.; Suarez, D.; García-Hernando, V.; Gisbert, J.P. Systematic review: Third-line susceptibility-guided treatment for *Helicobacter pylori* infection. *Therap. Adv. Gastroenterol.* **2016**, *9*, 437–448. [CrossRef]
59. Puig, I.; González-Santiago, J.M.; Molina-Infante, J.; Barrio, J.; Herranz, M.T.; Algaba, A.; Castro, M.; Gisbert, J.P.; Calvet, X. Fourteen-day high-dose esomeprazole, amoxicillin and metronidazole as third-line treatment for *Helicobacter pylori* infection. *Int. J. Clin. Pract.* **2017**, *71*, 9. [CrossRef]
60. Choi, J.H.; Yang, Y.J.; Bang, C.S.; Lee, J.J.; Baik, G.H. Current Status of the Third-Line *Helicobacter pylori* Eradication. *Gastroenterol. Res. Pract.* **2018**, *2018*, 6523653. [CrossRef] [PubMed]
61. Kanno, T.; Moayyedi, P. Who Needs Gastroprotection in 2020? *Curr. Treat Options Gastroenterol.* **2020**, *11*, 1–17. [CrossRef] [PubMed]
62. Zhu, X.Y.; Liu, F. Probiotics as an adjuvant treatment in *Helicobacter pylori* eradication therapy. *J. Dig. Dis.* **2017**, *18*, 195–202. [CrossRef] [PubMed]
63. Mladenova-Hristova, I. The inhibitory effect of genus Lactobacillus on *Helicobacter pylori* infection. *Trakia J. Sci.* **2013**, *11*, 299–303.
64. Hu, Y.; Zhu, Y.; Lu, N.H. Recent progress in *Helicobacter pylori* treatment. *Chin. Med. J.* **2020**, *133*, 335–343. [CrossRef]

65. Çekin, A.H.; Şahintürk, Y.; Harmandar, F.A.; Uyar, S.; Yolcular, B.O.; Çekin, Y. Use of probiotics as an adjuvant to sequential *H. pylori* eradication therapy: Impact on eradication rates, treatment resistance, treatment-related side effects, and patient compliance. *Turk. J. Gastroenterol.* **2017**, *28*, 3–11. [CrossRef] [PubMed]
66. Raymond, J.; Thiberge, J.M.; Dauga, C. Diagnosis of *Helicobacter pylori* recurrence: Relapse or reinfection? Usefulness of molecular tools. *Scand. J. Gastroenterol.* **2016**, *51*, 672–678. [CrossRef] [PubMed]
67. Kayali, S.; Manfredi, M.; Gaiani, F.; Bianchi, L.; Bizzarri, B.; Leandro, G.; Di Mario, F.; De'Angelis, G.L. *Helicobacter pylori*, transmission routes and recurrence of infection: State of the art. *Acta Biomed.* **2018**, *89*, 72–76.
68. Sun, Y.; Zhang, J. *Helicobacter pylori* recrudescence and its influencing factors. *J. Cell. Mol. Med.* **2019**, *23*, 7919–7925. [CrossRef]
69. Abadi, A.T.B. Vaccine against *Helicobacter pylori*: Inevitable approach. *World J. Gastroenterol.* **2016**, *22*, 3150–3157. [CrossRef] [PubMed]
70. Lehours, P.; Ferrero, R.L. Review: *Helicobacter*: Inflammation, immunology, and vaccines. *Helicobacter* **2019**, *24*, e12644. [CrossRef]
71. Stubljar, D.; Jukic, T.; Ihan, A. How far are we from vaccination against *Helicobacter pylori* infection? *Expert Rev. Vaccines* **2018**, *17*, 935–945. [CrossRef]
72. Sutton, P.; Boag, J.M. Status of vaccine research and development for *Helicobacter pylori*. *Vaccine* **2019**, *37*, 7295–7299. [CrossRef]
73. Ribaldone, D.G.; Fagoonee, S.; Astegiano, M.; Durazzo, M.; Morgando, A.; Sprujevnik, T.; Giordanino, C.; Baronio, M.; De Angelis, C.; Saracco, G.M.; et al. Rifabutin-based rescue therapy for *Helicobacter pylori* eradication: A long-term prospective study in a large cohort of difficult-to-treat patients. *J. Clin. Med.* **2019**, *8*, 199. [CrossRef] [PubMed]
74. Zeng, M.; Mao, X.; Li, J.; Tong, W.; Wang, B.; Zhang, Y.; Guo, G.; Zhao, Z.; Li, L.; Wu, D.; et al. Efficacy, safety, and immunogenicity of an oral recombinant *Helicobacter pylori* vaccine in children in China: A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* **2015**, *386*, 1457–1464. [CrossRef]

Journal of
Clinical Medicine

MDPI

Review

Autoimmune Diseases of Digestive Organs—A Multidisciplinary Challenge: A Focus on Hepatopancreatobiliary Manifestation

Lumir Kunovsky [1,2,3], Petr Dite [1,4,5], Petr Jabandziev [3,6,7,*], Zdenek Kala [2,3], Jitka Vaculova [1,3], Tomas Andrasina [3,8], Matej Hrunka [3,6], Martina Bojkova [4,5] and Jan Trna [1,3,9]

1 Department of Gastroenterology and Internal Medicine, University Hospital Brno, 625 00 Brno, Czech Republic; kunovsky.lumir@fnbrno.cz (L.K.); pdite.epc@gmail.com (P.D.); vaculova.jitka@fnbrno.cz (J.V.); jan.trna@seznam.cz (J.T.)
2 Department of Surgery, University Hospital Brno, 625 00 Brno, Czech Republic; kala.zdenek@fnbrno.cz
3 Faculty of Medicine, Masaryk University, 625 00 Brno, Czech Republic; andrasina.tomas@fnbrno.cz (T.A.); hrunka.matej@fnbrno.cz (M.H.)
4 Department of Gastroenterology and Internal Medicine, University Hospital Ostrava, 728 00 Ostrava, Czech Republic; martina.jelsikova@seznam.cz
5 Faculty of Medicine, University of Ostrava, 703 00 Ostrava, Czech Republic
6 Department of Pediatrics, University Hospital Brno, 613 00 Brno, Czech Republic
7 Central European Institute of Technology, Masaryk University, 625 00 Brno, Czech Republic
8 Department of Radiology and Nuclear Medicine, University Hospital Brno, 625 00 Brno, Czech Republic
9 Department of Gastroenterology and Digestive Endoscopy, Masaryk Memorial Cancer Institute, 602 00 Brno, Czech Republic
* Correspondence: jabandziev.petr@fnbrno.cz

Citation: Kunovsky, L.; Dite, P.; Jabandziev, P.; Kala, Z.; Vaculova, J.; Andrasina, T.; Hrunka, M.; Bojkova, M.; Trna, J. Autoimmune Diseases of Digestive Organs—A Multidisciplinary Challenge: A Focus on Hepatopancreatobiliary Manifestation. *J. Clin. Med.* **2021**, *10*, 5796. https://doi.org/10.3390/jcm10245796

Academic Editor: Marilena Durazzo

Received: 26 October 2021
Accepted: 7 December 2021
Published: 11 December 2021

Abstract: It is well known that some pathological conditions, especially of autoimmune etiology, are associated with the HLA (human leukocyte antigen) phenotype. Among these diseases, we include celiac disease, inflammatory bowel disease, autoimmune enteropathy, autoimmune hepatitis, primary sclerosing cholangitis and primary biliary cholangitis. Immunoglobulin G4-related diseases (IgG4-related diseases) constitute a second group of autoimmune gastrointestinal, hepatobiliary and pancreatic illnesses. IgG4-related diseases are systemic and rare autoimmune illnesses. They often are connected with chronic inflammation and fibrotic reaction that can occur in any organ of the body. The most typical feature of these diseases is a mononuclear infiltrate with IgG4-positive plasma cells and self-sustaining inflammatory response. In this review, we focus especially upon the hepatopancreatobiliary system, autoimmune pancreatitis and IgG4-related sclerosing cholangitis. The cooperation of the gastroenterologist, radiologist, surgeon and histopathologist is crucial for establishing correct diagnoses and appropriate treatment, especially in IgG4 hepatopancreatobiliary diseases.

Keywords: human leukocyte antigen; celiac disease; inflammatory bowel disease; autoimmune hepatitis; primary sclerosing cholangitis; primary biliary cholangitis; autoimmune pancreatitis; IgG4-related sclerosing cholangitis; IgG4-related hepatopathy

1. Introduction

Autoimmune diseases of digestive organs can be divided into three groups: (1) autoimmune digestive diseases associated with human leukocyte antigen (HLA), (2) immunoglobulin G4-related diseases (IgG4-RD) and (3) other autoimmune gastrointestinal diseases. It is well known that some pathological conditions, especially of autoimmune etiology, are associated with different HLA phenotypes [1,2]. Among these diseases, we include celiac disease, inflammatory bowel disease, autoimmune hepatitis, primary sclerosing cholangitis and primary biliary cholangitis. It is widely recognized that incidence of autoimmune diseases is generally increasing. We can demonstrate this in the incidence of autoimmune hepatitis (AIH). In an English study published by Grønbaek et al., the incidence of AIH grew between the years 1997 and 2015 from 1.27 to 2.56 per 100,000 population per year [3].

Very similar data were reported for a Danish cohort, in which case the incidence doubled between 1994 and 2012 [4]. This demonstrates the importance of our topic.

2. Autoimmune Digestive System Diseases Associated with HLA

2.1. Celiac Disease

Celiac disease is represented by a gluten-sensitive enteropathy. It develops in genetically susceptible individuals. The main role is played by T cell lymphocytes reactivity against gluten [5]. The diagnostic criteria are well defined in children; the last ESPGHAN (European Society for Pediatric Gastroenterology, Hepatology and Nutrition) guidelines admit the non-biopsy diagnostic approach, based only on serological tests (IgA antibodies against transglutaminase 2, IgA endomysial antibodies and total IgA antibodies). In defined cases, it is necessary to perform duodenal biopsy [6]. However, in adults the biopsy is necessary for the diagnosis of celiac disease [7]. Treatment is based upon a gluten-free diet. Celiac disease was first reported to be associated with HLA class 1 molecule B8. Later, association was shown with four haplotypes (celiac dimers) [8]. Celiac disease is an autoimmune illness and is today an important candidate for the clinical use of human leukocyte antigen isotype DQ (HLA-DQ) genotyping. The main determinants for genetic susceptibility are HLA-DQA1 and HLA-DQB1 genes encoding for HLA-DQ2 and HLA-DQ8 molecules [9]. In this context, HLA analysis seems to be an important resource in the diagnostic armamentarium for serving a population at high risk of celiac disease [10]. According to recent guidelines, HLA testing attained a new role in the diagnostic approach, because of its high negative predictive value [6]. Very interesting is the presence of autoantibodies against Saccharomyces cerevisiae (ASCA) in celiac patients, mainly before initiating a gluten-free diet. These autoantibodies are more specific for Crohn's disease. Granito et al. [11] reported, in their study, 59% of celiac patients having ASCA positivity. The potential explanation of these phenomenon is the immune response for small bowel inflammation [11,12].

2.2. Inflammatory Bowel Disease (IBD)

The role of HLA in patients with inflammatory bowel disease (IBD) remains uncertain. In addition, unclear is the etiology of IBD, which is assumed to include a combination of an individual's genetic background, alteration of gut microbiota, immune dysregulation and environmental factors [13]. HLA typing might be useful in discriminating Crohn's disease (CD) and ulcerative colitis (UC) [14,15] and its application may improve the sensitivity and specificity of serological markers. However, HLA typing is not routinely used in IBD patients. In clinical practice, we mainly use two serological markers of IBD, which are perinuclear anti-neutrophil cytoplasmic autoantibodies (pANCAs) and anti-Saccharomyces cerevisiae antibodies (ASCAs). ASCA, we mainly found in serum of CD patients, with sensitivity at 37–72% and specificity at 72–100%. ANCA are more common for UC patients with sensitivity reaching 70% and specificity almost 98% in some studies [16]. In a study by Bouzid et al. [17], patients with IBD showed significantly increased frequency of the homozygous DR Beta 1 (DRB1) 07 genotype [17].

The HLA system is also considered to be a major genetic marker and is associated with extraintestinal manifestation of IBD. For example, HLA-B27-positive patients with IBD have higher risk for developing ankylosing spondylitis. Primary sclerosing cholangitis, another autoimmune disease which is often coexisting with IBD, has also been associated with various HLA alleles. Generally, patients with IBD have increased an risk of various autoimmune and inflammatory diseases [18]. Treatment of IBD is a typical example of a multidisciplinary approach. The patient could be treated by aminosalicylates, corticosteroids, immunosuppressant, biologics or by surgery. Diet plays an especially important role in treating pediatric patients [19,20].

IBD is a diagnosis which offers wide scope for use of different biological drugs. They interfere with immune system on different levels. For example, in cytokine production, signaling pathway in T cell activation or inhibiting Januse kinase [21,22]. The most commonly

used are Tumor Necrosis Factor alfa antibodies (antiTNF-alfa) such as infliximab or adalimumab. These biologics have also been tried in the treatment of refractory autoimmune hepatitis [23] or primary sclerosing cholangitis [24]. However, antiTNF-alfa is currently not used in the treatment of autoimmune hepatitis, nor in primary sclerosing cholangitis. Despite the promise of biologics being able to target specific cellular and humoral pathways, results have been generally poor and safety has not been as expected [25].

We also have possibility to use some novel biologics for IBD treatment with promising therapeutic effects such as vedolizumab or ustekinumab [26,27].

2.3. Autoimmune Enteropathy (AIE)

Autoimmune enteropathy (AIE) in adults is a heterogeneous disease associated with a variety of circulating gut antibodies and predisposition to autoimmunity [28].

The pathophysiology of AIE is not exactly known. Dysfunction of $CD25^{+}CD4^{+}$ regulatory T cells probably plays an important role [29]. AIE is a result of humoral immune response involving anti-enterocyte antibodies (which have been detected in a majority of those affected) and anti-goblet cell antibodies [30]. Anti-enterocyte antibodies are not specific for AIE, as they have been described in such other diseases as allergic enteropathy, HIV infection and IBD [31]. On the other hand, other autoantibodies in patients with AIE are presented (e.g., antinuclear antibody or anti-smooth muscle antibodies) [32]. Antibodies against villin, a protein occurring in intestinal microvilli and proximal renal tubules, can be used in the diagnosis of immunodysregulation polyendocrinopathy enteropathy X-linked syndrome [33].

AIE is a rare condition, clinically connected with refractory diarrhea and malnutrition, mainly in children. Other typical changes are histological changes in small intestinal biopsy. In many patients, immunosuppressive therapies are principally used. Diagnostic criteria are detailed in Table 1 (adapted and modified from [28]).

Table 1. Proposed diagnostic criteria for adult autoimmune enteropathy (created in accordance with Akram et al. [28]).

1. Adult-onset chronic diarrhea (>6 weeks duration)
2. Malabsorption
3. Specific small bowel histology:
a. Partial/complete villous blunting
b. Deep crypt lymphocytosis
c. Increased crypt apoptotic bodies
d. Minimal intra-epithelial lymphocytosis
4. Exclusion of other causes of villous atrophy, including celiac disease, refractorysprue and intestinal lymphoma
5. Anti-enterocyte antibodies

Criteria 1–4 are required for a definite diagnosis of AIE. Presence of anti-enterocyte antibodies is an important diagnostic support, but their absence does not exclude the diagnosis of AIE. In the light of study from Biagi et al., we dare to remove anti-goblet cell antibodies from previous proposed diagnostic criteria. They are more unspecific than expected [34].

2.4. Autoimmune Hepatitis (AIH)

Autoimmune hepatitis (AIH) is a non-resolving inflammation of the liver. The disease reflects a complex interaction between triggering factors, autoantigens, genetic predisposition and immunoregulatory networks [35]. The disease usually is discriminated into three subtypes. Type 1 AIH is associated with antinuclear antibodies (ANAs) and/or smooth muscle antibodies (SMA). Type 2 AIH affects mostly children. Typical is a positivity of antibodies to liver-kidney microsome type 1 (LKM-1). Type 3 AIH is associated with soluble liver antigens. Diagnostic criteria for AIH are presented in Table 2.

Table 2. Simplified diagnostic criteria for AIH (created in accordance with Hennes et al. [36]).

Criteria	Cut-off	Points
ANA or SMA	≥1:40	1
ANA or SMA	≥1:80	
or LKM	≥1:40	2 (max. 2 points for all antibodies)
or SLA	Positive	
IgG	>Upper normal limit	1
	>1.10 times upper normal limit	2
Liver histology (evidence of hepatitis is a necessary condition)	Compatible with AIH	1
	Typical AIH	2
Absence of viral hepatitis	Yes	2
		≥6: probable AIH
		≥7: definite AIH

AIH is a global disease, occurring most frequently as type 1. HLA typing was part of original and revised diagnostic criteria of AIH [37], but now in simplified criteria is not accepted as a routine test for AIH, although it probably could be useful in distinguishing overlapping syndromes, in differentiating various types of autoimmune liver disease [38] or explaining regional differences in incidence of the disease [39]. Treatment of AIH includes using corticosteroids in induction therapy and azathioprine as a first-line maintenance treatment [40].

2.5. Primary Biliary Cholangitis (PBC)

Formerly known as primary biliary cirrhosis, primary biliary cholangitis (PBC) is a chronic cholestatic disease of unknown etiology and affecting mainly females. Essential to positive diagnosis is a combination of serological markers of cholestasis, the presence of autoantibodies such as antimitochondrial antibodies (AMA) and PBC specific antinuclear antibodies (ANA) anti-gp210 and anti-sp100 and imaging of liver and bile ducts. In unclear cases, we can perform liver biopsy [41]. The disease is characterized by the slow, progressive destruction of small intrahepatic bile ducts and by autoantibodies positivity. Ursodeoxycholic acid and obeticholic acid are available for managing PBC [41]. The end-point of this disease is liver cirrhosis and liver failure. For end-stage liver disease, liver transplant is the method of choice. The pathogenesis is multifactorial; genetic and environmental factors can induce an autoimmune reaction against bile ducts. The disease is strongly associated with several HLA haplotypes. According to a Scandinavian study, the most prominent risk HLA haplotypes are HLA-DRB1*13:01-DQA1*01:03-DQB1*06:03 [42].

Genetic factors are very important and documented by the high concordance rate of PBC among monozygotic twins [43]. This group of autoimmune gastrointestinal and hepatobiliary diseases is characterized not only by positivity of autoantigens, but also by positivity of HLA markers, genetic association and cytotoxic T cells population.

2.6. Primary Sclerosing Cholangitis (PSC)

Primary sclerosing cholangitis (PSC) is a rare, chronic cholestatic liver disease that can lead to liver fibrosis and cirrhosis. Pathologically, PSC is characterized by an inflammation and destruction of extra- and intrahepatic bile ducts. The etiology of this disease is unknown. Association between PSC and IBD has been described [44]. IBD was found in 80% of all patients with PSC, but PSC was found in just 5% of all IBD patients [45].

The pathogenetic mechanism is still unknown. Inflammatory changes are centered on the biliary epithelium and damage of the biliary tree is frequently observed. PSC is probably an immunologically mediated process with HLA association [46]. Experimental studies have shown that bacterial overgrowth also plays a role and there is a link with ulcerative colitis and gene mutations (e.g., *CFTR* mutation (cystic fibrosis transmembrane receptor mutation)) [47,48]. Subtypes of PSC are large-duct PSC, small-duct PSC, overlap syndrome with AIH and PSC with elevated IgG4 in serum and/or tissue [49]. Imaging

methods and estimation of serum alkaline phosphatase level play crucial roles in diagnosis. Liver biopsy is usually unnecessary, although this could be helpful in differential diagnosis (e.g., to determine AIH overlap syndrome or small-duct PSC). The prognosis for this disease varies and the course of the disease is connected with serum alkaline phosphatase level.

There is no effective pharmacological therapy. It remains unclear whether or not administering ursodeoxycholic acid can be effective. Liver transplantation is an effective treatment for end-stage liver disease [50].

3. Immunoglobulin G4-Related Diseases (IgG4-RD)

The second group of autoimmune gastrointestinal, hepatobiliary and pancreatic diseases is termed immunoglobulin G4-related diseases (IgG4-RD). IgG4-RD is a group of systemic and rare autoimmune diseases, often connected with chronic inflammation and fibrotic reaction that can occur in any organ of the body [51]. Etiology of IgG4-RD remains unclear. Current knowledge suggests that IgG4-RD are autoimmune disorders, where T and B cell lymphocytes are involved in pathophysiology [52]. The role of IgG4 antibodies has two explanations. The first is that IgG4 destroys tissues. The second is that high levels of IgG4 may reflect only overexpression of antibodies as a response to unknown inflammatory stimulus [53]. The most typical feature of these diseases is a mononuclear infiltrate with IgG4-positive plasma cells and self-sustaining inflammatory response.

IgG4 is an immunoglobulins fraction accounting for just 5.0% of the IgG pool. IgG4 is physiologically produced after a long-term exposure to food or environmental antigens. A current hypothesis is that the transformation from B cells to plasma cells and activation of eosinophilic granulocytes can probably be triggered by an initial Th1-type immune response via secretion of proinflammatory cytokines [36].

It is important that the level of plasmablast correlates with the diagnosis and disease activity much better than it does with serum IgG4 level. Plasmablast seems to be a precursor of tissue-resident antibody-producing plasma cells [54]. Oligoclonal cytotoxic T cell populations, such as CD4-positive cytotoxic T lymphocytes, correlate with disease activity and therefore may be better indicators of IgG4-RD activity, since serum IgG4 levels are not always increased. The cytotoxic T lymphocytes probably serve as vital antigen-presenting cells to rogue T cells, hence perpetuating the inflammation via secretion of profibrotic cytokines—growth factor-beta 1 and interleukin-1 beta—leading to chronic inflammation and fibrosis. A new biomarker consists in quantification of plasmablasts in peripheral blood. It has shown strong sensitivity and specificity for diagnosis of IgG4-RD [55]. Diagnostic criteria for IgG4-RD target organs are presented in Table 3.

Table 3. Diagnostic criteria for IgG4-RD target organs (created in accordance with Backhus and Löhr et al. [51,56]).

1. Clinical examination
a. Organ swelling
b. Pseudotumor
c. Jaundice
d. Minimal intra-epithelial lymphocytosis
2. Imaging
a. Diffuse or localized organ swelling
b. Pseudotumor
c. Pancreatic rim (in case of pancreatic involvement)
d. "Sausage-like" pancreas (in case of pancreatic involvement)
3. Assessing serum IgG4 concentration (upper level of normal = 135 mg/dL, but only levels higher than 4× the upper level seems to have clear diagnostic value)
4. Presence of 3 major histopathological characteristics
a. Lymphoplasmacellular infiltrate with IgG4+ plasma cells (ca 100%)
b. Storiform fibrosis (ca 75%)
c. Obliterative phlebitis (ca 45%) (see also Table 4)

3.1. Autoimmune Pancreatitis (AIP)

The most frequently encountered manifestation of IgG4-RD in the gastrointestinal system is autoimmune pancreatitis (AIP). Two subtypes of AIP have been described. Type 1 AIP is a typical pancreatic manifestation of IgG4-RD, while type 2 AIP is an autoimmune disease of the pancreas.

Type 1 AIP is known as lymphoplasmacytic sclerosing pancreatitis, or LPSP. There exists an international diagnostic consensus that can be followed when making the diagnosis according to the major IgG4-RD criteria. This consensus was published by Shimosegawa et al. [57] in 2011. Type 1 AIP is a rare disease, but in a German retrospective cross-sectional analysis the prevalence of AIP was 9.1%. All patients were nonalcoholic [58]. While type 2 AIP shares several features with type 1, a low amount or absence of IgG4 plasma cell infiltration and a presence of granulocytic epithelial lesions (GEL) are the most important diagnostic markers for type 2 AIP. Moreover, elevated serum IgG4 in type 2 AIP is very rare [59]. Diagnostic criteria for types 1 and type 2 AIP can be seen in Tables 4 and 5, respectively (adapted from [57]). Characteristics of types 1 and 2 AIP are summarized in Table 6 (adapted from [60]).

Table 4. Simplified international diagnostic criteria for type 1 autoimmune pancreatitis (created in accordance with Shimosegawa et al. [57]).

Criteria	Description
Pancreas histology (H)	Lymphoplasmacytic sclerosing pancreatitis (LPSP, core biopsy/resection). At least 3 of the following: periductal lymphoplasmacytic infiltrate without granulocytic infiltration, obliterative phlebitis, storiform fibrosis, abundant (>10 cells/high-power field) IgG4-positive cells
Parenchymal imaging (P)	Typical: diffuse enlargement with delayed enhancement (sometimes associated with ring-like enhancement)
Ductal imaging (D)	Long (>1/3 length of the main pancreatic duct) or multiple strictures without marked upstream dilation
Serology (S)	IgG4 > 2× upper normal limit
Other organ involvement (OOI)	1 or 2 1. Histology of extrapancreatic organs: • Lymphoplasmacytic infiltration with fibrosis and without granulocytic infiltration • Storiform fibrosis • Obliterative phlebitis • IgG4-positive plasma cells 2. Typical radiological evidence: • Segmental/multiple proximal bile duct stricture • Retroperitoneal fibrosis
Response to steroid therapy (Rt)	Rapid (≤2 weeks) radiologically demonstrable resolution or marked improvement in pancreatic/extrapancreatic manifestations

Table 5. Simplified international diagnostic criteria for type 2 autoimmune pancreatitis (created in accordance with Shimosegawa et al. [57]).

Criteria	Description
Histology (H)	Idiopathic duct centric pancreatitis (IDCP): Both of the following: • Granulocytic infiltration of duct wall (GEL) with or without granulocytic acinar inflammation • Absent or scant (0–10 cells/high-power field) IgG4-positive cells

Table 5. *Cont.*

Criteria	Description
Parenchymal imaging (P)	Typical: diffuse enlargement with delayed enhancement (sometimes associated with rim-like enhancement)
Ductal imaging (D)	Long (>1/3 length of the main pancreatic duct) or multiple strictures without marked upstream dilatation
Other organ involvement (OOI)	Clinically diagnosed inflammatory bowel disease
Response to steroid therapy (Rt)	Rapid (≤2 weeks) radiologically demonstrable resolution or marked improvement in pancreatic manifestations

Table 6. Characteristics of and fundamental differences between type 1 and type 2 autoimmune pancreatitis (created in accordance with Webster et al. [60]).

	Type 1 (LPSP)	Type 2 (IDCP)
IgG4-RD	Yes	No
Prevalence	Asia > USA/Europe	USA/Europe > Asia
Sex	M > F	M = F
Worldwide percentage (%)	>90	<10
Age predominance (years)	>50	30–50
Initial icterus (%)	>60	<30
Acute abdominal pain (%)	<30	>60
Elevated serum IgG4 (%)	>70	<10
Histopathology	Storiform fibrosis, LPSP, obliterative phlebitis	IDCP, GEL
Affection of other organs	Yes	No
Association with IBD (%)	<10	>40
Steroid response (%)	>90	>90
Relapse after steroid therapy (%)	>40	<10

AIP—autoimmune pancreatitis; IgG4-RD—IgG4-related disease; LPSP—lymphoplasmacytic sclerosing pancreatitis; IDCP—idiopathic duct centric pancreatitis; GEL—granulocytic epithelial lesions; IBD—Inflammatory bowel disease.

Despite international guidelines for diagnosing AIP, its differentiation from pancreatic cancer is still challenging [61]. In an interesting paper, Shih et al. report finding that patients with pancreatic cancer had significantly different profiling of IgG-glycosylation than did patients with AIP [62]. IgG glycosylation could probably be a useful marker in differentiating with high accuracy (sensitivity 94.6%, specificity 92.9%) between pancreatic ductal adenocarcinoma and focal form of AIP.

3.2. IgG4-Related Hepatobiliary Disease

The most common IgG4-RD among hepatobiliary diseases is IgG4-related sclerosing cholangitis. This disease is often associated with other organ manifestations of IgG4-related illnesses, most typically with type 1 AIP [63]. The disease is completely reversible under glucocorticoid therapy, which is typical for IgG4-RD. The most typical clinical symptoms in diagnosing IgG4-related sclerosing cholangitis are painless jaundice, pruritus, abdominal discomfort and oftentimes association with diabetes mellitus [64]. Biochemical markers of cholestasis are positive, the level of CA 19-9 could be very high (albeit with positive response to steroid therapy), serum IgG4 is elevated to >3 times the upper limit [65]. Histopathological criteria for IgG4-related sclerosing cholangitis are similar to those for AIP and are presented in Table 7.

Table 7. Histopathological criteria for IgG4-related sclerosing cholangitis (created in accordance with Deshpande et al. [66]).

1. Obliterative phlebitis
2. Storiform fibrosis
3. Lymphoplasmacellular infiltrate with more than 10 IgG4+ plasma cells per high- power field

In cholangiography, four typical images have been described and are classified by Nakazawa et al. [67]. The typical features are strictures of the lower part of the common bile duct, intrahepatic segmental or diffuse stricture, or a combination of hilar stricture with the lower part of common bile duct-related hepatopathy strictures. Figure 1 depicts IgG4-related sclerosing cholangitis classification with summary differential diagnosis (prepared in accordance with Nakazawa et al. [67]).

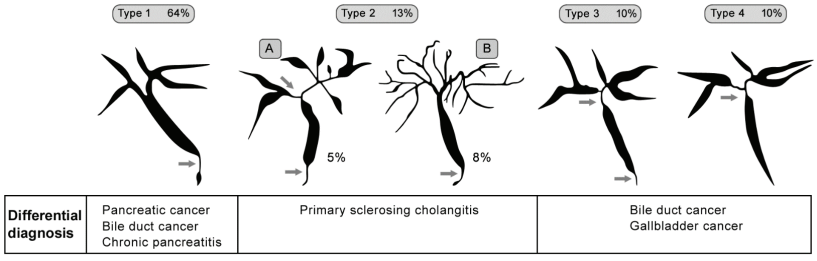


Figure 1. Classification of IgG4-related sclerosing cholangitis (prepared in accordance with Nakazawa et al. [67] and created in collaboration with the Service Center for E-Learning at Masaryk University, Faculty of Informatics).

In differential diagnoses, we must also always consider PSC. Figure 2 provides a comparison of the cholangiographic findings of IgG4-related sclerosing cholangitis and PSC (prepared in accordance with Ohara et al. [68]).

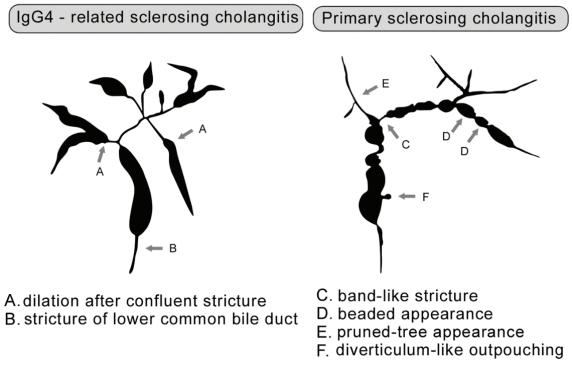


Figure 2. Comparison of cholangiographic findings of IgG4-related sclerosing cholangitis and PSC (prepared in accordance with Ohara et al. [68] and created in collaboration with the Service Center for E-Learning at Masaryk University, Faculty of Informatics).

In IgG4-related hepatopathy, five manifestations of liver involvement have been identified: (1) portal inflammation, (2) lobular hepatitis, (3) portal sclerosis, (4) lobular cholestasis and (5) bile duct obstruction [56]. The important diagnostic feature for IgG4-related hepatopathy is increasing IgG4 plasma cells. Nevertheless, the number of case reports on this topic is limited and the clinical relevance of IgG4-related AIH remains

unclear. Another problem is that different manifestations of hepatic changes in IgG4-RD have been reported in very small and retrospective studies [69,70].

3.3. Other IgG4-Related Gastrointestinal Diseases

Only a few clinical papers have been published about IgG4-related gastrointestinal diseases of the esophagus, stomach and bowel, with diffuse infiltration of the gastric mucosa by IgG4 + plasma cells being the most commonly described [71]. This infiltration does not fulfill the other histopathological criteria typical for IgG4-RD, but has been shown to disappear after oral therapy with steroids. In some patients, thickened (up to 15 mm) and nodular gastric mucosa can be seen [72].

In IgG4-related gastric lesions, focal polypoid lesions or focal masses up to 3 cm, gastric ulcer, diffuse thickening of the wall and association with local lymphadenopathies have been observed [73].

In colon biopsies from a set of 119 patients with IBD, Topal et al. found IgG4 positivity in 17.6% [74]. Obiorah et al. confirmed the diagnosis of IgG4-related esophagitis in 8 out of 18 patients [75]. Sporadic cases have also been described in the small bowel or rectum [76,77].

4. Other Autoimmune Gastrointestinal Diseases

Autoimmune Gastritis (AIG)

Autoimmune gastritis (AIG) is an immune-mediated chronic disease with mostly mild or non-specific clinical manifestation. It affects corpuscular acid-producing mucosa, especially parietal and chief cells, and leads to intrinsic factor deficiency and hypo- or achlorhydria [78]. AIG's etiology, which is not yet fully clarified, is marked by an important influence of genetic, hormonal and environmental factors in combination with immune dysregulation [79]. Although AIG is often reported to be a silent disease, we can encounter with it nonspecific gastrointestinal symptoms such as dyspepsia, postprandial fullness, nausea or early satiety. The most typical symptom is anemia, mainly from iron deficiency or as a pernicious anemia from vitamin B_{12} deficiency [80]. Essential to its diagnosis is upper gastrointestinal endoscopy with histological assessment of gastric biopsies. Anti-parietal cell antibodies provide a useful marker [78]. Treatment options for this condition are substantially limited and mainly focused on micronutrient supplementation. No anti-inflammatory, immunosuppressive or biological therapy is available [81].

5. Conclusions

Autoimmune diseases of the gastrointestinal organs constitute a huge gastroenterological challenge. Many gastrointestinal organs undergo biochemical and histopathological changes in connection with IgG4-RD or the HLA system. Diagnosis and therapy of these diseases require a multidisciplinary approach and cooperation among gastroenterologists, hepatologists, surgeons, immunologists, histopathologists and radiologists. There can be no doubt that autoimmune organ disorders have a place in the broad and multidisciplinary field of gastroenterology.

Author Contributions: Conceptualization, L.K. and P.D.; methodology, L.K.; literature search, J.V., T.A., M.H., M.B.; writing—original draft preparation, L.K, P.D., P.J., M.H., J.T.; writing—review and editing, Z.K., M.H., J.T.; supervision, Z.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Ministry of Health of the Czech Republic—conceptual development of research organization (FNBr, 65269705, Sup 3/21) and by the Ministry of Health of the Czech Republic, grant number NU20-03-00126.

Data Availability Statement: None.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Marsh, S.G.; Albert, E.D.; Bodmer, W.F.; Bontrop, R.E.; Dupont, B.; Erlich, H.A.; Geraghty, D.E.; Hansen, J.A.; Mach, B.; Mayr, W.R.; et al. Nomenclature for factors of the HLA system, 2002. *Tissue Antigens* **2002**, *60*, 407–464. [CrossRef]
2. Cassinotti, A.; Birindelli, S.; Clerici, M.; Trabattoni, D.; Lazzaroni, M.; Ardizzone, S.; Colombo, R.; Rossi, E.; Porro, G.B. HLA and autoimmune digestive disease: A clinically oriented review for gastroenterologists. *Am. J. Gastroenterol.* **2009**, *104*, 195–217, 194, 218. [CrossRef] [PubMed]
3. Grønbaek, L.; Otete, H.; Ban, L.; Crooks, C.; Card, T.; Jepsen, P.; West, J. Incidence, prevalence and mortality of autoimmune hepatitis in England 1997–2015. A population-based cohort study. *Liver Int.* **2020**, *40*, 1634–1644. [CrossRef] [PubMed]
4. Grønbæk, L.; Vilstrup, H.; Jepsen, P. Autoimmune hepatitis in Denmark: Incidence, prevalence, prognosis, and causes of death. A nationwide registry-based cohort study. *J. Hepatol.* **2014**, *60*, 612–617. [CrossRef] [PubMed]
5. Lundin, K.E.; Wijmenga, C. Coeliac disease and autoimmune disease-genetic overlap and screening. *Nat. Rev. Gastroenterol. Hepatol.* **2015**, *12*, 507–515. [CrossRef] [PubMed]
6. Husby, S.; Koletzko, S.; Korponay-Szabó, I.; Kurppa, K.; Mearin, M.L.; Ribes-Koninckx, C.; Shamir, R.; Troncone, R.; Auricchio, R.; Castillejo, G.; et al. European Society Paediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Coeliac Disease 2020. *J. Pediatr. Gastroenterol. Nutr.* **2020**, *70*, 141–156. [CrossRef]
7. Ludvigsson, J.F.; Bai, J.C.; Biagi, F.; Card, T.R.; Ciacci, C.; Ciclitira, P.J.; Green, P.H.; Hadjivassiliou, M.; Holdoway, A.; van Heel, D.A.; et al. Diagnosis and management of adult coeliac disease: Guidelines from the British Society of Gastroenterology. *Gut* **2014**, *63*, 1210–1228. [CrossRef]
8. Zubillaga, P.; Vidales, M.C.; Zubillaga, I.; Ormaechea, V.; García-Urkía, N.; Vitoria, J.C. HLA-DQA1 and HLA-DQB1 genetic markers and clinical presentation in celiac disease. *J. Pediatr. Gastroenterol. Nutr.* **2002**, *34*, 548–554. [CrossRef] [PubMed]
9. Sciurti, M.; Fornaroli, F.; Gaiani, F.; Bonaguri, C.; Leandro, G.; Di Mario, F.; De Angelis, G.L. Genetic susceptibilty and celiac disease: What role do HLA haplotypes play? *Acta Biomed.* **2018**, *89*, 17–21. [CrossRef]
10. Liu, E.; Dong, F.; Barón, A.E.; Taki, I.; Norris, J.M.; Frohnert, B.I.; Hoffenberg, E.J.; Rewers, M. High Incidence of Celiac Disease in a Long-term Study of Adolescents With Susceptibility Genotypes. *Gastroenterology* **2017**, *152*, 1329–1336.e1321. [CrossRef] [PubMed]
11. Granito, A.; Zauli, D.; Muratori, P.; Muratori, L.; Grassi, A.; Bortolotti, R.; Petrolini, N.; Veronesi, L.; Gionchetti, P.; Bianchi, F.B.; et al. Anti-Saccharomyces cerevisiae and perinuclear anti-neutrophil cytoplasmic antibodies in coeliac disease before and after gluten-free diet. *Aliment. Pharmacol. Ther.* **2005**, *21*, 881–887. [CrossRef]
12. Granito, A.; Muratori, L.; Muratori, P.; Guidi, M.; Lenzi, M.; Bianchi, F.B.; Volta, U. Anti-saccharomyces cerevisiae antibodies (ASCA) in coeliac disease. *Gut* **2006**, *55*, 296.
13. Zhang, Y.Z.; Li, Y.Y. Inflammatory bowel disease: Pathogenesis. *World J. Gastroenterol.* **2014**, *20*, 91–99. [CrossRef] [PubMed]
14. Oryoji, D.; Hisamatsu, T.; Tsuchiya, K.; Umeno, J.; Ueda, S.; Yamamoto, K.; Matsumoto, T.; Watanabe, M.; Hibi, T.; Sasazuki, T. Associations of HLA class I alleles in Japanese patients with Crohn's disease. *Genes Immun.* **2015**, *16*, 54–56. [CrossRef] [PubMed]
15. Mahdi, B.M. Role of HLA typing on Crohn's disease pathogenesis. *Ann. Med. Surg.* **2015**, *4*, 248–253. [CrossRef] [PubMed]
16. Kuna, A.T. Serological markers of inflammatory bowel disease. *Biochem. Med.* **2013**, *23*, 28–42. [CrossRef] [PubMed]
17. Bouzid, D.; Kammoun, A.; Amouri, A.; Mahfoudh, N.; Haddouk, S.; Tahri, N.; Makni, H.; Masmoudi, H. Inflammatory bowel disease: Susceptibility and disease heterogeneity revealed by human leukocyte antigen genotyping. *Genet. Test. Mol. Biomark.* **2012**, *16*, 482–487. [CrossRef] [PubMed]
18. Halling, M.L.; Kjeldsen, J.; Knudsen, T.; Nielsen, J.; Hansen, L.K. Patients with inflammatory bowel disease have increased risk of autoimmune and inflammatory diseases. *World J. Gastroenterol.* **2017**, *23*, 6137–6146. [CrossRef] [PubMed]
19. Torres, J.; Bonovas, S.; Doherty, G.; Kucharzik, T.; Gisbert, J.P.; Raine, T.; Adamina, M.; Armuzzi, A.; Bachmann, O.; Bager, P.; et al. ECCO Guidelines on Therapeutics in Crohn's Disease: Medical Treatment. *J. Crohns Colitis* **2020**, *14*, 4–22. [CrossRef]
20. Van Rheenen, P.F.; Aloi, M.; Assa, A.; Bronsky, J.; Escher, J.C.; Fagerberg, U.L.; Gasparetto, M.; Gerasimidis, K.; Griffiths, A.; Henderson, P.; et al. The Medical Management of Paediatric Crohn's Disease: An ECCO-ESPGHAN Guideline Update. *J. Crohns Colitis* **2021**, *15*, 171–194. [CrossRef]
21. Moroncini, G.; Calogera, G.; Benfaremo, D.; Gabrielli, A. Biologics in Inflammatory Immune-mediated Systemic Diseases. *Curr. Pharm. Biotechnol.* **2017**, *18*, 1008–1016. [CrossRef]
22. Baker, K.F.; Isaacs, J.D. Novel therapies for immune-mediated inflammatory diseases: What can we learn from their use in rheumatoid arthritis, spondyloarthritis, systemic lupus erythematosus, psoriasis, Crohn's disease and ulcerative colitis? *Ann. Rheum. Dis.* **2018**, *77*, 175–187. [CrossRef] [PubMed]
23. Weiler-Normann, C.; Schramm, C.; Quaas, A.; Wiegard, C.; Glaubke, C.; Pannicke, N.; Möller, S.; Lohse, A.W. Infliximab as a rescue treatment in difficult-to-treat autoimmune hepatitis. *J. Hepatol.* **2013**, *58*, 529–534. [CrossRef] [PubMed]
24. Björnsson, E.S.; Kalaitzakis, E. Recent advances in the treatment of primary sclerosing cholangitis. *Expert Rev. Gastroenterol. Hepatol.* **2021**, *15*, 413–425. [CrossRef]
25. Chang, C.; Tanaka, A.; Bowlus, C.; Gershwin, M.E. The use of biologics in the treatment of autoimmune liver disease. *Expert Opin. Investig. Drugs* **2020**, *29*, 385–398. [CrossRef] [PubMed]
26. Crooks, B.; Barnes, T.; Limdi, J.K. Vedolizumab in the treatment of inflammatory bowel disease: Evolving paradigms. *Drugs Context* **2020**, *9*, 2019-10-2. [CrossRef]

27. Feagan, B.G.; Sandborn, W.J.; Gasink, C.; Jacobstein, D.; Lang, Y.; Friedman, J.R.; Blank, M.A.; Johanns, J.; Gao, L.L.; Miao, Y.; et al. Ustekinumab as Induction and Maintenance Therapy for Crohn's Disease. *N. Engl. J. Med.* **2016**, *375*, 1946–1960. [CrossRef]
28. Akram, S.; Murray, J.A.; Pardi, D.S.; Alexander, G.L.; Schaffner, J.A.; Russo, P.A.; Abraham, S.C. Adult autoimmune enteropathy: Mayo Clinic Rochester experience. *Clin. Gastroenterol. Hepatol.* **2007**, *5*, 1282–1290, 1245. [CrossRef]
29. Russo, P.; Alvarez, F. Autoimmune enteropathy: A review. *Clin. Appl. Immunol. Rev.* **2002**, *2*, 203–216. [CrossRef]
30. Bishu, S.; Arsenescu, V.; Lee, E.Y.; Vargas, H.D.; de Villiers, W.J.; Arsenescu, R. Autoimmune enteropathy with a CD8+ CD7- T-cell small bowel intraepithelial lymphocytosis: Case report and literature review. *BMC Gastroenterol.* **2011**, *11*, 131. [CrossRef]
31. Montalto, M.; D'Onofrio, F.; Santoro, L.; Gallo, A.; Gasbarrini, A.; Gasbarrini, G. Autoimmune enteropathy in children and adults. *Scand. J. Gastroenterol.* **2009**, *44*, 1029–1036. [CrossRef]
32. Patey-Mariaud de Serre, N.; Canioni, D.; Ganousse, S.; Rieux-Laucat, F.; Goulet, O.; Ruemmele, F.; Brousse, N. Digestive histopathological presentation of IPEX syndrome. *Mod. Pathol.* **2009**, *22*, 95–102. [CrossRef]
33. Kobayashi, I.; Kubota, M.; Yamada, M.; Tanaka, H.; Itoh, S.; Sasahara, Y.; Whitesell, L.; Ariga, T. Autoantibodies to villin occur frequently in IPEX, a severe immune dysregulation, syndrome caused by mutation of FOXP3. *Clin. Immunol.* **2011**, *141*, 83–89. [CrossRef]
34. Biagi, F.; Bianchi, P.I.; Trotta, L.; Corazza, G.R. Anti-goblet cell antibodies for the diagnosis of autoimmune enteropathy? *Am. J. Gastroenterol.* **2009**, *104*, 3112. [CrossRef] [PubMed]
35. Czaja, A.J. Autoimmune hepatitis–approach to diagnosis. *MedGenMed* **2006**, *8*, 55.
36. Hennes, E.M.; Zeniya, M.; Czaja, A.J.; Parés, A.; Dalekos, G.N.; Krawitt, E.L.; Bittencourt, P.L.; Porta, G.; Boberg, K.M.; Hofer, H.; et al. Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology* **2008**, *48*, 169–176. [CrossRef] [PubMed]
37. Alvarez, F.; Berg, P.A.; Bianchi, F.B.; Bianchi, L.; Burroughs, A.K.; Cancado, E.L.; Chapman, R.W.; Cooksley, W.G.; Czaja, A.J.; Desmet, V.J.; et al. International Autoimmune Hepatitis Group Report: Review of criteria for diagnosis of autoimmune hepatitis. *J. Hepatol.* **1999**, *31*, 929–938. [CrossRef]
38. Sucher, E.; Sucher, R.; Gradistanac, T.; Brandacher, G.; Schneeberger, S.; Berg, T. Autoimmune Hepatitis-Immunologically Triggered Liver Pathogenesis-Diagnostic and Therapeutic Strategies. *J. Immunol. Res.* **2019**, *2019*, 9437043. [CrossRef]
39. Muratori, P.; Czaja, A.J.; Muratori, L.; Pappas, G.; Maccariello, S.; Cassani, F.; Granito, A.; Ferrari, R.; Mantovani, V.; Lenzi, M.; et al. Genetic distinctions between autoimmune hepatitis in Italy and North America. *World J. Gastroenterol.* **2005**, *11*, 1862–1866. [CrossRef]
40. Pape, S.; Schramm, C.; Gevers, T.J. Clinical management of autoimmune hepatitis. *United Eur. Gastroenterol. J.* **2019**, *7*, 1156–1163. [CrossRef]
41. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. *J. Hepatol.* **2017**, *67*, 145–172. [CrossRef] [PubMed]
42. Henriksen, E.K.K.; Viken, M.K.; Wittig, M.; Holm, K.; Folseraas, T.; Mucha, S.; Melum, E.; Hov, J.R.; Lazaridis, K.N.; Juran, B.D.; et al. HLA haplotypes in primary sclerosing cholangitis patients of admixed and non-European ancestry. *HLA* **2017**, *90*, 228–233. [CrossRef]
43. Selmi, C.; Mayo, M.J.; Bach, N.; Ishibashi, H.; Invernizzi, P.; Gish, R.G.; Gordon, S.C.; Wright, H.I.; Zweiban, B.; Podda, M.; et al. Primary biliary cirrhosis in monozygotic and dizygotic twins: Genetics, epigenetics, and environment. *Gastroenterology* **2004**, *127*, 485–492. [CrossRef] [PubMed]
44. Hirschfield, G.M.; Karlsen, T.H.; Lindor, K.D.; Adams, D.H. Primary sclerosing cholangitis. *Lancet* **2013**, *382*, 1587–1599. [CrossRef]
45. Gochanour, E.; Jayasekera, C.; Kowdley, K. Primary Sclerosing Cholangitis: Epidemiology, Genetics, Diagnosis, and Current Management. *Clin. Liver Dis. (Hoboken)* **2020**, *15*, 125–128. [CrossRef] [PubMed]
46. Lindor, K.D.; Kowdley, K.V.; Harrison, M.E. ACG Clinical Guideline: Primary Sclerosing Cholangitis. *Am. J. Gastroenterol.* **2015**, *110*, 646–659; quiz 660. [CrossRef] [PubMed]
47. Karlsen, T.H.; Folseraas, T.; Thorburn, D.; Vesterhus, M. Primary sclerosing cholangitis—A comprehensive review. *J. Hepatol.* **2017**, *67*, 1298–1323. [CrossRef]
48. Aabakken, L.; Karlsen, T.H.; Albert, J.; Arvanitakis, M.; Chazouilleres, O.; Dumonceau, J.M.; Färkkilä, M.; Fickert, P.; Hirschfield, G.M.; Laghi, A.; et al. Role of endoscopy in primary sclerosing cholangitis: European Society of Gastrointestinal Endoscopy (ESGE) and European Association for the Study of the Liver (EASL) Clinical Guideline. *Endoscopy* **2017**, *49*, 588–608. [CrossRef]
49. Culver, E.L.; Chapman, R.W. Systematic review: Management options for primary sclerosing cholangitis and its variant forms—IgG4-associated cholangitis and overlap with autoimmune hepatitis. *Aliment. Pharmacol. Ther.* **2011**, *33*, 1273–1291. [CrossRef]
50. Goode, E.C.; Clark, A.B.; Mells, G.F.; Srivastava, B.; Spiess, K.; Gelson, W.T.H.; Trivedi, P.J.; Lynch, K.D.; Castren, E.; Vesterhus, M.N.; et al. Factors Associated With Outcomes of Patients with Primary Sclerosing Cholangitis and Development and Validation of a Risk Scoring System. *Hepatology* **2019**, *69*, 2120–2135. [CrossRef]
51. Backhus, J.; Seufferlein, T.; Perkhofer, L.; Hermann, P.C.; Kleger, A. IgG4-Related Diseases in the Gastrointestinal Tract: Clinical Presentation, Diagnosis and Treatment Challenges. *Digestion* **2019**, *100*, 1–14. [CrossRef] [PubMed]
52. Yamamoto, M.; Takahashi, H. IgG4-Related Disease in Organs Other than the Hepatobiliary-Pancreatic System. *Semin. Liver Dis.* **2016**, *36*, 274–282. [CrossRef]
53. Mahajan, V.S.; Mattoo, H.; Deshpande, V.; Pillai, S.S.; Stone, J.H. IgG4-related disease. *Annu. Rev. Pathol.* **2014**, *9*, 315–347. [CrossRef] [PubMed]

54. Mattoo, H.; Mahajan, V.S.; Della-Torre, E.; Sekigami, Y.; Carruthers, M.; Wallace, Z.S.; Deshpande, V.; Stone, J.H.; Pillai, S. De novo oligoclonal expansions of circulating plasmablasts in active and relapsing IgG4-related disease. *J. Allergy Clin. Immunol.* **2014**, *134*, 679–687. [CrossRef]
55. Wallace, Z.S.; Mattoo, H.; Carruthers, M.; Mahajan, V.S.; Della Torre, E.; Lee, H.; Kulikova, M.; Deshpande, V.; Pillai, S.; Stone, J.H. Plasmablasts as a biomarker for IgG4-related disease, independent of serum IgG4 concentrations. *Ann. Rheum. Dis.* **2015**, *74*, 190–195. [CrossRef]
56. Löhr, J.M.; Beuers, U.; Vujasinovic, M.; Alvaro, D.; Frøkjær, J.B.; Buttgereit, F.; Capurso, G.; Culver, E.L.; de-Madaria, E.; Della-Torre, E.; et al. European Guideline on IgG4-related digestive disease—UEG and SGF evidence-based recommendations. *United Eur. Gastroenterol. J.* **2020**, *8*, 637–666. [CrossRef] [PubMed]
57. Shimosegawa, T.; Chari, S.T.; Frulloni, L.; Kamisawa, T.; Kawa, S.; Mino-Kenudson, M.; Kim, M.H.; Klöppel, G.; Lerch, M.M.; Löhr, M.; et al. International consensus diagnostic criteria for autoimmune pancreatitis: Guidelines of the International Association of Pancreatology. *Pancreas* **2011**, *40*, 352–358. [CrossRef]
58. Schneider, A.; Michaely, H.; Weiss, C.; Hirth, M.; Rückert, F.; Wilhelm, T.J.; Schönberg, S.; Marx, A.; Singer, M.V.; Löhr, J.M.; et al. Prevalence and Incidence of Autoimmune Pancreatitis in the Population Living in the Southwest of Germany. *Digestion* **2017**, *96*, 187–198. [CrossRef] [PubMed]
59. Zhang, L.; Chari, S.; Smyrk, T.C.; Deshpande, V.; Klöppel, G.; Kojima, M.; Liu, X.; Longnecker, D.S.; Mino-Kenudson, M.; Notohara, K.; et al. Autoimmune pancreatitis (AIP) type 1 and type 2: An international consensus study on histopathologic diagnostic criteria. *Pancreas* **2011**, *40*, 1172–1179. [CrossRef] [PubMed]
60. Webster, G.J. Autoimmune Pancreatitis—A Riddle Wrapped in an Enigma. *Dig. Dis.* **2016**, *34*, 532–539. [CrossRef] [PubMed]
61. Dickerson, L.D.; Farooq, A.; Bano, F.; Kleeff, J.; Baron, R.; Raraty, M.; Ghaneh, P.; Sutton, R.; Whelan, P.; Campbell, F.; et al. Differentiation of Autoimmune Pancreatitis from Pancreatic Cancer Remains Challenging. *World J. Surg.* **2019**, *43*, 1604–1611. [CrossRef] [PubMed]
62. Shih, H.C.; Chang, M.C.; Chen, C.H.; Tsai, I.L.; Wang, S.Y.; Kuo, Y.P.; Chang, Y.T. High accuracy differentiating autoimmune pancreatitis from pancreatic ductal adenocarcinoma by immunoglobulin G glycosylation. *Clin. Proteom.* **2019**, *16*, 1–10. [CrossRef]
63. Björnsson, E.; Chari, S.T.; Smyrk, T.C.; Lindor, K. Immunoglobulin G4 associated cholangitis: Description of an emerging clinical entity based on review of the literature. *Hepatology* **2007**, *45*, 1547–1554. [CrossRef]
64. Kamisawa, T.; Nakazawa, T.; Tazuma, S.; Zen, Y.; Tanaka, A.; Ohara, H.; Muraki, T.; Inui, K.; Inoue, D.; Nishino, T.; et al. Clinical practice guidelines for IgG4-related sclerosing cholangitis. *J. Hepatobiliary Pancreat. Sci.* **2019**, *26*, 9–42. [CrossRef] [PubMed]
65. Hubers, L.M.; Beuers, U. IgG4-related disease of the biliary tract and pancreas: Clinical and experimental advances. *Curr. Opin. Gastroenterol.* **2017**, *33*, 310–314. [CrossRef] [PubMed]
66. Deshpande, V.; Zen, Y.; Chan, J.K.; Yi, E.E.; Sato, Y.; Yoshino, T.; Klöppel, G.; Heathcote, J.G.; Khosroshahi, A.; Ferry, J.A.; et al. Consensus statement on the pathology of IgG4-related disease. *Mod. Pathol.* **2012**, *25*, 1181–1192. [CrossRef] [PubMed]
67. Nakazawa, T.; Naitoh, I.; Hayashi, K.; Okumura, F.; Miyabe, K.; Yoshida, M.; Yamashita, H.; Ohara, H.; Joh, T. Diagnostic criteria for IgG4-related sclerosing cholangitis based on cholangiographic classification. *J. Gastroenterol.* **2012**, *47*, 79–87. [CrossRef] [PubMed]
68. Ohara, H.; Okazaki, K.; Tsubouchi, H.; Inui, K.; Kawa, S.; Kamisawa, T.; Tazuma, S.; Uchida, K.; Hirano, K.; Yoshida, H.; et al. Clinical diagnostic criteria of IgG4-related sclerosing cholangitis 2012. *J. Hepatobiliary Pancreat. Sci.* **2012**, *19*, 536–542. [CrossRef]
69. Chung, H.; Watanabe, T.; Kudo, M.; Maenishi, O.; Wakatsuki, Y.; Chiba, T. Identification and characterization of IgG4-associated autoimmune hepatitis. *Liver Int.* **2010**, *30*, 222–231. [CrossRef] [PubMed]
70. Umemura, T.; Zen, Y.; Hamano, H.; Joshita, S.; Ichijo, T.; Yoshizawa, K.; Kiyosawa, K.; Ota, M.; Kawa, S.; Nakanuma, Y.; et al. Clinical significance of immunoglobulin G4-associated autoimmune hepatitis. *J. Gastroenterol.* **2011**, *46* (Suppl. S1), 48–55. [CrossRef] [PubMed]
71. Ciccone, F.; Ciccone, A.; Di Ruscio, M.; Vernia, F.; Cipolloni, G.; Coletti, G.; Calvisi, G.; Frieri, G.; Latella, G. IgG4-Related Disease Mimicking Crohn's Disease: A Case Report and Review of Literature. *Dig. Dis. Sci.* **2018**, *63*, 1072–1086. [CrossRef] [PubMed]
72. Koizumi, S.; Kamisawa, T.; Kuruma, S.; Tabata, T.; Chiba, K.; Iwasaki, S.; Endo, Y.; Kuwata, G.; Koizumi, K.; Shimosegawa, T.; et al. Immunoglobulin G4-related gastrointestinal diseases, are they immunoglobulin G4-related diseases? *World J. Gastroenterol.* **2013**, *19*, 5769–5774. [CrossRef]
73. Sánchez-Oro, R.; Alonso-Muñoz, E.M.; Martí Romero, L. Review of IgG4-related disease. *Gastroenterol. Hepatol.* **2019**, *42*, 638–647. [CrossRef] [PubMed]
74. Topal, F.; Sarıtaş Yüksel, E.; Ekinci, N.; Pekdiker, M.; Cakalağaoğlu, F.; Alper, E.; Unsal, B. The prevalence of IgG4-positive plasma cell infiltrates in inflammatory bowel disease patients without autoimmune pancreatitis. *Turk. J. Gastroenterol.* **2014**, *25*, 558–562. [CrossRef]
75. Obiorah, I.; Hussain, A.; Palese, C.; Azumi, N.; Benjamin, S.; Ozdemirli, M. IgG4-related disease involving the esophagus: A clinicopathological study. *Dis. Esophagus* **2017**, *30*, 1–7. [CrossRef] [PubMed]
76. Ko, Y.; Woo, J.Y.; Kim, J.W.; Hong, H.S.; Yang, I.; Lee, Y.; Hwang, D.; Min, S.J. An immunoglobulin G4-related sclerosing disease of the small bowel: CT and small bowel series findings. *Korean J. Radiol.* **2013**, *14*, 776–780. [CrossRef] [PubMed]
77. Choi, S.B.; Lim, C.H.; Cha, M.G.; Kang, W.K. IgG4-related disease of the rectum. *Ann. Surg. Treat. Res.* **2016**, *90*, 292–295. [CrossRef] [PubMed]
78. Hall, S.N.; Appelman, H.D. Autoimmune Gastritis. *Arch. Pathol. Lab. Med.* **2019**, *143*, 1327–1331. [CrossRef] [PubMed]

79. Neumann, W.L.; Coss, E.; Rugge, M.; Genta, R.M. Autoimmune atrophic gastritis–pathogenesis, pathology and management. *Nat. Rev. Gastroenterol. Hepatol.* **2013**, *10*, 529–541. [CrossRef] [PubMed]
80. Lenti, M.V.; Rugge, M.; Lahner, E.; Miceli, E.; Toh, B.H.; Genta, R.M.; De Block, C.; Hershko, C.; Di Sabatino, A. Autoimmune gastritis. *Nat. Rev. Dis. Primers* **2020**, *6*, 56. [CrossRef] [PubMed]
81. Lahner, E.; Zagari, R.M.; Zullo, A.; Di Sabatino, A.; Meggio, A.; Cesaro, P.; Lenti, M.V.; Annibale, B.; Corazza, G.R. Chronic atrophic gastritis: Natural history, diagnosis and therapeutic management. A position paper by the Italian Society of Hospital Gastroenterologists and Digestive Endoscopists [AIGO], the Italian Society of Digestive Endoscopy [SIED], the Italian Society of Gastroenterology [SIGE], and the Italian Society of Internal Medicine [SIMI]. *Dig. Liver Dis.* **2019**, *51*, 1621–1632. [CrossRef]

Journal of
Clinical Medicine

MDPI

Article

Effect of Vedolizumab on Anemia of Chronic Disease in Patients with Inflammatory Bowel Diseases

Patrizio Scarozza [1], Elena De Cristofaro [1], Ludovica Scucchi [1], Irene Rocchetti [2], Irene Marafini [1], Benedetto Neri [1], Silvia Salvatori [1], Livia Biancone [1], Emma Calabrese [1] and Giovanni Monteleone [1,*]

[1] Department of Systems Medicine, University of Rome "Tor Vergata", 00133 Rome, Italy; scarozzapatrizio@gmail.com (P.S.); elena_decr@hotmail.it (E.D.C.); ludovicascucchi@yahoo.it (L.S.); irene.marafini@gmail.com (I.M.); benedettoneri@gmail.com (B.N.); silviasalvatori23@gmail.com (S.S.); biancone@med.uniroma2.it (L.B.); emma.calabrese@uniroma2.it (E.C.)

[2] Statistical Office, Superior Council of Judiciary, 00185 Rome, Italy; irene.rocchetti@gmail.com

* Correspondence: gi.monteleone@med.uniroma2.it; Tel.: + 39-06-72596158; Fax: + 39-06-72596391

Received: 29 May 2020; Accepted: 3 July 2020; Published: 6 July 2020

Abstract: Background: Anemia of Chronic Disease (ACD) can negatively influence the clinical course of Inflammatory Bowel Disease (IBD) patients. The aim of this study was to evaluate the effect of Vedolizumab on ACD in IBD. Methods: Clinical data of 75 IBD patients (25 Crohn's disease (CD) and 50 Ulcerative Colitis (UC)) receiving Vedolizumab in a tertiary referral IBD center were retrospectively evaluated and the effect of the drug on ACD was ascertained at weeks 14 and 24. Results: ACD was diagnosed in 35 (11 CD and 24 UC) out of 75 (47%) IBD patients. At both week 14 and week 24, improvements and resolutions of ACD were achieved by 13/35 (37%) and 11/35 (31%) patients, respectively. Baseline demographic/clinical characteristics did not differ between patients with ACD improvements/resolutions and those with persistent ACD. Clinical response occurred more frequently in patients who achieved ACD resolution (10/11, 91%) than in those without ACD improvement (5/11, 45%, $p = 0.022$). When analysis was restricted to anemic patients, ACD resolution was documented in 10/22 patients (45%) achieving clinical response and 1/13 of non-responders (8%; $p = 0.02$). Conclusions: ACD occurs in half of the IBD patients and, in nearly two thirds of them, Vedolizumab treatment associates with ACD resolution/improvement.

Keywords: Crohn's disease; ulcerative colitis; biologics; anemia

1. Introduction

Anemia of chronic disease (ACD) (also referred to as anemia of inflammation) is a form of anemia, which develops in the context of systemic inflammation because of reduced production of erythrocytes, accompanied by a modest reduction in erythrocyte survival [1]. In ACD, the erythrocytes are generally normal and not small (low mean corpuscular volume) and hemoglobin-deficient (low mean corpuscular hemoglobin concentration), as seen in iron-deficiency anemia (IDA). However, erythrocytes can become small in the cases of ACD, in which iron deficiency coexists or develops as a complication. Similar to IDA, ACD is characterized by low serum iron levels, but it differs from IDA in that iron stores are preserved in macrophages [1]. Therefore, ACD is primarily a disorder of iron distribution and cellular metabolism.

Iron metabolism and homeostasis are tightly controlled phenomena, which are mainly under the control of hepcidin, produced by hepatocytes, and ferroportin, which is both the hepcidin receptor and the sole cellular iron exporter expressed on the cell surface of macrophages, hepatocytes and enterocytes, through which iron is transferred from the intracellular compartment to the blood [2–4]. Hepcidin inhibits the activation of ferroportin, thereby promoting the accumulation of iron in

iron-recycling macrophages. During chronic inflammation, cytokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF), enhance production of hepcidin, with the downstream effect of limiting the availability of iron for bone marrow erythropoiesis [1,5]. TNF can also convert erythropoiesis to myelopoiesis in human hematopoietic stem/progenitor cells, further contributing to ACD [6]. Moreover, chronic inflammatory processes are associated with the diminished renal production of erythropoietin and a decreased expression of erythropoietin receptors on erythroid progenitors [7]. The hyperactivation of macrophages by inflammatory stimuli induces hemophagocytosis and, consequently, a diminished erythrocyte lifespan [1].

Both ACD and IDA are common systemic complications of inflammatory bowel diseases (IBD) and have been associated with restless leg syndrome, fatigue, impaired physical function, decreased quality of life (QoL) and cognitive function in IBD patients [2]. Indeed, therapeutic interventions aimed at increasing hemoglobin resulted in improved QoL scores, independent of IBD activity [8]. On the other hand, it has been shown that the prevalence and severity of anemia are related to IBD activity and treatments used to attenuate the IBD-associated mucosal inflammation (i.e., TNF blockers) can improve ACD [9–11].

Vedolizumab, a gut-selective humanized monoclonal antibody that binds to the $\alpha 4\beta 7$ integrin and selectively reduces intestinal immune cell trafficking, is a safe and effective treatment option for patients with IBD [12–17]. In both ulcerative colitis (UC) and Crohn's disease (CD), Vedolizumab is effective in inducing and maintaining clinical and endoscopic/histologic remission [12–17]. However, to the best of our knowledge, no study has yet evaluated the effect of Vedolizumab on ACD, although, at least in CD, anemia at baseline has been associated with lower durability of treatment [18]. We here examine the effect of Vedolizumab on ACD.

2. Materials and Methods

2.1. Study Design

This was a retrospective study conducted on IBD patients treated with Vedolizumab at the Tor Vergata University Hospital (Rome, Italy). Patients' data were retrospectively collected between April 2018 and October 2019 and, after a de-identification process, registered into an electronic database. The primary objective of the study was to evaluate the effect of Vedolizumab on ACD improvement and resolution at weeks 14 and 24 of therapy. The week 24 was selected because the data came from clinical charts, which were completed between infusion sessions at weeks 22 and 30. The study was approved by the local Ethics Committee (CEI Policlinico Tor Vergata, Rome) (code 0024988/2019; 14 January 2020).

2.2. Patients

Inclusion criteria included: a confirmed diagnosis of CD or UC [19,20]; a clinically active disease at baseline (regardless of the grade) requiring Vedolizumab treatment; available data on clinical outcome at baseline and at weeks 14 and 24 of therapy. Patients were excluded if they were in clinical remission at baseline, had unclassified/indeterminate colitis or pouchitis and if the clinical data at the indicated time points were not available.

For each patient, several demographic and clinical variables were considered for the analysis, as shown in Table S1. Clinical disease activity for UC was evaluated by the partial Mayo (pMayo) score (mild activity: pMayo of 2–4; moderate activity: pMayo of 5–7; severe activity: pMayo > 7) [21] and for CD by the Harvey–Bradshaw index (HBI) (mild activity: HBI of 5–7; moderate activity: HBI of 8–16; severe activity: HBI > 16) [22]. Clinical response was defined as a reduction of a minimum of three points of the pMayo score for UC and HBI for CD. Endoscopic activity at baseline was evaluated by the endoscopic Mayo score for UC [23] and the Simple Endoscopic Score for CD (SES-CD) (mild activity: SES-CD score of 3–6; moderate activity: SES-CD of 7–15; severe activity: SES-CD > 15) [24].

ACD was defined as the presence of clinical evidence of inflammation with a hemoglobin level <13 gr/dL (for males) and <12 gr/dL (for females) and a serum ferritin >100 µg/L and transferrin saturation (TfS) <20% [2]. Mixed type anemia was defined as the presence of the abovementioned criteria associated with a serum ferritin level between 30 and 100 µg/L [2]. ACD improvement was defined as the increase in hemoglobin level by at least 1 gr/dL. ACD resolution was defined as the achievement of a normal value of hemoglobin (≥12 gr/dL for females and ≥13 gr/dL for males).

2.3. Statistical Analysis

Continuous variables were reported as median with interquartile range (IQR) and categorical variables were expressed as percentage. The patients without anemia improvement or resolution were considered as the group of comparison; the distribution of the variables between patients with ACD improvement or resolution (considered separately) and patients with persistent ACD at week 14 and week 24 were evaluated by binomial analysis, using the χ^2 or Fisher exact test for the categorical variables and with Mann–Whitney test for the continuous variables. A $p < 0.05$ level was considered for statistical significance.

3. Results

3.1. Frequency of ACD in IBD

Seventy-five IBD patients (25 CD and 50 UC) were enrolled. Patients had a median duration of disease longer than 10 years and most of them (68%) had been previously exposed to TNF-α antagonists, as shown in Table S1.

ACD was diagnosed in 35/75 (47%) patients (11 CD and 24 UC). Fifteen out of 35 patients (43%) had pure ACD, while the remaining 20 (57%) had mixed type anemia (ACD combined with IDA). Among the anemic patients, anemia was mild (≥9.5 gr/dl) in 31 patients (88%) and moderate (8–9.5 gr/dL) in the remaining patients (12%); no cases of severe anemia (<8 gr/dl) were recorded.

Demographic and clinical characteristics at baseline did not differ between patients with ACD and those without ACD as well as between patients with pure ACD and those with mixed type anemia, as shown in Table 1 and Table S2, except for a higher level of both ferritin and transferrin saturation in the group of patients without ACD, as shown in Table 1. Concomitant immune–inflammatory disorders did not differ between patients with ACD and those without ACD, as shown in Table 1.

Table 1. Distribution of baseline demographic/clinical characteristics and clinical response to Vedolizumab in patients with ACD and those without ACD.

	Patients with ACD (35/75)	Patients without ACD (40/75)	*p* Value
Male gender	15 (43%)	21 (52%)	$p = 0.695$
Age < 65 years	31 (88%)	31 (77%)	$p = 0.206$
Crohn's disease	11 (31%)	14 (35%)	$p = 0.743$
Ulcerative colitis	24 (69%)	26 (65%)	$p = 0.743$
Current smokers	3 (8%)	6 (15%)	$p = 0.392$
Previous anti-TNF	26 (74%)	25 (62%)	$p = 0.275$
Concomitant steroids	18 (51%)	23 (57%)	$p = 0.598$
Concomitant immunosuppressors	1 (3%)	5 (12%)	$p = 0.124$
Concomitant immuno-inflammatory disorders §	5 (14%)	4 (10%)	$p = 0.324$
Severe clinical activity	3 (8%)	1 (2%)	$p = 0.243$
Moderate clinical activity	23 (66%)	29 (73%)	$p = 0.524$
Mild clinical activity	9 (26%)	10 (25%)	$p = 0.943$

Table 1. *Cont.*

	Patients with ACD (35/75)	Patients without ACD (40/75)	*p* Value
Severe endoscopic activity *	21 (66%)	23 (66%)	$p = 0.993$
Moderate endoscopic activity	3 (9%)	9 (26%)	$p = 0.081$
Mild endoscopic activity	8 (25%)	3 (8%)	$p = 0.069$
Hemoglobin (median, IQR) (gr/dL)	10.9 (10.35–12.7)	13.8 (13.2–15.1)	$p = 0.0002$
Ferritin value (median, IQR) (μg/L)	86 (45–143)	103 (84.7–189)	$p = 0.019$
Transferrin saturation (median, IQR) (%)	16 (13.2–20)	22 (21.6–37.1)	$p = 0.002$
CRP > 5 mg/L	23 (66%)	20 (50%)	$p = 0.169$
CRP value (median, IQR) (mg/L)	10 (3.75–56.3)	6.8 (2.85–52)	$p = 0.849$
IBD clinical response to Vedolizumab at week 14	22 (63%)	22 (55%)	$p = 0.490$
IBD clinical response to Vedolizumab at week 24	22 (63%)	21 (52%)	$p = 0.365$

ACD: Anemia of Chronic Disease; Anti-TNF: Anti-Tumor Necrosis Factor; CRP: C reactive protein; IBD: Inflammatory Bowel Disease; § Concomitant immuno-inflammatory disorders included three Hashimoto thyroiditis, one autoimmune pancreatitis and one erythema nodosum in the group of ACD and one rheumatoid arthritis, one Basedow disease, one ankylosing spondylitis and one Hashimoto thyroiditis in the group of patients without anemia; * Endoscopic data available in 32/35 patients with ACD and in 35/40 patients without ACD; IQR: interquartile range.

3.2. Effect of Vedolizumab on ACD Course

The clinical response to Vedolizumab was documented in 44/75 (59%; 11 CD and 33 UC) patients at week 14 and in 43/75 (57%) (11 CD and 32 UC) patients at week 24. At week 14, clinical response was observed in 22/35 (63%) patients with ACD and 22/40 (55%) of those without ACD, as shown in Table 1. At week 24, the percentage of responders did not differ between patients with ACD (22/35 (63%)) and those without ACD (21/40 (52%)), as shown in Table 1.

ACD improvement occurred in 13/35 (37%) patients at week 14 and was maintained in all of them at week 24. ACD improvement was documented in seven out of 15 patients with pure ACD (47%) and six out of 20 patients with mixed type anemia (30%) ($p = 0.312$), as shown in Table S2. Patients with no improvement of ACD at week 14 remained anemic at week 24. There was no difference in terms of demographic and clinical characteristics between patients with and without ACD improvement, except for a higher CRP value at baseline in the group of patients with ACD improvement, as shown in Table 2.

ACD resolution was achieved by 11/35 (31%) patients at both week 14 and week 24. Four out of 15 patients with pure ACD (27%) and seven out of 20 patients with mixed type anemia (35%) achieved a resolution of anemia ($p = 0.599$), as shown in Table S2. The baseline clinical and demographic characteristics did not differ between patients with ACD resolution and those with persistence of ACD, following Vedolizumab treatment, as shown in Table 3.

In line with the above results, the median values of hemoglobin increased following Vedolizumab treatment, even though a statistically significant difference was seen between baseline (median value: 10.9; interquartile range: 10.3–12.7) and week 14 (median value: 12; interquartile range: 11–13.5; $p = 0.016$) but not week 24 (median value: 11.9; interquartile range: 11.2–13.8; $p = 0.186$), as shown in Figure 1.

Table 2. Distribution of baseline demographic/clinical characteristics and clinical response to Vedolizumab in patients with ACD improvement and those without ACD improvement.

Variable	Patients with ACD Improvement (13/35)	Patients without ACD Improvement (11/35)	*p* Value
Male gender	7 (54%)	4 (36%)	$p = 0.391$
Age < 65 years	10 (77%)	11 (100%)	$p = 0.222$
Crohn's Disease	7 (54%)	2 (18%)	$p = 0.072$
Ulcerative colitis	6 (46%)	9 (82%)	$p = 0.072$
Current smokers	1 (8%)	0	$p = 1$
Previous anti-TNF	11 (85%)	7 (64%)	$p = 0.236$
Concomitant steroids	8 (61%)	5 (45%)	$p = 0.430$
Concomitant immunosuppressors	1 (8%)	0	$p = 1$
Hemoglobin (median, IQR) (gr/dL)	10.9 (8.7–12.7)	11.4 (8.9–12.3)	$p = 0.865$
Mild anemia (hemoglobin ≥ 9.5 gr/dL)	11 (85%)	10 (91%)	$p = 0.642$
Moderate anemia (hemoglobin 8–9.5 gr/dL)	2 (15%)	1 (9%)	$p = 0.642$
Severe clinical activity ∫	1 (8%)	2 (18%)	$p = 0.438$
Moderate clinical activity	10 (77%)	5 (45%)	$p = 0.112$
Mild clinical activity	2 (15%)	4 (36%)	$p = 0.236$
Severe endoscopic activity *	7 (58%)	9 (82%)	$p = 0.221$
Moderate endoscopic activity	5 (42%)	1 (9%)	$p = 0.075$
Mild endoscopic activity	0	1 (9%)	$p = 0.478$
CRP > 5 mg/L	10 (77%)	5 (45%)	$p = 0.112$
CRP value (median, IQR) (mg/L)	10.5 (6–28)	3.9 (1.8–40)	$p = 0.033$
Iron therapy for anemia	6 (46%)	7 (64%)	$p = 0.391$
IBD clinical response to Vedolizumab at week 14	7 (54%)	5 (45%)	$p = 0.682$
IBD clinical response to Vedolizumab at week 24	7 (54%)	5 (45%)	$p = 0.682$

ACD: Anemia of Chronic Disease; Anti-TNF: Anti-Tumor Necrosis Factor. CRP; C reactive protein; IBD: Inflammatory Bowel Disease; IQR: Interquartile range; ∫ Clinical activity was classified with partial Mayo Score for ulcerative colitis (mild activity: pMayo of 2–4; moderate activity: pMayo of 5–7; severe activity: pMayo > 7) and with Harvey–Bradshaw Index (HBI) for Crohn's disease (mild activity: HBI of 5–7; moderate activity: HBI of 8–16; severe activity: HBI > 16). * Endoscopic data classified with Endoscopic Mayo Score for ulcerative colitis and Simple Endoscopic Score for Crohn's disease (SES-CD) available in 12/13 patients with ACD improvement and in 11/11 patients without ACD improvement. A SES-CD score of 3–6 was considered as mild endoscopic activity, 7–15 as moderate endoscopic activity and > 15 as severe endoscopic activity.

Table 3. Distribution of baseline demographic/clinical characteristics and clinical responses to Vedolizumab in patients with ACD resolution, as compared to patients without ACD improvement.

Variable	Patients with ACD Resolution (11/35)	Patients without ACD Improvement (11/35)	*p* Value
Male gender	4 (36%)	4 (36%)	$p = 1$
Age < 65 years	10 (91%)	11 (100%)	$p = 1$
Crohn's Disease	2 (18%)	2 (18%)	$p = 1$
Ulcerative colitis	9 (82%)	9 (82%)	$p = 1$
Current smokers	1 (9%)	2 (18%)	$p = 0.534$
Previous anti-TNF	8 (73%)	7 (64%)	$p = 0.647$
Concomitant steroids	5 (45%)	5 (45%)	$p = 1$
Concomitant immunosuppressors	0	0	$p = 1$
Hemoglobin (median, IQR) (gr/dL)	10.9 (8.3–12.5)	11.4 (8.9–12.3)	$p = 0.373$

Table 3. *Cont.*

Variable	Patients with ACD Resolution (11/35)	Patients without ACD Improvement (11/35)	*p* Value
Mild anemia (hemoglobin ≥ 9.5 gr/dL)	10 (91%)	10 (91%)	$p = 1$
Moderate anemia (hemoglobin 8–9.5 gr/dL)	1 (9%)	1 (9%)	$p = 1$
Severe clinical activity ∫	0	2 (18%)	$p = 0.476$
Moderate clinical activity	8 (73%)	5 (45%)	$p = 0.193$
Mild clinical activity	3 (27%)	4 (36%)	$p = 0.647$
Severe endoscopic activity *	5 (56%)	9 (82%)	$p = 0.202$
Moderate endoscopic activity	2 (22%)	1 (9%)	$p = 0.413$
Mild endoscopic activity	2 (22%)	1 (9%)	$p = 0.413$
CRP > 5 mg/L	8 (73%)	5 (45%)	$p = 0.193$
CRP value (median, IQR) (mg/L)	13.3 (5.65–56.3)	3.9 (1.8–40)	$p = 0.138$
Iron therapy for anemia	4 (36%)	7 (64%)	$p = 0.200$
IBD clinical response to Vedolizumab at week 14	10 (91%)	5 (45%)	$p = 0.022$
IBD clinical response to Vedolizumab at week 24	10 (91%)	5 (45%)	$p = 0.022$

ACD: Anemia of Chronic Disease; Anti-TNF: Anti-Tumor Necrosis Factor; CRP: C reactive protein; IBD: Inflammatory Bowel Disease; IQR: Interquartile range. ∫ Clinical activity was classified with partial Mayo Score for ulcerative colitis (mild activity: pMayo of 2–4; moderate activity: pMayo of 5–7; severe activity: pMayo > 7) and with Harvey–Bradshaw Index (HBI) for Crohn's disease (mild activity: HBI of 5–7; moderate activity: HBI of 8–16; severe activity: HBI > 16). * Endoscopic data (classified with Endoscopic Mayo Score for ulcerative colitis and Simple Endoscopic Score for Crohn's disease) available in 9/11 patients with ACD resolution and in 11/11 patients without ACD improvement. A SES-CD score of 3–6 was considered as mild endoscopic activity, 7–15 as moderate endoscopic activity and > 15 as severe endoscopic activity.

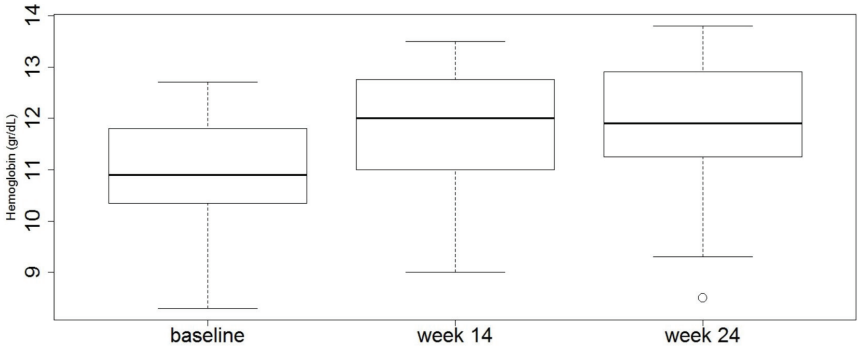


Figure 1. Vedolizumab treatment enhances hemoglobin values in patients with inflammatory bowel disease. The box-plots show the median values and the interquartile ranges at baseline and at week 14 and week 24 following Vedolizumab treatment in the total ACD population. Baseline vs. week 14, $p = 0.016$; baseline vs. week 24, $p = 0.18$.

3.3. Relationship between Clinical Response and ACD Resolution

At both week 14 and week 24, clinical response occurred more frequently in patients who achieved ACD resolution (10/11, 91%) than in those without anemia improvement (5/11, 45%, $p = 0.022$) (Table 3). When analysis was restricted to the 35 anemic patients, ACD resolution was documented in 10 out of the 22 patients (45%) achieving clinical response and 1 out of the 13 non-responders (8%; $p = 0.02$). Among the 22 patients with clinical response to the drug there was no difference in the frequency of concomitant immuno-inflammatory disorders between patients achieving anemia improvement/resolution and those without anemia improvement (2/17 (12%) vs 1/5 (20%), $p = 0.637$).

4. Discussion

ACD is a condition that can accompany immune–inflammatory diseases, in which there is a decrease in hemoglobin, hematocrit and erythrocyte counts due to a complex process, usually initiated by cellular immunity mechanisms and inflammatory cytokines. Biologics used to treat such disorders are supposed to improve ACD due to their systemic activity [1,9]. This study was undertaken to evaluate the impact of Vedolizumab therapy on the course of ACD in IBD. Indeed, it is well known that ACD can complicate the course of both CD and UC and circumstantial evidence indicates that the development of ACD is mainly related to the IBD-associated inflammation [2]. We found a 47% prevalence of ACD. Most patients had mild anemia and this relies probably on the fact that the study was focused on IBD outpatients, thus excluding the most severe cases of ACD requiring hospitalization. ACD was more frequent in UC than in CD, consistent with the demonstration that circulating levels of hepcidin in UC patients are similar or higher than those in Crohn's disease [25,26].

As with IBD, other chronic immuno-inflammatory disorders can be associated with ACD [27]. However, the frequency of concomitant immuno-inflammatory pathologies did not differ between patients with ACD and those without anemia.

The induction of clinical response at week 14 was achieved by half of the patients treated with Vedolizumab, thus confirming previous real-life study findings [28–41]. A substantial positive effect of Vedolizumab on ACD was seen in two thirds of the anemic patients. The resolution or improvement of ACD were observed at week 14 and maintained at week 24. The baseline clinical and demographic characteristics of the patients did not influence the effect of Vedolizumab on ACD, even though we cannot exclude the possibility that some associations could be masked by the small number of patients analyzed.

Previous studies have evaluated the therapeutic effect of TNF blockers on hemoglobin levels and anemia in IBD patients. In a study on 18 CD patients with anemia, treatment with infliximab improved hemoglobin levels in nearly two thirds of the cases [9]. Similarly, Lönnkvist and colleagues reported that CD responders to infliximab exhibited increased hemoglobin levels [42]. In contrast, no significant difference in hemoglobin levels was found between responders and non-responders to anti-TNF treatment (infliximab or adalimumab) in pediatric IBD [43]. In line with the latter results, Koutroubakis and colleagues showed that anti-TNF therapy had only a modest effect on patients' hemoglobin levels, despite significant beneficial effects on disease activity and clinical outcomes [44]. The factors accounting for such discrepancies remain unknown, but differences in the patient selection and types of anemia considered might have contributed. In contrast to other chronic inflammatory disorders where anemia results mainly from the action of inflammatory molecules on hemopoiesis, anemia in IBD also relies on mucosal blood loss and impaired iron absorption. Our analysis was restricted to ACD, even though in more than 50% of the ACD patients there was a concomitant iron deficiency anemia. Nonetheless, the data indicate that ACD improvement and resolution following Vedolizumab treatment occurred independently of the baseline hemoglobin value and concomitant iron supplementation therapy, suggesting that the positive effect of the drug on ACD is secondary to the control of the ongoing mucosal inflammation. Indeed, we found a strong association between ACD resolution and clinical response to the drug, as 10/11 patients with ACD resolution achieved clinical response following Vedolizumab treatment. However, at both week 14 and week 24, in nearly half of the patients achieving clinical response, we documented no effect of the drug on ACD. Although it remains to be clarified, it is plausible that the persistence of ACD in such patients relies on the inability of the drug to fully halt the mucosal inflammation. It is also unlikely that the persistence of anemia in patients with clinical response was due to a concomitant immuno-inflammatory disorder because such pathologies were equally distributed between patients with ACD resolution/improvement and those with no ACD improvement.

This study has some limitations. It was a retrospective study based on data collected from the medical records of a small group of IBD patients and, therefore, we cannot exclude the fact that it was subjected to some selection biases, which could have either overestimated or underestimated the

relationship between Vedolizumab treatment and ACD improvement. The effectiveness of Vedolizumab treatment was determined using only clinical scores as data on endoscopic/histological response to the treatment, and changes in inflammatory markers, such as serum CRP and fecal calprotectin, were not available. Therefore, it remains possible that some of the positive effects of the treatment on the clinical symptoms were not paralleled by a concomitant suppression of the ongoing mucosal inflammation, thereby introducing a bias in the definition of response/remission to Vedolizumab. Moreover, we had no blood samples to measure the circulating levels of hepcidin and to assess whether the effect of Vedolizumab on ACD was paralleled by the decreased synthesis of this protein. The fact that virtually all the patients had mild anemia may have also led to an overestimation of the effectiveness of the drug in ACD. Further limitations would include the relatively medium-term outcomes (i.e., 24 weeks) of the study and the lack of data on the relationships between the resolution of ACD and improvement in the quality of life of the patients, as it was proven that ACD significantly worsens the quality of life of patients with chronic diseases [2]. Therefore, we are aware that the present data may be used as the initial study generating hypotheses to be studied further by larger prospective studies.

In conclusion, this is the first study suggesting a positive effect of Vedolizumab on ACD course.

Supplementary Materials: The following are available online at http://www.mdpi.com/2077-0383/9/7/2126/s1, Table S1. Clinical and demographic characteristics of the patients, Table S2. Distribution of baseline demographic/clinical characteristics and clinical response to Vedolizumab in patients with pure ACD and patients with mixed type anemia.

Author Contributions: Conceptualization, P.S.; data curation, P.S.; formal analysis, P.S.; funding acquisition, P.S.; investigation, P.S. and I.R.; methodology, P.S. and I.R.; project administration, P.S., I.R. and G.M.; resources, E.D.C., L.S., B.N. and S.S.; software, E.D.C. and I.R.; supervision, L.B., E.C. and G.M.; validation, L.B., E.C. and G.M.; visualization, I.M., B.N., S.S., L.B., E.C. and G.M.; writing—original draft, P.S.; writing—review and editing, G.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ganz, T. Anemia of inflammation. *N. Engl. J. Med.* **2019**, *381*, 1148–1157. [CrossRef] [PubMed]
2. Dignass, A.U.; Gasche, C.; Bettenworth, D.; Birgegard, G.; Danese, S.; Gisbert, J.P.; Gomollon, F.; Iqbal, T.; Katsanos, K.; Koutroubakis, I.; et al. European consensus on the diagnosis and management of iron deficiency and anemia in inflammatory bowel diseases. *J. Crohns Colitis* **2015**, *99*, 211–222. [CrossRef] [PubMed]
3. Ganz, T. Systemic iron homeostasis. *Physiol. Rev.* **2013**, *93*, 1721–1741. [CrossRef]
4. Wang, C.Y.; Babitt, J.L. Liver iron sensing and body iron homeostasis. *Blood* **2019**, *133*, 18–29. [CrossRef] [PubMed]
5. Nemeth, E.; Rivera, S.; Gabayan, V.; Keller, C.; Taudorf, S.; Pedersen, B.K.; Ganz, T. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J. Clin. Investig.* **2004**, *113*, 1271–1276. [CrossRef] [PubMed]
6. Orsini, M.; Chateauvieux, S.; Rhim, J.; Gaigneaux, A.; Cheillan, D.; Christov, C.; Dicato, M.; Morceau, F.; Diederich, M. Sphingolipid-mediated inflammatory signaling leading to autophagy inhibition converts erythropoiesis to myelopoiesis in human hematopoietic stem/progenitor cells. *Cell Death Differ.* **2019**, *26*, 1796–1812. [CrossRef] [PubMed]
7. Macdougall, I.C.; Cooper, A.C. Erythropoietin resistance: The role of inflammation and pro-inflammatory cytokines. *Nephrol. Dial. Transplant.* **2002**, *17*, 39–43. [CrossRef]
8. Gasché, C.; Dejaco, C.; Waldhoer, T.; Tillinger, W.; Reinisch, W.; Fueger, G.F.; Gangl, A.; Lochs, H. Intravenous iron and erythropoietin for anemia associated with Crohn disease. A randomized, controlled trial. *Ann. Intern. Med.* **1997**, *126*, 782–787. [CrossRef]
9. Bergamaschi, G.; Di Sabatino, A.; Albertini, R.; Ardizzone, S.; Biancheri, P.; Bonetti, E.; Cassinotti, A.; Cazzola, P.; Markopoulos, K.; Massari, A.; et al. Prevalence and pathogenesis of anemia in inflammatory bowel disease. Influence of anti-tumor necrosis factor-alpha treatment. *Haematologica* **2010**, *95*, 199–205. [CrossRef]

10. Rubin, D.T.; Mulani, P.; Chao, J.; Pollack, P.F.; Bensimon, A.G.; Yu, A.P.; Ghosh, S. Effect of adalimumab on clinical laboratory parameters in patients with Crohn's disease: Results from the CHARM trial. *Inflamm. Bowel Dis.* **2012**, *18*, 818–825. [CrossRef]
11. Peyrin-Biroulet, L.; Van Assche, G.; Gómez-Ulloa, D.; García-Álvarez, L.; Lara, N.; Black, C.M.; Kachroo, S. Systematic review of tumor necrosis factor antagonists in extraintestinal manifestations in inflammatory bowel disease. *Clin. Gastroenterol. Hepatol.* **2017**, *15*, 25–36. [CrossRef] [PubMed]
12. Fedyk, E.R.; Wyant, T.; Yang, L.-L.; Csizmadia, V.; Burke, K.; Yang, H.; Kadambi, V.J. Exclusive antagonism of the α4β7 integrin by vedolizumab confirms the gut-selectivity of this pathway in primates. *Inflamm. Bowel Dis.* **2012**, *18*, 2107–2119. [CrossRef]
13. Feagan, B.G.; Greenberg, G.R.; Wild, G.; Fedorak, R.N.; Paré, P.; McDonald, J.W.D.; Dubé, R.; Cohen, A.; Steinhart, H.A.; Landau, S.; et al. Treatment of ulcerative colitis with a humanized antibody to the alpha4beta7 integrin. *N. Engl. J. Med.* **2005**, *352*, 2499–2507. [CrossRef] [PubMed]
14. Feagan, B.G.; Rutgeerts, P.; Sands, B.E.; Fedorak, R.N.; Paré, P.; McDonald, J.W.; Dubé, R.; Cohen, A.; Steinhart, A.H.; Landau, S.; et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. *N. Engl. J. Med.* **2013**, *369*, 699–710. [CrossRef] [PubMed]
15. Sandborn, W.J.; Feagan, B.G.; Rutgeerts, P.; Hanauer, S.; Colombel, J.F.; Sands, B.E.; Lukas, M.; Fedorak, R.N.; Lee, S.; Bressler, B.; et al. Vedolizumab as induction and maintenance therapy for Crohn's disease. *N. Engl. J. Med.* **2013**, *369*, 711–721. [CrossRef]
16. Sands, B.E.; Feagan, B.G.; Rutgeerts, P.; Colombel, J.F.; Sandborn, W.J.; Sy, R.; D'Haens, G.; Ben-Horin, S.; Xu, J.; Rosario, M.; et al. Effects of vedolizumab induction therapy for patients with Crohn's disease in whom tumor necrosis factor antagonist treatment had failed. *Gastroenterology* **2014**, *147*, 618–627. [CrossRef]
17. Engel, T.; Ungar, B.; Yung, D.E.; Ben-Horin, S.; Eliakim, R.; Kopylov, U. Vedolizumab in IBD-lessons from real-world experience; a systematic review and pooled analysis. *J. Crohns Colitis* **2018**, *12*, 245–257. [CrossRef]
18. Chaparro, M.; Garre, A.; Ricart, E.; Iborra, M.; Mesonero, F.; Vera, I.; Riestra, S.; García-Sánchez, V.; Luisa De Castro, M.; Martin-Cardona, A.; et al. Short and long-term effectiveness and safety of vedolizumab in inflammatory bowel disease: Results from the ENEIDA registry. *Aliment. Pharmacol. Ther.* **2018**, *48*, 839–851. [CrossRef] [PubMed]
19. Gomollón, F.; Dignass, A.; Annese, V.; Tilg, H.; Van Assche, G.; Lindsay, J.O.; Peyrin-Biroulet, L.; Cullen, G.J.; Daperno, M.; Kucharzik, T.; et al. 3rd European evidence-based consensus on the diagnosis and management of Crohn's Disease 2016: Part 1: Diagnosis and medical management. *J. Crohns Colitis* **2017**, *11*, 3–25. [CrossRef]
20. Magro, F.; Gionchetti, P.; Eliakim, R.; Ardizzone, S.; Armuzzi, A.; Barreiro-de Acosta, M.; Burisch, J.; Gecse, K.B.; Hart, A.L.; Hindryckx, P.; et al. Third European evidence-based consensus on diagnosis and management of ulcerative Colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. *J. Crohns Colitis* **2017**, *11*, 649–670. [CrossRef]
21. Lewis, J.D.; Chuai, S.; Nessel, L. Use of the noninvasive components of the Mayo score to assess clinical response in ulcerative colitis. *Inflamm. Bowel Dis.* **2008**, *14*, 1660–1666. [CrossRef] [PubMed]
22. Harvey, R.F.; Bradshaw, J.M. A simple index of Crohn's disease activity. *Lancet* **1980**, *1*, 514. [CrossRef]
23. Mohammed Vashist, N.; Samaan, M.; Mosli, M.H.; Parker, C.E.; MacDonald, J.K.; Nelson, S.A.; Zou, G.Y.; Feagan, B.G.; Khanna, R.; Jairath, V. Endoscopic scoring indices for evaluation of disease activity in ulcerative Colitis. *Cochrane Database Syst. Rev.* **2018**, *16*, CD011450. [CrossRef] [PubMed]
24. Daperno, M.; D'Haens, G.; Van Assche, G.; Parker, C.E.; MacDonald, J.K.; Nelson, S.A.; Zou, G.Y.; Feagan, B.G.; Khanna, R.; Jairath, V. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: The SES-CD. *Gastrointest. Endosc.* **2004**, *60*, 505–512. [CrossRef]
25. Pakoz, Z.B.; Çekiç, C.; Arabul, M.; Sarıtaş Yuksel, E.; Ipek, S.; Vatansever, S.; Unsal, B. An evaluation of the correlation between hepcidin serum levels and disease activity in inflammatory Bowel disease. *Gastroenterol. Res. Pract.* **2015**, *2015*, 810942. [CrossRef]
26. Mecklenburg, I.; Reznik, D.; Fasler-Kan, E.; Drewe, J.; Beglinger, C.; Hruz, P.; Swiss IBD Cohort Study Group. Serum hepcidin concentrations correlate with ferritin in patients with inflammatory Bowel disease. *J. Crohns Colitis* **2014**, *8*, 1392–1397. [CrossRef]
27. Masson, C. Rheumatoid anemia. *Jt. Bone Spine* **2011**, *78*, 131–137. [CrossRef]

28. Baumgart, D.C.; Bokemeyer, B.; Drabik, A.; Stallmach, A.; Schreiber, S.; Vedolizumab Germany Consortium. Vedolizumab induction therapy for inflammatory bowel disease in clinical practice—A nationwide consecutive German cohort study. *Aliment. Pharmacol. Ther.* **2016**, *43*, 1090–1102. [CrossRef]
29. Eriksson, C.; Marsal, J.; Bergemalm, D.; Vigren, L.; Björk, J.; Eberhardson, M.; Karling, P.; Söderman, C.; SWIBREG Vedolizumab Study Group; Myrelid, P.; et al. Long-term effectiveness of vedolizumab in inflammatory bowel disease: A national study based on the Swedish National Quality Registry for Inflammatory Bowel Disease (SWIBREG). *Scand. J. Gastroenterol.* **2017**, *52*, 722–729. [CrossRef]
30. Amiot, A.; Grimaud, J.C.; Peyrin-Biroulet, L.; Filippi, J.; Pariente, B.; Roblin, X.; Buisson, A.; Stefanescu, C.; Trang-Poisson, C.; Altwegg, R.; et al. Effectiveness and safety of vedolizumab induction therapy for patients with inflammatory bowel disease. *Clin. Gastroenterol. Hepatol.* **2016**, *14*, 1593–1601. [CrossRef]
31. Kopylov, U.; Ron, Y.; Avni-Biron, I.; Koslowsky, B.; Waterman, M.; Daher, S.; Ungar, B.; Yanai, H.; Maharshak, N.; Ben-Bassat, O.; et al. Efficacy and safety of vedolizumab for induction of remission in inflammatory bowel disease-the Israeli real-world experience. *Inflamm. Bowel Dis.* **2017**, *23*, 404–408. [CrossRef] [PubMed]
32. Vivio, E.E.; Kanuri, N.; Gilbertsen, J.J.; Monroe, K.; Dey, N.; Chen, C.H.; Gutierrez, A.M.; Ciorba, M.A. Vedolizumab effectiveness and safety over the first year of use in an IBD clinical practice. *J. Crohns Colitis* **2016**, *10*, 402–409. [CrossRef] [PubMed]
33. Dulai, P.S.; Singh, S.; Jiang, X.; Peerani, F.; Narula, N.; Chaudrey, K.; Whitehead, D.; Hudesman, D.; Lukin, D.; Swaminath, A.; et al. The real-world effectiveness and safety of vedolizumab for moderate-severe Crohn's disease: Results from the US VICTORY consortium. *Am. J. Gastroenterol.* **2016**, *111*, 1147–1155. [CrossRef] [PubMed]
34. Narula, N.; Peerani, F.; Meserve, J.; Kochhar, G.; Chaudrey, K.; Hartke, J.; Chilukuri, P.; Koliani-Pace, J.; Winters, A.; Katta, L.; et al. Vedolizumab for ulcerative colitis: Treatment outcomes from the VICTORY consortium. *Am. J. Gastroenterol.* **2018**, *113*, 1345–1354. [CrossRef]
35. Vermeire, S.; Loftus, E.V.; Colombel, J.F.; Feagan, B.; Sandborn, W.; Danese, S.; D'Haens, G.; Kaser, A.; Panaccione, R.; Rubin, D.; et al. Long-term effectiveness and safety of vedolizumab in patients with Crohn's disease: 5-year cumulative exposure of GEMINI 2 completers rolling into the GEMINI open-label extension study. *Gastroenterology* **2017**, *152*, S601.
36. Amiot, A.; Serrero, M.; Peyrin-Biroulet, L.; Filippi, J.; Pariente, B.; Roblin, X.; Buisson, A.; Stefanescu, C.; Trang-Poisson, C.; Altwegg, R.; et al. One-year effectiveness and safety of vedolizumab therapy for inflammatory bowel disease: A prospective multicenter cohort study. *Aliment. Pharmacol. Ther.* **2017**, *46*, 310–321. [CrossRef]
37. Amiot, A.; Serrero, M.; Peyrin-Biroulet, L.; Filippi, J.; Pariente, B.; Roblin, X.; Buisson, A.; Stefanescu, C.; Trang-Poisson, C.; Altwegg, R.; et al. Three-year effectiveness and safety of vedolizumab therapy for inflammatory bowel disease: A prospective multi-center cohort study. *Aliment. Pharmacol. Therap.* **2019**, *50*, 40–53. [CrossRef]
38. Stallmach, A.; Langbein, C.; Atreya, R.; Bruns, T.; Dignass, A.; Ende, K.; Hampe, J.; Hartmann, F.; Neurath, M.F.; Maul, J.; et al. Vedolizumab provides clinical benefit over 1 year in patients with active inflammatory Bowel disease—A prospective multicenter observational study. *Aliment. Pharmacol. Ther.* **2016**, *44*, 1199–1212. [CrossRef]
39. Danese, S.; Sandborn, W.J.; Colombel, J.F.; Vermeire, S.; Glover, S.C.; Rimola, J.; Siegelman, J.; Jones, S.; Bornstein, J.D.; Feagan, B.G. Endoscopic, radiologic, and histologic healing with vedolizumab in patients with active Crohn's disease. *Gastroenterology* **2019**, *157*, 1007–1018. [CrossRef]
40. Löwenberg, M.; Vermeire, S.; Mostafavi, N.; Hoentjen, F.; Franchimont, D.; Bossuyt, P.; Hindryckx, P.; Rispens, T.; de Vries, A.; van der Woude, C.J.; et al. Vedolizumab induces endoscopic and histologic remission in patients with Crohn's disease. *Gastroenterology* **2019**, *157*, 997–1006.
41. Scarozza, P.; Marafini, I.; Laudisi, F.; Troncone, E.; Schmitt, H.; Lenti, M.V.; Costa, S.; Rocchetti, I.; De Cristofaro, E.; Salvatori, S.; et al. Extent of mucosal inflammation in ulcerative colitis influences the clinical remission induced by vedolizumab. *J. Clin. Med.* **2020**, *9*, 385. [CrossRef]
42. Lönnkvist, M.H.; Befrits, R.; Lundberg, J.O.; Lundahl, J.; Fagerberg, U.L.; Hjortswang, H.; van Hage, M.; Hellström, P.M. Infliximab in clinical routine: Experience with Crohn's disease and biomarkers of inflammation over 5 years. *Eur. J. Gastroenterol. Hepatol.* **2009**, *21*, 1168–1176. [CrossRef] [PubMed]

43. Assa, A.; Hartman, C.; Weiss, B.; Broide, E.; Rosenbach, Y.; Zevit, N.; Bujanover, Y.; Shamir, R. Long-term outcome of tumor necrosis factor alpha antagonist's treatment in pediatric Crohn's disease. *J. Crohns Colitis* **2013**, *77*, 369–376. [CrossRef] [PubMed]
44. Koutroubakis, I.E.; Ramos-Rivers, C.; Regueiro, M.; Koutroumpakis, E.; Click, B.; Schwartz, M.; Swoger, J.; Baidoo, L.; Hashash, J.G.; Barrie, A.; et al. The influence of anti-tumor necrosis factor agents on hemoglobin levels of patients with inflammatory Bowel disease. *Inflamm. Bowel Dis.* **2015**, *21*, 1587–1593. [CrossRef] [PubMed]

Journal of
Clinical Medicine

MDPI

Article

Determinants of Sleep Quality in Inflammatory Bowel Diseases

Marcin Sochal [1,*], Ewa Małecka-Panas [2], Agata Gabryelska [1], Renata Talar-Wojnarowska [2], Bartosz Szmyd [1], Monika Krzywdzińska [2] and Piotr Białasiewicz [1]

[1] Department of Sleep Medicine and Metabolic Disorders, Medical University of Lodz, 92-215 Lodz, Poland; agata.gabryelska@gmail.com (A.G.); bartoszmyd@gmail.com (B.S.); piotr.bialasiewicz@umed.lodz.pl (P.B.)
[2] Department of Digestive Tract Diseases, Medical University of Lodz, 90-153 Lodz, Poland; ewuncia@poczta.onet.pl (E.M.-P.); r-wojnarowska@wp.pl (R.T.-W.); monika.krzywdzinska@wp.pl (M.K.)
* Correspondence: sochalmar@gmail.com

Received: 24 August 2020; Accepted: 4 September 2020; Published: 10 September 2020

Abstract: The causes of disordered sleep, frequently reported by patients with inflammatory bowel diseases (IBD), are poorly understood. The study aimed to evaluate sleep quality in IBD patients and to identify factors affecting their sleep. IBD patients (n = 133) and healthy controls (HC; n = 57) were included in the study and completed sleep questionnaires (Pittsburgh Sleep Quality Index (PSQI), Athens insomnia scale (AIS), and Epworth sleepiness scale (ESS)), Beck Depression Inventory (BDI), and pain scales (Visual Analogue Scale and Laitinen Pain Scale). IBD patients attained higher scores in all sleep questionnaires compared to HC: PSQI, AIS, and ESS (all $p < 0.001$). They also had prolonged sleep latency ($p < 0.001$) with reduced sleep efficiency ($p < 0.001$). Patients in exacerbation of IBD had higher scores in PSQI ($p = 0.008$), ESS ($p = 0.009$), but not in AIS, compared to those in remission. Participants with comorbid chronic diseases had higher scores in PSQI and AIS, but not in ESS, compared to others. Multiple regression revealed that the sleep questionnaire results were significantly affected by mood level (BDI), but not by the aforementioned pain scales. Sleep impairment in IBD patients is a common problem that deserves attention in everyday clinical practice and mood level seems to be the main factor affecting the quality of sleep in IBD patients.

Keywords: inflammatory bowel diseases; sleep disorders; sleep medicine

1. Introduction

Inflammatory bowel diseases (IBD) represent the group of chronic gastrointestinal tract diseases, including Crohn's disease (CD) and ulcerative colitis (UC) [1–3]. Impaired regulation of local and systemic immunologic reactions, as well as changes in the intestinal microbiome, have been implicated in their etiology [4]. Due to the high prevalence of IBD in developed countries, it is proposed that such factors as stress, obesity, lack of exercise, anxiety, or sleep disorders may be important in the IBD clinical course and etiopathogenesis [5].

Sleep is a physiological state in which people spend over one-third of their life, but so far it is poorly understood [6,7]. The association between IBD and sleep disturbances is bidirectional, i.e., disease exacerbation leads to sleep disturbances, and the latter increases the disease activity [8]. Sleep may interfere with the gut-brain axis. This axis can be affected by changes in cortisol levels that reach a nadir at the beginning of sleep and a peak before awakening [9]. Moreover, during sleep, the activity of the complement system is increased, and immunological memory is formed [10]. In the dextran sodium sulfate-induced mouse model of UC, the increase in colonic inflammation has been observed following intermittent sleep deprivation [11]. It has been proven that partial sleep deprivation affects the immune system, increasing susceptibility to infection and reducing the immune response to vaccinations [12]. Conversely, inflammation can affect the quality of sleep. For instance,

administration of interleukin-1-β (IL-1-β), tumor necrosis factor (TNF), or interferon-α (IFN-α) into the cerebral ventricle of rabbits enhances the non-rapid eye movement sleep (NREMS) phase [13].

Numerous studies on sleep quality among IBD patients have been published [14–16]. There have been, however, limited reports comparing disordered sleep in IBD and healthy control (HC). Insomnia symptoms among IBD patients have not been investigated by a dedicated scale yet. Most researchers used only one scale to assess disordered sleep, e.g., Pittsburgh Sleep Quality Index (PSQI), which is a validated and widely used questionnaire; however, the overall score does not indicate the plausible causes of sleep disturbances, such as difficulty in falling asleep, waking up at night, or reduction of sleep efficiency [17]. There have been only a few reports on the origin of disordered sleep among IBD patients, which may contribute to the more effective treatment thereof in this group of patients [15,18].

Therefore, the aims of the study were assessment of sleep quality and the search for factors affecting sleep among IBD patients.

2. Experimental Section

2.1. Sample

There were 190 study participants recruited for the study in the Department of Digestive Tract Diseases, Medical University of Lodz, Poland, which included 133 IBD patients (68 with CD and 65 with UC) and 57 apparently healthy subjects serving as control (HC).

The severity of the disease was assessed by clinical scales: Harvey–Bradshaw Index (HBI) for CD and Partial Mayo Score (PMS) for UC. Disease remission was defined as a score below 5 points according to HBI and 2 points according to PMS [19,20].

Information on the course of the disease, previous gastrointestinal operations, the presence of fistulas (perianal, enteroureteral, enterovaginal, and/or enteroenteric), perianal fissures, abscesses, the disease onset, extraintestinal complications (arthritis, iritis and scleritis, acute pancreatitis, hepatitis or erythema nodosum), information about the current treatment, smoking and other chronic diseases (such as asthma, compensated hypothyroidism, migraine, musculoskeletal system diseases, diabetes, endometriosis, rheumatoid arthritis, and psoriasis) were collected.

The inclusion criteria comprised of the signing of informed consent to participate in the study, age over 18 and under 65, and diagnosis of IBD based on clinical, radiological, endoscopic, and histopathological criteria. Exclusion criteria were previous abdominal and thoracic surgery in the last six months, active malignant disease except for skin basal cell carcinoma, addiction to alcohol or other psychoactive substances, diagnosed and treated psychiatric disorders.

Healthy volunteers were recruited for the study according to the snowball sampling method [21]. The additional criteria for inclusion in the healthy group included: no history of chronic diseases (apart from hypertension or hypercholesterolemia), especially those related to the digestive system, and no history of hypnotic drugs use. Health control was matched to IBD patients in terms of sex, age, and BMI.

The study protocol was accepted by the Ethical Committee of the Medical University of Lodz, Poland (number: RNN/433/18/KE). All respondents received information for the patient and were asked to sign informed consent to participate in the study.

2.2. Questionnaires

Respondents completed questionnaires of sleep quality, mood level, and pain intensity. The study was conducted in a ward and outpatient clinic, which are part of the Department of Digestive Tract Diseases. The researchers provided participants with instructions regarding the questionnaires. All patients who consented to participate in the study, completed all questionnaires. Sleep quality was measured by PSQI [17], Athens Insomnia Scale (AIS) [22,23], and Epworth sleepiness scale (ESS) [24]. Obtaining 6 or more points in PSQI was considered as reduced sleep quality, >5 in AIS as mild insomnia, and >10 points in ESS as drowsiness. Sleep efficiency was expressed as a total sleep time related to time

spent in bed. To assess the subjective severity of pain, Visual Analogue Scale (VAS), and Laitinen Pain Scale (LPS) were used [25,26]. The mood level was measured by the Beck Depression Inventory (BDI).

2.3. Statistical Analysis

Statistical analysis was performed using Statistica 13.1PL (StatSoft, Tulsa, OK, USA). Obtained data had non-normal distribution (Shapiro–Wilk test, $p < 0.05$). Thus, they were presented as median with interquartile range (IQR: first–third quartile) and non-parametric tests were used: Mann–Whitney U test for two independent samples, and Spearman's rank correlation for two continuous variables. Fisher's exact test ($n_{min} < 5$), $\chi 2$ with Yates' correction ($5 \leq n_{min} < 15$), and $\chi 2$ ($n_{min} \geq 15$) were used for testing dependencies between nominal data. Moreover, we decided to conduct a multiple regression analysis (a forward stepwise model) to determine the impact of dependent data, including BDI and pain parameters (LPS, VAS), on selected sleep questionnaires (PSQI, AIS, and ESS). A p-value less than 0.05 was considered as statistically significant; only for multiple testing between dependent subgroups the Bonferroni correction was used (p-value divided by the number of tests between subgroups).

3. Results

Eighty-three patients (62.4%) suffered from active IBD, while 50 participants (37.6%) were in remission. The characteristics of the study participants are presented in Table 1.

Table 1. Characteristics of the participants.

Parameter	IBD			HC	p	
	All	Exacerbation	Remission			
n (%)	133 (70%)	83 (44%)	50 (26%)	57 (30%)	-	
CD UC	68 (51%) 65 (49%)	40 (59%) 42 (65%)	28 (41%) 23 (35%)	-	-	
HBI PMS	5 (2–8) 3 (1–4)	7 (6–9) 4 (3–5.75)	2 (1–3) 1 (0–1)	-	-	
n women (%)	73 (55%)	40 (48%)	33 (66%)	33 (58%)	[1] 0.702 [3] 0.259	[2] 0.422 [4] 0.752
Age	37.0 (30.0–47.0)	36.0 (30.0–46.5)	38.0 (28.5–46.8)	38.0 (28.0–50.0)	[1] 0.899 [3] 0.882	[2] 0.754 [4] 0.958
BMI (kg/m^2)	23.5 (20.5–26.5)	23.5 (20.8–27.4)	23.2 (20.3–26.1)	24.2 (21.8–25.7)	[1] 0.394 [3] 0.546	[2] 0.736 [4] 0.341
Hypnotic drugs	18 (13.5%)	15 (18.1%)	3 (6.0%)	-		[2] 0.066
Steroids treatment	33 (25%)	33 (40%)	0 (0%)	-	-	
Immuno-modulators	45 (34%)	28 (34%)	17 (34%)	-	-	
Anti-TNF therpay	29 (22%)	29 (35%)	0 (0%)	-	-	

Data are presented as median (IQR) unless otherwise indicated. In bold—statistically significant differences regarding multiple testing correction between subgroups; [1] All vs HC; [2] Exacerbation vs Remission; [3] Exacerbation vs HC; [4] Remission vs HC; Abbreviations: BMI: body mass index; CD: Crohn's disease; ESS: Epworth sleepiness scale; HBI: Harvey–Bradshaw Index; HC: health control; IBD: inflammatory bowel diseases; IQR: interquartile range; n: number; PMS: Partial Mayo Score; TNF: tumour necrosis factor; UC: ulcerative colitis.

IBD patients attained higher scores in all sleep questionnaires compared to HC. They also had prolonged sleep latency, more often complained of nocturnal defecation, and their sleep efficiency was reduced compared to HC (Table 2). Six or more points in the PSQI scale were obtained by 43.6% of IBD patients (n = 58) vs. 26.3% of HC (n = 15, $p = 0.025$). Similarly, higher percentage of IBD patients scored more than 5 points in AIS, namely 49.6% (n = 66) vs. 26.3% of HC (n = 15, $p = 0.003$). Further, at least 11 points in ESS was attained by 21.1% of IBD patients (n = 28) while only by 5.3% of HC (n = 4, $p = 0.004$).

Table 2. Comparison of the results of questionnaires/scales between study groups.

Parameter	IBD			HC	*p*	
	All	Exacerbation	Remission			
PSQI	5 (4–7)	5 (4–8)	5 (3–6.8)	4 (2–6)	[1] **<0.001**	[2] **0.008**
					[3] **<0.001**	[4] 0.067
ESS	6 (4–9)	6 (4–10.5)	5 (3–7)	3 (2–7)	[1] **<0.001**	[2] **0.009**
					[3] **<0.001**	[4] 0.105
AIS	5.0 (4.0–8.0)	6.0 (4.0–9.0)	5.0 (3.0–7.0)	3.0 (1.0–6.0)	[1] **<0.001**	[2] 0.021
					[3] **<0.001**	[4] 0.019
BDI	7.0 (4.0–11.0)	8.0 (5.0–12.0)	5.0 (2.0–9.8)	2.0 (1.0–4.0)	[1] **<0.001**	[2] **0.001**
					[3] **<0.001**	[4] **0.001**
VAS	4.0 (0–5.0)	5.0 (2.0–5.5)	2.8 (0–4.9)	0 (0–3.0)	[1] **<0.001**	[2] **0.004**
					[3] **<0.001**	[4] **0.009**
LPS	3.0 (0.0–5.0)	4.0 (2.0–6.0)	2.0 (0.0–3.0)	1.0 (0.0–3.0)	[1] **0.004**	[2] **0.001**
					[3] **<0.001**	[4] 0.617
Sleep latency [min]	15 (10–30)	15 (10–35)	15 (10–30)	10 (5–15)	[1] **<0.001**	[2] 0.352
					[3] **<0.001**	[4] **0.009**
Hours of sleep	7.0 (6.0–7.8)	7.0 (6.0–8.0)	7.0 (6.0–7.5)	6.8(6.0–7.3)	[1] 0.304	[2] 0.968
					[3] 0.381	[4] 0.352
Hours spent in bed	8.0 (7.0–8.5)	8.0 (7.0–8.5)	8.0 (7.0–8.5)	7.0 (6.5–8.0)	[1] **<0.001**	[2] 0.824
					[3] **<0.001**	[4] **0.002**
Sleep efficiency (%)	88.9 (83.2–94.7)	88.9 (81.8–95.5)	89.4 (86.2–94.4)	97.2 (92.9–98.6)	[1] **<0.001**	[2] 0.662
					[3] **<0.001**	[4] **<0.001**
Waking up in the night n (%)	94 (70.7%)	66 (79.5%)	28 (56.0%)	24 (42.1%)	[1] **<0.001**	[2] **0.004**
					[3] **<0.001**	[4] 0.151

Data are presented as median (IQR) unless otherwise indicated. In bold—statistically significant differences regarding multiple testing correction between subgroups; [1] All vs HC; [2] Exacerbation vs Remission; [3] Exacerbation vs HC; [4] Remission vs HC; Abbreviations: AIS: Athens Insomnia Scale; BDI: Beck Depression Inventory; ESS: Epworth sleepiness scale; HC: health control; IBD: inflammatory bowel diseases; IQR: interquartile range; LPS: Laitinen Pain Scale; n: number; PSQI: Pittsburgh Sleep Quality Index; VAS: Visual Analogue Scale.

Results of sleep questionnaires were similar in CD and UC patients: PSQI (5, 4–8 vs. 5, 4–7, $p = 0.740$), AIS (6, 4–8 vs. 5, 3–8, $p = 0.335$), and ESS (6, 4–8.25 vs. 6, 4–9, $p = 0.692$), respectively.

Patients in exacerbation of IBD had higher scores in PSQI and ESS, but not in the AIS, compared to the remission. Sleep latency and efficiency were similar in both groups, but patients in exacerbation were more often presented with nocturnal defecation, Table 2.

Patients in exacerbation of IBD had higher scores in sleep questionnaires, longer sleep latency time, lower sleep efficiency, and more often waking up in the night than HC, Table 2.

Patients in IBD remission had similar sleep questionnaire scores and the frequency of waking up in the night, but have longer sleep latency and their sleep efficiency was lower compared to HC, Table 2.

HBI values (estimated for CD patients) were positively correlated with PSQI (r = 0.390, $p = 0.001$), but not with AIS (r = 0.163, $p = 0.183$), nor ESS (r = 0.183, $p = 0.135$). There were also positive correlations between PMS (estimated for UC patients) and PSQI (r = 0.256, $p = 0.040$), AIS (r = 0.370, $p = 0.002$), as well as ESS (r = 0.276, $p = 0.026$). The age of IBD patients did not correlate with the PSQI (r = 0.091, $p = 0.288$), AIS (r = 0.042, $p = 0.629$), and ESS (r = −0.049, $p = 0.569$) scores.

The following factors that may have affected the results of sleep questionnaires were identified: Patients with comorbid chronic diseases and taking hypnotic drugs had higher scores in PSQI and AIS, but not in ESS, compared to others. Steroid therapy did not affect the results of PSQI, AIS, and ESS. There was no difference in the results of PSQI, AIS, and ESS among patients with previous abdominal surgery, perianal fistulas, and treated with immunomodulatory drugs (Table 3).

Table 3. Factors affecting the quality of sleep.

Factor		PSQI		AIS		ESS	
Steroids treatment	Yes No	5.0 (4.0–9.0) 5.0 (4.0–7.0)	$p = 0.210$	6.0 (4.0–9.0) 5.0 (3.0–8.0)	$p = 0.410$	6.0 (4.0–8.0) 6.0(3.0–9.0)	$p = 0.395$
Hypnotic drugs	Yes No	10.0 (7.0–15.0) 5.0 (4.0–7.0)	$p < 0.001$	7.0 (5.0–10.0) 5.0 (3.0–8.0)	$p = 0.040$	6.0 (3.0–8.0) 6.0 (4.0–9.0)	$p = 0.808$
Abdominal surgery	Yes No	5.0 (4.0–8.0) 5.0 (4.0–7.0)	$p = 0.501$	7.0 (4.0–9.0) 5.0 (3.3–8.0)	$p = 0.306$	5.0 (4.0–8.0) 6.0 (4.0–9.8)	$p = 0.424$
Comorbid chronic diseases *	Yes No	5.0 (4.0–7.0) 5.0 (3.0–7.0)	$p = 0.044$	6.0 (3.0–8.0) 5.0 (2.5–7.0)	$p = 0.049$	6.0 (3–8.5) 5.0 (3.0–8.0)	$p = 0.191$
Fistulas	Yes No	5.0 (3.0–12.0) 5.0 (4.0–7.0)	$p = 0.630$	6.0 (4.0–8.5) 5.0 (4.0–8.0)	$p = 0.880$	4.0 (3.0–5.0) 6.0 (4.0–9.0)	$p = 0.057$
Immuno-modulators	Yes No	5.0 (4.0–7.0) 5.0 (4.0–7.25)	$p = 0.652$	6.0 (4.0–7.0) 5.0 (3.0–9.0)	$p = 0.841$	6.0(4.0–11.0) 6.0 (4.0–8.0)	$p = 0.532$
Anti-TNF therapy	Yes No	5.0 (4.0–8.0) 5.0 (4.0–7.0)	$p = 0.863$	5.0 (4.0–8.0) 5.5 (4.0–8.0)	$p = 0.575$	6.0(4.0–9.0) 6.0 (4.0–8.25)	$p = 0.833$

Data are presented as median (IQR). * such as asthma, compensated hypothyroidism, hypertension, migraine, musculoskeletal system diseases, diabetes, endometriosis, rheumatoid arthritis, and psoriasis; Abbreviations: AIS: Athens Insomnia Scale; ESS: Epworth sleepiness scale; PSQI: Pittsburgh Sleep Quality Index; TNF: tumour necrosis factor.

Positive correlations have been shown between scores of sleep scales (PSQI, AIS, and ESS) and the severity of depression according to BDI as well as pain according to VAS and LPS, Table 4. Multiple regression, using the progressive step method, revealed that sleep questionnaire results were significantly affected by BDI mood level, but not by the aforementioned pain scales scores. Obtained models explain 49.0% variability of PSQI, 39.1% of AIS, and 9.2% of ESS (Table 4).

Table 4. Association between sleep questionnaires results, pain scales, and depression rate.

	Correlation		Regression	
PSQI				
BDI	r = 0.540	$p < 0.001$	b = 0.316	$p < 0.001$
VAS	r = 0.286	$p = 0.001$		
LPS	r = 0.324	$p < 0.001$		
	$R^2 = 0.490$, $b = 3.264$, $p < 0.001$			
AIS				
BDI	r = 0.533	$p < 0.001$	b = 0.276	$p < 0.001$
VAS	r = 0.327	$p < 0.001$	b = 0.139	$p = 0.195$
LPS	r = 0.367	$p < 0.001$		
	$R^2 = 0.391$, $b = 3.318$, $p < 0.001$			
ESS				
BDI	r = 0.296	$p = 0.001$	b = 0.129	$p = 0.027$
VAS	r = 0.239	$p = 0.006$		
LPS	r = 0.245	$p = 0.004$	b = 0.187	$p = 0.238$
	$R^2 = 0.092$, $b = 5.182$, $p < 0.001$			

AIS: Athens Insomnia Scale; BDI: Beck Depression Inventory; ESS: Epworth sleepiness scale; LPS: Laitinen Pain Scale: Pittsburgh Sleep Quality Index; VAS: Visual Analogue Scale.

4. Discussion

The problem of disordered sleep in immune diseases has been recently widely studied [14,16,27,28]. To date, only a few studies have been published assessing sleep quality among IBD patients using mainly PSQI [14,29]. The impact of factors such as pain and depression on sleep in patients with IBD has not been extensively evaluated yet.

In our study, patients with CD and UC had similar sleep quality measured with PSQI and AIS, and daytime sleepiness according to ESS. Sobolewska et al. also did not notice differences

in the quality of sleep between CD and UC on a small group of patients measured by PSQI [14]. Interestingly, Ananthakrishnan et al. showed, that reduced quality of sleep in CD patients was a risk factor of exacerbation within 6 months, in contrast to UC patients, in whom no such association was demonstrated. CD patients had a slightly higher risk of developing disordered sleep compared to UC [15].

We showed, that IBD patients had greater sleep-related problems measured by PSQI compared to HC. The severity of the disease had an impact on the results of PSQI and patients in exacerbation had worse sleep quality compared to those in remission and HC. In another study, the decreased quality of sleep measured by PSQI in CD patients compared to healthy controls was also noted and the correlation between the severity of the disease and the PSQI score was observed [18]. We did not notice the difference in the sleep duration either between patients with IBD and HC or between patients in disease exacerbation and remission. On the other hand, Gingold-Belfer et al. observed differences in the length of sleep duration between patients in exacerbation, remission, and HC. In contrast to our study, there was no difference in sleep latency and sleep efficiency [18]. We observed that sleep efficiency was worse in IBD than HC, but did not differ between the exacerbation and remission subgroups. It should be noted that the results obtained can be significantly biased by the subjective nature of the data. Paixão et al. showed in a small group of IBD patients (n = 20) differences in sleep efficiency between patients in exacerbation and remission assessed by PSQI, but not in polysomnography examination [30]. Polysomnography is not dedicated to the diagnosis of all sleep disorders, i.e., sleep diary, questionnaires, and actigraphy could be more helpful in the diagnosis of insomnia [31]. However, polysomnography examination on a large group of IBD could provide additional information about the nature of the sleep disturbances in this group of patients.

Additionally, we noticed more frequent sleep interruption by getting up to the toilet in IBD patients compared to HC, as well as among patients in IBD exacerbation than in remission. Therefore, unsurprisingly, diarrhea may be one of the main causes of disordered sleep.

Although many guides for patients highlights the problem of insomnia in IBD, the studies using scales dedicated to its evaluation are few. In the Canadian Community Health Survey, the likelihood of bowel disorders increased with the frequency of insomnia problems; however, the severity of insomnia was measured using questions elaborated by the authors [32]. Recently, we noticed a positive correlation between brain-derived neurotrophic factor serum level and the results of the AIS questionnaires among CD patients [33]. In another study in young patients, no differences in the severity of insomnia were observed in relation to the clinical state of IBD [34]. However, to assess insomnia, the Women's Health Initiative Insomnia Rating Scale was used, which was validated only in postmenopausal women [35]. In our study, the symptoms of insomnia (according to the AIS) were present more often among IBD patients than in HC. AIS results correlated with the clinical severity of CD, but not UC. Some authors postulate a two-way interaction between sleep quality and disease severity [32,36]. The colitis mouse model suggests that insomnia may exacerbate the disease. Acute sleep deprivation increased disease severity measured with tissue myeloperoxidase and chronic intermittent sleep deprivation caused both worsening of histological as well as clinical manifestations of colitis [11]. A study of a cohort group including 151,871 women shown that the duration of sleep between 6 and 9 h per day reduced risk for the development of UC, but interestingly, not for CD [16]. Shorter sleep time is associated with higher TNF level, while longer sleep time with an increased level of C-reactive protein and IL-6 [8,37]. Sleep deprivation causes many immunological phenomena that can affect the course of IBD; for example, TNF is secreted during sleep deprivation, which is a target for effective anti-TNF therapy of IBD [38–40].

Patients in our study attained higher median results of ESS reflecting daytime sleepiness than HC. However, only 21% of IBD patients scored 11 or more points (which characterizes at least mild sleepiness). Iskandar et al. did not notice the difference in ESS between CD and HC, but patients with active disease had higher scores than in the remission. There were also no differences in the results of actigraphy and urine melatonin levels between CD and controls [27]. In another study, there were no

differences in ESS depending on the severity of the disease, while actigraphy has shown decreased sleep efficiency in moderate and severe CD group compared to patients in remission [34]. It is worth mentioning that urine melatonin levels and actigraphy are not dedicated methods to assess sleepiness. Clinical scales are used to screen for hypersomnia, but the gold standard is the Multiple Sleep Latency Test [41]. Perhaps ESS is not a fully reliable tool for assessing sleepiness in IBD patients. This scale was created to assess drowsiness in the course of sleep disorders, such as narcolepsy, other hypersomnias of the central origin, or obstructive sleep apnea [24,42]. On the other hand, ESS is simple and easy to use in everyday clinical practice.

Sleep is affected by many factors, such as mood, stress, and medications. One of them is the use of glucocorticosteroids, their negative effects on sleep have been reported [43]. Nevertheless, we found that patients on steroid therapy had similar sleep quality according to PSQI, AIS, and ESS. On the other hand, Ananthakrishnan et al. noticed the negative effect of steroids on the quality of sleep in IBD patients [15]. Additionally, we found that IBD patients with coexisting chronic diseases had decreased quality of sleep, which is expected as multiple comorbidities are a known risk factor for reduced sleep quality [44]. Surprisingly, the presence of fistulas and history of abdominal surgery did not affect the results obtained in the sleep quality questionnaires.

Disordered sleep may also be affected by the concurrent depression, which in the patients with IBD is more often diagnosed than in the general population [45–47]. Numerous studies have been published assessing the frequency of depression in IBD patients [46,47]. This disorder had been often diagnosed one year before IBD diagnosis [46]. In our study, patients with a history of mental illness were excluded from the analysis, but many of them might have undiagnosed disorders, especially depression. We noticed a strong correlation between the level of depression measured with the BDI questionnaire and the scores of other sleep scales—PSQI, AIS, and ESS. It has been previously shown that patients with depressive symptoms had an almost three-fold increased risk of sleep disturbances, which is consistent with the results of our study [15].

Pain also represents an important factor affecting sleep quality in the general population [48]. We have also shown that the subjective severity of pain was associated with higher sleep questionnaire scores. The resulting multiple regression model explained almost 50% of the PSQI variability with the mood level as a significant variable, while, quite surprisingly, the reported severity of pain did not affect sleep quality.

The limitation of this study was the use of subjective methods to assess the quality of sleep. On the other hand, the questionnaires used were validated and are still widely used. The use of three questionnaires assessing sleep in its various aspects increased the value of information obtained. However, future research should also include polysomnography and actigraphy to better understand the nature of sleep disturbances in IBD patients. The division of patients by clinical condition based on subjective scales (HBI and PMS) was also a limitation to this study. However, these scales are based on the complaints reported by patients that have an important impact on their perception of the quality of life.

5. Conclusions

Sleep impairment in IBD patients is a common problem that deserves attention in everyday clinical practice. Adequate sleep counseling can not only improve the quality of life of these patients, but also can have a positive effect on the course of the disease. Mood level, but not pain, is the main factor affecting the quality of sleep in IBD patients. Future research should focus on the search for causes of reduced quality of sleep and their impact on the risk of morbidity or exacerbation of the disease.

Author Contributions: Conceptualization, M.S.; methodology, M.S.; formal analysis, B.S. and M.S.; investigation, M.S., M.K. and R.T.-W.; writing—original draft preparation, M.S.; writing—review and editing, E.M.-P., A.G., R.T.-W. and P.B.; supervision, P.B.; funding acquisition, M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Science Centre, Poland (#2018/31/N/NZ5/03715 to MS).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Burisch, J.; Jess, T.; Martinato, M.; Lakatos, P.L. The burden of inflammatory bowel disease in Europe. *J. Crohns Colitis.* **2013**, *7*, 322–337. [CrossRef]
2. Łodyga, M.; Eder, P.; Bartnik, W.; Gonciarz, M.; Kłopocka, M.; Linke, K.; Małecka-Panas, E.; Radwan, P.; Reguła, J.; Rydzewska, G. Guidelines for the management of Crohn's disease. Recommendations of the Working Group of the Polish National Consultant in Gastroenterology and the Polish Society of Gastroenterology. *Gastroenterol. Rev.* **2012**, *6*, 317–338. [CrossRef]
3. Eder, P.; Łodyga, M.; Łykowska-Szuber, L.; Bartnik, W.; Durlik, M.; Gonciarz, M.; Kłopocka, M.; Linke, K.; Małecka-Panas, E.; Radwan, P.; et al. Guidelines for the management of ulcerative colitis. Recommendations of the Working Group of the Polish National Consultant in Gastroenterology and the Polish Society of Gastroenterology. *Gastroenterol. Rev.* **2013**, *1*, 1–20. [CrossRef]
4. Ganji-Arjenaki, M.; Rafieian-Kopaei, M. Probiotics are a good choice in remission of inflammatory bowel diseases: A meta analysis and systematic review. *J. Cell. Physiol.* **2018**, *233*, 2091–2103. [CrossRef]
5. Sgambato, D.; Miranda, A.; Ranaldo, R.; Federico, A.; Romano, M. The Role of Stress in Inflammatory Bowel Diseases. *Curr. Pharm. Des.* **2017**, *23*. [CrossRef]
6. Hirshkowitz, M.; Whiton, K.; Albert, S.M.; Alessi, C.; Bruni, O.; DonCarlos, L.; Hazen, N.; Herman, J.; Katz, E.S.; Kheirandish-Gozal, L.; et al. National Sleep Foundation's sleep time duration recommendations: Methodology and results summary. *Sleep Health* **2015**, *1*, 40–43. [CrossRef]
7. Hertenstein, E.; Gabryelska, A.; Spiegelhalder, K.; Nissen, C.; Johann, A.F.; Umarova, R.; Riemann, D.; Baglioni, C.; Feige, B. Reference Data for Polysomnography-Measured and Subjective Sleep in Healthy Adults. *J. Clin. Sleep Med.* **2018**, *15*, 523–532. [CrossRef]
8. Swanson, G.R.; Burgess, H.J.; Keshavarzian, A. Sleep disturbances and inflammatory bowel disease: A potential trigger for disease flare? *Expert Rev. Clin. Immunol.* **2011**, *7*, 29–36. [CrossRef]
9. Kaseva, N.; Pyhälä, R.; Pesonen, A.K.; Räikkönen, K.; Järvenpää, A.L.; Andersson, S.; Eriksson, J.G.; Hovi, P.; Kajantie, E. Diurnal cortisol patterns and dexamethasone suppression test responses in healthy young adults born preterm at very low birth weight. *PLoS ONE* **2016**, *11*. [CrossRef]
10. Reis, E.S.; Lange, T.; Köhl, G.; Herrmann, A.; Tschulakow, A.V.; Naujoks, J.; Born, J.; Köhl, J. Sleep and circadian rhythm regulate circulating complement factors and immunoregulatory properties of C5a. *Brain Behav. Immun.* **2011**, *25*, 1416–1426. [CrossRef]
11. Tang, Y.; Preuss, F.; Turek, F.W.; Jakate, S.; Keshavarzian, A. Sleep deprivation worsens inflammation and delays recovery in a mouse model of colitis. *Sleep Med.* **2009**, *10*, 597–603. [CrossRef] [PubMed]
12. Besedovsky, L.; Lange, T.; Born, J. Sleep and immune function. *Pflug. Arch. Eur. J. Physiol.* **2012**, *463*, 121–137. [CrossRef]
13. Shoham, S.; Davenne, D.; Cady, A.B.; Dinarello, C.A.; Krueger, J.M. Recombinant tumor necrosis factor and interleukin 1 enhance slow-wave sleep. *Am. J. Pychsiol.* **1987**, *253*, R142–R149. [CrossRef] [PubMed]
14. Sobolewska-Wlodarczyk, A.; Wlodarczyk, M.; Banasik, J.; Gasiorowska, A.; Wisniewska-Jarosinska, M.; Fichna, J. Sleep disturbance and disease activity in adult patients with inflammatory bowel diseases. *J. Physiol. Pharmacol.* **2018**, *69*. [CrossRef]
15. Ananthakrishnan, A.N.; Long, M.D.; Martin, C.F.; Sandler, R.S.; Kappelman, M.D. Sleep disturbance and risk of active disease in patients with Crohn's disease and ulcerative colitis. *Clin. Gastroenterol. Hepatol.* **2013**, *11*, 965–971. [CrossRef] [PubMed]
16. Ananthakrishnan, A.N.; Khalili, H.; Konijeti, G.G.; Higuchi, L.M.; de Silva, P.; Fuchs, C.S.; Richter, J.M.; Schernhammer, E.S.; Chan, A.T. Sleep Duration Affects Risk for Ulcerative Colitis: A Prospective Cohort Study. *Clin. Gastroenterol. Hepatol.* **2014**, *12*, 1879–1886. [CrossRef]

17. Buysse, D.J.; Reynolds, C.F.; Monk, T.H.; Berman, S.R.; Kupfer, D.J. The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. *Psychiatry Res.* **1989**, *28*, 193–213. [CrossRef]
18. Gingold-Belfer, R.; Peled, N.; Levy, S.; Katz, N.; Niv, Y.; Fass, R.; Fraser, G.; Dickman, R. Impaired sleep quality in Crohn's disease depends on disease activity. *Dig. Dis. Sci.* **2014**, *59*, 146–151. [CrossRef]
19. Harvey, R.F.; Bradshaw, J.M. A simple index of Crohn's-disease activity. *Lancet* **1980**, *1*, 514. [CrossRef]
20. Schroeder, K.; Tremanie, W.; Ilstrup, D. Coated Oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *N. Engl. J. Med.* **1987**, 1625–1629. [CrossRef]
21. Naderifar, M.; Goli, H.; Ghaljaei, F. Snowball Sampling: A Purposeful Method of Sampling in Qualitative Research. *Stud. Dev. Med. Educ.* **2017**, *14*, e67670. [CrossRef]
22. Fornal-Pawłowska, M.; Wołyńczyk-Gmaj, D.; Szelenberger, W. Validation of the Polish version of the Athens Insomnia Scale | Walidacja Ateńskiej Skali Bezsenności. *Psychiatr. Pol.* **2011**, *45*.
23. Soldatos, C.R.; Dikeos, D.G.; Paparrigopoulos, T.J. Athens Insomnia Scale: Validation of an instrument based on ICD-10 criteria. *J. Psychosom. Res.* **2000**, *48*, 555–560. [CrossRef]
24. Johns, M.W. A new method for measuring daytime sleepiness: The Epworth sleepiness scale. *Sleep* **1991**, *14*, 540–545. [CrossRef] [PubMed]
25. Aitken, R.C. Measurement of feelings using visual analogue scales. *Proc. R. Soc. Med.* **1969**, *62*, 989–993. [PubMed]
26. Laitinen, J. Acupuncture and transcutaneous electric stimulation in the treatment of chronic sacrolumbalgia and ischialgia. *Am. J. Chin. Med.* **1976**, *4*, 169–175. [CrossRef]
27. Iskandar, H.N.; Linan, E.E.; Patel, A.; Moore, R.; Lasanajak, Y.; Gyawali, C.P.; Sayuk, G.S.; Ciorba, M.A. Self-reported sleep disturbance in Crohn's disease is not confirmed by objective sleep measures. *Sci. Rep.* **2020**, *10*, 1980. [CrossRef]
28. Gabryelska, A.; Sochal, M.; Wasik, B.; Białasiewicz, P. Patients With Obstructive Sleep Apnea Are Over Four Times More Likely to Suffer From Psoriasis Than the General Population. *J. Clin. Sleep Med.* **2018**, *14*, 153. [CrossRef]
29. Ranjbaran, Z.; Keefer, L.; Farhadi, A.; Stepanski, E.; Sedghi, S.; Keshavarzian, A. Impact of sleep disturbances in inflammatory bowel disease. *J. Gastroenterol. Hepatol.* **2007**, *22*, 1748–1753. [CrossRef]
30. Paixão, D.L.; Poyares, D.; De Paula, M.S.; Duarte, J.W.; Castelo, P.M.; Ambrogini-Júnior, O.; Miszputen, S.J.; Oshima, C.T.F.; Chagas, J.R.; Paiotti, A.P.R. Evaluation of home polysomnography findings, quality of sleep, and fatigue in inflammatory bowel disease: A case series. *J. Clin. Sleep Med.* **2019**, *15*, 39–45. [CrossRef]
31. Krystal, A.D.; Prather, A.A.; Ashbrook, L.H. The assessment and management of insomnia: An update. *World Psychiatry* **2019**, *18*, 337–352. [CrossRef] [PubMed]
32. Hon, C.Y.; Nicol, A.M. Examining the Association Between Insomnia and Bowel Disorders in Canada: Is There a Trend? *UBC Med. J.* **2010**, *2*, 11–15.
33. Sochal, M.; Małecka-Panas, E.; Gabryelska, A.; Fichna, J.; Talar-Wojanrowska, R.; Szymd, B.; Białasiewicz, P. Brain-derived neurotrophic factor is elevated in the blood serum of Crohn's disease patients, but is not influenced by anti-TNF-α treatment—A pilot study. *Neurogastroenterol. Motil.* **2020**. [CrossRef]
34. Qazi, T.; Verma, R.; Hamilton, M.J.; Kaplan, E.R.; Redline, S.; Burakof, R. The use of actigraphy differentiates sleep disturbances in active and inactive Crohn's disease. *Inflamm. Bowel Dis.* **2019**, *25*, 1044–1053. [CrossRef] [PubMed]
35. Levine, D.W.; Dailey, M.E.; Rockhill, B.; Tipping, D.; Naughton, M.J.; Shumaker, S.A. Validation of the Women's Health Initiative Insomnia Rating Scale in a multicenter controlled clinical trial. *Psychosom. Med.* **2005**, *67*, 98–104. [CrossRef]
36. Sonnenberg, A. Occupational distribution of inflammatory bowel disease among German employees. *Gut* **1990**, *31*, 1037–1040. [CrossRef] [PubMed]
37. Eissa, N.; Mujawar, Q.; Abdul-Salam, T.; Zohni, S.; El-Matary, W. The Immune-Sleep Crosstalk in Inflammatory Bowel Disease. *Sleep Med.* **2020**. [CrossRef] [PubMed]
38. Chennaoui, M.; Sauvet, F.; Drogou, C.; Van Beers, P.; Langrume, C.; Guillard, M.; Gourby, B.; Bourrilhon, C.; Florence, G.; Gomez-Merino, D. Effect of one night of sleep loss on changes in tumor necrosis factor alpha (TNF-α) levels in healthy men. *Cytokine* **2011**, *56*, 318–324. [CrossRef] [PubMed]
39. Sochal, M.; Krzywdzinska, M.; Gabryelska, A.; Talar-Wojnarowska, R.; Malecka-Panas, E. Efficiency and safety of one-year anti-TNF-α treatment in Crohn's disease: A Polish single-centre experience. *Gastroenterol. Rev.* **2020**, *15*, 156–160. [CrossRef] [PubMed]

40. Sochal, M.; Krzywdzinska, M.; Gabryelska, A.; Talar-Wojnarowska, R.; Białasiewicz, P.; Malecka-Panas, E. A simple index to predict the efficiency of adalimumab treatment in Crohn's disease with a limited duration of therapy. *Pol. Arch. Intern. Med.* **2020**. [CrossRef] [PubMed]
41. Littner, M.R.; Kushida, C.; Wise, M.; Davila, D.G.; Morgenthaler, T.; Lee-Chiong, T.; Hirshkowitz, M.; Loube, D.L.; Bailey, D.; Berry, R.B.; et al. Practice parameters for clinical use of the multiple sleep latency test and the maintenance of wakefulness test. *Sleep* **2005**, *28*, 113–121. [CrossRef] [PubMed]
42. Lee, S.J.; Kang, H.W.; Lee, L.H. The relationship between the Epworth Sleepiness Scale and polysomnographic parameters in obstructive sleep apnea patients. *Eur. Arch. Oto-Rhino-Laryngol.* **2012**, *269*, 1143–1147. [CrossRef] [PubMed]
43. Moser, N.J.; Phillips, B.A.; Guthrie, G.; Barnett, G. Effects of dexamethasone on sleep. *Pharmacol. Toxicol.* **1996**, *79*, 100–102. [CrossRef] [PubMed]
44. Foley, D.; Ancoli-Israel, S.; Britz, P.; Walsh, J. Sleep disturbances and chronic disease in older adults: Results of the 2003 National Sleep Foundation Sleep in America Survey. *J. Psychosom. Res.* **2004**, *56*, 497–502. [CrossRef] [PubMed]
45. Franzen, P.L.; Buysse, D.J. Sleep disturbances and depression: Risk relationships for subsequent depression and therapeutic implications. *Dialogues Clin. Neurosci.* **2008**, *10*, 473–481.
46. Kurina, L.M.; Goldacre, M.J.; Yeates, D.; Gill, L.E. Depression and anxiety in people with inflammatory bowel disease. *J. Epidemiol. Community Health* **2001**, *55*, 716–720. [CrossRef]
47. Byrne, G.; Rosenfeld, G.; Leung, Y.; Qian, H.; Raudzus, J.; Nunez, C.; Bressler, B. Prevalence of anxiety and depression in patients with inflammatory bowel disease. *Can. J. Gastroenterol. Hepatol.* **2017**, *2017*. [CrossRef]
48. Keilani, M.; Crevenna, R.; Dorner, T.E. Sleep quality in subjects suffering from chronic pain. *Wien. Klin. Wochenschr.* **2018**, *130*, 31–36. [CrossRef]

Journal of
Clinical Medicine

MDPI

Article

Proton Pump Inhibitor Use May Increase the Risk of Diverticulitis but Not It's Severity among Patients with Colonic Diverticulosis: A Multicenter Study

Wisam Sbeit [1,†], Tawfik Khoury [1,†], Anas Kadah [1], Waseem Asadi [1], Amir Shahin [1], Ahmad Lubany [2], Mohammed Safadi [2], Haya Haddad [2], Ruba Abu Ahmad [2], Sami Abu El Hija [2], Rand Abboud [2], Mahmud Mahamid [3], Rinaldo Pellicano [4] and Amir Mari [5,*]

1 Department of Gastroenterology, Galilee Medical Center, Nahariya, Israel, Faculty of Medicine in the Galilee, Bar-Ilan University, Safed 13100, Israel; wisams@gmc.gov.il (W.S.); tawfikkhoury1@hotmail.com (T.K.); anask@gmc.gov.il (A.K.); wassim_assadi@yahoo.com (W.A.); amir.schahin@gmail.com (A.S.)
2 Surgery Department, The Nazareth Hospital, EMMS, Nazareth 1613101, Israel; ahmadlubany@gmail.com (A.L.); dr.m.safadi99@gmail.com (M.S.); haya_haddad@hotmail.com (H.H.); Rubaabuahmad@gmail.com (R.A.A.); sami.813@hotmail.com (S.A.E.H.); rand.abboud@gmail.com (R.A.)
3 Faculty of Medicine, Hebrew University of Jerusalem, Department of Gastroenterology and Liver Diseases, Shaare Zedek Medical Center, Jerusalem 9103401, Israel; mahmudmahamid@yahoo.com
4 Gastroenterology Unit, Molinette Hospital, 10125 Turin, Italy; rinaldo_pellican@hotmail.com
5 Gastroenterology and Endoscopy United, The Nazareth Hospital, EMMS, Faculty of medicine, Bar-Ilan University, Nazareth 1613101, Israel
* Correspondence: Amir.mari@hotmail.com
† First two authors contributed equally.

Received: 13 August 2020; Accepted: 10 September 2020; Published: 14 September 2020

Abstract: Colonic diverticular disease, especially diverticulitis constitutes a major cause of hospitalization and an economic burden in developed countries. Proton pump inhibitors (PPIs) are among the commonest drugs used to treat several diseases affecting the upper gastrointestinal tract. A few studies have reported that the use of Proton Pump Inhibitors PPIs caused dysbiosis. In this study, we searched for a relationship between PPI use and the onset and severity of diverticulitis in patients with colonic diverticulosis. In a retrospective study, patients who were hospitalized for documented diverticulitis were enrolled as cases and compared with a control group of patients with uncomplicated diverticulosis. Overall, 613 patients who had a diagnosis of diverticulosis were included in the study, 217 of whom had diverticulitis. After multivariate analysis, the non-modifiable risk factors associated with diverticulitis included: age ($p < 0.0001$), hypertension ($p < 0.0001$), chronic renal failure ($p = 0.007$), diabetes mellitus ($p < 0.0001$), and left colon location ($p = 0.02$). However, among the modifiable factors, only PPI use ($p < 0.0001$) showed a significant association. Advanced disease severity (according to Hinchey classification of diverticulitis stages II–IV) was associated with aspirin use ($p = 0.0004$) and pan-colonic location ($p = 0.02$). PPI use was the only modifiable factor significantly associated with diverticulitis, but not with its severity, among patients with diverticulosis. This observation should be confirmed in future multicenter prospective studies.

Keywords: PPI; diverticulosis; diverticulitis; risk factors

1. Introduction

Colonic diverticular disease, especially when complicated by diverticulitis and bleeding, constitutes a major cause of hospitalization and an economic burden in developed countries [1,2]. Complications, such as diverticulitis, bleeding, and perforation occur in about 10–20% of patients with colonic diverticulosis [3]. The incidence of diverticulitis has been increasing, as reported by a

nationwide study of hospitalizations performed in the United States that revealed a 26% increase in admissions from 1998 to 2005 [4]. Well-known risk factors for diverticulitis include high intake of red meat or fat, low fiber intake, high body mass index (BMI), lack of physical activity, smoking habits [5], and nonsteroidal anti-inflammatory drug (NSAID) use [6]. The main pathogenetic mechanism seems to involve inspissated fecal material that leads to mucus secretion and eventual bacterial overgrowth within the diverticulum inducing inflammation, focal necrosis, and micro- or macroperforation [7]. Due to their high efficacy and low toxicity, proton pump inhibitors (PPIs) are the most common drug used to treat gastroesophageal reflux disease, peptic ulcer disease (PUD), and to prevent NSAID and aspirin associated peptic ulcer disease [8]. Their use, especially long-term use, has increased significantly [9]. Alongside the gastric H^+/K^+-ATPase (proton pump) blocked by PPIs, several bacteria within the gastrointestinal tract produce acid and contain ATPase enzymes. Hence, it is believed that PPIs can inadvertently affect these bacteria directly by targeting their proton pumps and indirectly by changing the pH with an effect on the gut flora [10]. In fact, several studies have reported that PPI use has led to dysbiosis [9,11–13]. The role of gut microbiota in host resistance against colonization by exogenous enteric microbes and overgrowth of indigenous commensals is well known [14]. Accordingly, PPI-induced dysbiosis can increase the risk of bacterial enteric infections and translocation [9,15].

In this multicenter retrospective study, we aimed to study whether or not PPI use was associated with increased risk of diverticulitis development among patients with diverticulosis, as well as to assess whether PPI use was related to diverticulitis severity.

2. Materials and Methods

This study was performed using the databases of three Israeli academic medical centers (Galilee Medical Center, EMMS Nazareth Hospital, and Sharee Zedek Medical Center), from January 2010 to December 2019. The group consisted of 613 patients, and included patients with diverticulosis who lacked signs of inflammation as confirmed in computed tomography (CT) scans that had been performed electively for various causes and diverticulosis and that had been documented in the final report diagnosis. A second search was performed among these patients to detect patients who had been hospitalized at a later time (more than 6 months from the first elective CT), with the clinical diagnosis of diverticulitis (based on history, physical examination, and inflammatory markers) that had been confirmed by computed tomography (CT) scan, during the study period when they were eligible for enrollment in the study. The group of patients with uncomplicated diverticulosis served as the control.

PPI use was defined as taking PPIs for more than one month during an interval of 6 months prior to diverticulitis.

The extracted data included demographic variables (age and gender), smoking and alcohol drinking habits, as well as PPI, NSAID, aspirin, and statin use. Exclusion criteria resulted in excluding the following patients: 6 patients with a concomitant diagnosis of colorectal cancer and 2 patients who presented with colonic perforation and underwent surgery. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki [16], and was approved by each institution's human research committee. Written informed consent was waived by the local ethical committee due to the retrospective non-interventional nature of the study.

2.1. Study Aims

The primary aim of the study was to elucidate whether or not PPI use increased the risk of developing an episode of acute diverticulitis among patients with diverticulosis. The secondary aim was to identify predictors of increased diverticulitis severity, as assessed by the Hinchey classification on the basis of CT scan results which were reported as follows: stage 0, mild clinical diverticulitis with mild bowel wall inflammation; stage I, localized abscess (para-colonic); stage II, pelvic abscess; stage III, purulent peritonitis; and stage IV, fecal peritonitis [17]. Patients who presented with Hinchey

grade 0 and I were considered to have mild disease, while those with Hinchey grades II, III, and IV (generalized fecal peritonitis) were considered to have moderate to severe complicated diverticulitis.

2.2. Statistical Analysis

Chi-square and Fisher's exact tests were used to analyze the association between two categorical variables (presented as frequencies and percentages), while either the two-sample t-test or the Mann–Whitney U test was used to compare continuous variables (reported as mean ± standard deviation (SD)). To measure the association between PPI exposure and the risk and severity of diverticulitis, a univariate model analysis was performed and variables with statistically significant p values (<0.05) by univariate analysis were entered into the multiple logistic regression analysis which reported odds ratios (OR) and confidence intervals (CI). Statistical analyses were carried out with commercial software, Statistical Package for Social Science (SPSS version 24.0, IBM, Chicago, IL, USA).

3. Results

3.1. Demographics, Clinical, and Laboratory Characteristics

Overall, 613 patients with a confirmed diagnosis of diverticulosis were included in the study. Among them, 217 patients had at least one diverticulitis episode (group A), as compared with 396 patients who did not develop diverticulitis (group B). The mean age was 60.7 ± 14.7 and 70.4 ± 12.3 years in groups A and B, respectively. In group A, 66.8% were males as compared with 59.6% in group B. Notably, among the patients in group A, 120 (55.3%), 13 (6%), 81 (37.3%), and 85 (39.2%) of them were on chronic treatment (more than 1 month) with PPIs, NSAIDs, statins, and aspirin, respectively, as compared with 111 (28%), 23 (5.8%), 186 (47%), and 178 (45%) patients in group B. In all groups, the most common location of diverticulosis was the sigmoid colon (53% vs. 58.8% in groups A and B, respectively). Table 1 shows the demographics and baseline characteristics of the study cohort.

Table 1. Demographics and baseline characteristics of study cohort.

Parameters	Patients with Diverticulitis	Patients without Diverticulitis
Number of Patients	217	396
Age (Years), Mean ±SD (Range)	60.7 ± 14.7 (24–88)	70.4 ± 12.3 (18–97)
Gender n (%)		
Male	145(66.8)	236(59.6)
Female	72(33.2)	160(40.4)
Medical History, n (%)		
Hypertension	103(47.5)	279(70.5)
Diabetes Mellitus	110(50.7)	103(26)
Smoking	58(26.7)	90(22.7)
Ischemic Heart Disease	34(15.7)	81(20.5)
Chronic Renal Failure	5(2.3)	34(8.6)
Heart Failure	5(2.3)	33(8.3)
Obesity (BMI > 30)	109(50.2)	112(28.3)
Proton Pump Inhibitors Use, *n* (%)	120(55.3)	111(28)
Non-Steroidal Anti-Inflammatory Drugs Use, *n* (%)	13(6)	23(5.8)
Statins Use, *n* (%)	81(37.3)	186(47)
Aspirin Use, *n* (%)	8(39.2)	178(45)
Site of Diverticulosis, n (%)		
Sigmoid	115(53)	233(58.8)
Left Colon	42(19.3)	112(28.3)
Transverse Colon	0	11(2.8)
Right Colon	20(9.2)	29(7.3)
Pan-Colonic	43(19.8)	81(20.4)

3.2. Parameters Associated with Diverticulitis in the Univariate and Multivariate Analysis

We identified several parameters associated with the development of diverticulitis (Table 2). The univariate model analysis indicated that age (OR 0.95, 95% CI 0.94–0.96, $p < 0.0001$), hypertension (OR 0.38, 95% CI 0.27–0.54, $p < 0.0001$), chronic renal failure (OR 0.27, 95% CI 0.11–0.68, $p = 0.005$), heart failure (OR 0.28, 95% CI 0.11–0.71, $p = 0.007$), diabetes mellitus (OR 2.92, 95% CI 2.06–4.13, $p < 0.0001$), obesity (OR 2.55, 95% CI 1.81–3.60, $p < 0.0001$), left colon location (OR 0.61, 95% CI 0.41–0.92, $p = 0.016$), statin use (OR 0.67, 95% CI 0.48–0.95, $p = 0.02$), and PPI use (OR 2.77, 95% CI 1.45–5.32, $p = 0.002$) were statistically significant. Conversely, there was no effect of other parameters including smoking ($p = 0.12$), NSAID use ($p = 0.89$), and aspirin use ($p = 0.17$). In the multivariate logistic regression analysis, age (OR 0.94, 95% CI 0.92–0.95, $p < 0.0001$), hypertension (OR 0.25, 95% CI 0.15–0.44, $p < 0.0001$), chronic renal failure (OR 0.23, 95% CI 0.08–0.68, $p = 0.007$), diabetes mellitus (OR 5.73, 95% CI 3.11–10.52, $p < 0.0001$), and left colon location (OR 0.58, 95% CI 0.35–0.94, $p = 0.02$) remained significantly correlated with diverticulitis, whereas among the modifiable risk factors, only PPI use (OR 3.94, 95% CI 2.26–6.86, $p < 0.0001$) was significantly associated with diverticulitis (Table 3).

Table 2. Univariate analysis of parameters associated with diverticulitis.

Parameter	Odds Ratio	95% Confidence Interval	*p* Value
Age (Years)	0.95	0.94–0.96	*<0.0001*
Gender (Male Vs. Female)	1.36	0.96–1.93	0.08
Hypertension	0.38	0.27–0.54	*<0.0001*
Chronic Renal Failure	0.27	0.11–0.68	*0.005*
Heart Failure	0.28	0.11–0.71	*0.007*
Ischemic Heart Disease	0.73	0.47–1.13	0.16
Diabetes Mellitus	2.92	2.06–4.13	*<0.0001*
Obesity	2.55	1.81–3.60	*<0.0001*
Smoking	1.33	0.91–1.95	0.15
Statins Use	0.67	0.48–0.95	*0.02*
Non-Steroidal Anti-Inflammatory Drugs Use	1.05	0.52–2.11	0.89
Aspirin Use	0.79	0.56–1.11	0.17
Proton Pump Inhibitors Use	2.77	1.45–5.32	*0.002*
Sigmoid Location	0.79	0.57–1.10	0.16
Left Colon Location	0.61	0.41–0.92	0.016
Transverse Colon Location	0.08	0.004–1.49	0.09
Right Colon Location	1.29	0.71–2.34	0.4
Pan-Colonic Location	0.97	0.64–1.46	0.87

Table 3. Multivariate analysis of parameters associated with diverticulitis.

Non- Modifiable Risk Factors			
Parameter	**Odds Ratio**	**95% Confidence Interval**	***p* Value**
Age (Years)	0.94	0.92–0.95	<0.0001
Hypertension	0.25	0.15–0.44	*<0.0001*
Chronic Renal Failure	0.23	0.08–0.68	0.007
Diabetes Mellitus	5.73	3.11–10.52	<0.0001
Left Colon Location	0.58	0.35–0.94	0.02
Modifiable Risk Factors			
Parameter	**Odds Ratio**	**95% Confidence Interval**	***p* value**
Proton Pump Inhibitors Use	3.94	2.26–6.86	<0.0001

3.3. Parameters Associated With Diverticulitis Severity

Overall, 217 patients had an episode of diverticulitis, however, since two patients had missing data, the final analysis was performed on 215 patients. Among them, 59 patients (27.2%) had moderate to severe diverticulitis defined by Hinchey stage II–IV (group C) as compared with 156 patients (72.8%) who had mild diverticulitis (stage 0–I) (group D). The mean ages for groups C and D were 65.8 ± 11.8 and 58.8 ± 15.3, respectively. Male gender was similar between the groups (67.8% vs. 66.7%, for groups C and D, respectively). Table 4 shows the baseline characteristics of the cohort with diverticulitis according to severity.

Table 4. Demographics and baseline characteristics of the study cohort with diverticulitis.

Parameters	**Severe Diverticulitis (Hinchey Stage II-IV)**	**Mild Diverticulitis (Hinchey Stage 0-I)**
Number of Patients	59	156
Age (Years), Mean ±SD (Range)	65.8 ± 11.8(25–88)	58.8 ± 15.3(24–87)
Gender n (%)		
Male	40(67.8)	104(66.7)
Female	19(32.2)	52(33.3)
Medical History, n (%)		
Hypertension	41(69.5)	60(38.5)
Diabetes Mellitus	44(74.6)	65(41.7)
Smoking	18(30.5)	39(25)
Ischemic Heart Disease	13(22)	20(12.8)
Chronic Renal Failure	3(5.1)	2(1.3)
Heart Failure	3(5.1)	2(1.3)
Obesity	44(74.6)	64(41)
Proton Pump Inhibitors Use, n (%)	43(72.9)	76(487)
Non-Steroidal Anti-Inflammatory Drugs Use, n (%)	7(11.9)	6(3.9)
Statins Use, n (%)	27(45.8)	53(34)
Aspirin Use, n (%)	38(64.4)	46(29.5)
Site of Diverticulosis, n (%)		
Sigmoid	25(42.4)	89(57.1)
Left Colon	9(15.3)	31(19.9)
Right Colon	3(5.1)	17(10.9)
Pan-Colonic	22(37.3)	21(13.5)

3.4. Parameters Associated With Diverticulitis Severity in the Univariate and Multivariate Analysis

The univariate analysis indicated that age (OR 1.04, 95% CI 1.01–1.06, $p = 0.002$), hypertension (OR 3.58, 95% CI 1.89–6.77, $p < 0.0001$), diabetes mellitus (OR 4.01, 95% CI 2.07–7.78, $p < 0.0001$), obesity (OR 4.12, 95% CI 2.12–7.99, $p < 0.0001$), aspirin use (OR 4.26, 95% CI 2.26–8.01, $p < 0.0001$), NSAID use (OR 3.31, 95% CI 1.06–10.28, $p = 0.04$), PPI use (OR 2.77, 95% CI 1.45–5.32, $p = 0.002$), and pan-colonic diverticulosis (OR 3.78. 95% CI 1.88–7.61, $p = 0.0002$) were associated with more severe diverticulitis (Hinchey stage II–IV) (Table 5). After multivariate analysis, only aspirin use (OR 3.4, 95% CI 1.73–6.63, $p = 0.0004$) and pan-colonic location (OR 2.41, 95% CI 1.13–5.13, $p = 0.02$) remained significantly associated with severe diverticulitis (Hinchey stage II–IV) (Table 5), whereas there was no effect of NSAID use ($p = 0.2$), PPI use ($p = 0.11$), age ($p = 0.7$), hypertension ($p = 0.1$), diabetes mellitus ($p = 0.25$), and obesity ($p = 0.1$).

Table 5. Univariate and multivariate analyses of parameters associated with higher diverticulitis severity (Hinchey stage II–IV).

Univariate Analysis			
Parameter	**Odds Ratio**	**95% Confidence Interval**	***p* Value**
Age (Years)	1.04	1.01–1.06	*0.002*
Gender Male Vs. Female	1.04	0.55–1.97	0.9
Hypertension	3.58	1.89–6.77	*<0.0001*
Chronic Renal Failure	3.83	0.63–23.25	0.14
Heart Failure	3.83	0.63–23.25	0.14
Ischemic Heart Disease	1.93	0.89–4.19	0.09
Diabetes Mellitus	4.01	2.07–7.78	*<0.0001*
Obesity	4.12	2.12–7.99	*<0.0001*
Smoking	1.24	0.64–2.42	0.52
Statins Use	1.64	0.89–3.01	0.11
Non-Steroidal Anti-Inflammatory Drugs Use	3.31	1.06–10.28	*0.04*
Aspirin Use	4.26	2.26–8.01	*<0.0001*
Proton Pump Inhibitors Use	2.77	1.45–5.32	*0.002*
Sigmoid Location	0.56	0.31–1.02	0.058
Left Colon Location	0.75	0.34–1.68	0.48
Right Colon Location	0.49	0.15–1.67	0.25
Pan-Colonic Location	3.78	1.88–7.61	*0.0002*
Multivariate Logistic Regression Analysis			
Parameter	**Odds Ratio**	**95% Confidence Interval**	***p* value**
Aspirin Use	3.4	1.73–6.63	*0.0004*
Pan-Colonic Location	2.41	1.13–5.13	*0.02*

4. Discussion

Colonic diverticulosis is becoming one the most common diseases worldwide [18,19]. Although most patients have a silent asymptomatic course throughout their life, an increasing prevalence of diverticulitis with increasing morbidity has been encountered, posing a significant burden on health service systems [20], with approximately 20% of patients needing surgical intervention for diverticulitis at first presentation [21,22]. The increased prevalence of diverticulitis cannot be attributed solely to the known risk factors including Western diet (with high red meat and fat intake and low fiber intake), obesity, lack of physical activity, smoking, and NSAID consumption. Therefore, other modifiable risk

factors, including medications, could be involved in the development of diverticulitis. Due to the widespread availability and the increased use of PPIs, and the increasing prevalence of diverticulitis, and because both are associated with enteric bacterial overgrowth and infections, a possible causal relationship between PPI use and the development of diverticulitis has been hypothesized. Medications which suppress gastric acid production increase the risk of infections by different pathogens [23], due to hypochlorhydria when the gastric pH exceeds 4.0, enabling pathogens to escape the defense mechanism of the acidic stomach which leads to colonization and bacterial overgrowth, in addition to reduced gastrointestinal host defense originating from delayed gastric emptying, increased bacterial translocation, decreased gastric mucus viscosity, changes in normal microbial flora [24], and blocking the bacterial proton pumps [10]. It has been shown that gastric acid inhibitors increase the risk of community-acquired pneumonia and acute gastroenteritis in children, as a result of changes in the normal gut flora and leukocyte dysfunction [15]. An association between dosage and treatment duration of PPI therapy and increased risk of gastrointestinal infections has been shown [25].

In our study, we showed a possible association between PPI use and the risk of diverticulitis that remained significant in multivariate analysis ($p > 0.001$, OR 3.94, 95% CI 2.26–6.86). This observation was not documented in the English literature hitherto. Notably, major points should be discussed regarding this possible association since demographics such as age and background illnesses could have contributed to this observed association. It is well known that age is a risk factor for diverticulosis and its complications including diverticulitis [6]. The patients in the diverticulitis group were older by about 10 years as compared with the diverticulosis group, a fact that could raise an option of possible confounding. Importantly, the association of PPI use with acute diverticulitis was observed in the multivariate analysis as well. Moreover, the patients in the different groups still belonged to approximately the same age group (60–70), therefore generally speaking an elderly patient group. The only report that has analyzed this matter was a nested case-control study which enrolling 690 patients based on the National Health Insurance Research Database in Taiwan, in which the authors showed that the use of PPIs did not increase the risk of diverticulitis. However, among the limitations of this study, the authors stated that the results could not be applied to Western societies as colonic diverticular disease is not as common in Asian as compared with Western societies [24]. A finding of our multicenter study is that PPI use did not affect the severity of diverticulitis. However, a recent study by Tursi et al. showed that PPI use was directly related to the severity of diverticular disease [26].

Conversely, in our study, some known risk factors for diverticulitis including smoking, as well as NSAID and aspirin use did not affect the rate of diverticulitis. In a study on 47,210 U.S. men included in the Health Professionals Follow-up Study cohort, Strate et al. reported that regular use of aspirin or NSAIDs increased the risk of diverticulitis [6]. Two studies by Laine et al. reported diverticulitis among the commonest serious lower gastrointestinal side effects of NSAIDs [27,28]. These difference with respect to our findings could be explained considering the small number of NSAID users (6%) included in our cohort. However, patients could have used over-the-counter NSAIDs, a fact that could result in data bias.

Furthermore, we found several other known non-modifiable risk factors for the development of diverticulitis in multivariate analysis including advanced age, hypertension, chronic renal failure, diabetes mellitus, and left colon location. Our results were in agreement with the evidence published throughout the literature showing that age [29], hypertension [30], and chronic kidney disease [31] were significantly associated with increased prevalence of diverticulitis. Although there was no data for the association of diabetes mellitus with diverticulitis, diabetes mellitus was associated with advanced disease severity as assessed by Hinchey and Ambrosetti scores [32]. Some possible explanations of this observation are the probable coexistence of DM with metabolic syndrome that can induce the development of diverticulitis through the release of cytokines that promote inflammation in the subcutaneous abdominal fat, as well as alterations in gut microbiota that can play a key role in inducing diverticulitis [33].

Our finding that left colon location had a significant association with diverticulitis is similar to findings reported by previous studies performed in Western countries [34]. Although previous evidence showed that obesity, smoking, and statin use increased the prevalence and severity of diverticular disease and its related complications [35,36], our results did not support the aforementioned findings, suggesting that more studies are warranted to exactly assess this association.

Moreover, although aspirin use did not affect the prevalence of diverticulitis in our study, it was associated with higher diverticulitis severity, as shown by higher Hinchey classification. Potential mechanisms for aspirin and NSAID diverticular complications are supposed to result from direct topical injury to the colon and impaired prostaglandin synthesis which compromise mucosal integrity, increase permeability, and enable the influx of bacteria and toxins [37]. Therefore, more data from laboratory research and prospective studies are needed to elucidate the exact effect of aspirin and NSAIDs on the prevalence and severity of diverticulitis.

The main limitation of our study is its retrospective design that could cause data and information bias. Ideally, the possible causal relationship between PPI and diverticulitis should be assessed in a prospective study design rather than retrospectively. However, a strength of our study included limited potential data heterogeneity, due to the fact that, in all three involved centers, all the physicians followed homogeneous protocols for both data collection and diagnostic algorithm. Furthermore, during the last 20 years, all consultations had been recorded in a computerized data bank, and therefore our data were complete and homogeneous.

Another main strength of this study was the multicenter design and the relatively large number of patients included.

5. Conclusions

In conclusion, several modifiable and non-modifiable factors were found to be associated with an increased prevalence of colonic diverticulitis. PPI use seems to be associated with acute diverticulitis but not its severity. Moreover, disease severity was mainly affected by aspirin use and pan-colonic location. Larger prospective studies are warranted in order to confirm our data.

Author Contributions: W.S., T.K., and A.M. contributed to the study concept and design; W.S., T.K., M.M., and A.M. contributed to data analysis; R.P. contributed to intellectual content support; W.S. and T.K. wrote the final draft of the manuscript; T.K., A.M., R.P., and W.S. contributed to critical revision and all authors approved the final version to be published. All authors contributed to data collection. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest regarding this manuscript.

Abbreviations

PPIs	Proton Pump Inhibitors
BMI	Body Mass Index
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
PUD	Peptic Ulcer Disease
CT	Computed Tomography
OR	Odds Ratio
CI	Confidence Interval
DM	Diabetes Miletus

References

1. Everhart, J.E.; Ruhl, C.E. Burden of digestive diseases in the United States Part III: Liver, biliary tract, and pancreas. *Gastroenterology* **2009**, *136*, 1134–1144. [CrossRef] [PubMed]

2. Shaheen, N.J.; Hansen, R.A.; Morgan, D.R.; Gangarosa, L.M.; Ringel, Y.; Thiny, M.T.; Russo, M.W.; Sandler, R.S. The burden of gastrointestinal and liver diseases, 2006. *Am. J. Gastroenterol.* **2006**, *101*, 2128–2138. [CrossRef] [PubMed]
3. Stollman, N.; Raskin, J.B. Diverticular disease of the colon. *Lancet* **2004**, *363*, 631–639. [CrossRef]
4. Etzioni, D.A.; Mack, T.M.; Beart, R.W., Jr.; Kaiser, A.M. Diverticulitis in the United States: 1998–2005: Changing patterns of disease and treatment. *Ann. Surg.* **2009**, *249*, 210–217. [CrossRef] [PubMed]
5. Liu, P.H.; Cao, Y.; Keeley, B.R.; Tam, I.; Wu, K.; Strate, L.L.; Giovannucci, E.L.; Chan, A.T. Adherence to a Healthy Lifestyle is Associated With a Lower Risk of Diverticulitis among Men. *Am. J. Gastroenterol.* **2017**, *112*, 1868–1876. [CrossRef] [PubMed]
6. Strate, L.L.; Liu, Y.L.; Huang, E.S.; Giovannucci, E.L.; Chan, A.T. Use of aspirin or nonsteroidal anti-inflammatory drugs increases risk for diverticulitis and diverticular bleeding. *Gastroenterology* **2011**, *140*, 1427–1433. [CrossRef]
7. Szojda, M.M.; Cuesta, M.A.; Mulder, C.M.; Felt-Bersma, R.J. Review article: Management of diverticulitis. *Aliment. Pharmacol. Ther.* **2007**, *26* (Suppl. 2), 67–76. [CrossRef]
8. Savarino, V.; Marabotto, E.; Zentilin, P.; Furnari, M.; Bodini, G.; De Maria, C.; Pellegatta, G.; Coppo, C.; Savarino, E. The appropriate use of proton-pump inhibitors. *Minerva Med.* **2018**, *109*, 386–399. [CrossRef]
9. Hojo, M.; Asahara, T.; Nagahara, A.; Takeda, T.; Matsumoto, K.; Ueyama, H.; Matsumoto, K.; Asaoka, D.; Takahashi, T.; Nomoto, K.; et al. Gut Microbiota Composition Before and After Use of Proton Pump Inhibitors. *Dig. Dis. Sci.* **2018**, *63*, 2940–2949. [CrossRef]
10. Vesper, B.J.; Jawdi, A.; Altman, K.W.; Haines, G.K., 3rd; Tao, L.; Radosevich, J.A. The effect of proton pump inhibitors on the human microbiota. *Curr. Drug Metab.* **2009**, *10*, 84–89. [CrossRef]
11. Jackson, M.A.; Goodrich, J.K.; Maxan, M.E.; Freedberg, D.E.; Abrams, J.A.; Poole, A.C.; Sutter, J.L.; Welter, D.; Ley, R.E.; Bell, J.T.; et al. Proton pump inhibitors alter the composition of the gut microbiota. *Gut* **2016**, *65*, 749–756. [CrossRef]
12. Imhann, F.; Bonder, M.J.; Vich Vila, A.; Fu, J.; Mujagic, Z.; Vork, L.; Tigchelaar, E.F.; Jankipersadsing, S.A.; Cenit, M.C.; Harmsen, H.J.; et al. Proton pump inhibitors affect the gut microbiome. *Gut* **2016**, *65*, 740–748. [CrossRef]
13. Takagi, T.; Naito, Y.; Inoue, R.; Kashiwagi, S.; Uchiyama, K.; Mizushima, K.; Tsuchiya, S.; Okayama, T.; Dohi, O.; Yoshida, N.; et al. The influence of long-term use of proton pump inhibitors on the gut microbiota: An age-sex-matched case-control study. *J. Clin. Biochem. Nutr.* **2018**, *62*, 100–105. [CrossRef] [PubMed]
14. Kamada, N.; Chen, G.Y.; Inohara, N.; Nunez, G. Control of pathogens and pathobionts by the gut microbiota. *Nat. Immunol.* **2013**, *14*, 685–690. [CrossRef]
15. Canani, R.B.; Cirillo, P.; Roggero, P.; Romano, C.; Malamisura, B.; Terrin, G.; Passariello, A.; Manguso, F.; Morelli, L.; Guarino, A.; et al. Therapy with gastric acidity inhibitors increases the risk of acute gastroenteritis and community-acquired pneumonia in children. *Pediatrics* **2006**, *117*, e817–e820. [CrossRef]
16. World Medical, A. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA* **2013**, *310*, 2191–2194. [CrossRef]
17. Hinchey, E.J.; Schaal, P.G.; Richards, G.K. Treatment of perforated diverticular disease of the colon. *Adv. Surg.* **1978**, *12*, 85–109.
18. Cirocchi, R.; Nascimbeni, R.; Binda, G.A.; Vettoretto, N.; Cuomo, R.; Gambassi, G.; Amato, A.; Annibale, B. Surgical treatment of acute complicated diverticulitis in the elderly. *Minerva Chir.* **2019**, *74*, 465–471. [CrossRef]
19. Elisei, W.; Brandimarte, G.; Tursi, A. Diverticulosis today. *Minerva Gastroenterol. Dietol.* **2019**, *65*, 42–52. [CrossRef]
20. Elisei, W.; Brandimarte, G.; Tursi, A. Management of diverticulosis: What's new? *Minerva Med.* **2017**, *108*, 448–463. [CrossRef]
21. Regenbogen, S.E.; Hardiman, K.M.; Hendren, S.; Morris, A.M. Surgery for diverticulitis in the 21st century: A systematic review. *JAMA Surg.* **2014**, *149*, 292–303. [CrossRef] [PubMed]
22. Sandler, R.S.; Everhart, J.E.; Donowitz, M.; Adams, E.; Cronin, K.; Goodman, C.; Gemmen, E.; Shah, S.; Avdic, A.; Rubin, R. The burden of selected digestive diseases in the United States. *Gastroenterology* **2002**, *122*, 1500–1511. [CrossRef] [PubMed]
23. Martinsen, T.C.; Bergh, K.; Waldum, H.L. Gastric juice: A barrier against infectious diseases. *Basic Clin. Pharmacol. Toxicol.* **2005**, *96*, 94–102. [CrossRef] [PubMed]

24. Ho, B.L.; Hu, H.Y.; Chang, S.S. Association between use of proton pump inhibitors and occurrence of colon diverticulitis. *J. Chin. Med. Assoc.* **2016**, *79*, 5–10. [CrossRef] [PubMed]
25. Garcia Rodriguez, L.A.; Ruigomez, A.; Panes, J. Use of acid-suppressing drugs and the risk of bacterial gastroenteritis. *Clin. Gastroenterol. Hepatol.* **2007**, *5*, 1418–1423. [CrossRef]
26. Tursi, A.; Violi, A.; Cambie, G.; Franceschi, M.; Baldassarre, G.; Rodriguez, K.I.; Miraglia, C.; Brandimarte, G.; Elisei, W.; Picchio, M.; et al. Risk factors for endoscopic severity of diverticular disease of the colon and its outcome: A real-life case-control study. *Eur. J. Gastroenterol. Hepatol.* **2020**, *32*, 1123–1129. [CrossRef]
27. Laine, L.; Connors, L.G.; Reicin, A.; Hawkey, C.J.; Burgos-Vargas, R.; Schnitzer, T.J.; Yu, Q.; Bombardier, C. Serious lower gastrointestinal clinical events with nonselective NSAID or coxib use. *Gastroenterology* **2003**, *124*, 288–292. [CrossRef]
28. Laine, L.; Curtis, S.P.; Langman, M.; Jensen, D.M.; Cryer, B.; Kaur, A.; Cannon, C.P. Lower gastrointestinal events in a double-blind trial of the cyclo-oxygenase-2 selective inhibitor etoricoxib and the traditional nonsteroidal anti-inflammatory drug diclofenac. *Gastroenterology* **2008**, *135*, 1517–1525. [CrossRef]
29. Reichert, M.C.; Krawczyk, M.; Appenrodt, B.; Casper, M.; Friesenhahn-Ochs, B.; Grunhage, F.; Jungst, C.; Zimmer, V.; Lammert, F.; Dauer, M. Selective association of nonaspirin NSAIDs with risk of diverticulitis. *Int. J. Colorectal Dis.* **2018**, *33*, 423–430. [CrossRef]
30. Rosemar, A.; Angeras, U.; Rosengren, A. Body mass index and diverticular disease: A 28-year follow-up study in men. *Dis. Colon Rectum* **2008**, *51*, 450–455. [CrossRef]
31. Morris, C.R.; Harvey, I.M.; Stebbings, W.S.; Hart, A.R. Incidence of perforated diverticulitis and risk factors for death in a UK population. *Br. J. Surg.* **2008**, *95*, 876–881. [CrossRef] [PubMed]
32. Cologne, K.G.; Skiada, D.; Beale, E.; Inaba, K.; Senagore, A.J.; Demetriades, D. Effects of diabetes mellitus in patients presenting with diverticulitis: Clinical correlations and disease characteristics in more than 1000 patients. *J. Trauma Acute Care Surg.* **2014**, *76*, 704–709. [CrossRef] [PubMed]
33. Munie, S.T.; Nalamati, S.P.M. Epidemiology and Pathophysiology of Diverticular Disease. *Clin. Colon Rectal Surg.* **2018**, *31*, 209–213. [CrossRef] [PubMed]
34. Nguyen, G.C.; Sam, J.; Anand, N. Epidemiological trends and geographic variation in hospital admissions for diverticulitis in the United States. *World J. Gastroenterol.* **2011**, *17*, 1600–1605. [CrossRef]
35. Manousos, O.; Day, N.E.; Tzonou, A.; Papadimitriou, C.; Kapetanakis, A.; Polychronopoulou-Trichopoulou, A.; Trichopoulos, D. Diet and other factors in the aetiology of diverticulosis: An epidemiological study in Greece. *Gut* **1985**, *26*, 544–549. [CrossRef]
36. Humes, D.J.; Fleming, K.M.; Spiller, R.C.; West, J. Concurrent drug use and the risk of perforated colonic diverticular disease: A population-based case-control study. *Gut* **2011**, *60*, 219–224. [CrossRef]
37. Lanas, A.; Sopena, F. Nonsteroidal anti-inflammatory drugs and lower gastrointestinal complications. *Gastroenterol. Clin. N. Am.* **2009**, *38*, 333–352. [CrossRef]

Journal of
Clinical Medicine

MDPI

Article

Abdominal Symptoms and Colonic Diverticula in Marfan's Syndrome: A Clinical and Ultrasonographic Case Control Study

Giovanni Maconi [1,*], Alessandro Pini [2], Elia Pasqualone [1], Sandro Ardizzone [1] and Gabrio Bassotti [3]

[1] Gastroenterology Unit, Department of Biomedical and Clinical Sciences, ASST Fatebenefratelli-Sacco, University of Milan, 20157 Milan, Italy; pasqualone.elia@gmail.com (E.P.); sandro.ardizzone1@unimi.it (S.A.)
[2] Cardiovascular-Genetic Center, IRCCS Policlinico San Donato, 20097 San Donato Milanese, Italy; alessandro.pini@grupposandonato.it
[3] Gastroenterology & Hepatology Section, Department of Medicine, University of Perugia Medical School, 06122 Perugia, Italy; gabassot@tin.it
* Correspondence: Giovanni giovanni.maconi@unimi.it; Tel.: +39-023-904-3164

Received: 6 August 2020; Accepted: 11 September 2020; Published: 28 September 2020

Abstract: Background: Marfan's syndrome (MFS) seems to be frequently associated with colonic diverticulosis, but the prevalence of diverticula and symptoms evocative of diverticular disease in this population are still unknown. Methods: This prospective case control study included 90 consecutive patients with MFS, 90 unselected controls, and 90 asymptomatic subjects. The clinical characteristics, including lower gastrointestinal symptoms, and ultrasonographic features of the bowel, including diverticula and thickening of the muscularis propria of the sigmoid colon, were investigated. In addition, the genotype of MFS patients was assessed. The characteristics of patients and controls were compared using parametric tests. Results: Complaints of abdominal symptoms were made by 23 (25.6%) patients with MFS and 48 (53%) control subjects ($p < 0.01$). Constipation and bloating were reported less frequently by MFS patients than controls (constipation: 13.3% vs. 26.6%, $p = 0.039$; bloating: 3.3% vs. 41.1%, $p < 0.0001$), while other symptoms were not significantly different. Sigmoid diverticulosis was detected in 12 (12.3%) patients with MFS, as well as in 3 (3.3%) asymptomatic healthy subjects and 4 (4.4%) random controls ($p = 0.0310$). The genetic variants of MFS were not correlated with symptoms or diverticula. Conclusion: Patients with MFS have a greater prevalence of diverticula, although less abdominal symptoms, compared to the general population. Symptoms and diverticula in MFS are not correlated with any genetic variant.

Keywords: Marfan's syndrome; diverticula; abdominal symptoms; ultrasound

1. Introduction

Marfan's syndrome (MFS) is an autosomal dominant hereditary disorder, characterized by an altered production of connective tissue—particularly fibrillin—a major constituent of elastic tissue. Its estimated frequency in the general population is approximately 2–3/10,000 [1].

Patients with MFS may display a wide range of clinical manifestations involving different organs and tissues. For example, major clinical manifestations occur in the cardiovascular (e.g., mitral valve prolapse and aortic root aneurysm and dissection), musculoskeletal, and ocular (e.g., ectopia lentis and myopia) systems. Skin, pulmonary, and neurological systems can also be affected. The phenotype is highly variable, even within affected families.

Marfan syndrome is the result of a mutation in FBN1, the gene encoding the fibrillin-1 protein. Fibrillin-1 belongs to the family of fibrillins, a group of large extracellular proteins that form the core

of microfibrils. The latter are ubiquitously distributed, regulate the bioavailability of transforming growth factor beta (TGFB) and bone morphogenic proteins, and provide a scaffold for elastogenesis in the majority of elastic tissues [2,3].

The ubiquitous distribution of the fibrillin-1 protein explains why MFS displays multisystemic involvement. However, it is generally thought that gastrointestinal (GI) tract complications are not commonly associated with MFS. A review of the literature shows instances of diaphragmatic hernia, inguinal hernia, and abdominal wall defects due to the diastasis recti and laparocele of the abdominal rectus muscles.

Alteration in FBN1 might also cause GI manifestations. In fact, the disrupted connective tissue could potentially result in a structural defect in the GI system and an increased prevalence of diverticulosis in both the colon and the small bowel. Diverticula formation, in turn, might then represent a risk factor for bacterial contamination of the small bowel, intussusceptions, and volvulus [4]. In addition, the abnormalities of the connective tissue could also explain some non-specific GI symptoms in MFS.

It is worth noting that, at present, the GI manifestations of MFS are poorly documented. Thus far, in the literature, there are only case series reporting the association between MFS, abdominal pain and altered evacuation, and, in particular, cases of severe acute diverticulitis or complications of diverticulosis, for which MFS is historically considered a risk factor [4–12]. Inayet et al. [13] and Nee et al. [14] recently evaluated the prevalence of functional GI diseases and pelvic floor symptoms in a cohort of MFS patients compared to patients with Ehlers–Danlos syndrome, a group of inherited heterogenous multisystem disorders often in differential diagnosis with MFS (as far as skin hyperextensibility, atrophic scarring, joint hypermobility, and generalized tissue fragility are concerned). Furthermore, a recent work [15] described the case of a 68-year-old man with MFS and a history of diffuse diverticulosis of the small bowel leading to a perforated distal jejunum.

To date, the prevalence of GI symptoms in MFS, particularly those evocative of diverticular disease, is unknown. Considering that MFS represents a risk factor for diverticulosis even at a young age, and that patients with diverticula are at higher risk of perforation during colonoscopy [16], it would be interesting to develop a noninvasive diagnostic method to better estimate the actual prevalence of morphologic changes in the bowel and the association with GI symptoms in MFS. This would allow optimizing the diagnostic work-up and follow-up on these patients.

Intestinal ultrasound (IUS) has been proposed in recent years as a non-invasive method for the diagnosis of many GI diseases. Of interest, some international guidelines recommend IUS as the first diagnostic technique in patients with suspected acute diverticulitis [17,18]. Moreover, IUS is able to diagnose left-sided colonic diverticulosis in 85% of patients [19] by detecting diverticula as protrusions of the intestinal wall containing fecaliths, combined with an increased thickness of the walls of the sigmoid and left colon, generally due to hypertrophy of muscularis propria [20].

Thus far, the data regarding the prevalence of GI symptoms and the morphological features of the colon, including the presence of diverticula of the sigmoid colon in MFS, are scant, as well as their potential correlation with the genetic variability of this syndrome.

In this prospective case–control study, we assessed: (1) The prevalence of lower GI symptoms; (2) the presence of sonographic changes of the bowel wall—including diverticula—in a series of consecutive patients with MFS, compared to a sex- and age-matched sample of the general population; (3) possible correlations between the clinical and ultrasound features of the sigmoid wall and the genotype of MFS.

2. Experimental Section

2.1. Study Design

From November 2016 to March 2017, we conducted a prospective case–control observational study on a series of consecutive patients with MFS regularly attending the Marfan Clinic at Luigi Sacco

Hospital, Milan, which currently includes approximately 350 patients. All patients met the revised Ghent criteria of 2010 for a clinical diagnosis of MFS [21].

The clinical characteristics and ultrasonographic features of the bowels of these patients, including the prevalence of colonic diverticulosis, were compared to those of non-selected subjects matched by sex and age, recruited among students, technicians, nurses, and physicians attending our department and the biochemical laboratory of our hospital. The sonographic features of the sigmoid wall were also compared to those of asymptomatic subjects, identified from the same non-selected population matched for age (±5 years) and gender. The two control groups were conceived to address the following aims: (1) The assessment of prevalence of lower GI symptom had as controls a random population (which may have abdominal symptoms likely due to IBS, diverticular disease and other causes); (2) the assessment of sonographic changes of the bowel wall (diverticula and thickening of the muscularis propria of the sigmoid colon) had as control group asymptomatic subjects (likely healthy and without intestinal diseases).

The study protocol was approved by the local ethics committee (p.n. 19-ST-042) and all patients gave their written informed consent to participate in the study.

2.2. Patients

Patients with the following criteria were considered eligible: MFS diagnosed using the Ghent criteria [21]; good clinical conditions and autonomous walking ability (therefore able to perform ambulatory visits); aged between 7 and 70 years; able to provide informed consent or parents' consent in the case of a minor patient. Patients with major GI surgery were excluded.

2.3. Clinical Evaluation

Patients and controls were administered a questionnaire (Supplementary Materials Appendix 1) to investigate previous or current lower GI symptoms and/or diagnoses. The questionnaire included the collection of biographical (i.e., sex age), biometrics (i.e., body mass index (BMI)), and clinical data. The latter specifically assessed: (1) Previous (last year) or current GI symptoms lasting more than one week, including abdominal pain, bloating or abdominal distension, or changes in bowel habit (i.e., diarrhea and/or constipation), severe enough to prompt diagnostic evaluation or treatment; (2) previous colonoscopy, barium enema, or virtual colonoscopy; (3) previous fecal occult blood test for the screening of colorectal cancer; (4) minor GI surgery (e.g., appendectomy, cholecystectomy, and abdominal laparoplasty).

2.4. Ultrasound Evaluation

In all recruited subjects, IUS was carried out to detect the morphological characteristics of the small bowel and the colon. All procedures were done during fasting by a sonographer with particular expertise in the bowel (>10,000 investigations) using an ultrasound machine (Hitachi Logos HiVision C, Steinhausen, CH) with a low-frequency convex ultrasound probe (3.5–5 MHz) and a microconvex high-frequency probe (4–8 MHz), as per standard protocol.

The following parameters were retrieved: Dilatation of the small bowel >3 cm; thickening of any intestinal wall segment >3 mm; enlarged lymph nodes (shorter in diameter than >7 mm), mesenteric hypertrophy, or peri-intestinal effusion; sigmoid wall thickness; thickness of the muscularis propria of the sigmoid colon; the presence of diverticula and their complications, if any.

2.5. Genetic Characteristics

The following genetic variables, assessed in the population at diagnosis and therefore prior to the beginning of the study, were taken into consideration: (1) Positivity of the genetic test; (2) gene mutations; (3) number of mutations; (4) exons; (5) haploinsufficiency/dominant negative mutation. The genomic DNA of each patient was extracted from peripheral blood lymphocytes using a Gene Catcher gDNA 96 × 10 mL Automated Blood Kit (Invitrogen, Life Technologies™, Carlsbad, CA, USA). The genetic

test was performed using the next-generation sequencing (NGS) technique. A panel composed of 11 genes (i.e., FBN1, TGFBR1, TGFBR2, COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, MYH11, NOTCH1, and ACTA2) known to be associated with MFS and Marfan-like phenotypes (such as Ehlers–Danlos syndromes (EDS), Loeys–Doetz syndrome (LDS), and thoracic aortic aneurysm (TAAD)) were analyzed. An Illumina TruSeq Custom Amplicon Kit (Illumina, Inc. San Diego, CA, USA) was used to capture all exons, intron–exon boundaries, and at least 50 bp flanking sequences of target genes (RefSeq database, hg19 assembly). The data collected from the NGS experiments were analyzed in order to identify single-nucleotide variants and small insertions/deletions [22].

2.6. Statistical Analysis

Data are presented as means ± standard deviation (SD). The clinical and sonographic characteristics of patients and controls were compared with parametric tests (i.e., the *t*-test and the chi-square test). The association between the clinical and sonographic parameters and the genetic variables of patients was assessed by analysis of variance. A value of $p < 0.05$ was considered significant.

3. Results

In the study period, 90 patients with MFS (48 men; aged 36.4 ± 16.4 years; BMI 22.2 ± 4.4) and 180 control subjects, including 90 subjects from a random sample of the general population (48 men; aged 36.55 ± 16.7 years; BMI 22.9 ± 3.4) and 90 asymptomatic healthy subjects (48 men; aged 37.6 ± 16.8 years; BMI 22.6 ± 3.1), were recruited.

3.1. Clinical Features

Overall, past or current complaints of abdominal symptoms were made by 23 (25.6%) patients with MFS and 48 (53%) control subjects ($p < 0.01$). In particular, constipation and bloating/abdominal distension were reported less frequently by patients with MFS compared to the controls (constipation: 13.3% vs. 26.6%, $p = 0.039$; bloating/abdominal distension: 3.3% vs. 41.1%, $p < 0.0001$). Meanwhile, diarrhea was reported by 4.4% of patients with MFS and 8.8% of the controls ($p = 0.371$), and abdominal pain by 18.8% of patients with MFS and 21.1% of the controls ($p = 0.852$) (Table 1). Nine patients with MFS and 20 controls had previous endoscopic or radiologic investigations of the colon, mainly for prevention of colorectal cancer (15), rectal bleeding (6), or abdominal complaints (abdominal pain and/or diarrhea) (8). No complications were reported during endoscopic procedures in MFS patients.

Table 1. Abdominal symptoms in patients with Marfan's syndrome (MFS) and control subjects.

	MFS *n* (%)	Random Controls *n* (%)	*p*-Value
Diarrhea	4 (4.4%)	8 (8.8%)	0.371
Constipation	12 (13.3%)	24 (26.6%)	0.039
Abdominal pain	17 (18.8%)	19 (21.1%)	0.852
Bloating	3 (3.3%)	37 (41.1%)	<0.0001

Asymptomatic subjects did not have at present any symptom and did not complain symptoms in the last year.

3.2. Sonographic Features

Overall, sigmoid diverticulosis was detected in 12 (12.3%) patients with MFS, 3 (3.3%) asymptomatic healthy subjects, and four random controls ($p = 0.0310$). The rate of diverticulosis increased with age, starting from 30 years old with greater prevalence over 50 years (Table 2).

Table 2. Sonographic prevalence of diverticula in patients with MFS and the controls.

Age Class (Number of Patients)	MFS Patients *n* (%)	Asymptomatic Controls *n* (%)	Random Controls *n* (%)
<15 years (*n* = 7)	0 (0)	0 (0)	0 (0)
15–29 years (*n* = 25)	0 (0)	0 (0)	0 (0)
30–44 years (*n* = 21)	2 (9.5)	0 (0)	0 (0)
45–59 years (*n* = 31)	7 (25.9) [§]	1 (3.2)	2 (7.1)
≥60 years (*n* = 6)	3 (50.0)	2 (33.3)	2 (40.0)
Total (*n* = 90)	12 (13.3) [#]	3 (3.3)	4 (4.4)

[§] $p = 0.0310$ for asymptomatic controls and random controls; [#] $p = 0.0160$ for asymptomatic controls and random controls.

Thickening of the muscularis propria of the sigmoid colon increased progressively with age, but it was not statistically different between patients with MFS and healthy subjects (1.38 ± 0.55 mm vs. 1.42 ± 0.4 mm; $p = 0.723$) or in any age group (Table 3). No other bowel abnormalities were assessed by IUS in the study population, apart from the presence of enlarged mesenteric lymph nodes found in two patients with MFS and four in control subjects. In particular, no significant abnormalities (including bowel wall thickening, abnormal dilatation, or diverticula) were detected in the small bowel.

Table 3. Sonographic thickening of the muscularis propria in patients with MFS and the asymptomatic controls.

Age Class (Number of Patients)	MFS Patients *n* (%)	Asymptomatic Controls *n* (%)
<15 years (*n* = 7)	0.86 ± 0.33	1.21 ± 0.23
15–29 years (*n* = 25)	1.10 ± 0.40	1.20 ± 0.28
30–44 years (*n* = 21)	1.41 ± 0.47	1.25 ± 0.29
45–59 years (*n* = 31)	1.69 ± 0.54	1.57 ± 0.36
≥60 years (*n* = 6)	1.61 ± 0.44	1.38 ± 0.62
Total (*n* = 90)	1.39 ± 0.55	1.38 ± 0.40

The *p*-value was not significant for any comparison.

3.3. Genetic Features and Sonographic Findings

Of the subjects, 97.1% were found to harbor a pathogenetic variant in the FBN1 gene. This is in line with the data from the literature, according to which states that not all subjects with a clinical diagnosis of MFS receive a positive genetic FBN1 test.

In one subject without mutation of the FBN1 gene, a pathogenetic variant of the TGFBR2 gene (a gene involved in transforming the growth factor β (TGF-β) signaling pathway) was present; this led to a diagnosis of LDS, a genetic condition overlapping MSF in an aortic root aneurysm and risk of dissection, in skeletal features and habitus [23]. The suggested follow-up in LDS syndrome is the same as for MFS. In eight patients (8.9%), two genetic variants were identified: A pathogenetic variant in FBN1 and another variant of uncertain significance in the COL1A2, COL5A1, or COL5A2 genes. These genes, when functionally altered, cause Ehlers–Danlos syndrome—the classic type and, on the basis of a recent study [23], could act as modifiers of phenotypes. The presence of diverticula was found in eight out of the 62 (12.9%) patients with a single mutation and in one out of eight (12.5%) patients with a double mutation ($p > 0.99$).

The thickness of the muscularis propria in patients with a single or double mutation was similar (1.39 ± 0.57 mm vs. 1.38 ± 0.53 mm; $p = 0.951$), and the association between the number of mutations and the thickness of the muscularis propria was not statistically significant ($p = 0.92$). Investigation of haploinsufficiency was available for 65 patients with a mutated FBN1 gene: 20 (33.3%) had a mutually

unstable mutation and 45 (66.6%) had a dominant negative mutation. The presence of diverticula was found in two patients (10%) with a mutually tolerant mutation and in seven patients (15.5%) with a dominant negative mutation ($p = 0.709$). The average thickness of the muscularis propria of the sigmoid colon in patients with a mutually tolerant mutation (1.21 ± 0.52 mm) and those with a dominant negative mutation (1.46 ± 0.58 mm) was not statistically different ($p = 0.091$). The type of mutation in the FBN1 gene or the number of genes involved were not correlated with any specific GI symptom.

4. Discussion

MFS, together with EDS and LDS, are multisystemic genetic disorders that affect the soft connective tissue, and potentially even the GI tract. However, whereas joint hypermobility syndrome and EDS display a high prevalence of functional GI disorders, in MFS, the prevalence of GI disorders is still disputed [13,14,24,25]. To date, GI disease associated with MFS has been reported only in case series, highlighting, in particular, its association with complications of intestinal diverticula, a condition for which MFS is historically considered a genetic risk factor [4–12]. Furthermore, the level of evidence supporting the relationship between MFS and diverticulosis, and its potentially related symptoms, is still unsatisfactory.

Our study, carried out in a consecutive and unselected series of patients with MFS, showed that despite a higher prevalence of colonic diverticulosis, patients with MFS have a low prevalence of GI symptoms, inferior to that of the general population comparable by gender and age, particularly regarding constipation and bloating. In this regard, we assumed that subjects attending and working at our hospital department were representative of the general population. Despite the fact that this could be questionable, it has to be acknowledged that subjects of this group were recruited merely by opportunity and based on prompt availability criteria and selected only according to their sex and age so as to be comparable to the MFS patients. In particular, none of them were selected according to any symptoms, visits, or examinations previously performed, and all invited subjects agreed to take part in the study. The protocol did not foresee any additional investigation besides ultrasound, and given the features of the symptoms, none of them were submitted to further diagnostic examinations. Therefore, it was not possible to verify whether symptoms were due to diverticulosis.

The prevalence of GI symptoms in cases of MFS has been recently investigated using an electronically mailed questionnaire sent to members of the local and national MFS and EDS societies in the USA. Despite the lack of a clear comparison with a matched control population, the study reported that functional GI and pelvic floor symptoms were significantly higher in Ehlers Danlos Syndrome (EDS) patients than in MFS patients [14]. However, the prevalence of some functional GI disorders complained of by MFS patients, such as constipation (5.3%), diarrhea (1.5%), abdominal pain and overall IBS symptoms (27%), and bloating (16%), were less than half of those of HDS patients, but similar, if not lower, than those usually found in patients referred to a luminal gastroenterology clinic or encountered in the general population [26].

At present, the reasons for the discrepancy between the prevalence of symptoms and functional GI disorders in HDS and MFS patients remains unknown. In addition, the discrepancies in the prevalence of some symptoms (constipation and bloating) found in our study between MFS patients and the controls are difficult to explain. The assessment of symptoms in a series of consecutive patients (and not selected from among those who responded to a survey) and the selection of a control group among personnel attending hospital and their relatives might have influenced the low and slightly high rate of symptoms in the MFS patients and the controls, respectively.

Interestingly, on account of the low prevalence of GI symptoms, it is unlikely that stress and anxiety, frequently encountered in patients with MFS due to the severe cardiovascular complications of this syndrome [27,28], are predisposing factors of GI functional syndromes such as irritable bowel syndrome in MFS.

We also investigated the relationship between MFS and diverticulosis, and, in particular, assessed the hypothesis of the syndrome as a genetic predisposition for this condition (verifying the results against asymptomatic subjects) by using IUS. Ultrasound of the GI tract is a well-recognized tool to investigate chronic inflammatory conditions of the gut, including acute diverticulitis, and it is also able to define the anatomical features of the bowel, particularly the thickening of its layers. Given the association between colon diverticulosis and the thickness of the muscularis propria, we assessed the sonographic features of these conditions in a non-selected population of patients with MFS, compared with those of a population of healthy asymptomatic controls. We found that patients with MFS have a high prevalence of colonic diverticulosis, also at younger age, although the associated thickening of the muscularis propria is comparable to that of the general population.

According to the literature, a fibrillin disorder, which is the cause of MFS, should cause higher levels of TGF-ß. This extracellular mediator interferes with the production of collagen, particularly type I and type III, and this should theoretically be translated into an overall thinning of the organ, particularly the muscularis propria. Our study showed that the thickness of the sigmoid wall of MFS patients is quite similar to that of healthy asymptomatic subjects. The evaluation of any correlation between the clinical and ultrasound characteristics of the sigmoid colon and the patient's genotype (number of mutations, the lack of a genotype, or a negligent dominance) did not show any statistically significant results, in contrast to those characteristics that most often correlate with a severe clinical (cardiovascular and musculoskeletal) picture.

However, it should be noted that ultrasound, although considered to be a very good diagnostic test, comparable to computed tomography (CT) for acute diverticulitis, is not the same for non-complicated diverticulosis (sensitivity and specificity approximately 85%) [19].

Of course, this study has limitations. First, ultrasound is not the gold standard for diagnosing diverticulosis, and detection can be hampered by gas or patient habitus, as well as by the site and size of diverticula. However, it is the preferred method for the non-invasive investigation of a consecutive and unselected series of patients, including asymptomatic and young subjects. Other techniques such as CT colonography would be more appropriate and accurate, but, given their invasiveness and radiation exposure, their use as screening methods should be justified by clinical conditions. The number of patients we investigated was not large enough for an epidemiological study, but it represents the largest series of patients investigated so far, and we feel that this cohort was sufficient to suggest that other diagnostic investigation to assess the presence of diverticulosis and its complications was not clinically justified, due to the paucity of symptoms in the population and the absence of sonographic signs suggestive of complications of diverticulosis. Third, the IUS assessment of diverticula and intestinal features was not performed blindly with respect to the clinical condition; this was an ineludible condition. On the other hand, a control group was necessary to assess the validity of our findings in the study population. Last, but not least, the assessment of symptoms has been done by using a very simple and not validated questionnaire, not specifically designed to assess diverticular disease, which investigated symptoms (only presence/absence) occurred for more than 1 week in the last year, without considering their duration and severity.

5. Conclusions

In conclusion, the results of this study indicate that MFS is associated with an increased prevalence of colonic diverticulosis, without sonographic signs of diverticulitis or symptoms suggestive of diverticular disease. Of interest, although the population studied is rather young, and therefore with lower risk of symptoms and complications, the patients seem to have less abdominal symptoms and, despite the presence—and likely underestimated prevalence—of diverticulosis, a thickening of the muscularis propria comparable to that of healthy subjects.

Supplementary Materials: The following are available online at http://www.mdpi.com/2077-0383/9/10/3141/s1, Appendix 1: Questionnaire.

Author Contributions: Conceptualization and methodology, G.M. and G.B.; software, G.M.; formal analysis, G.M.; investigation, G.M., E.P., and A.P.; data curation, G.M. and E.P.; writing—original draft preparation, G.M.; writing—review and editing, S.A. and G.B.; supervision, S.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Groth, K.A.; Hove, H.; Kyhl, K.; Folkestad, L.; Gaustadnes, M.; Vejlstrup, N.; Stochholm, K.; Østergaard, J.R.; Andersen, N.H.; Gravholt, C.H. Prevalence, incidence, and age at diagnosis in Marfan Syndrome. *Orphanet J. Rare Dis.* **2015**, *10*, 153. [CrossRef] [PubMed]
2. Piha-Gossack, A.; Sossin, W.; Reinhardt, D.P. The evolution of extracellular fibrillins and their functional domains. *PLoS ONE* **2012**, *7*, e33560. [CrossRef] [PubMed]
3. Thomson, J.; Singh, M.; Eckersley, A.; Cain, S.A.; Sherratt, M.J.; Baldock, C. Fibrillin microfibrils and elastic fibre proteins: Functional interactions and extracellular regulation of growth factors. *Semin. Cell Dev. Biol.* **2019**, *89*, 109–117. [CrossRef] [PubMed]
4. McLean, A.M.; Paul, R.E.; Kritzman, J.; Farthing, M.J. Malabsorption in Marfan (Ehlers-Danlos) Syndrome. *J. Clin. Gastroenterol.* **1985**, *7*, 304–308. [CrossRef] [PubMed]
5. Suster, S.M.; Ronnen, M.; Bubis, J.J. Diverticulosis coli in association with Marfan's Syndrome. *Arch. Intern. Med.* **1984**, *144*, 203. [CrossRef] [PubMed]
6. Clunie, G.J.; Mason, J.M. Visceral diverticula and the Marfan Syndrome. *Br. J. Surg.* **1962**, *50*, 51–52. [CrossRef]
7. Samet, J.D.; Johnson, P.T.; Horton, K.M.; Fishman, E.K. Diverticulitis complicated by fistulous communication of sigmoid colon with anterior sacral meningocele in a patient with Marfan Syndrome. *Radiol. Case Rep.* **2015**, *7*, 442. [CrossRef]
8. Slesser, A.A.P.; Patel, P.H.; Das, S.C.; Leahy, A.; Livingstone, J.; Riaz, A.A. A Rare case of segmental small bowel pneumatosis intestinalis: A case report. *Int. J. Surg. Case Rep.* **2011**, *2*, 185–187. [CrossRef]
9. Thomas, G.P.; Purkayastha, S.; Athanasiou, T.; Darzi, A. General surgical manifestations of Marfan's Syndrome. *Br. J. Hosp. Med. (Lond.)* **2008**, *69*, 270–274. [CrossRef]
10. Santin, B.J.; Prasad, V.; Caniano, D.A. Colonic diverticulitis in adolescents: An index case and associated syndromes. *Pediatr. Surg. Int.* **2009**, *25*, 901–905. [CrossRef]
11. Eliashar, R.; Sichel, J.Y.; Biron, A.; Dano, I. multiple gastrointestinal complications in Marfan Syndrome. *Postgrad. Med. J.* **1998**, *74*, 495–497. [CrossRef] [PubMed]
12. Junpaparp, P.; Chayanupatkul, M.; Buppajarntham, S.; Phowthongkum, P. Sigmoid volvulus: Is it related to Marfan Syndrome? *Int. J. Colorectal Dis.* **2014**, *29*, 771–772. [CrossRef] [PubMed]
13. Inayet, N.; Hayat, J.O.; Kaul, A.; Tome, M.; Child, A.; Poullis, A. Gastrointestinal symptoms in Marfan Syndrome and hypermobile Ehlers-Danlos Syndrome. *Gastroenterol. Res. Pract.* **2018**, *2018*, 485470. [CrossRef]
14. Nee, J.; Kilaru, S.; Kelley, J.; Oza, S.S.; Hirsch, W.; Ballou, S.; Lembo, A.; Wolf, J. Prevalence of functional GI diseases and pelvic floor symptoms in Marfan Syndrome and Ehlers-Danlos Syndrome: A national cohort study. *J. Clin. Gastroenterol.* **2019**, *53*, 653–659. [CrossRef]
15. Robey, B.S.; Peery, A.F.; Dellon, E.S. Small bowel diverticulosis and jejunal perforation in Marfan Syndrome. *ACG Case Rep. J.* **2018**, *5*, e5. [CrossRef] [PubMed]
16. Loffeld, R.J.L.F.; Engel, A.; Dekkers, P.E.P. Incidence and causes of colonoscopic perforations: A single-center case series. *Endoscopy* **2011**, *43*, 240–242. [CrossRef] [PubMed]
17. Cuomo, R.; Barbara, G.; Pace, F.; Annese, V.; Bassotti, G.; Binda, G.A.; Casetti, T.; Colecchia, A.; Festi, D.; Fiocca, R.; et al. Italian consensus conference for colonic diverticulosis and diverticular disease. *United Eur. Gastroenterol. J.* **2014**, *2*, 413–442. [CrossRef]
18. Galetin, T.; Galetin, A.; Vestweber, K.-H.; Rink, A.D. Systematic review and comparison of national and international guidelines on diverticular disease. *Int. J. Colorectal. Dis.* **2018**, *33*, 261–272. [CrossRef]
19. Hollerweger, A.; Macheiner, P.; Hübner, E.; Brunner, W.; Gritzmann, N. Colonic diverticulosis: A comparison between sonography and endoscopy. *Ultraschall Med.* **2002**, *23*, 41–46. [CrossRef]
20. Maconi, G.; Carmagnola, S.; Guzowski, T. Intestinal ultrasonography in the diagnosis and management of colonic diverticular disease. *J. Clin. Gastroenterol.* **2016**, *50*, S20–S22. [CrossRef]

21. Loeys, B.L.; Dietz, H.C.; Braverman, A.C.; Callewaert, B.L.; De Backer, J.; Devereux, R.B.; Hilhorst-Hofstee, Y.; Jondeau, G.; Faivre, L.; Milewicz, D.M.; et al. The revised ghent nosology for the Marfan Syndrome. *J. Med. Genet.* **2010**, *47*, 476–485. [CrossRef] [PubMed]
22. Gentilini, D.; Oliveri, A.; Fazia, T.; Pini, A.; Marelli, S.; Bernardinelli, L.; Di Blasio, A.M. NGS analysis in Marfan Syndrome spectrum: Combination of rare and common genetic variants to improve genotype-phenotype correlation analysis. *PLoS ONE* **2019**, *14*, e0222506. [CrossRef]
23. Camerota, L.; Ritelli, M.; Wischmeijer, A.; Majore, S.; Cinquina, V.; Fortugno, P.; Monetta, R.; Gigante, L.; Sangiuolo, F.C.; Novelli, G.; et al. Genotypic categorization of Loeys-Dietz Syndrome based on 24 novel families and literature data. *Genes* **2019**, *10*, 764. [CrossRef] [PubMed]
24. Beckers, A.B.; Keszthelyi, D.; Fikree, A.; Vork, L.; Masclee, A.; Farmer, A.D.; Aziz, Q. Gastrointestinal disorders in joint hypermobility syndrome/Ehlers-Danlos Syndrome hypermobility type: A review for the gastroenterologist. *Neurogastroenterol. Motil.* **2017**, *29*. [CrossRef] [PubMed]
25. Castori, M.; Morlino, S.; Pascolini, G.; Blundo, C.; Grammatico, P. Gastrointestinal and nutritional issues in joint hypermobility syndrome/Ehlers-Danlos Syndrome, hypermobility type. *Am. J. Med. Genet. C Semin. Med. Genet.* **2015**, *169*, 54–75. [CrossRef]
26. Shivaji, U.N.; Ford, A.C. Prevalence of functional gastrointestinal disorders among consecutive new patient referrals to a gastroenterology clinic. *Frontline Gastroenterol.* **2014**, *5*, 266–271. [CrossRef]
27. Benke, K.; Ágg, B.; Pólos, M.; Sayour, A.A.; Radovits, T.; Bartha, E.; Nagy, P.; Rákóczi, B.; Koller, Á.; Szokolai, V.; et al. The effects of acute and elective cardiac surgery on the anxiety traits of patients with Marfan Syndrome. *BMC Psychiatry* **2017**, *17*, 253. [CrossRef]
28. Schneider, M.B.; Davis, J.G.; Boxer, R.A.; Fisher, M.; Friedman, S.B. Marfan Syndrome in adolescents and young adults: Psychosocial functioning and knowledge. *J. Dev. Behav. Pediatr.* **1990**, *11*, 122–127. [CrossRef]

Journal of
Clinical Medicine

MDPI

Article

Eosinophil-Derived Neurotoxin, Tumor Necrosis Factor Alpha, and Calprotectin as Non-Invasive Biomarkers of Food Protein-Induced Allergic Proctocolitis in Infants

Artur Rycyk *, Beata Cudowska and Dariusz M. Lebensztejn

Department of Pediatrics, Gastroenterology, Hepatology, Nutrition and Allergology, Medical University of Bialystok, 15-089 Bialystok, Poland; beata.cudowska@umb.edu.pl (B.C.); dariusz.lebensztejn@umb.edu.pl (D.M.L.)

* Correspondence: artur.rycyk@umb.edu.pl; Tel.: +48-85-7450-779

Received: 22 August 2020; Accepted: 25 September 2020; Published: 29 September 2020

Abstract: Diagnosis of non-IgE mediated food allergy presents a special challenge due to lack of a single, non-invasive diagnostic method. We selected three fecal biomarkers of allergic inflammation of gastrointestinal origin in order to improve the diagnostic process. Twenty-seven infants with symptoms of hematochezia were prospectively enrolled into this study. All patients underwent a complete differential diagnosis of rectal bleeding. Non-IgE mediated food allergy was confirmed by an open, oral food challenge. The control group included twenty-five infants with functional gastrointestinal disorders. Eosinophil-derived neurotoxin (EDN), tumor necrosis factor alpha (TNFα), and calprotectin concentration were measured in stools of all children by enzyme-linked immunosorbent assays (ELISA) using commercial kits. Median eosinophil-derived neurotoxin and calprotectin fecal levels were significantly higher in the study group than in the control group ($p < 0.05$). The difference of fecal tumor necrosis factor alpha concentration between both groups was not statistically significant ($p > 0.05$). The best diagnostic performance was reached in a combination of fecal calprotectin (fCal) and EDN i.e., 88.9% and 84%, respectively. Fecal EDN and fCAl are reliable tools in differentiating between food protein-induced allergic proctocolitis and gastrointestinal functional disorders in infants.

Keywords: infant; food allergy; fecal biomarkers; tumor necrosis factor α; eosinophil derived neurotoxin; calprotectin

1. Introduction

Food allergy (FA) is defined as an occurrence of reproducible, clinical symptoms caused by an abnormal, immune response to food components [1]. Based on the pathophysiologic background, immune hypersensitivity reactions to food are categorized into two main groups: IgE-mediated and non-IgE-mediated, with both requiring a different diagnostic approach.

The diagnosis of an IgE-mediated FA begins with a history of clinical symptoms, based on which skin pricks tests (SPT) and serum specific-IgE (sIgE) with potential allergens are carried out. Both methods present good sensitivity, but poor specificity [2]. Similarly, the application of a highly sensitive component-resolved diagnosis (CRD) is limited to immediate reactions exclusively [2]. What is more, the panel of sIgE presents an additional value in predicting polysymptomatic allergy development [3].

Non-IgE-mediated allergic reactions account for approximately 40% of cow's milk protein (CMP) allergy in infants and young children. Broad clinical manifestations include food protein-induced enterocolitis syndrome (FPIES), food protein-induced allergic proctocolitis (FPIAP),

food protein-induced enteropathy (FPIE), Heiner's syndrome (pulmonary hemosiderosis) and CMP-induced iron deficiency anemia [4]. FPIAP is the most common entity, with a relatively prominent symptomatology of intermittent blood-streaked normal to moderately loose stools, which accounts for 18% to 64% of the infants with rectal bleeding [5,6].

Diagnosis of non-IgE-mediated FA is more challenging, because of the delayed onset of symptoms and blurred symptomatology. Since available data from the application of atopy patch tests (APT) are limited and indicate their poor sensitivity, they are not being recommended in the routine management [2]. Due to the lack of a single, reliable, diagnostic method, oral food challenge (OFC) remains the gold standard in FA [7].

Although most reliable, OFC has considerable limitations: time and resource consumption, potential risk of anaphylaxis, involvement of qualified medical practitioners, and stress for the patient [8].

In order to improve the management of FA, non-IgE in particular, alternative diagnostic methods have been studied: affinity of IgE binding, cytokines profile, T-cell number and function, B-cell activity, DNA methylation signatures, and so called "omics" which have already been shown to be useful in other gastrointestinal inflammatory diseases [9,10]. In comparison, fecal biomarkers are especially promising due to ease of collection, good correlation with the severity of inflammation in the intestinal wall (their place of origin), and specificity for intestine inflammation. Fecal calprotectin (fCal) has already found a reliable place in gastroenterology to diagnose and monitor inflammatory diseases [11]. Studies on pathophysiology of an allergic reaction have indicated the potential role of eosinophil-derived neurotoxin (EDN), representing the activation and degranulation of eosinophils and tumor necrosis factor α (TNFα) involved in the process of intestinal epithelial cell damage, increased intestinal permeability, and mucosal infiltration by leukocytes [12–15]. The combination of all three parameters might be a promising method in FA diagnostics. To the authors' best knowledge, there has only been one study which targeted a similar biomarker profile, by Kalach et al. [16].

The aim of this study was to assess the usefulness of the simultaneous measurement of three non-invasive fecal biomarkers: EDN, fCal, TNFα in the diagnosis of non-IgE-mediated food allergy in children.

2. Materials and Methods

2.1. Patients and Study Design

Thirty-one children aged between 1 and 12 months, admitted to the hospital with symptoms of hematochezia, were prospectively enrolled into the study. The presence of blood in the stool was verified by macroscopic evaluation or a positive occult blood test. All patients underwent the standard protocol for differential diagnosis of infancy rectal bleeding. Schematic diagnostic procedures are shown in Figure 1. Children were excluded if they were diagnosed with: perianal excoriations or anal fissures (physical examination), anatomical abnormalities of the gastrointestinal tract (abdominal sonography and/or radiography), history of abdominal surgery, coagulation disorders (prothrombin time [PT] ≥ 17 s, international normalized ratio [INR] ≥ 1.5, activated partial thromboplastin time [APTT] ≥ 60 s, platelet count < 100,000 × 10^9/L), vitamin K deficiency (INR ≥ 1.4), parasitic or infectious gastroenteritis (positive stool analysis or culture), necrotizing enterocolitis (NEC) (combination of physical examination, laboratory tests, and imaging characteristics with Bell staging), or sepsis (procalcitonin [PCT] ≥ 2.0 ng/mL). None of the selected patients required endoscopy in order to exclude inflammatory bowel disease.

Further steps the in allergic work-up followed the current Guideline of The European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) [7]. In order to exclude IgE-mediated mechanisms, the serum-specific IgE panel with food allergens was carried out, followed by fecal marker measurement i.e., calprotectin, EDN and TNFα. Afterwards, all children in the study group were introduced to a diagnostic elimination diet. Depending on the clinical manifestation,

patients were given an elimination diet i.e., either extensively hydrolyzed milk formula (eHF), amino acid-based formula (AAF), or egg-free/cow's milk protein free dietary restrictions for children and their breastfeeding mothers. After a hematochezia resolution, followed by an elimination period of 2–4 weeks, all patients were subjected to an open, OFC according to ESPGHAN protocol.

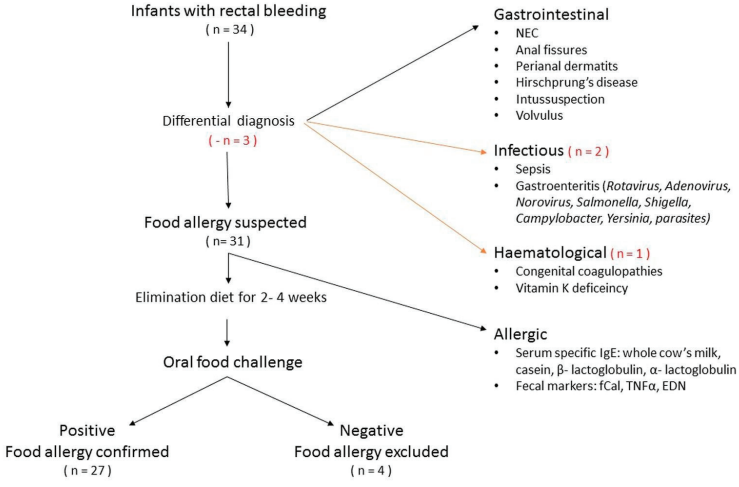


Figure 1. Study design. EDN, eosinophil derived neurotoxin; fCal, fecal calprotectin; NEC, necrotizing enterocolitis; TNFα, tumor necrosis factor α.

Simultaneously with the study cohort, twenty-five children matched by gender and age, diagnosed at the same hospital with functional gastrointestinal disorders (according to revised IV Rome criteria) were selected as the control group. Patients with functional gastrointestinal disorders were included, due to the fact that, such diagnosis requires exclusion of an organic background.

A written informed consent was obtained from the parents of all patients. The study protocol was approved by the Local Ethics Committee under the registration number R-I-002/106/2017.

2.2. Serum-Specific IgE Measurement

Antigen-specific IgE were measured using PolyCheck® RAST tests (Biocheck GmbH, Münster, Germany) calibrated according to the manufacturer's titers. The IgE cut-off value was accessed upon 0.15 kU/L. The main food allergens were tested: cow's milk protein (f02), egg white and egg yolk (f01, f35), soybean (f14), rice (f09), peanut (f13), flour mix (fx10), hazelnut (f17), and codfish (f03).

2.3. Open Oral Food Challenge

The open milk OFC was conducted according to the ESPGHAN guidelines during a one-day hospital stay [7]. The challenge used a standard cow's milk based infant formula. Stepwise increasing doses (1, 3, 10, 30, 100 mL) were given in 30-min intervals and the tolerance was monitored by parental record of the child's symptoms. The challenge was discontinued when adverse reactions were noted. Infants without symptoms continued to receive the formula at home with doses appropriate for age. To recognize the delayed onset of symptoms, parents were contacted on day 5, or earlier if requested. The test was considered positive and the challenge discontinued if any of the initial symptoms recurred (i.e., bloody or/and mucousy stools). Infants with clinical manifestation continued on a CMP-free diet. Finally, CMP allergy was diagnosed as the resolution of symptoms on the elimination diet and their relapse during the challenge. In all cases, in which the challenge proved positive and mothers wished to continue breastfeeding, calcium supplements were prescribed (i.e., 1000 mg/day) with subsequent dietetic counseling.

The egg OFC was introduced to the patients with positive clinical history and no improvement after initial cow's milk protein elimination diet. The egg OFC was conducted according to a three-level stepwise manner [17]. In STEP 0, patients were given approximately one quarter of a boiled egg yolk (i.e., 3.5 g), which contains about 1.8 mg of egg protein. The tolerance was monitored by parental record of the child's symptoms, similar to the one described above. Patients who tolerated STEP 0 were subjected to low-dose OFC (STEP 1—Pumpkin cake containing one heated egg yolk i.e., 213 mg of egg protein), medium-dose OFC (STEP 2—Pumpkin cake containing 1/4 heated whole egg i.e., 1550 mg), and finally, high-dose (STEP 3—One scrambled egg i.e., 6200 mg).

2.4. Fecal Markers Measurement

Fecal samples were collected from the diaper immediately after defecation, as a part of allergic work-up, before introducing an elimination diet and OFC. fCal was tested on the same day. The remaining stool sample was frozen and stored at −20 °C until assayed. Frozen stool samples were thawed before analysis. Feces samples were weighed (15 mg) on an assay balance. Afterwards, a buffer solution (0.75 mL of 1:10 diluted WASHBUF (wash buffer concentrate) for TNFα measurement; or 1.5 mL of 1:2.5 diluted IDK Extract®) was added, and the sample was vortex-mixed for 10 min. The samples were centrifuged (1000 rpm, 5 min) and subsequently allowed to stand for approximately 10 min for sediment to settle. For analysis the amount of 0.1 mL per well was used. The feces samples were tested using an IDK® Immunodiagnostic AG Bensheim Germany ELISA kit for EDN and TNFα. EUROIMMUN Medizinische Labordiagnostica AG Lübeck Germany ELISA kit was used for fCal detection. The detection limits for each parameter were the following: TNFα = 10 pg/mL, EDN = 0.164 ng/mL, fCal = 6.5 μg/g.

2.5. Statistical Analysis

Statistical tests, computing and graphics were performed using the STATISTICA 13 software (TIBCO Software Inc., Palo Alto, CA, USA). Variables with a normal distribution are expressed as means ± standard deviations (SD), whereas variables with a non-normal distribution are expressed as medians and ranges. In order to investigate whether the biomarker's distribution is similar to the normal distribution, the Shapiro–Wilk test was performed. Differences between quantitative parameters were analyzed using the non-parametric Mann–Whitney U-test. Differences between qualitative parameters were calculated by the X^2-test. The non-parametric Spearman's test was employed for determining the correlations. Cut-off levels, specificity and sensitivity were calculated using the receiver operating characteristic (ROC) analysis. To determine the diagnostic usefulness of combined markers, synthetic indicators were developed. These indicators are linear combinations of selected variables. These synthetic indicators were used to construct ROC curves and calculate the area under the curves (AUCs). To calculate the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of synthetic indicators, cut-off values were selected, based on the criterion of maximization of Youden's J statistic and the criterion of minimization of Euclidean distance to perfect classifier. p-value of <0.05 was considered statistically significant.

3. Results

Between December 2017 and March 2019, a total number of 59 children were enrolled into the study i.e., 34 infants with gastrointestinal bleeding and 25 patients with functional disorders as a control group. Standard diagnostic work-ups identified 2 children with acute gastroenteritis and one with Wiskott–Aldrich syndrome. OFC was negative in 4 patients (Figure 1). Detailed patients' characteristics are provided in Table 1.

Patients diagnosed with FA encompassed 73.5% of all children with hematochezia referred to the hospital. The offending food was identified as CMP in 85% (23/27) of patients and hen's egg in 15% (4/27) of cases. On follow-up, at approximately 12–16 months of age, 85% (23/27) of children gained tolerance to the allergen.

Children with FA demonstrated significantly higher levels of fCal and EDN, compared with the controls ($p < 0.05$) (Table 1, Figure 2).

Table 1. Background data, median (min-max; first, third quartile) values of fecal biomarkers among study and control groups. Significance is accepted at $p < 0.05$.

	Group		*p*-Value
	Study	Control	
Total number	27	25	NS
Age (median, months) (range; first, third quartile)	4 (1–11; 2, 6)	5 (2–10; 3, 6)	
Sex			<0.05
Girls	9	11	
Boys	18	14	
Primary diet			NS
Breast- fed	10	10	
Milk formula	17	15	
Biomarker			
fCal (μg/g)	651.1 (88–2755; 491, 934)	332 (74–759; 218, 384)	<0.05
EDN (ng/mL)	1450.8 (75.6–4146; 725, 2985)	471 (109–1446; 251, 749)	<0.05
TNFα (pg/mL)	472 (148–1772; 320, 913)	444 (121–1303; 288, 503)	NS
Treatment diet		N/A	
Maternal diet	7	–	
Casein eHF	10	–	
Whey eHF	1	–	
AAF	5	–	
Egg free diet	4	–	
Gained tolerance		N/A	
Yes	23	–	
No	4	–	

Abbreviations: AAF, amino acid formula; EDN, eosinophil derived neurotoxin; eHF, extensively hydrolyzed protein formula; fCal, fecal calprotectin; N/A, not applicable; NS, not significant; TNFα, tumor necrosis factor α.

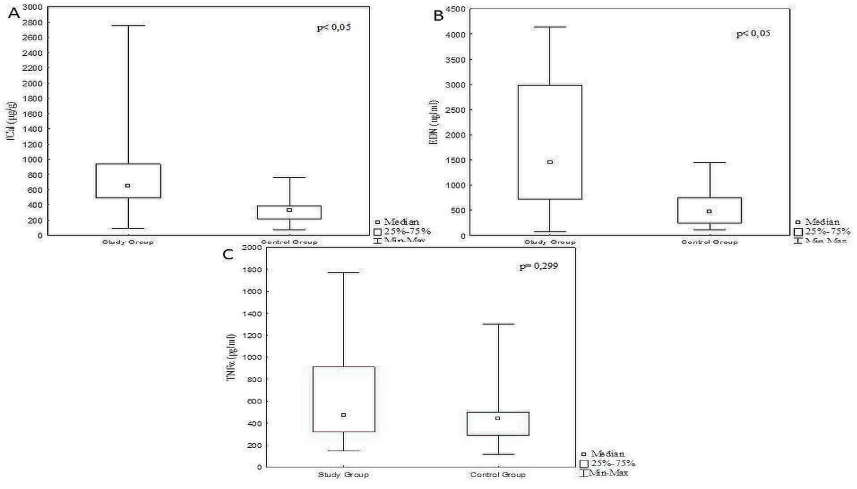


Figure 2. Differences in the concentration of fecal calprotectin (**A**), eosinophil derived neurotoxin (**B**) and tumor necrosis factor α (**C**) in infants with food protein induced allergic proctocolitis (study) and control groups.

In order to select the best marker's combination for the discrimination of children with FA from control subjects, an ROC curve analysis was performed. Although the AUC values for fCal and EDN were above 0.8 (0.803 and 0.8119 respectively), the specificity of the test was the highest for fCal solely i.e., 92% for cut-off 486 ug/g (Figure 3).

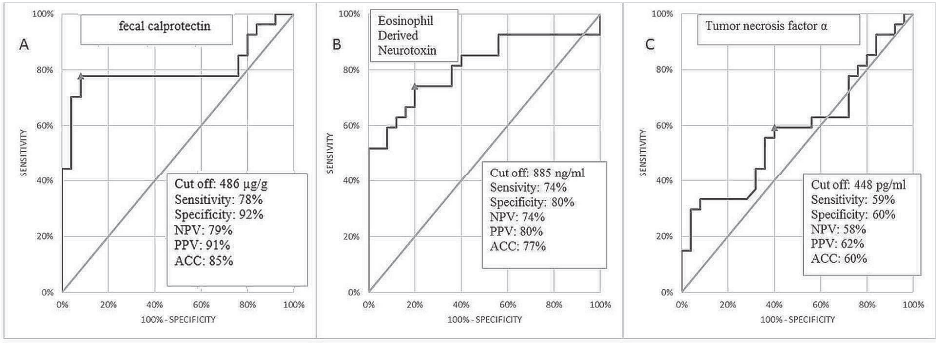


Figure 3. Receiver operating characteristics (ROC) curve of fecal calprotectin (**A**), eosinophil derived neurotoxin (**B**) and TNFα (**C**) for prediction of FPIAP. Abbreviations: NPV, negative predictive values; PPV, positive predictive values; ACC, accuracy.

The best diagnostic performance, regarded as significant increase in AUC, sensitivity, and specificity was reached in a combination of fCal and EDN (Table 2). Adding TNFα did not improve the diagnostic usefulness. Detailed statistical analyses of markers concentration in both groups are presented in Table 2 and Figure 3.

Table 2. Area under the curve (AUC), standard error (SE), confidence interval, sensitivity (sen), specificity (spec) of eosinophil derived neurotoxin (EDN), tumor necrosis factor α (TNFα), and fecal calprotectin (fCal) among study group.

Variable	AUC	SE	95% C.I. (AUC)	*p*-Value (AUC = 0.5)	Minimum Euclidean Distance Classifier			Youden's Index		
					Cut-off	Sen	Spec	Cut-off	Sen	Spec
EDN (ng/mL)	0.8119	0.0625	(0.689–0.934)	0.0000	>884	74%	80%	>884	74%	80%
TNFα (pg/mL)	0.5844	0.0809	(0.426–0.743)	0.2966	>448	59%	60%	>733	30%	96%
fCal (µg/g)	0.803	0.0687	(0.668–938)	0.0000	>486	78%	92%	>486	78%	92%
EDN/TNFα	0.8141	0.062	(0.693–0.936)	0.0000		74%	84%		67%	92%
EDN/fCal	0.8778	0.0524	(0.775–0.98)	0.0000		89%	84%		89%	84%
TNFα/fCal	0.8044	0.0693	(0.669–0.94)	0.0000		78%	96%		78%	96%
EDN/TNFα/fCal	0.8756	0.0539	(0.77–0.981)	0.0000		89%	84%		89%	84%

Abbreviations: AUC, area under the curve; C.I, confidence interval; EDN, eosinophil derived neurotoxin; fCal, fecal calprotectin; SE, sensitivity; Sen, sensitivity; Spec, specificity; TNFα, tumor necrosis factor α;.

4. Discussion

This prospective study presents a potential role of three selected non- invasive, fecal biomarkers measurement in improving the diagnosis of FPIAP in children. The study provides the following new information: fCal and fecal EDN concentration proved to be significantly higher in children with FPIAP than with the gastrointestinal functional disorders and thus potentially useful in differentiating between two clinical entities; both biomarkers presented mutual correlation indicating simultaneous involvement of neutrophils and eosinophils in pathophysiology of FPIAP; a combined measurement of fCal and EDN presents better diagnostic performance than testing each biomarker solely; sensitivity and specificity of combined fCal and EDN testing reached 88.9% and 84%, respectively.

In relation to the current state of knowledge on FPIAP, the study cohort might seem unusual with relatively low breastfeeding percentage (37%), which is more typical for FPIES. Indeed FPIES, especially

chronic type, might present only with a single reaction accompanied by bloody and/or mucous stools [18]. We retrospectively reanalyzed the study cohort and found no FPIES cases fulfilling the diagnostic criteria by Nowak-Wegrzyn et al. [19]. It can be speculated that the low number of breastfed children in our study might be a result of parental distress caused by lack of immediate improvement after initial, maternal, dietary restrictions, leading to introducing hydrolyzed milk formula.

A number of studies have shown that fCal is a sensitive marker for inflammation within gastrointestinal tract [20]. What is more, it has shown a good correlation with other inflammatory markers, such as plasma C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), providing additional value in diagnosis and management of patients with IBD 10. Ezri et al. indicated that fCal values are age-dependent and estimated cut-off levels upon <350 μg/g in the first year of life [21]. A recent study has presented the normogram for fCal concentration, in which the 95th percentile being 910.3 mg/kg for 0–12 months of age [22]. In our study, the median fCal value was 331.7 μg/g, 74–751 μg/g in 25 controls below 1 year of age, similar to Ezir et al. results and significantly lower than the Roca et al. study. Discrepancies might be a result of including neonates into the study group, in which fCal levels tend to be higher due to a more permeable small bowel [23]. The study by Oord and Hornung presented that the 97th percentile for fCal was 538 mg/kg in the group 1 to 6 months and 162 mg/kg in the group 6 to 12 months [24]. On the contrary, a recent study conducted in healthy infants aged 0–12 months revealed a median fCal concentration of 313 μg/g [25]. In our opinion, every arbitrary approach to age group will involve the risk of bias. What is more, intestinal epithelial homeostasis in children is particularly susceptible to the effect of intestinal microbiome, which may result in great variety of fCal concentrations [26]. Finally, different biomarkers' concentration in studies might result from preanalytical errors i.e., various time of sample collection. Olafsdottir et al. reported that stool collected from the diaper had up to 30% higher calprotectin concentration due to water absorption [27]. In relation to allergy, Beser et al. revealed that in non-IgE mediated type, fCal tends to present significantly higher levels than in IgE-mediated ones (886 ± 278 μg/g vs. 392 ± 209 μg/g, respectively) [28]. In the presented study, patients with FPIAP demonstrated similar results of fCal concentration: median value 651.1 μg/g, 88.2–2755.4 μg/g. In a similar study group, infants with hematochezia and presumptive allergic colitis, Baldasare et al. described lower mean values of fCal (325.89 ± 152.31 μg/g) than in our study [29]. In a recent study, the mean fCal level in a group of 40 infants with cow's milk protein allergy (CMPA) was 442 μg/g, whereas in the control group—100 μg/g, leading to the conclusion that optimal cut-off point for fCal should be 138 μg/g [30]. However, we suggest a cut-off point for fCal upon 485.65 μg/g, with the sensitivity of 77.8% and specificity of 92%.

Studies suggest the usefulness of fCal measurement in follow up in children with diagnosed CMPA [28,29]. However contrary evidence also exists [31]. An increased level of fCal has also been described in gastrointestinal malignancies, infections, polyps, and in nonsteroidal anti-inflammatory drug-therapies and therefore is not specific to allergic reaction [32].

During an effector phase of an allergic reaction, recruitment of mononuclear inflammatory cells leads to the release of a number of proinflammatory cytokines-including EDN [33]. Previous studies concerning children with atopic dermatitis and suspected food allergy revealed the usefulness of fecal eosinophil-derived proteins (eosinophil cationic protein [ECP], eosinophil protein-X [EPX]) in diagnostic workup [34]. Further studies by Wada et al. revealed fecal EDN concentration changes in response to control allergen stimuli in 8 patients with non-IgE-mediated food allergy [12]. Baseline levels of biomarker were variable, however after OFC, a significant increase was noted in all patients and a maximum concentration after 24 h (mean 33.244 ng/mL) of exposure. Among children with gastrointestinal allergy, those with hematochezia exhibited higher values of fecal EDN [13]. Kalach et al. studied a group of children with CMPA, in which fecal EDN in a single spot sample was measured [16]. Although fecal EDN did not differ significantly between both groups ($p = 0.06$), it was positively correlated with an allergic condition, with cut-off value for CMPA of 2818 ng/g, sensitivity of 54.5% and specificity of 85.7%. In our study, the mean EDN concentration in stool was significantly higher in the study group than in controls ($p < 0.05$), sensitivity reached 74.1%, specificity 80% with cut off value 884.45 ng/mL. The before mentioned study, by Roca et al., estimated the 95th percentile for EDN upon 7.4 mg/kg in infants, which remains in the agreement with our results [22].

TNF-α is involved in pathophysiology of gastrointestinal allergy by initiating the process of increased intestinal permeability [35]. Although Wada et al. reported that levels of fecal TNF-α were not significantly elevated between patients positive to OFC and control group, they remained increased for one month's time after the oral challenges [14]. In the presented study, a comparison of TNFα levels in both groups revealed no significant differences ($p = 0.299$). In the cohort of healthy children, TNFα levels below 90 pg/g were considered normal [15]. In our study, the median biomarker value in the control group was higher, reaching 443.6 pg/g, 120.5–1302.7 pg/g. In the case of allergic disorders, Majamaa et al. indicated that a particularly high concentrations of TNFα were found in patients manifesting delayed-onset allergy reactions [36]. However, Kalach et al. found fecal TNFα levels below detection range of 30 pg/g in all patients with CMPA [16]. The wide range of results might arise from the fact that TNFα is closely correlated with the inflammatory activity within intestine mucosa and susceptible to degranulation, which makes the timing of measurement critical [37,38]. It might be speculated that fecal TNFα measurement might be useful in differentiation between FA and inflammatory bowel disease in children.

Several limitations of the study require consideration. Firstly, only children below 12 months of age, with particular symptoms and diagnosed in hospital exclusively, were enrolled, which makes the study susceptible to selection biases, and thus not representative of the general population of patients with FA. Secondly, OFC was performed only in the study group, leaving potentially, subclinical cases of FA undetected and thus potentially interfering with the results. Thirdly, as blood carries neutrophils, bloody stools might overestimate the true value of fecal calprotectin resulting from allergic, gut inflammation. In addition, the cohort of patients might be considered as modest, however it is comparable to other studies of similar interest in the pediatric population. Moreover, a single spot biomarkers' measurement allows only for the assessment of diagnostic usefulness, but not for the follow up process. Further studies in larger cohorts of patients are required in this field.

From a practical point of view, a fecal biomarkers measurement is cheaper, faster, and more patient-friendly than the standard diagnostic work-up. Simultaneous testing for fCal and EDN might differentiate patients with FPIAP from infants with, common in this age, gastrointestinal, functional disorders. Furthermore, it can possibly shorten diagnosis, as their laboratory measurement is faster than typical time of 72–96 h for resolution of symptoms. Moreover, due to their relatively simplicity in laboratory analysis, their testing might enhance differential diagnosis on outpatient's basis, limiting unnecessary hospital admissions and lowering financial burden on health insurance systems. In doubtful cases (i.e., lack of improvement after initial treatment, polyvalent allergy suspected), fCal and EDN might support diagnostic decisions by avoidance furthers steps in differential diagnostics. In terms of science, fCal and EDN might be useful in research on pathophysiology of controversial conditions, like food protein induced constipation.

Author Contributions: Conceptualization, B.C. and D.M.L.; methodology, B.C.; software, A.R.; validation, A.R., B.C. and D.M.L.; formal analysis, A.R.; investigation, A.R. and B.C.; resources, A.R. and B.C.; data curation, A.R.; writing—original draft preparation, A.R.; writing—review and editing, B.C. and D.M.L.; visualization, A.R.; supervision, D.M.L.; project administration, B.C.; funding acquisition, B.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by The Medical University of Bialystok, grant number N/ST/ZB/17/005/1143.

Acknowledgments: We would like to express our sincere appreciation to Irena Werpachowska for the excellent laboratory assistance and to Urszula Daniluk for writing assistance, technical editing, language editing and proofreading of the manuscript.

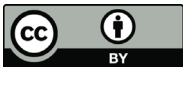
Conflicts of Interest: The authors declare no conflict of interest.

References

1. Johansson, S.G.O.; Bieber, T.; Dahl, R.; Friedmanm, P.S.; Lanier, B.; Lockey, R.; Motala, C.; Ortega Martel, J.A.; Platts-Mills, T.A.E.; Ring, J.; et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J. Allergy Clin. Immunol.* **2004**, *113*, 832–836. [CrossRef] [PubMed]

2. Soares-Weiser, K.; Takwoingi, Y.; Panesar, S.S.; Muraro, A.; Werfel, T.; Hoffmann-Sommergruber, K.; Roberts, G.; Halken, S.; Poulsen, L.; van Ree, R.; et al. The diagnosis of food allergy: A systematic review and meta-analysis. *Allergy* **2014**, *69*, 76–86. [CrossRef] [PubMed]
3. Daniluk, U.; Alifier, M.; Kaczmarski, M.; Stasiak-Barmuta, A.; Lebensztejn, D. Longitudinal observation of children with enhanced total serum IgE. *Ann. Allergy Asthma Immunol.* **2015**, *114*, 404–410. [CrossRef] [PubMed]
4. Nowak-Węgrzyn, A. Food protein-induced enterocolitis syndrome and allergic proctocolitis. *Allergy Asthma Proc.* **2015**, *36*, 172–184. [CrossRef]
5. Arvola, T.; Ruuska, T.; Keränen, J.; Hyöty, H.; Salminen, S.; Isolauri, E. Rectal bleeding in infancy: Clinical, allergological, and microbiological examination. *Pediatrics* **2006**, *117*, 760–768. [CrossRef]
6. Xanthakos, S.A.; Schwimmer, J.B.; Melin-Aldana, H.; Rothenberg, M.E.; Witte, D.P.; Cohen, M.B. Prevalence and outcome of allergic colitis in healthy infants with rectal bleeding: A prospective cohort study. *J. Pediatr. Gastroenterol. Nutr.* **2005**, *41*, 16–22. [CrossRef]
7. Koletzko, S.; Niggemann, B.; Arato, A.; Dias, J.A.; Heuschkel, R.; Husby, S.; Mearin, M.L.; Papadopoulou, A.; Ruemmele, F.M.; Staiano, A.; et al. Diagnostic approach and management of cow's-milk protein allergy in infants and children: ESPGHAN GI Committee practical guidelines. *J. Pediatr. Gastroenterol. Nutr.* **2012**, *55*, 221–229. [CrossRef]
8. Yum, H.Y.; Yang, H.J.; Kim, K.W. Oral food challenges in children. *Korean J. Pediatr.* **2011**, *54*, 6–10. [CrossRef]
9. Sicherer, S.H.; Sampson, H.A. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. *J. Allergy Clin. Immunol.* **2018**, *141*, 41–58. [CrossRef]
10. Filimoniuk, A.; Daniluk, U.; Samczuk, P.; Wasilewska, N.; Jakimiec, P.; Kucharska, M.; Lebensztejn, D.M.; Ciborowski, M. Metabolomic profiling in children with inflammatory bowel disease. *Adv. Med. Sci.* **2020**, *65*, 65–70. [CrossRef]
11. Daniluk, U.; Daniluk, J.; Krasnodebska, M.; Lotowska, J.M.; Sobaniec-Lotowska, M.E.; Lebensztejn, D.M. The combination of fecal calprotectin with ESR, CRP and albumin discriminates more accurately children with Crohn's disease. *Adv. Med. Sci.* **2019**, *64*, 9–14. [CrossRef] [PubMed]
12. Wada, T.; Toma, T.; Muraoka, M.; Matsuda, Y.; Yachie, A. Elevation of fecal eosinophil-derived neurotoxin in infants with food protein-induced enterocolitis syndrome. *Pediatr. Allergy Immunol.* **2014**, *25*, 617–619. [CrossRef] [PubMed]
13. Morita, H.; Nomura, I.; Shoda, T. Fecal Eosinophil-derived neurotoxin is significantly elevated in non-IgE dependent Gastrointestinal allergies, especially in subtypes showing bloody stool [abstract]. *J. Allergy Clin. Immunol.* **2012**, *129*, A353.
14. Wada, H.; Horisawa, T.; Inoue, M.; Yoshida, T.; Toma, T.; Yachie, A. Sequential measurement of fecal parameters in a case of non-immunoglobulin E-mediated milk allergy. *Pediatr. Int.* **2007**, *49*, 109–111.
15. Kapel, N.; Roman, C.; Caldari, D. Fecal tumor necrosis factor-alpha and calprotectin as differential diagnostic markers for severe diarrhea of small infants. *J. Pediatr. Gastroenterol. Nutr.* **2005**, *41*, 396–400. [CrossRef]
16. Kalach, N.; Kapel, N.; Waligora-Dupriet, A.J.; Castelain, M.C.; Cousin, M.O.; Sauvage, C.; Ba, F.; Nicolis, I.; Campeotto, F.; Butel, M.J.; et al. Intestinal permeability and fecal eosinophil-derived neurotoxin are the best diagnosis tools for digestive non-IgE-mediated cow's milk allergy in toddlers. *Clin. Chem. Lab. Med.* **2013**, *51*, 351–361. [CrossRef]
17. Yanagida, N.; Minoura, T.; Kitaoka, S.; Ebisawa, M. A three-level stepwise oral food challenge for egg, milk, and wheat allergy. *J Allergy Clin. Immunol. Pract.* **2018**, *6*, 658–660. [CrossRef]
18. Nowak-Wegrzyn, A.; Berin, M.C.; Mehr, S. Food Protein-Induced Enterocolitis Syndrome. *J. Allergy Clin. Immunol. Pract.* **2020**, *8*, 24–35. [CrossRef]
19. Nowak-Węgrzyn, A.; Chehade, M.; Groetch, M.E.; Spergel, J.M.; Wood, R.A.; Allen, K.; Atkins, D.; Bahna, S.; Barad, A.V.; Berin, C.; et al. International consensus guidelines for the diagnosis and management of food protein-induced enterocolitis syndrome: Executive summary-Workgroup Report of the Adverse Reactions to Foods Committee, American Academy of Allergy, Asthma & Immunology. *J. Allergy Clin. Immunol.* **2017**, *139*, 1111–1126.e4. [CrossRef]
20. Summerton, C.B.; Longlands, M.G.; Wiener, K.; Shreeve, D.R. Faecal calprotectin: A marker of inflammation throughout the intestinal tract. *Eur. J. Gastroenterol. Hepatol.* **2002**, *14*, 841–845. [CrossRef]
21. Ezri, J.; Nydegger, A. Pediatrics. Fecal calprotectin in children: Use and interpretation. *Rev. Med. Suisse* **2011**, *7*, 69–70.

22. Roca, M.; Rodriguez Varela, A.; Donat, E.; Cano, F.; Hervas, D.; Armisen, A.; Vaya, M.J.; Sjölander, A.; Ribes-Koninckx, C. Fecal Calprotectin and Eosinophil-derived Neurotoxin in Healthy Children Between 0 and 12 Years. *J. Pediatr. Gastroenterol. Nutr.* **2017**, *65*, 394–398. [CrossRef] [PubMed]
23. Anderson, R.C.; Dalziel, J.E.; Gopal, P.K.; Bassett, A.E.; Roy, N.C. *The Role of Intestinal Barrier Function in Early Life in the Development of Colitis*; Fukata, C., Ed.; InTech: Rijeka, Croatia, 2012. [CrossRef]
24. Oord, T.; Hornung, N. Fecal calprotectin in healthy children. *Scand. J. Clin. Lab. Investig.* **2014**, *74*, 254–258. [CrossRef] [PubMed]
25. Günaydın Şahin, B.S.; Keskindemirci, G.; Özden, T.A.; Durmaz, Ö.; Gökçay, G. Faecal calprotectin levels during the first year of life in healthy children. *J. Paediatr. Child. Health* **2020**. [CrossRef] [PubMed]
26. Daniluk, U.; Alifier, M.; Kaczmarski, M. Probiotic-induced apoptosis and its potential relevance to mucosal inflammation of gastrointestinal tract. *Adv. Med. Sci.* **2012**, *57*, 175–182. [CrossRef]
27. Olafsdottir, E.; Aksnes, L.; Fluge, G.; Berstad, A. Faecal calprotectin levels in infants with infantile colic, healthy infants, children with inflammatory bowel disease, children with recurrent abdominal pain and healthy children. *Acta Paediatr.* **2002**, *91*, 45–50. [CrossRef] [PubMed]
28. Beşer, O.F.; Sancak, S.; Erkan, T.; Kutlu, T.; Cokuğraş, H.; Cokuğraş, F.Ç. Can Fecal Calprotectin Level Be Used as a Markers of Inflammation in the Diagnosis and Follow-Up of Cow's Milk Protein Allergy? *Allergy Asthma Immunol. Res.* **2014**, *6*, 33–38. [CrossRef]
29. Baldassarre, M.E.; Laforgia, N.; Fanelli, M.; Laneve, A.; Grosso, R.; Lifschitz, C. Lactobacillus GG improves recovery in infants with blood in the stools and presumptive allergic colitis compared with extensively hydrolyzed formula alone. *J. Pediatr.* **2010**, *156*, 397–401. [CrossRef]
30. Trillo Belizón, C.; Ortega Páez, E.; Medina Claros, A.F.; Rodríguez Sánchez, I.; Reina González, A.; Vera Medialdea, R.; Ramón Salguero, J.M. Faecal calprotectin as an aid to the diagnosis of non-IgE mediated cow's milk protein allergy. *An. Pediatr. (Barc.)* **2016**, *84*, 318–323. [CrossRef]
31. Ataee, P.; Zoghali, M.; Nikkhoo, B. Diagnostic Value of Fecal Calprotectin in Response to Mother's Diet in Breast-Fed Infants with Cow's Milk Allergy Colitis. *Iran J. Pediatr.* **2018**, *28*, e66172. [CrossRef]
32. Pathirana, W.G.W.; Chubb, S.P.; Gillett, M.J.; Vasikaran, S.D. Faecal Calprotectin. *Clin. Biochem. Rev.* **2018**, *39*, 77–90. [PubMed]
33. Rosenberg, H.F. Eosinophil-derived neurotoxin/RNase 2: Connecting the past, the present and the future. *Curr. Pharm. Biotechnol.* **2008**, *9*, 135–140. [CrossRef] [PubMed]
34. Majamaa, H.; Laine, S.; Miettinen, A. Eosinophil protein X and eosinophil cationic protein as indicators of intestinal inflammation in infants with atopic eczema and food allergy. *Clin. Exp. Allergy* **1999**, *29*, 1502–1506. [CrossRef] [PubMed]
35. Chung, H.L.; Hwang, J.B.; Park, J.J.; Kim, S.G. Expression of transforming growth factor beta1, transforming growth factor type I and II receptors, and TNF-alpha in the mucosa of the small intestine in infants with food protein-induced enterocolitis syndrome. *J. Allergy Clin. Immunol.* **2002**, *109*, 150–154. [CrossRef]
36. Majamaa, H.; Miettinen, A.; Laine, S.; Isolauri, E. Intestinal inflammation in children with atopic eczema: Faecal eosinophil cationic protein and tumour necrosis factor alpha as non-invasive indicators of food allergy. *Clin. Exp. Allergy* **1996**, *26*, 181–187. [CrossRef] [PubMed]
37. Braegger, C.P.; Nicholls, S.; Murch, S.H.; Stephens, S.; MacDonald, T.T. Tumour necrosis factor alpha in stool as a marker of intestinal inflammation. *Lancet* **1992**, *339*, 89–91. [CrossRef]
38. Soulé, J.C.; Andant, C.; Kapel, N. Evaluation of fecal tumor necrosis factor alpha (TNFα) as a marker of Crohn's disease (CD) activity. *Gastroenterology* **1998**, *114*, A1089. [CrossRef]



Journal of
Clinical Medicine

MDPI

Article

Short-Term Outcomes of Polycarbophil and *Propionibacterium acnes* Lysate Gel after Open Hemorrhoidectomy: A Prospective Cohort Study

Gaetano Gallo [1,*,†], Ugo Grossi [2,†], Gian Luca Di Tanna [3], Giulio Aniello Santoro [2], Gilda De Paola [1], Giuseppe Clerico [4], Alberto Realis Luc [4], Mario Trompetto [4] and Giuseppe Sammarco [5]

1 Department of Medical and Surgical Sciences, University of Catanzaro, Viale Europa, 88100 Catanzaro, Italy; gilda_depaola@libero.it
2 Tertiary Referral Pelvic Floor Center, IV Surgery Unit, Treviso Regional Hospital, DISCOG, University of Padua, 31100 Treviso, Italy; grossiugo@gmail.com (U.G.); giulioasantoro@yahoo.com (G.A.S.)
3 Statistics Division, The George Institute for Global Health, Faculty of Medicine, University of New South Wales, Sidney, NSW 2042, Australia; glditanna@gmail.com
4 Department of Colorectal Surgery, S. Rita Clinic, 13100 Vercelli, Italy; clerico.giuseppe@gmail.com (G.C.); alberto.realisluc@libero.it (A.R.L.); trompetto.mario@libero.it (M.T.)
5 Department of Health Sciences, University of Catanzaro, Viale Europa, 88100 Catanzaro, Italy; sammarco@unicz.it
* Correspondence: gallog@unicz.it
† Shared first author position.

Received: 31 October 2020; Accepted: 7 December 2020; Published: 10 December 2020

Abstract: Background: Pain is the most common complication after open excisional hemorrhoidectomy (OEH). We assessed the effectiveness of polycarbophil and *Propionibacterium acnes* lysate gel (Emorsan®Gel) on pain control after OEH. Research design and methods: Fifty consecutive patients undergoing OEH were included. All patients received stool softeners and oral analgesia in the post-operative period. Emorsan®Gel was also used topically by the last 25 patients (Emorsan®Gel group (EG)) until Post-Operative Day 20 (POD 20). The primary outcome was the effectiveness of Emorsan®Gel on pain relief using an 11-point visual analogue scale (VAS). Morbidity, wound healing (WH), and time to work were documented at POD 1, POD 10, POD 20, and POD 40. Results: Of the 50 patients enrolled, twenty-eight (56%) were males; median age, 49 (range, 28–73) years. The VAS score decreased over time in all patients, with significantly lower scores at POD 20 in the EG (1.44 (SD, 1.16) vs. 2.12 (0.93) in the control group (CG); $p = 0.045$). All patients in the EG achieved complete WH at last follow-up, compared to only 17 (68%) in the CG ($p = 0.004$). The likelihood of WH was 66% higher in the EG (OR, 1.66 [95%CI, 0.80–3.44; $p = 0.172$). Conclusions: Emorsan®Gel is safe and effective at reducing pain after EOH, promoting earlier WH compared to standard care treatment.

Keywords: Emorsan®Gel; hemorrhoidal disease; hemorrhoidectomy; pain; wound healing

1. Introduction

Open excisional hemorrhoidectomy is the gold standard treatment of III and IV degree hemorrhoidal disease (HD) [1]. However, despite technological advances (e.g., radiofrequency and ultrasound devices) [2,3], the post-operative period remains a very delicate phase that can deeply affect patient's quality of life. Bleeding, pain, and anal stricture are among the complications of hemorrhoidectomy, which may require re-intervention in the short or long term.

Post-operative pain represents a major burden for patients. It is frequently experienced during the first 7–10 days after surgery [4] and may arise from the incorporation of sensitive anal mucosa or fibers

of the internal anal sphincter into stitches, delayed wound healing, hard stool consistency, or edema of the mucocutaneous bridges. Technical advice allied with the optimization of post-operative analgesia may help prevent some of these triggers [5,6]. In a single-blind randomized trial comparing pedicle coagulation vs. ligation during excisional hemorrhoidectomy, a better control of post-operative pain was observed after pedicle coagulation, demonstrated by a reduced number of required analgesics [7].

Emorsan®Gel (Depofarma S.p.A, Treviso, Italy) is a topical gel rich in dimethicone and cyclopentasiloxane. These substances protect the skin and mucous membranes from external agents [8,9]. Their property of forming occlusive barriers on the epidermis helps to reduce inflammation and itching, while promoting the healing process [10]. Furthermore, lactic acid can restore the local pH to physiological levels, with a positive impact on wound healing [11].

The aim of the present study is to assess the short-term outcomes of Emorsan®Gel in the post-operative management after open diathermy excisional hemorrhoidectomy.

2. Patients and Methods

Between January and December 2018, fifty consecutive patients were prospectively included in the "EMORGEL Study", approved by regional ethics committee "Sezione Area Centro, Regione Calabria"; Approval Code 84/19). Written informed consent was obtained from all patients.

Consecutive subjects aged between 18 and 75 years, undergoing open diathermy excisional hemorrhoidectomy for Goligher III or IV degree HD were included in this study. The procedures were performed according to the PROSPECT (PROcedure-SPECific post-operative pain management) evidence [12], and neither ligation of the vascular pedicle, nor concomitant internal sphincterotomy were applied [13].

Pre-specified exclusion criteria were: pregnancy, current use of specific medications (e.g., psychopharmaceuticals, antibiotics, antimycotics, immunomodulators, corticosteroids, or other immunosuppressive drugs), active cancer, previous open hemorrhoidectomy, known allergy to product components.

A block enrolment strategy was used with two cohorts of 25 patients (control group (CG) and Emorsan®Gel group (EG)) enrolled at intervals of 6 months (from January to June and from July to December 2018, respectively). All patients received standard care treatment in the immediate post-operative period, consisting of stool softeners and oral analgesia (a recommended oral dose of ketorolac tromethamine of 10 mg every 4–6 h as needed, not exceeding 40 mg per day for 5 consecutive days, according to the short-term management of moderate/severe acute post-operative pain). Only the last 25 patients enrolled (i.e., in the second half of 2018) also received Emorsan®Gel topically every 12 h (2 mL per dose) for 20 consecutive days.

All patients were followed up at 4 time points: T1, Post-Operative Day 1 (POD 1); T2, POD 10; T3, POD 20; T4, POD 40. The following data were recorded on each visit: pain severity (spontaneous and/or on defecation) using an 11-point visual analogue scale (VAS); presence of thrombosis (defined as one or more swollen painful piles at the site of the mucocutaneous bridge); hemorrhage; wound status (granulating; healed); use of analgesia; grade of satisfaction on a 5-point scale (insufficient; sufficient; more than sufficient; good; excellent). Data on bowel habit were also recorded at T2, T3, and T4 using three patterns of the Bristol stool scale (hard, 1–2; normal, 3–5; non-formed, 6–7) [14]. During the same appointments, patients were asked to report the grade of activities they could sustain using a 4-item scale: complete inactivity, total autonomy at home, ability to drive, or return to normal activities (i.e., autonomy at home, driving, and working).

Any adverse events related to the use of Emorsan®Gel were recorded at T2, T3, and T4 from the last 25 patients enrolled, using a 6-point score: 0, no erythema; 1, very mild erythema; 2, moderate erythema without edema; 3, moderate erythema with edema without papules; 4, severe erythema with edema +/− papules; 5, severe erythema with edema and vesicles.

Primary outcome was the effectiveness of Emorsan®Gel on pain relief. Secondary outcomes were post-operative morbidity, wound healing, and time to work and social life.

All data were recorded anonymously on a prospectively built electronic database.

The study is reported in accordance to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines [15].

Statistical Analysis

Continuous variables are presented as the mean and standard deviation, while binary variables as proportions. Comparisons across groups were made using ANOVA and Fisher's exact tests, respectively. *p*-values were reported at their nominal value. Uni- and multi-variable logistic regressions were performed with a pre-defined covariate set, which included age and gender. All statistical analyses were performed using Stata 16 (College Station, TX: StataCorp LLC, Texas, TX, USA).

3. Results

Of the 50 patients enrolled, twenty-eight (56%) were males. Median age was 49 (interquartile range (IQR), 43.5–56) years. Gender (males, 60% vs. 52%, respectively; $p = 0.776$) and age (median, 51 vs. 48, respectively; $p = 0.899$) were similarly distributed in the two groups (Table 1).

Table 1. Patients' characteristics.

	Emorsan®Gel ***n* = 25**	**Controls** ***n* = 25**	***p*-Value**
Females (%)	10 (40)	12 (48)	0.776
Age (IQR)	51 (13)	48 (13)	0.899
Goligher grade			
III	12	13	0.898
IV	13	14	
ASA scoring system			
I	18	17	
II	4	4	0.997
III	3	4	
Ketorolac tromethamine (median daily dose in mg, IQR)			
POD 1	10 (12.5)	10 (12.5)	0.878
POD 2	5 (10)	5 (7.5)	0.795
POD 3	0 (5)	0 (5)	0.897
POD 4	0 (0)	0 (0)	0.921
POD 5	0 (0)	0 (0)	0.932

ASA: American Society of Anesthesiology; IQR: interquartile range; POD: post-operative day.

Mean operative time was 24.3 (SD, standard deviation 6.4; range 17–37) minutes in the EG and 25.3 (SD, 6.5; range 18–39) minutes in the CG. Morbidity occurred in six (12%) patients: bleeding ($n = 4$, requiring reintervention in two patients, one per group) and urinary retention ($n = 2$, one per group).

Daily use of analgesia up to POD 5 was also similar, with a significant drop after POD 1.

The VAS score decreased likewise over time (Figure 1). However, patients in the EG had a significantly lower mean score at POD 20 (1.44 (standard deviation, 1.16), compared to 2.12 (0.93) in the CG; $p = 0.045$). Although not reaching statistical difference, the VAS mean score over time was 0.1 points lower in the EG vs. CG (Table 2).

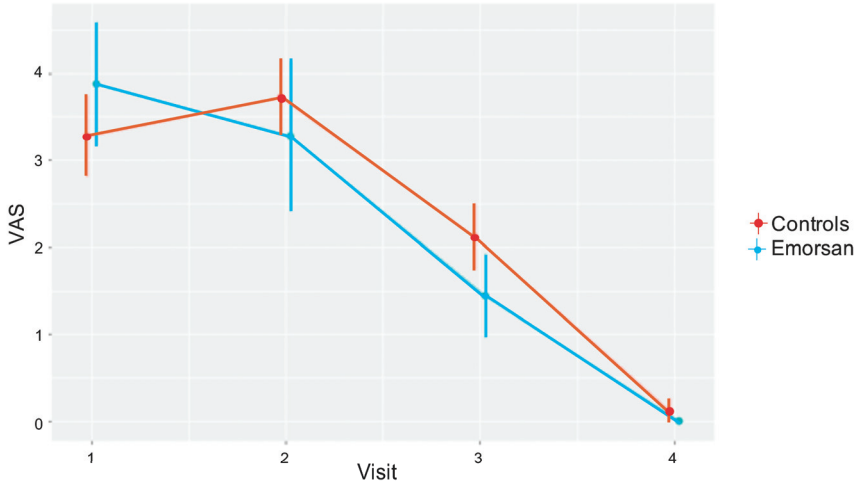


Figure 1. Visual analogue scale (VAS) mean scores in the two groups at each follow up visit.

Table 2. Restricted maximum likelihood mixed model of the visual analogue scale (VAS) scores (between groups).

Δ VAS score	Coefficient	95% CI		*p*-Value
		Lower	Upper	
Emorsan®Gel	–0.09	–0.96	0.79	0.845
Age	0.01	–0.04	0.51	0.779
Gender	0.71	–0.81	0.95	0.874

CI: confidence interval.

Thrombosis was observed at T2 and T3 in two (8%) patients in the CG and spontaneously resolved at T4. None of the patients in the EG experienced this complication, nor adverse events. No cases of hemorrhage were noted.

All patients in the EG achieved complete wound healing at last follow-up, compared to only 17 (68%) in the CG ($p = 0.004$). The likelihood of wound healing was 66% higher in the EG, although not reaching statistical significance (odds ratio (OR), 1.66 (95% confidence interval, CI, 0.80–3.44; $p = 0.172$)) (Table 3).

Table 3. Mixed-effects logistic regression exploring the likelihood of wound healing controlling for age and gender.

Complete Wound Healing	OR	95% CI		*p*-Value
		Lower	Upper	
Emorsan®Gel	1.66	0.80	3.44	0.172
Age	0.99	0.96	1.03	0.798
Gender	1.53	0.73	3.24	0.262

OR: odds ratio; CI: confidence interval.

Bowel habit similarly improved in both groups from T2 to T4: the number of patients achieving normal stool consistency passed from 19 (76%) to 20 (80%) in the EG and from 18 (72%) to 22 (88%) in the CG ($p = 0.846$).

On last visit, all patients in both groups had returned to normal activities. A high level of satisfaction was reported by all patients (mean EG, 4.76 (0.52); mean CG, 4.60 (0.58)).

4. Discussion

Several medical and surgical strategies have been developed to improve the post-operative management of patients undergoing open excisional hemorrhoidectomy [4,16–24].

Post-operative pain is one of the oldest and most debated problems after hemorrhoidectomy. Although the VAS mean score was not statistically significantly lower over time in the EG vs. the CG, at POD 20 (T3), twenty patients in the former group achieved a higher benefit compared to the controls. A similar outcome was observed in a previous prospective multicenter study on the efficacy of mesoglycan in pain control after excisional hemorrhoidectomy [4]. The highest improvement achieved in the intervention group in post-operative pain symptoms at POD 20 was determinant for a faster return to work.

While burdened with higher recurrence rates, a better control of pain and faster recovery were observed after transanal hemorrhoidal dearterialization (THD) compared to open and closed excisional hemorrhoidectomies [16]. The absence of a surgical wound after THD may well explain these findings. On the other hand, patients totally unkeen to accept a risk of recurrence may reluctantly undergo THD.

Our results demonstrated that the use of Emorsan®Gel benefited all patients receiving the product in terms of wound healing at 40 days after surgery. While higher in the EG, the likelihood of wound healing did not reach statistical significance, possibly due to the small sample size. However, earlier wound healing observed in the EG may explain the better pain control at 20 days after surgery.

In a previous work, we highlighted the role of inflammation in the pathogenesis of HD by demonstrating a high level of matrix metalloproteinases in patients with III and IV degree hemorrhoids [25]. In this context, Emorsan®Gel forms a tight epidermal barrier and may reduce local oxidative stress through the action of polycarbophil and *Propionibacterium acnes* extract. Indeed, the radical scavenging property of *Propionibacterium* lysate prevents cell damage from oxygen free radicals, thus reducing the inflammatory process.

Such anti-inflammatory properties could contribute to limit post-operative edema that may eventually cause thrombosis. This often involves the muco-cutaneous bridges and represents the most frequent cause of pain. Of note, none of the patients in the EG experienced this complication, as opposed to 8% of subjects in the CG.

This study has some limitations including the non-randomization design and the small sample size. However, patients were recruited consecutively to mitigate the selection bias. We also acknowledge the lack of a standardized thrombosis-measuring tool.

5. Conclusions

The results of this study support the safety and effectiveness of Emorsan®Gel on pain control after open hemorrhoidectomy, promoting earlier wound healing compared to standard care treatment. Larger trials are needed to confirm such findings.

Author Contributions: Conceptualization, G.G. and U.G.; methodology, software, validation, formal analysis, resources G.G., U.G. and G.L.D.T.; investigation, G.G., U.G.; data curation, G.G., U.G., G.C. and A.R.L.; writing—original draft preparation, G.G. and U.G.; writing—review and editing, G.G., U.G., G.L.D.T. and G.D.P.; visualization, All authors; supervision, G.A.S., M.T. and G.S.; project administration, G.G. and M.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: All authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

References

1. Gallo, G.; Martellucci, J.; Sturiale, A.; Clerico, G.; Milito, G.; Marino, F.; Cocorullo, G.; Giordano, P.; Mistrangelo, M.; Trompetto, M. Consensus statement of the Italian society of colorectal surgery (SICCR): Management and treatment of hemorrhoidal disease. *Tech. Coloproctology* **2020**, *24*, 145–164. [CrossRef] [PubMed]
2. Altomare, D.F.; Milito, G.; Andreoli, R.; Arcanà, F.; Tricomi, N.; Salafia, C.; Segre, D.; Pecorella, G.; D'urso, A.P.; Cracco, N.; et al. Ligasure™ Precise vs. Conventional Diathermy for Milligan-Morgan Hemorrhoidectomy: A Prospective, Randomized, Multicenter Trial. *Dis. Colon Rectum* **2008**, *51*, 514–519. [CrossRef] [PubMed]
3. Abo-Hashem, A.; Sarhan, A.; Aly, A. Harmonic Scalpel compared with bipolar electro-cautery hemorrhoidectomy: A randomized controlled trial. *Int. J. Surg.* **2010**, *8*, 243–247. [CrossRef] [PubMed]
4. Gallo, G.; Mistrangelo, M.; Passera, R.; Testa, V.; Pozzo, M.; Perinotti, R.; Lanati, I.; Lazzari, I.; Tonello, P.; Ugliono, E.; et al. Efficacy of Mesoglycan in Pain Control after Excisional Hemorrhoidectomy: A Pilot Comparative Prospective Multicenter Study. *Gastroenterol. Res. Pr.* **2018**, *2018*, 1–8. [CrossRef]
5. Chierici, A.; Frontali, A. Post Hemorrhoidectomy Pain Management: The Latest News. *Rev. Recent Clin. Trials* **2020**, *15*, 1–19. [CrossRef]
6. Elbetti, C.; Caminati, F.; Giani, I.; Feroci, F.; Zalla, T.; Calussi, M.; Dreoni, P.; Talamo, G. Tailored anal block (TAB): A new anesthesia procedure for surgical treatment of hemorrhoids in an outpatient setting. *Tech. Coloproctology* **2019**, *23*, 497–500. [CrossRef]
7. Bessa, S.S. Diathermy Excisional Hemorrhoidectomy: A Prospective Randomized Study Comparing Pedicle Ligation and Pedicle Coagulation. *Dis. Colon Rectum* **2011**, *54*, 1405–1411. [CrossRef]
8. Nair, B. Cosmetic Ingredients Review Expert P. Final report on the safety assessment of stearoxy dimethicone, dimethicone, methicone, amino bispropyl dimethicone, aminopropyl dimethicone, amodimethicone, amodimethicone hydroxystearate, behenoxy dimethicone, C24-28 alkyl methicone, C30-45 alkyl methicone, C30-45 alkyl dimethicone, cetearyl methicone, cetyl dimethicone, dimethoxysilyl ethylenediaminopropyl dimethicone, hexyl methicone, hydroxypropyldimethicone, stearamidopropyl dimethicone, stearyl dimethicone, stearyl methicone, and vinyldimethicone. *Int. J. Toxicol.* **2003**, *22*, 11–35.
9. Fiume, M.M.; Bergfeld, W.F.; Belsito, D.V.; Hill, R.A.; Klaassen, C.D.; Liebler, D.C.; Marks, J.J.G.; Shank, R.C.; Slaga, T.J.; Snyder, P.W.; et al. Safety Assessment of PEGylated Alkyl Glycerides as Used in Cosmetics. *Int. J. Toxicol.* **2020**, *39*, 26S–58S. [CrossRef]
10. Becker, L.C.; Bergfeld, W.F.; Belsito, D.V.; Hill, R.A.; Klaassen, C.D.; Liebler, D.C.; Marks, J.G., Jr.; Shank, R.C.; Slaga, T.J.; Snyder, P.W.; et al. Safety Assessment of Dimethicone Crosspolymers as Used in Cosmetics. *Int. J. Toxicol.* **2014**, *33*, 65–115. [CrossRef]
11. Percival, S.L.; Mccarty, S.; Hunt, J.A.; Woods, E.J. The effects of pH on wound healing, biofilms, and antimicrobial efficacy. *Wound Repair Regen.* **2014**, *22*, 174–186. [CrossRef] [PubMed]
12. Sammour, T.; on behalf of the PROSPECT group (Collaborators); Barazanchi, A.; Hill, A.G. Evidence-Based Management of Pain After Excisional Haemorrhoidectomy Surgery: A Prospect Review Update. *World J. Surg.* **2017**, *41*, 603–614. [CrossRef] [PubMed]
13. Gallo, G.; Luc, A.R.; Clerico, G.; Trompetto, M. Diathermy excisional haemorrhoidectomy—Still the gold standard—A video vignette. *Color. Dis.* **2018**, *20*, 1154–1156. [CrossRef] [PubMed]
14. Lewis, S.J.; Heaton, K.W. Stool Form Scale as a Useful Guide to Intestinal Transit Time. *Scand. J. Gastroenterol.* **1997**, *32*, 920–924. [CrossRef] [PubMed]
15. Von Elm, E.; Douglas, G.; Altman, D.G.; Egger, M.; Pocock, S.J.; Gøtzsche, P.C.; Vandenbroucke, J.P. STROBE Initiative. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: Guidelines for reporting observational studies. *Lancet* **2007**, *370*, 1453–1457. [CrossRef]
16. Simillis, C.; Thoukididou, S.N.; Slesser, A.; Rasheed, S.; Tan, E.; Tekkis, P.P. Systematic review and network meta-analysis comparing clinical outcomes and effectiveness of surgical treatments for haemorrhoids. *BJS* **2015**, *102*, 1603–1618. [CrossRef] [PubMed]
17. Giannini, I.; Pecorella, G.; Pennisi, D.; Santangelo, G.; Digennaro, R.; Latorre, F.; Giuliani, G.; Altomare, D.F. Control of post-hemorrhoidectomy symptoms and wound healing by Triclosan: A randomized, double-blind, controlled trial. *Minerva Chir.* **2014**, *69*, 75–82. [PubMed]
18. Gosselink, M.P.; Harman, A.N.; Ctercteko, G. Why is a haemorrhoid never infected? *Color. Dis.* **2019**, *21*, 385–386. [CrossRef]

19. Sturiale, A.; Gallo, G.; Brusciano, L.; Cacace, C.; Cafaro, D.; Porzio, F.C.; Naldini, G. Safety and Efficacy of Proctosoll Allevia in the Management of Haemorrhoidal Disease in Adults: A Prospective Randomized Clinical Trial. *Rev. Recent Clin. Trials* **2020**, *15*, 152–159. [CrossRef]
20. Balta, A.Z.; Ozdemir, Y.; Sucullu, I.; Filiz, A.I.; Yucel, E.; Akin, M.L. The effect of early warm plastic bag application on postoperative pain after hemorrhoidectomy: A prospective randomized controlled trial. *Am. Surg.* **2015**, *8*, 182–186. [CrossRef]
21. Ratansingham, K.; Uzzaman, M.; Andreani, S.; Light, D.; Patel, B. Meta-analysis of the use of glyceryl trinitrate ointment after haemorrhoidectomy as an analgesic and in promoting wound healing. *Int. J. Surg.* **2010**, *8*, 606–611. [CrossRef] [PubMed]
22. Ala, S.; Alvandipour, M.; Saeedi, M.; Hamidian, M.; Shiva, A.; Rahmani, N.; Faramarzi, F. Effects of Topical Atorvastatin (2%) on Posthemorrhoidectomy Pain and Wound Healing: A Randomized Double-Blind Placebo-Controlled Clinical Trial. *World J. Surg.* **2017**, *41*, 596–602. [CrossRef] [PubMed]
23. Patti, R.; Almasio, P.L.; Muggeo, V.M.R.; Buscemi, S.; Arcara, M.; Matranga, S.; Di Vita, G. Improvement of Wound Healing After Hemorrhoidectomy: A Double-Blind, Randomized Study of Botulinum Toxin Injection. *Dis. Colon Rectum* **2005**, *48*, 2173–2179. [CrossRef] [PubMed]
24. Gupta, P.J.; Heda, P.S.; Kalaskar, S.; Tamaskar, V.P. Topical Sucralfate Decreases Pain After Hemorrhoidectomy and Improves Healing: A Randomized, Blinded, Controlled Study. *Dis. Colon Rectum* **2008**, *51*, 231–234. [CrossRef] [PubMed]
25. Serra, R.; Gallelli, L.; Grande, R.; Amato, B.; De Caridi, G.; Sammarco, G.; Ferrari, F.; Butrico, L.; Gallo, G.; Rizzuto, A.; et al. Hemorrhoids and matrix metalloproteinases: A multicenter study on the predictive role of biomarkers. *Surgery* **2016**, *159*, 487–494. [CrossRef]

Journal of
Clinical Medicine

MDPI

Article

Anxiety and Gastrointestinal Symptoms Related to COVID-19 during Italian Lockdown

Ludovico Abenavoli [1,*], Pietro Cinaglia [2], Giuditta Lombardo [3], Eduardo Boffoli [4], Miriam Scida [4], Anna Caterina Procopio [1], Tiziana Larussa [1], Luigi Boccuto [5], Christian Zanza [6,7], Yaroslava Longhitano [7], Sharmila Fagoonee [8] and Francesco Luzza [1]

[1] Department of Health Sciences, University "Magna Graecia", 88100 Catanzaro, Italy; procopioannacaterina@gmail.com (A.C.P.); tarussa@unicz.it (T.L.); luzza@unicz.it (F.L.)
[2] Department of Surgical and Medical Sciences, University "Magna Graecia", 88100 Catanzaro, Italy; cinaglia@unicz.it
[3] Independent Researcher, 88100 Catanzaro, Italy; giuditta.lombardo@libero.it
[4] School of Medicine, University "Magna Graecia", 88100 Catanzaro, Italy; eduardo.boffoli96@gmail.com (E.B.); scidamiriam0@gmail.com (M.S.)
[5] School of Nursing, College of Behavioral, Social and Health Sciences, Clemson University, Clemson, SC 29634, USA; lboccut@clemson.edu
[6] Department of Anesthesia, Critical Care Medicine and Emergency Medicine, Michele and Pietro Ferrero Hospital, 12060 Verduno, Italy; christian.zanza@live.it
[7] Department of Anesthesia and Critical Care Medicine, AON SS Antonio e Biagio e Cesare Arrigo, 15121 Alessandria, Italy; lon.yaro@gmail.com
[8] Institute of Biostructure and Bioimaging, National Research Council, Molecular Biotechnology Center, 10135 Turin, Italy; sharmila.fagoonee@unito.it
* Correspondence: l.abenavoli@unicz.it; Tel.: +39-0961-3694387

Citation: Abenavoli, L.; Cinaglia, P.; Lombardo, G.; Boffoli, E.; Scida, M.; Procopio, A.C.; Larussa, T.; Boccuto, L.; Zanza, C.; Longhitano, Y.; et al. Anxiety and Gastrointestinal Symptoms Related to COVID-19 during Italian Lockdown. *J. Clin. Med.* **2021**, *10*, 1221. https://doi.org/10.3390/jcm10061221

Academic Editors: Marilena Durazzo and John F. Mayberry

Received: 3 February 2021
Accepted: 11 March 2021
Published: 16 March 2021

Abstract: The first case of infection by SARS-CoV-2 (i.e., COVID-19) was officially recorded by the Italian National Health Service on 21 February 2020. Respiratory tract manifestations are the most common symptoms, such as gastrointestinal symptoms (GISs) like nausea or sickness, diarrhea, and anorexia, and psychological effects may be reported in affected individuals. However, similar symptoms may be observed in healthy people as a consequence of an anxiety state. Methods: We analyzed GISs and anxiety state during the COVID-19 lockdown period; from 9 March 2020 to 4 May 2020. A web-based survey consisting of 131 items was administered to 354 students affiliated with the School of Medicine of the University "Magna Graecia" of Catanzaro; Italy. A set of statistical analyses was performed to analyze the relationships among the answers to assess a correlation between the topics of interest. Results: The statistical analysis showed that 54.0% of interviewed reported at least one GISs, 36.16% of which reported a positive history for familial GISs (FGISs). The 354 subjects included in our cohort may be stratified as follows: 25.99% GISs and FGISs, 27.97% GISs and no-FGISs, 10.17% no-GISs and FGISs, 35.87% no-GISs and no-FGISs. Results indicated an anxiety state for 48.9% of respondents, of which 64.74% also presented GISs. In addition, considered dietary habits, we detect the increased consumption of hypercaloric food, sweetened drinks, and alcoholic beverages. Conclusions: The increase of GISs during the lockdown period in a population of medical students, may be correlated to both dietary habits and anxiety state due to a concern for one's health.

Keywords: medical students; public health; social media; dietary habit; pandemic

1. Introduction

The World Health Organization (WHO) defines Coronavirus Disease 2019 (COVID-19) as a serious challenge to the world public health. On 31 December 2019, a cluster of pneumonia cases of unknown etiology was reported in Wuhan, a Chinese city in the Hubei Province [1]. COVID-19 involves principally respiratory tract, and the clinical presentation was very similar to that recorded during the Severe Acute Respiratory Syndrome (SARS)

outbreak of 2003 [2,3]. The virus rapidly spread worldwide, and it is responsible for a pandemic state with relevant clinical, psychological and economic consequences [4–6].

In Italy, the first case of infection by SARS Coronavirus 2 (SARS-CoV-2) was officially recorded on 21 February 2020 and on 11 March 2020, the WHO declared the COVID-19 outbreak a global pandemic and the Italian government enforced a lockdown measure, which is defined as a state of isolation and restricted access [7,8]. The lockdown period in Italy extended from 11 March to the following two months exactly until 4 May 2020.

The COVID-19 pandemic has influenced all aspects of human life with an unprecedented global crisis characterized by drastic changes in social life, personal freedom, and economic activities and has created distress, as well as exacerbation of mental health issues in a traumatic stress context, especially for healthcare workers [9].

Literature supports the association between anxiety and gastrointestinal symptoms (GISs) [10,11]. In subjects with functional gastrointestinal disorders, such as dyspepsia and irritable bowel syndrome, the presence of anxiety seems to play a major role in the genesis and the perception of symptoms [12]. The gastrointestinal tract and the nervous system are intimately connected via bidirectional signaling mechanisms characterized by neural, endocrine, and immune pathways, in a context knows as the "gut–brain axis" [13]. In particular, the central stress circuitry is the neural network that receives input from the somatic and visceral afferent systems and also from the visceral motor cortex and generates the stress response [14]. In this way, stress conditions could increase gastrointestinal motility and visceral sensitivity [15].

It has been proven that anxiety can affect many university students, particularly the ones affiliated with medical schools [16]. Indeed, medical students differ from the general population in terms of the high academic and professional standards placed on them. They are under constant stress due to the duration of their study plan characterized by higher work overload with physical and mental exhaustion. Recent studies conducted among medical students clearly report their psychological status concerning the present pandemic [17–19].

In this way, our study aimed to analyze GISs before and during the Italian lockdown for SARS-CoV-2 and the possible correlations of the lockdown on both the anxiety state and the referred changes in dietary habits in a population of students affiliated with the School of Medicine at the University "Magna Graecia" (UMG) of Catanzaro, Italy.

2. Materials and Methods

2.1. Study Design

We designed a web-based survey and eventually administered it to 354 medical students. Data has been acquired by using an online recruitment strategy over a period between 9 March 2020 and 4 May 2020. Our study was performed on 354 medical students, of which 111 males (31.4%) and 243 females (68.6%).

The following exclusion criteria were applied: (i) self-reported positivity to COVID-19, and (ii) subject not affiliated with the School of Medicine of the UMG, Italy. Both have been used to deny access to the survey, therefore, only the subjects who satisfied these criteria were allowed to complete the survey, while the others were discarded and unregistered. The second criterion has been checked limiting the access to the email domain released by UMG in order to check their affiliation. Our survey consists of 131 items, and it was designed as follow:

- 8 questions related to socio-demographic characteristics;
- 17 questions related to past medical history for GISs;
- 2 sets (before and during lockdown, respectively) of 17 questions related to the presence of GISs;
- 2 sets (before and during lockdown, respectively) of 10 questions to evaluate dietary habits;
- 2 sets (before and during lockdown, respectively) of 26 questions for anxiety evaluation, of these 12 indicate the common self-reported symptoms related to anxiety

and 14 represent the Short Health Anxiety Inventory (SHAI) test, a psychometrically sound tool for assessing health anxiety [20].

According to SHAI, the related answers are based on a Likert-type scale representing a score defined in a range 0–3 for never, rarely, sometimes, and often, respectively.

It was intended to collect data related to (i) socio-demographic characteristics, (ii) GISs, and (iii) psychometric parameters. An exhaustive representation of our questionnaire has been reported in Table S1 (see Supplementary Materials).

2.2. Statistical Analysis

Statistical analyses have been performed both to explore the relationships among the answers and to assess a correlation between the topics of interest over the SARS-CoV-2 lockdown: occurrence of GISs, and the influence of anxiety state on these symptoms. All the computations were carried out by using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). Each test is chosen according to the type of data representing the information of interest, as appropriate.

Likert-type data may be analyzed by using nonparametric tests such as Spearman's correlation, and Wilcoxon signed-rank test. Latter represents an optimal solution when independent variables consist of two categorical and related groups that make the application of a classic dependent *t*-test inappropriate. For instance, we use this test to correlate the GISs with the dietary habits within the same period. Otherwise, *t*-test and ANOVA have been used to analyze the changes over time; for instance, to evaluate the changes from pre-lockdown to during-lockdown, we use a Paired-samples *t*-test for scale data (e.g., level anxiety), and ANOVA for ordinal data (e.g., values in Likert-type scale). Each test has been chosen according to the type of data (e.g., ordinal, nominal, scale) to be analyzed and the period, as appropriate [21].

Furthermore, a preliminary assessment has been performed by using a descriptive analysis for summarizing data frequency and tendency. We codified the multiple-choice from raw text into integer values in order to define the related Likert-type scale assigning a progressive score for each answer. An independent-sample *t*-test has been performed on the anxiety level by using GISs as a grouping variable. It reports both the *t*-test for equality of means and Levene's test for equality of variances. Therefore, the anxiety state has been correlated with the presence of GISs by performing a bivariate correlation and a test of significance for two-tailed. Spearman's correlation has been used to study the psychometric parameters to evaluate the related anxiety state. For all subjects studied, a mathematical function has been applied in order to calculate the anxiety level by assigning to each answer a score; the final score is the sum of all scores related to psychometric evaluation. According to SHAI, we assumed the existence of an anxiety state for an anxiety level with a final score greater than or equal to 18. The periods before and during SARS-CoV-2 lockdown have been analyzed by performing an ANOVA test, to evaluate also how data changes within groups as changes in dietary habits, while a paired-samples *t*-test. Graphical representation of the figures is depicted by using contingency tables or crosstabs, in order to summarize the relationship between several categorical variables. Contingency tables do not report the statistical significance, as well as the bar chart used to summarize results in the before-during analysis. Therefore, the statistical significance has been evaluated by using the statistical tests described above for each set of data separately. We assume a value as statistically significant for a p-value less than 0.05.

2.3. Ethical Considerations

The study was conducted in accordance with the Helsinki Declaration. The participants received oral and written information regarding the study. All participants were informed that participation was voluntary and that they could withdraw at any time without consequences. The study protocol was approved by the local Research Ethics Committee (n.221/2020), and informed consent was obtained from all participants.

3. Results

Table S2 (see Supplementary Materials) summarizes demographic data related to subjects recruited for this study.

The statistical analysis conducted on our cohort showed that 54.0% of subjects interviewed, self-reported GISs. A clustered bar chart based on the contingency table related to the interactions between these symptoms is depicted in Figure 1 and shows 36.16% of the cohort students having positive history for familial GISs (FGISs). The 354 subjects in our sample may be grouped as follows: 25.99% GISs and FGISs, 27.97% GISs and no-FGDs, 10.17% no-GISs and FGISs, 35.87% no-GISs and no-FGISs. A nonparametric correlation based on the Spearman method has been performed to analyze the relationship between GISs and FGISs. Our results showed a high statistical significance between these parameters.

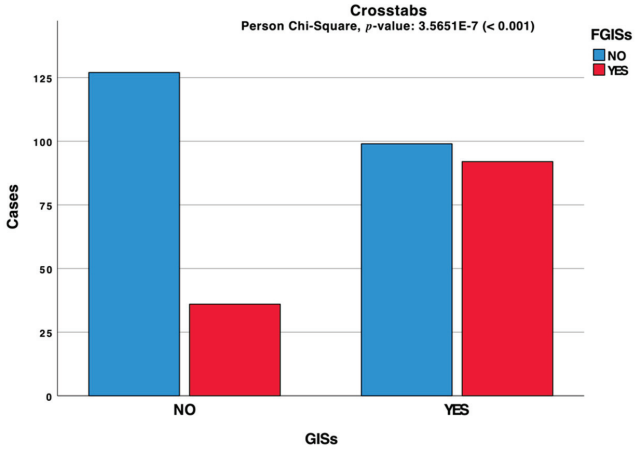


Figure 1. Clustered bar chart based on the contingency table related to the interactions between GISs and FGISs during the lockdown period.

The collected data confirmed a relationship between GISs and FGISs. A detailed analysis assessed an anxiety state for 48.9% of interviewed, of which 64.74% reported also GISs (Figure 2).

Other analyses have been performed to study the relationships between anxiety level and the presence of GISs, as well as between the latter and the anxiety state. Table 1 reports the frequency analysis for the related anxiety state during the lockdown. Table 2 reports the correlation between this one and the presence of GISs, during the lockdown.

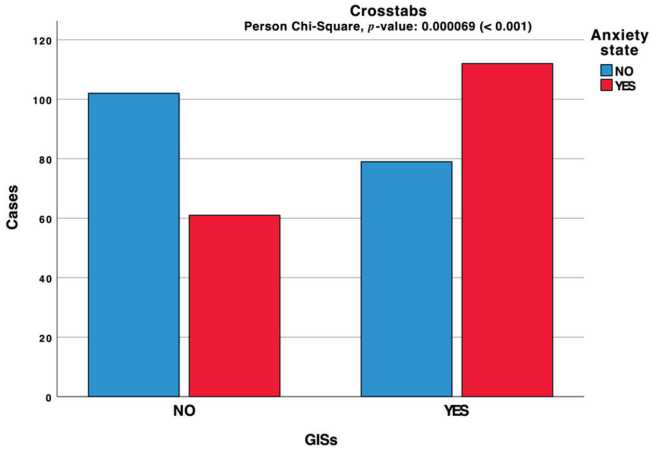


Figure 2. Clustered bar chart based on the contingency table related to the interactions between GISs and anxiety state during the lockdown period.

Table 1. Anxiety state: frequency analysis.

Group		Frequency	Percent	Valid Percent	Cumulative Percent
Before	NO	331	93.5	93.5	93.5
	YES	23	6.5	6.5	100.0
	Total	354	100.0	100.0	
During	NO	181	51.1	51.1	51.1
	YES	173	48.9	48.9	100.0
	Total	354	100.0	100.0	

Table 2. Correlation between anxiety state and presence of GISs.

			Presence of GISs
Spearman's rho	**Anxiety state**	**Correlation Coefficient**	0.212
		Sig. (2-tailed)	<0.001
		n	354

The anxiety level analysis (Table 3) shows that its mean value was 13.85 in pre-lockdown, while it increased to 18.18 during-lockdown (+31.26%); this increase was statistically significant.

Table 3. Anxiety level before and during the lockdown period.

Group		*n*	Mean	Std. Deviation	Sig. (2-Tailed)
Before	Anxiety level	354	13.85	2.997	<0.001
	Valid N (listwise)	354			
During	Anxiety level	354	18.12	7.290	
	Valid N (listwise)	354			

Furthermore, it has been correlated with the presence of GISs during the lockdown by performing an independent-sample t-test. Table 4 shows the related group statistics, while Table 5 shows both equality of means and Levene's test for equality of variances. GISs have been analyzed to study their evolution during the SARS-CoV-2 lockdown period. The

information related to the GISs and dietary habits has been evaluated before and during the SARS-CoV-2 lockdown period and correlated by performing a Wilcoxon signed-rank test.

Table 4. Correlation between anxiety level and presence of GISs during the lockdown: group statistics; see Table 5 for statistical significance.

	GISs	*n*	Mean	Std. Deviation	Std. Error Mean
Anxiety level	NO	163	16.08	6.357	0.498
	YES	191	19.85	7.595	0.550

Table 5. Correlation between anxiety level and presence of GISs during the lockdown: independent samples.

		Levene's Test for Equality of Variances		*t*-Test for Equality of Means				
		F	Sig.	t	df	Sig. (2-Tailed)	Mean Difference	Std. Error Difference
Anxiety level	Equal variances assumed	6.527	0.011	−5.018	352	<0.001	−3.774	0.752
	Equal variances not assumed			−5.089	351.874	<0.001	−3.774	0.742

The latter has been structured on two sets of answers, each one concerning the period before and during the lockdown. Tables 6 and 7 show the resultant output. For each pair of questions has been calculated if the correlation between these ones is statistically significant, thus if it may be used to provide evidence concerning the likelihood of their change during the lockdown period. Table 6 show that correlations between GISs before and during the SARS-CoV-2 lockdown are all statistically significant, while Table 7 show an increase in the consumption of specific food as meat, pizza, pre-cooked food, alcohol beverages, and sweetened drinks.

Table 6. Correlation between GISs before and during the SARS-CoV-2 lockdown.

GISs	*p*-Value
Diarrhea	<0.001
Constipation	<0.001
Pain During Defecation	<0.001
Blood in feces	0.003
Nausea	<0.001
Sickness	<0.001
Indigestion	<0.001
Heartburn (Pre-Meal)	<0.001
Heartburn (After-Meal)	<0.001
Stomachache	<0.001
Fullness and early safety	<0.001
Hypophagia	0.001
Hyperphagia	0.026
Dyspepsia	0.007
Abdominal inflation	<0.001
Abdominal cramps	<0.001

Table 7. Correlation between dietary habits before and during the SARS-CoV-2 lockdown period.

Dietary Items	*p*-Value
Fruits/Vegetables	0.183
Meat	0.015
Pasta	0.088
Pizza	<0.001
Desserts	0.505
Pre-Cooked Food	<0.001
Alcohol Beverages	<0.001
Soda	<0.001
Cheese and sausages	0.015

Furthermore, Figure 3 shows the data related to dietary habits, while the consequent onset of GISs is reported in Figure 4. Statistical significance related to before-during comparison has been reported in Table 7 for Figure 3, and in Table 6 for Figure 4. Data are in Likert-type scale, which represents the related score in a range 0–5 from "Never" to "Always", an increase in the score represents a worsening; for each alteration, the related *p*-value has also been reported.

Table 8 reports the analysis conducted on anxiety state before and during the lockdown. Anxiety level and anxiety state have been reported above within Tables 1–5.

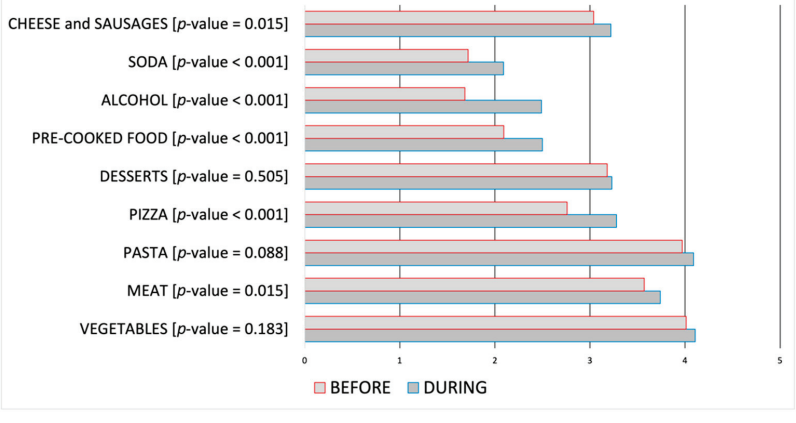


Figure 3. Dietary habits before and during the SARS-CoV-2 lockdown period, within the range 0 (never) to 5 (always).

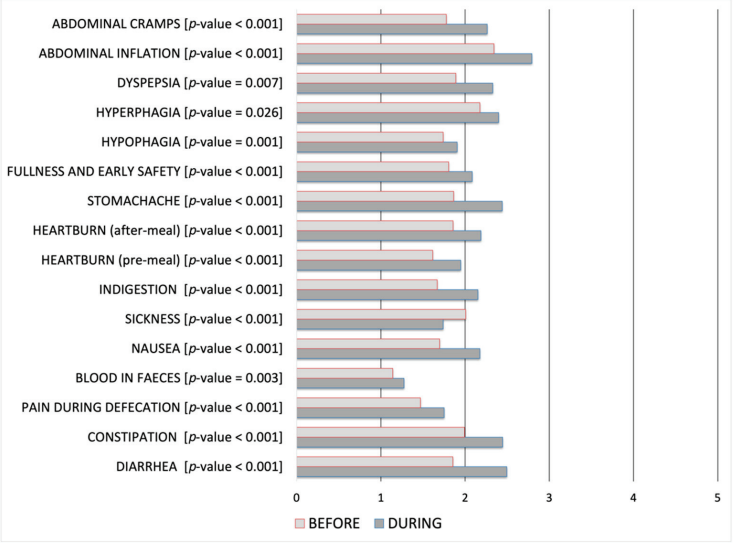


Figure 4. GISs before and during the SARS-CoV-2 lockdown period, within the range 0 (never) to 5 (always).

Table 8. Correlation between psychometric parameters before and during the SARS-CoV-2 lockdown period.

		t	df	Sig. (2-Tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Headache	Equal variances assumed	−9.955	706	<0.001	−0.805	0.081	−0.964	−0.646
	Equal variances not assumed	−9.955	697.555	<0.001	−0.805	0.081	−0.964	−0.646
Fainting or dizziness	Equal variances assumed	1.723	706	0.085	0.124	0.072	−0.017	0.266
	Equal variances not assumed	1.723	630.337	0.085	0.124	0.072	−0.017	0.266
Chest pain or discomfort	Equal variances assumed	<0.001	706	1.000	<0.001	0.074	−0.145	0.145
	Equal variances not assumed	<0.001	647.664	1.000	<0.001	0.074	−0.145	0.145
Back pain	Equal variances assumed	−8.094	706	<0.001	−0.686	0.085	−0.853	-0.520
	Equal variances not assumed	−8.094	704.141	<0.001	−0.686	0.085	−0.853	−0.520
Stomach pain and nausea	Equal variances assumed	−5.172	706	<0.001	−0.421	0.081	−0.581	−0.261
	Equal variances not assumed	−5.172	705.819	<0.001	−0.421	0.081	-0.581	−0.261
Muscle aches	Equal variances assumed	−14.273	706	<0.001	−0.975	0.068	−1.109	−0.841
	Equal variances not assumed	−14.273	672.848	<0.001	−0.975	0.068	−1.109	−0.841
Shortness of breath	Equal variances assumed	−8.587	706	<0.001	−0.556	0.065	−0.684	−0.429
	Equal variances not assumed	−8.587	701.109	<0.001	−0.556	0.065	−0.684	−0.429
Hot/Cold flash	Equal variances assumed	−8.408	706	<0.001	−0.551	0.066	−0.679	−0.422
	Equal variances not assumed	−8.408	701.436	<0.001	−0.551	0.066	−0.679	−0.422
Lump in the throat	Equal variances assumed	−8.393	706	<0.001	−0.554	0.066	−0.683	−0.424
	Equal variances not assumed	−8.393	695.004	<0.001	−0.554	0.066	−0.683	−0.424
Weakness	Equal variances assumed	−11.863	706	<0.001	−0.799	0.067	−0.932	−0.667
	Equal variances not assumed	−11.863	674.446	<0.001	−0.799	0.067	−0.932	−0.667
Feeling that your arms or legs are heavy	Equal variances assumed	−9.521	706	<0.001	−0.661	0.069	-0.797	-0.525
	Equal variances not assumed	−9.521	681.280	<0.001	−0.661	0.069	−0.797	−0.525
Abnormal sensations of numbness and tingling	Equal variances assumed	−19.020	706	<0.001	−1.130	0.059	−1.247	−1.013
	Equal variances not assumed	−19.020	519.731	<0.001	−1.130	0.059	−1.247	−1.013

4. Discussion

The COVID-19 pandemic represents the most important public health emergency of the contemporary era. As a consequence of this new global scenario, many people have been suffering from a spike of excruciating psychological issues [22]. The social distancing and quarantine during the COVID-19 pandemic in general and the lockdown period in particular, have limited the activities of the population with increased prevalence of mental disorders [23]. Depression and anxiety are the most common mental illnesses that impact negatively on the quality of life [24]. Several studies indicate a high prevalence of psychological disturbances among medical and non-medical populations [16,17,25]. A study including 1210 respondents from 194 cities in China found that 54% of respondents rated the psychological impact of the COVID-19 outbreak as moderate or severe, 29% reported moderate to severe anxiety symptoms, and 17% reported moderate to severe depressive symptoms [26].

On this basis, the aim of the present study was to investigate self-reported functional GISs of a medical student's population before and during the first Italian lockdown period and its potential correlation with the anxiety level and dietary habits. In this context, it is important to highlight that Italy was the first European country to announce severe nationwide limits on travel, as the government struggled to stem the spread of a COVID-19 outbreak. In our study, a set of 131 questions was performed on 354 medical students, of which 111 of whom males (31.4%) and 243 females (68.6%). Table S2 (see Supplementary Materials) summarizes demographic data of subjects recruited for this study. Two main reasons can explain the fact that the main part of our cohort is represented by females: (1) in Italy, the majority of medical students are women [27], and (2) females are more active in computer-mediated communication than men [28].

Our statistical analysis showed an anxiety state for 48.9% of interviewed, of which 64.74% report the association with GISs. Medical students show, in general, higher baseline rates of stress and anxiety, compared to the general population [16]. A systematic review of the prevalence of anxiety among this population outside of North America found a large range of prevalence between 7.7% and 65.5% [2]. A more recent meta-analysis analyzes the data from sixty-nine studies comprising 40,348 medical students and found that the global prevalence rate of anxiety among them was 33.8% [15]. Anxiety was most prevalent in the Middle East and Asia subjects, and the subgroup analyses by gender and year of study do not found statistical differences. Moreover, preliminary reports from the literature highlight how anxiety rates remain stable over the pandemic time among medical students or decrease when compared to non-medical ones [29]. This can be explained by a better knowledge of these subjects about incidence, prevalence, diagnosis, and treatments available for SARS-CoV-2 infection versus the non-medical students.

Also, we report statistically significant correlations between GISs before and during the SARS-CoV-2 lockdown for all evaluated symptoms (Table 6). Available literature data indeed note elevated psychiatric symptoms among university students, including anxiety during the lockdown period [30,31]. Our results confirm that stressful life events deeply influence the onset of anxiety in association with GISs. In particular, we found that all the GISs evaluated are statistically significant if compared before and during the lockdown. However, it must be taken into account that a pandemic is an exceptional situation that involves not only a single person or a community, but the worldwide population. Its long time influences and consequences on psychological and functional conditions of the global community are unknown.

Considered dietary habits, we detected not only the increased consumption of hypercaloric food as meat, pizza, pre-cooked food, and sweetened drinks but also alcoholic beverages, with a reduction in the intake of fruits and vegetables. Some recent studies carried out in different countries have reported modification in alimentary profiles associated with the lockdown. In particular, increased consumption of foods with high sugar content, such as chocolate and salty snacks, has been reported [32,33]. The possible explanation of these alimentary regimen changes can be explained to the increased levels

of anxiety and the difficulties to find open grocery stores close to home [34]. In addition, some evidence from the scientific literature indicates that GISs, and in particular abdominal pain and discomfort, are exacerbated by the intake of specific nourishment as foods rich in carbohydrates, fatty food agents such as alcohol beverages and spices [35]. In this way, we identify in our cohort an unhealthy alimentary pattern profile, that can be associated with the increased perception of GISs.

Finally, our study was conducted during the critical time of lockdown and social distancing, and to collect the data quickly, we selected a web-based survey method. This solution allows the direct guidance of respondents to a uniform resource locator and the students preferred online surveys [36]. Furthermore, it presents the advantages of saving time in its ease of use with limited cost, the ability to prevent errors, and finally, the rapid transmission of survey results [37]. An online survey may not allow the generalization of results, especially since anxiety and GISs may be due to many other factors other than the pandemic; it is also necessary to consider that the same nature of self-reported data, may result in response biases and in particular for anxiety assessment which may not always be as accurate as being assessed by a mental health professional. However, despite these limitations, our study provides information about the immediate psychological profile of Italian medical students to the COVID-19 pandemic in the gastroenterology setting.

5. Conclusions

Our data highlight the pivotal role of the gut-brain axis in human health and its interaction with the alimentary regimen and stressogenic life events to induce GISs. In particular, we highlight the role of stress conditions related to the lockdown, and we found that the concern for one's health is directly related to a worsening of the GISs in medical students. The data presented show the indirect effects of the COVID-19 pandemic on the gastrointestinal tract and emphasize the role of a prolonged stress condition on psychological health status. Our data provides evidence that a large percentage of medical students have been suffering from anxiety symptoms and changed their dietary habits, during the ongoing pandemic. In this way, a cumulative and critical analysis of the data published in the literature on this topic can be helpful to better define the factors based on GISs and anxiety onset. In the future, the COVID-19 picture may continue to influence people's lives, and the results of this study may assist in identifying medical students with functional problems, including GISs, so that they can be supported to cognitive-behavioral therapy.

Supplementary Materials: The following are available online at https://www.mdpi.com/2077-0383/10/6/1221/s1, Table S1: The survey consists of the following main categories: socio-demographic characteristics, GISs and psychometric parameters, Table S2: Descriptive characteristics of the respondent cohort.

Author Contributions: Conceptualization, L.A. and G.L.; methodology, L.A.; software, P.C.; validation, L.A., L.B. and P.C.; formal analysis, P.C.; investigation, L.A.; resources, E.B. and M.S.; data curation, P.C. and A.C.P.; writing—original draft preparation, P.C.; writing—review and editing, L.A., L.B. and S.F.; visualization, P.C., T.L., Y.L. and C.Z.; supervision, F.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Research Ethics Committee of University "Magna Graecia", Catanzaro, Italy (n.221/2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy limitation.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hui, D.S.C.; Zumla, A. Severe Acute Respiratory Syndrome: Historical, Epidemiologic, and Clinical Features. *Infect. Dis. Clin. N. Am.* **2019**, *33*, 869–889. [CrossRef]
2. Rabi, F.A.; Al Zoubi, M.S.; Kasasbeh, G.A.; Salameh, D.M.; Al-Nasser, A.D. SARS-CoV-2 and Coronavirus Disease 2019: What We Know So Far. *Pathogens* **2020**, *9*, 231. [CrossRef] [PubMed]
3. Malik, Y.S.; Kumar, N.; Sircar, S.; Kaushik, R.; Bhat, S.; Dhama, K.; Gupta, P.; Goyal, K.; Singh, M.P.; Ghoshal, U.; et al. Coronavirus Disease Pandemic (COVID-19): Challenges and a Global Perspective. *Pathogens* **2020**, *9*, 519. [CrossRef] [PubMed]
4. Słomka, A.; Kowalewski, M.; Żekanowska, E. Coronavirus Disease 2019 (COVID-19): A Short Review on Hematological Manifestations. *Pathogens* **2020**, *9*, 493. [CrossRef] [PubMed]
5. Gencer, S.; Lacy, M.; Atzler, D.; van der Vorst, E.P.C.; Döring, Y.; Weber, C. Immunoinflammatory, Thrombohaemostatic, and Cardiovascular Mechanisms in COVID-19. *Thromb. Haemost.* **2020**, *120*, 1629–1641. [CrossRef] [PubMed]
6. Nicola, M.; Alsafi, Z.; Sohrabi, C.; Kerwan, A.; Al-Jabir, A.; Iosifidis, C.; Agha, M.; Agha, R. The socio-economic implications of the coronavirus pandemic (COVID-19): A review. *Int. J. Surg.* **2020**, *78*, 185–193. [CrossRef] [PubMed]
7. Cucinotta, D.; Vanelli, M. WHO Declares COVID-19 a Pandemic. *Acta Biomed.* **2020**, *91*, 157–160. [CrossRef]
8. Abenavoli, L.; Cinaglia, P.; Luzza, F.; Gentile, I.; Boccuto, L. Epidemiology of Coronavirus Disease Outbreak: The Italian Trends. *Rev. Recent Clin. Trials.* **2020**, *15*, 87–92. [CrossRef]
9. D'Ettorre, G.; Ceccarelli, G.; Santinelli, L.; Vassalini, P.; Innocenti, G.P.; Alessandri, F.; Koukopoulos, A.E.; Russo, A.; d'Ettorre, G.; Tarsitani, L. Post-Traumatic Stress Symptoms in Healthcare Workers Dealing with the COVID-19 Pandemic: A Systematic Review. *Int. J. Environ. Res. Public Health* **2021**, *18*, 601. [CrossRef] [PubMed]
10. Addolorato, G.; Mirijello, A.; D'Angelo, C.; Leggio, L.; Ferrulli, A.; Abenavoli, L.; Vonghia, L.; Cardone, S.; Leso, V.; Cossari, A.; et al. State and trait anxiety and depression in patients affected by gastrointestinal diseases: Psychometric evaluation of 1641 patients referred to an internal medicine outpatient setting. *Int. J. Clin. Pract.* **2008**, *62*, 1063–1069. [CrossRef] [PubMed]
11. Larussa, T.; Flauti, D.; Abenavoli, L.; Boccuto, L.; Suraci, E.; Marasco, R.; Imeneo, M.; Luzza, F. The Reality of Patient-Reported Outcomes of Health-Related Quality of Life in an Italian Cohort of Patients with Inflammatory Bowel Disease: Results from a Cross-Sectional Study. *J. Clin. Med.* **2020**, *9*, 2416. [CrossRef]
12. Söderquist, F.; Syk, M.; Just, D.; Kurbalija Novicic, Z.; Rasmusson, A.J.; Hellström, P.M.; Ramklint, M.; Cunningham, J.L. A cross-sectional study of gastrointestinal symptoms, depressive symptoms and trait anxiety in young adults. *BMC Psychiatry* **2020**, *20*, 535. [CrossRef]
13. Tait, C.; Sayuk, G.S. The Brain-Gut-Microbiotal Axis: A framework for understanding functional GI illness and their therapeutic interventions. *Eur. J. Intern. Med.* **2021**, *84*, 1–9. [CrossRef]
14. Fung, C.; Vanden Berghe, P. Functional circuits and signal processing in the enteric nervous system. *Cell. Mol. Life Sci.* **2020**, *77*, 4505–4522. [CrossRef]
15. Huerta-Franco, M.R.; Vargas-Luna, M.; Montes-Frausto, J.B.; Morales-Mata, I.; Ramirez-Padilla, L. Effect of psychological stress on gastric motility assessed by electrical bio-impedance. *World J. Gastroenterol.* **2012**, *18*, 5027–5033. [CrossRef]
16. Quek, T.T.; Tam, W.W.; Tran, B.X.; Zhang, M.; Zhang, Z.; Ho, C.S.; Ho, R.C. The Global Prevalence of Anxiety Among Medical Students: A Meta-Analysis. *Int. J. Environ. Res. Public Health* **2019**, *16*, 2735. [CrossRef]
17. Almazan, A.N.; Chun, A.S.; Perez-Urbano, I. The Medical Student Response to the Mental Health Consequences of COVID-19. *Acad. Psychiatry* **2020**, *44*, 689–690. [CrossRef] [PubMed]
18. Sun, S.; Goldberg, S.B.; Lin, D.; Qiao, S.; Operario, D. Psychiatric symptoms, risk, and protective factors among university students in quarantine during the COVID-19 pandemic in China. *Glob. Health* **2021**, *17*, 15. [CrossRef] [PubMed]
19. Sheraton, M.; Deo, N.; Dutt, T.; Surani, S.; Hall-Flavin, D.; Kashyap, R. Psychological effects of the COVID 19 pandemic on healthcare workers globally: A systematic review. *Psychiatry Res.* **2020**, *292*, 113360. [CrossRef] [PubMed]
20. Salkovskis, P.M.; Rimes, K.A.; Warwick, H.M.; Clark, D.M. The Health Anxiety Inventory: Development and validation of scales for the measurement of health anxiety and hypochondriasis. *Psychol. Med.* **2002**, *32*, 843–853. [CrossRef]
21. Norman, G. Likert scales, levels of measurement and the "laws" of statistics. *Adv. Health Sci. Educ. Theory Pract.* **2010**, *15*, 625–632. [CrossRef] [PubMed]
22. Pfefferbaum, B.; North, C.S. Mental Health and the CoVid-19 Pandemic. *N. Engl. J. Med.* **2020**, *383*, 510–512. [CrossRef] [PubMed]
23. Hu, S.; Tucker, L.; Wu, C.; Yang, L. Beneficial Effects of Exercise on Depression and Anxiety During the Covid-19 Pandemic: A Narrative Review. *Front. Psychiatry* **2020**, *11*, 587557. [CrossRef]
24. Hohls, J.K.; König, H.H.; Quirke, E.; Hajek, A. Association between anxiety, depression and quality of life: Study protocol for a systematic review of evidence from longitudinal studies. *BMJ Open* **2019**, *9*, e027218. [CrossRef] [PubMed]
25. Tung, Y.J.; Lo, K.K.H.; Ho, R.C.M.; Tam, W.S.W. Prevalence of depression among nursing students: A systematic review and meta-analysis. *Nurse Educ. Today* **2018**, *63*, 119–129. [CrossRef]
26. Wang, C.; Pan, R.; Wan, X.; Tan, Y.; Xu, L.; Ho, C.S.; Ho, R.C. Immediate Psychological Responses and Associated Factors during the Initial Stage of the 2019 Coronavirus Disease (COVID-19) Epidemic among the General Population in China. *Int. J. Environ. Res. Public Health* **2020**, *17*, 1729. [CrossRef] [PubMed]
27. AlmaLaurea. National Survey of College Graduates. 2019. Available online: https://www.almalaurea.it (accessed on 3 March 2021).

28. Sun, B.; Mao, H.; Yin, C. Male and Female Users' Differences in Online Technology Community Based on Text Mining. *Front. Psychol.* **2020**, *11*, 806. [CrossRef]
29. Hope, V.; Henderson, M. Medical student depression, anxiety and distress outside North America: A systematic review. *Med. Educ.* **2014**, *48*, 963–979. [CrossRef]
30. Giusti, L.; Salza, A.; Mammarella, S.; Bianco, D.; Ussorio, D.; Casacchia, M.; Roncone, R. #Everything Will Be Fine. Duration of Home Confinement and "All-or-Nothing" Cognitive Thinking Style as Predictors of Traumatic Distress in Young University Students on a Digital Platform During the COVID-19 Italian Lockdown. *Front. Psychiatry* **2020**, *11*, 574812. [CrossRef]
31. Lasheras, I.; Gracia-García, P.; Lipnicki, D.M.; Bueno-Notivol, J.; López-Antón, R.; de la Cámara, C.; Lobo, A.; Santabárbara, J. Prevalence of Anxiety in Medical Students during the COVID-19 Pandemic: A Rapid Systematic Review with Meta-Analysis. *Int. J. Environ. Res. Public Health* **2020**, *17*, 6603. [CrossRef]
32. Miguel, A.Q.C.; Tempski, P.; Kobayasi, R.; Mayer, F.B.; Martins, M.A. Predictive factors of quality of life among medical students: Results from a multicentric study. *BMC Psychol.* **2021**, *9*, 36. [CrossRef] [PubMed]
33. Pérez-Rodrigo, C.; Gianzo Citores, M.; Hervás Bárbara, G.; Ruiz-Litago, F.; Casis Sáenz, L.; Arija, V.; López-Sobaler, A.M.; Martínez de Victoria, E.; Ortega, R.M.; Partearroyo, T.; et al. Patterns of Change in Dietary Habits and Physical Activity during Lockdown in Spain Due to the COVID-19 Pandemic. *Nutrients* **2021**, *13*, 300. [CrossRef] [PubMed]
34. Scarmozzino, F.; Visioli, F. Covid-19 and the Subsequent Lockdown Modified Dietary Habits of Almost Half the Population in an Italian Sample. *Foods* **2020**, *9*, 675. [CrossRef] [PubMed]
35. Okawa, Y.; Fukudo, S.; Sanada, H. Specific foods can reduce symptoms of irritable bowel syndrome and functional constipation: A review. *Biopsychosoc. Med.* **2019**, *13*, 10. [CrossRef] [PubMed]
36. McLean, S.F. Case-Based Learning and its Application in Medical and Health-Care Fields: A Review of Worldwide Literature. *J. Med. Educ. Curric. Dev.* **2016**, *3*, JMECD–S20377. [CrossRef] [PubMed]
37. Geldsetzer, P. Use of Rapid Online Surveys to Assess People's Perceptions During Infectious Disease Outbreaks: A Cross-sectional Survey on COVID-19. *J. Med. Internet Res.* **2020**, *22*, e18790. [CrossRef] [PubMed]

Journal of
Clinical Medicine

MDPI

Article

Carotid Plaque Assessment Reclassifies Patients with Inflammatory Bowel Disease into Very-High Cardiovascular Risk

Alejandro Hernández-Camba [1,†], Marta Carrillo-Palau [2,†], Laura Ramos [2], Noemi Hernández Alvarez-Buylla [2], Inmaculada Alonso-Abreu [2], Anjara Hernández-Pérez [2], Milagros Vela [1], Laura Arranz [1], Manuel Hernández-Guerra [2], Miguel Ángel González-Gay [3,4,5,‡] and Iván Ferraz-Amaro [6,*,‡]

1 Division of Gastroenterology, Hospital Universitario de Nuestra Señora de la Candelaria, 38010 Tenerife, Spain; dr.alejandrohc@gmail.com (A.H.-C.); milvelillas@yahoo.es (M.V.); lauraarranz@gmail.com (L.A.)
2 Division of Gastroenterology, Hospital Universitario de Canarias, 38320 Tenerife, Spain; martacarry@yahoo.es (M.C.-P.); laura7ramos@gmail.com (L.R.); noehdz78@hotmail.com (N.H.A.-B.); macuaa@hotmail.com (I.A.-A.); anjarahdez@gmail.com (A.H.-P.); mhernand@ull.edu.es (M.H.-G.)
3 Division of Rheumatology, Hospital Universitario Marqués de Valdecilla, Universidad de Cantabria, 39008 Santander, Spain; miguelaggay@hotmail.com
4 Epidemiology, Genetics and Atherosclerosis Research Group on Systemic Inflammatory Diseases, Hospital Universitario Marqués de Valdecilla, IDIVAL, 39008 Santander, Spain
5 Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg 2193, South Africa
6 Division of Rheumatology, Hospital Universitario de Canarias, 38320 Tenerife, Spain
* Correspondence: iferrazamaro@hotmail.com
† These authors contributed equally to this work and both share first authorship.
‡ These authors share senior authorship.

Citation: Hernández-Camba, A.; Carrillo-Palau, M.; Ramos, L.; Hernández Alvarez-Buylla, N.; Alonso-Abreu, I.; Hernández-Pérez, A.; Vela, M.; Arranz, L.; Hernández-Guerra, M.; González-Gay, M.Á.; et al. Carotid Plaque Assessment Reclassifies Patients with Inflammatory Bowel Disease into Very-High Cardiovascular Risk. *J. Clin. Med.* **2021**, *10*, 1671. https://doi.org/10.3390/jcm10081671

Academic Editor: Marilena Durazzo

Received: 13 March 2021
Accepted: 7 April 2021
Published: 13 April 2021

Abstract: The addition of carotid ultrasound into cardiovascular (CV) risk scores has been found to be effective in identifying patients with chronic inflammatory diseases at high-CV risk. We aimed to determine if its use would facilitate the reclassification of patients with inflammatory bowel disease (IBD) into the very high-CV-risk category and whether this may be related to disease features. In this cross-sectional study encompassing 186 IBD patients and 175 controls, Systematic Coronary Risk Evaluation (SCORE), disease activity measurements, and the presence of carotid plaques by ultrasonography were assessed. Reclassification was compared between patients and controls. A multivariable regression analysis was performed to evaluate if the risk of reclassification could be explained by disease-related features and to assess the influence of traditional CV risk factors on this reclassification. After evaluation of carotid ultrasound, a significantly higher frequency of reclassification was found in patients with IBD compared to controls (35% vs. 24%, $p = 0.030$). When this analysis was performed only on subjects included in the SCORE low-CV-risk category, 21% IBD patients compared to 11% controls ($p = 0.034$) were reclassified into the very high-CV-risk category. Disease-related data, including disease activity, were not associated with reclassification after fully multivariable regression analysis. Traditional CV risk factors showed a similar influence over reclassification in patients and controls. However, LDL-cholesterol disclosed a higher effect in controls compared to patients (beta coef. 1.03 (95%CI 1.02–1.04) vs. 1.01 (95%CI 1.00–1.02), interaction $p = 0.035$) after adjustment for confounders. In conclusion, carotid plaque assessment is useful to identify high-CV risk IBD patients.

Keywords: inflammatory bowel disease; SCORE; carotid plaques; cardiovascular risk

1. Introduction

Inflammatory bowel disease (IBD), which includes both Crohn's disease (CD) and ulcerative colitis (UC), is characterized by chronic inflammation of the gastrointestinal tract. There is growing evidence that IBD patients have a higher incidence of cardiovascular

(CV) events compared to the general population [1–4]. This occurs despite having a lower burden of traditional CV risk factors [5]. Systemic inflammation load may play an important role in the process of accelerated atherosclerosis in these patients [6]. It leads to increased oxidative stress and elevated levels of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), yielding phenotypic changes in smooth muscle cells and promoting the development of atherosclerosis and CV disease in IBD patients [7]. In addition to the endogenous factors and cytokines, it has been suggested that due to the compromised intestinal mucosal barrier, endotoxins, and bacterial lipopolysaccharides produced by intestinal microflora can enter into circulation and activate inflammatory responses that lead to atherosclerosis [8].

Predictive scoring algorithms for CV disease, such as the European Systematic Coronary Risk Assessment (SCORE), have been found to underestimate the actual CV risk of patients with chronic inflammatory diseases [9,10]. In this sense, the 2016 European Guidelines for the Prevention of Cardiovascular Diseases in Clinical Practice proposed the evaluation of the plaque of the carotid artery by ultrasound as a practical way to reclassify those people for whom the SCORE may underestimate their actual CV risk [11]. This is because the presence of carotid plaque is considered an excellent tool to identify high-CV risk in patients included in the SCORE category of moderate CV risk. This is due to the fact that the assessment of carotid plaque burden with ultrasound has been demonstrated to be predictive of CV events because peripheral arterial disease represents an independent risk factor for CV death. For this reason, reclassifying the CV risk category, and identifying patients at a very high risk, would allow more intense preventive measures to be taken. In this regard, previous studies on rheumatoid arthritis, the prototype of chronic inflammatory disease, demonstrated that patients categorized as moderate-CV risk according to the SCORE often present with carotid plaques and, consequently, they should be included in the very high-CV risk category [12,13].

To the best of our knowledge, there is no extensive information on the predictive value of risk charts algorithms, in particular the SCORE, to identify IBD patients at high-CV risk [14,15]. This is also the case for the possible relevance of the presence of carotid plaques to reclassify patients with IBD as having high-CV risk. Taking together all these considerations, in the present study, we aimed to determine how the carotid ultrasound assessment may help identify IBD patients who were included in the categories of low and intermediate-CV risk when the SCORE was applied. We additionally assessed if the reclassification into the very-high CV risk due to the presence of carotid plaques may be influenced by disease characteristics, in particular by the activity of the disease.

2. Materials and Methods

2.1. Study Participants

The cross-sectional study included 186 consecutive patients with IBD and 175 controls. All of them were 18 years old or older and had a clinical diagnosis of IBD based upon clinical, endoscopic, and histological criteria at least within the previous 12 months. They had been diagnosed by gastroenterologists and were periodically followed-up at the Gastroenterology Outpatient Clinics of Hospital Universitario de Canarias and Hospital Universitario Nuestra Señora de La Candelaria. For the purpose of inclusion in the present study, IBD disease duration had to be $\geq$1 year. Although long-term anti-TNF-α therapy has been associated with a decreased risk of acute arterial events in patients with IBD [7], those undergoing anti-TNF-α, or other biological therapies, were not excluded from the present study. Likewise, since glucocorticoids are used in the management of IBD, patients taking prednisone were not excluded. The controls were community-based, recruited by general practitioners in primary health centers. Controls with family history of any inflammatory bowel disease or other autoimmune disorder were excluded. None of the patients and controls had established CV disease such as coronary heart disease (angina or myocardial infarction) or heart failure, strokes or transient ischemic attack, peripheral arterial disease, and aortic disease such as aortic aneurysm. Since diabetes mellitus is

considered an equivalent of very-high CV risk, diabetic patients and controls were also excluded. The study protocol was approved by the Institutional Review Committee at Hospital Universitario de Canarias and Hospital Universitario Nuestra Señora de La Candelaria, both in Spain, and all subjects provided informed written consent (approval no. CHUC_2019_103). Research carried out with human subjects was in compliance with the Helsinki Declaration.

2.2. Assessments and Data Collection

Surveys in IBD patients and controls were performed to assess CV risk factors and medication. Hypertension was defined as a systolic or a diastolic blood pressure higher than 140 and 90 mmHg, respectively. Dyslipidemia was defined if one of the following factors was present: total cholesterol >200 mg/dL, triglycerides >150 mg/dL, HDL-cholesterol <40 mg/dL in men or <50 mg/dL in women, or LDL-cholesterol >130 mg/dL. Standard techniques were used to measure serum lipids, high-sensitivity C-reactive protein (CRP), and fecal calprotectin. Additionally, it was registered if the patient had a recent colonoscopy or magnetic resonance enterography. Disease activity in CD was assessed through Crohn's Disease Activity Index (CDAI) and the Harvey-Bradshaw Index (HBI) [16]. CDAI was broken down into asymptomatic remission (0 to 149 points), mildly to moderately active (150 to 220), moderately to severely active (221 to 450 points), and severely active to fulminant disease (451 to 1100 points) categories as previously described [17]. Similarly, the Harvey–Bradshaw Index was categorized as remission (0 to 4 points), mildly active disease (5 to 7 points), moderately active disease (8 to 16 points), and severely active disease (17 to 100 points) [16]. Disease activity in UC was calculated through the partial Mayo Clinic score [18]. In patients with IBD, physical activity was assessed through the International Physical Activity Questionnaire (IPAQ) short form, and data was presented as metabolic equivalent-of-task (MET)-minutes per week [19]. All data acquisition regarding fecal calprotectin assessment and questionnaires evaluation, including carotid ultrasound, were performed in the same visit day.

2.3. Carotid Ultrasound Assessment

Carotid ultrasound was performed to determine carotid intima media thickness (cIMT) in the common carotid artery and to detect focal plaques in the extracranial carotid tree both in patients with IBD and in controls [12,13]. A commercially available scanner, Mylab 70, (Esaote, Genoa, Italy) equipped with a 7–12 MHz linear transducer and an automated software-guided radiofrequency technique—Quality Intima Media Thickness in real-time (QIMT, Esaote, Maastricht, Holland)—was used for this purpose. Based on the Mannheim consensus, plaque criteria in the accessible extracranial carotid tree (common carotid artery, bulb, and internal carotid artery) were defined as follows: a focal protrusion in the lumen measuring at least cIMT >1.5 mm; a protrusion at least 50% greater than the surrounding cIMT; or an arterial lumen encroaching >0.5 mm [20].

2.4. Statistical Analysis

Patients and controls with carotid plaques based on ultrasound assessment were reclassified into very-high CV risk category. Subjects without plaques were maintained in their original SCORE category. cIMT was not used to determine reclassification because according to current guidelines [11], cIMT is not considered an unequivocal CV disease on imaging. Demographic and clinical characteristics were shown as frequencies for binary variables. Continuous variables data were expressed as mean ± standard deviation (SD) or as a median and interquartile range (IQR) for non-normally distributed variables. Univariable differences between patients and controls were assessed through Student's *t*-test, U Mann–Whitney, Chi squared, or Fisher Exact tests according to normal distribution or the number of subjects. Logistic regression analysis adjusted for the variables with a *p*-value below 0.20 in the univariate analysis was performed to assess the relation of IBD disease-related data with the presence of reclassification. Interaction factors were added to

the regression models when we addressed the comparison of the effect (beta coefficients) between controls and IBD patients. All of the analyses used a 5% two-sided significance level and were performed using SPSS software, v. 25 (IBM, Chicago, IL, USA) and STATA software, v.15/SE (Stata Corp., College Station, TX, USA). A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Demographic, Laboratory, and Disease-Related Data

A total of 186 IBD patients and 175 sex-matched controls with a mean ± SD age of 45 ± 12 and 48 ± 10 years, respectively, were included in this study. Demographic and disease-related characteristics of the participants are shown in Table 1. Body mass index (27 ± 5 vs. 26 ± 4 kg/m^2, p = 0.008) and waist circumference (93 ± 12 vs. 90 ± 14 cm, p = 0.048) were higher in IBD patients than controls. In this regard, whereas there were not differences in the prevalence of smoking or hypertension, patients with IBD were more commonly obese (27% vs. 13%, p = 0.001) and more frequently met the definition for dyslipidemia (73% vs. 56%, p = 0.017).

Table 1. Characteristics of patients with inflammatory bowel disease (IBD) and controls.

IBD Patients	Controls (n = 175)	IBD Patients (n = 186)	p-Value
Age, years	45 ± 12	48 ± 10	**0.009**
Male, n (%)	89 (51)	85 (46)	0.30
Body mass index, kg/m^2	26 ± 4	27 ± 5	**0.008**
Abdominal circumference, cm	90 ± 14	93 ± 12	0.066
Systolic blood pressure, mmHg	125 ± 15	125 ± 19	0.83
Diastolic blood pressure, mmHg	78 ± 9	74 ± 12	**0.000**
Cardiovascular co-morbidity			
Current smokers, n (%)	32 (18)	36 (19)	0.84
Hypertension, n (%)	20 (11)	31 (17)	0.16
Dyslipidemia, n (%)	98 (56)	136 (73)	**0.004**
Obesity, n (%)	22 (13)	50 (27)	**0.001**
Laboratory and lipid profile			
CRP, mg/L	0.8 (0.5–2.0)	1.8 (0.9–3.6)	**0.030**
Cholesterol, mg/dL	200 ± 34	204 ± 49	0.37
Triglycerides, mg/dL	103 ± 55	147 ± 88	**0.000**
HDL-cholesterol, mg/dL	58 ± 17	57 ± 18	0.53
LDL-cholesterol, mg/dL	120 ± 31	117 ± 40	0.44
LDL:HDL cholesterol ratio	2.22 ± 0.87	2.17 ± 0.86	0.60
Non-HDL cholesterol, mg/dL	142 ± 36	146 ± 43	0.25
Atherogenic index	3.64 ± 1.06	3.77 ± 1.16	0.27
IBD related data			
Crohn's disease, n (%)		127 (68)	
Ulcerative colitis, n (%)		59 (32)	
Disease duration since diagnosis, years		12 (8–19)	
Crohn's Disease related data, n (%)			
A1 below 16 years		19 (15)	
A2 between 17 and 40 years		79 (62)	
A3 above 40 years		55 (43)	
L1 ileal		55 (43)	
L2 colonic		23 (18)	
L3 ileocolonic		49 (39)	
L4 isolated upper disease		11 (9)	
B1 non-stricturing, non-penetrating		71 (56)	
B2 stricturing		45 (35)	
B3 penetrating		14 (11)	

Table 1. *Cont.*

IBD Patients	Controls (n = 175)	IBD Patients (n = 186)	p-Value
CDAI score		39 (7–85)	
Asymptomatic remission		113 (90)	
Mildly to moderately active CD		10 (8)	
Moderately to severely active CD		3 (2)	
Severely active to fulminant disease		0 (0)	
Harvey-Bradshaw Index		2 (0–4)	
Clinical remission		103 (82)	
Mildly active disease		14 (11)	
Moderately active disease		8 (6)	
Severely active disease		1 (1)	
Ulcerative Colitis related data, *n* (%)			
Proctosigmoiditis		6 (10)	
Left-sided colitis		22 (37)	
Pancolitis		29 (49)	
Partial Mayo score		1 (0–1)	
<2		45 (76)	
≥2		14 (24)	
Fecal calprotectin, mcg/g			
<120		58 (31)	
≥120		68 (37)	
Perianal disease, *n* (%)		22 (12)	
Previous surgery, *n* (%)		54 (29)	
Extraintestinal manifestations		53 (28)	
Arthritis, *n* (%)		34 (18)	
Uveitis, *n* (%)		4 (2)	
Erythema nodosum, *n* (%)		4 (2)	
Psoriasis, *n* (%)		5 (3)	
Current prednisone, *n* (%)		6 (3)	
Prednisone, mg/day		8 (5–20)	
Oral Mesalazine, *n* (%)		60 (32)	
Methotrexate, *n* (%)		21 (11)	
Azathioprine, *n* (%)		58 (31)	
Anti-TNF therapy, *n* (%)		56 (30)	
Adalimumab, n (%)		23 (12)	
Infliximab, n (%)		33 (18)	
Ustekinumab, *n* (%)		8 (4)	
Vedolizumab, *n* (%)		5 (3)	
Tofacitinib, *n* (%)		4 (2)	
Certolizumab, *n* (%)		1 (1)	
Carotid intima media assessment			
Carotid plaque, *n* (%)	43 (25)	62 (33)	0.067
bilateral, *n* (%)	19 (11)	30 (16)	0.14
cIMT, microns	604 ± 115	641 ± 137	**0.006**

Data represent means ± SD or median (interquartile range) when data were not normally distributed. BMI: body mass index; CRP: C reactive protein; LDL: low-density lipoprotein. HDL: high-density lipoprotein; TNF: tumor necrosis factor; cIMT: carotid intima media. CDAI: Crohn's Disease Activity Index. Dyslipidemia was defined if one of the following was present: total cholesterol > 200 mg/dL, triglycerides > 150 mg/dL, HDL-cholesterol < 40 in men or <50 mg/dL in women, or LDL-cholesterol > 130 mg/dL. CDAI was categorized as 0 to 149: Asymptomatic remission; 150 to 220 points: Mildly to moderately active; 221 to 450 points: Moderately to severely active; 451 to 1100 points: Severely active to fulminant disease. Harvey–Bradshaw Index was categorized as 0 to 4 points Clinical remission; 5 to 7 points: Mildly active disease; 8 to 16 points: Moderately active disease; 17 to 100 points: Severely active disease. Extraintestinal manifestations refer to those related to musculoskeletal, dermatological or ocular systems. Significant p-values are depicted in bold.

The median disease duration of IBD was 12 years (IQR 8–19). CD patients had mostly the ileal and non-stricturing, non-penetrating, types. Median CDAI score was 39 (IQR 7–85), and 89% of the patients were considered to be in the asymptomatic remission category. Similarly, the Harvey–Bradshay Index was 2 (IQR 0–4), and most of the patients (81%) were in the remission category of this index. Regarding UC, 49% were pancolitis, and 76% of the

patients had a partial Mayo score inferior to 2 points. Additional information regarding disease-related data is shown in Table 1.

Concerning carotid ultrasound assessment, 33% of the IBD patients had carotid plaques compared to 25% of controls (p = 0.067). The average cIMT in patients and controls was 641 ± 137 mm and 604 ± 115 mm, respectively (p = 0.006).

3.2. SCORE Risk Category Reclassification after Carotid Sonography

Following SCORE risk chart stratification, 124 (67%) patients and 133 (76%) controls were included in the low-CV risk category. Only 2 controls and 1 patient fulfilled the definition for very high-CV risk when the risk charts were applied (Table 2). Interestingly, carotid ultrasound assessments disclosed a significantly higher frequency of reclassification in IBD patients compared to controls (34% vs. 24%, p = 0.030). In this regard, 26 of the 124 patients (21%) and 15 of the 133 controls (11%) who met the definition of low CV risk, according to the SCORE risk tables, had carotid plaques; consequently, they were reclassified in the very high-risk category (21% vs. 11%, p = 0.034). Thirty of 55 patients (55%) and 17 of 28 controls (61%) (p = 0.59) included in the moderate-CV risk SCORE category had carotid plaques and were also reclassified into the very high-CV risk category. Similarly, 8 of 11 IBD patients (73%) and 6 of 6 controls (100%) included in the high-CV risk SCORE category prior to carotid ultrasound assessment were reclassified into the very high-risk category once that this test was performed (p = 0.52) (Table 2).

Table 2. Systematic Coronary Risk Evaluation (SCORE) risk category reclassification after carotid ultrasound assessment in patients with inflammatory bowel disease (IBD) and controls.

Initial Score Risk Category		Risk Category after Carotid Ultrasound Assessment				% Patients Reclassified	p
		Low	Moderate	High	Very High		
Controls							
Low	133	118			15	11%	
Moderate	28		11		17	61%	
High	11			3	8	73%	
Very High	2				2	-	
Total	174	118	11	3	42	24%	
IBD patients							
Low	124	98			26	21%	**0.034**
Moderate	55		25		30	55%	0.59
High	6			0	6	100%	0.52
Very High	1				1	-	-
Total	186	98	25	0	63	34%	**0.030**

SCORE: Systematic Coronary Risk Evaluation; IBD: inflammatory bowel disease. *P*-values in every risk category represent the comparison between patients and controls for that category. Significant *p*-values are given in bold.

3.3. Differences in the Effect of Traditional CV Risk Factors on Reclassification in Controls and IBD Patients

Most of the demographics, traditional CV risk factors, and lipid profile-related molecules were associated with reclassification in both patients and controls (Table 3). However, the magnitude of these relations differed between patients and controls. In this sense, the beta coefficients of male gender, body mass index (BMI), systolic blood pressure, and dyslipidemia, in their relation to reclassification, were higher in controls when compared to patients with IBD. However, when differences between populations were assessed through the addition of interaction factors into the regression model, beta coefficients were not found to be different.

Table 3. Differences in the effect of traditional cardiovascular risk factors on reclassification in IBD patients and controls.

IBD Patients	Reclassified after Carotid Ultrasound			
	OR (95% CI), *p*		Interaction	
	Controls	IBD	Univariable	Adjusted
Demographics				
Age, years	**1.13 (1.08–1.18), 0.000**	**1.13 (1.08–1.18), 0.000**	0.94	
Male, *n* (%)	**2.79 (1.31–5.95), 0.008**	1.58 (0.85–2.91), 0.15	0.25	
Body mass index, kg/m^2	**1.12 (1.03–1.21), 0.010**	1.02 (0.96–1.08), 0.59	0.078	0.79
Abdominal circumference, cm	**1.05 (1.02–1.08), 0.001**	**1.03 (1.00–1.06), 0.034**	0.29	
Systolic blood pressure, mmHg	**1.04 (1.01–1.06), 0.003**	**1.03 (1.01–1.04), 0.005**	0.44	
Diastolic blood pressure, mmHg	1.04 (1.00–1.08), 0.065	1.02 (1.00–1.05), 0.087	0.58	
Cardiovascular co-morbidity				
Smoking, *n* (%)	1.13 (0.46–2.75), 0.80	1.57 (0.74–3.30), 0.24	0.58	
Hypertension, *n* (%)	2.52 (0.95–6.69), 0.063	**2.97 (1.35–6.53), 0.007**	0.80	
Dyslipidemia, *n* (%)	**2.47 (1.11–5.50), 0.026**	2.01 (0.94–4.28), 0.071	0.71	
Obesity, *n* (%)	0.72 (0.23–2.25), 0.57	1.04 (0.53–2.07), 0.91	0.58	
Laboratory and lipid profile				
CRP, mg/L	0.96 (0.85–1.09), 0.55	0.99 (0.92–1.06), 0.77	0.71	
Cholesterol, mg/dL	**1.02 (1.01–1.03), 0.001**	**1.01 (1.00–1.01), 0.035**	**0.044**	0.086
Triglycerides, mg/dL	**1.01 (1.00–1.01), 0.020**	**1.00 (1.00–1.01), 0.013**	0.43	
HDL cholesterol, mg/dL	**0.97 (0.95–0.99), 0.044**	0.99 (0.97–1.01), 0.29	0.30	
LDL cholesterol, mg/dL	**1.03 (1.02–1.04), 0.000**	1.01 (1.00–1.02), 0.057	**0.009**	**0.035**
LDL:HDL cholesterol ratio	**2.62 (1.62–4.18), 0.000**	**1.91 (1.31–2.79), 0.001**	0.32	
Non-HDL cholesterol, mg/dL	**1.02 (1.01–1.04), 0.000**	**1.01 (1.00–1.02), 0.005**	0.059	0.21
Atherogenic index	**2.00 (1.38–2.90), 0.000**	**1.61 (1.21–2.14), 0.001**	0.36	

Reclassification is considered the dependent variable in the logistic regression analysis. Interaction is adjusted for age and sex. Significant *p*-values are given in bold. BMI: body mass index; CRP: C reactive protein; LDL: low-density lipoprotein. HDL: high-density lipoprotein; TNF: tumor necrosis factor; cIMT: carotid intima media. Dyslipidemia was defined if one of the following was present: total cholesterol > 200 mg/dL, triglycerides > 150 mg/dL, HDL-cholesterol < 40 in men or <50 mg/dL in women, or LDL-cholesterol > 130 mg/dL.

Almost all the lipid profile related-molecules were associated with reclassification in both patients and controls. In general, beta coefficients were higher in controls compared to patients, but statistical significance was not reached. Only the effect of LDL-cholesterol on reclassification was found to be greater in controls compared to IBD patients (beta coef. 1.03 (95%CI 1.02–1.04) vs. 1.01 (95%CI 1.00–1.02), interaction $p = 0.035$) after multivariable analysis adjusting for age and sex (Table 3).

3.4. IBD-Related Features Association with Reclassification

Disease-related features were not related to reclassification (Table 4). In this sense, neither disease duration, disease activity scores, laboratory data such as calprotectin, nor the different treatments used were not associated with reclassification. Only the onset of CD after 40 years and the use of methotrexate were significantly associated with reclassification in the univariable analysis. However, after adjustment in a fully multivariable analysis, these relationships were lost.

Table 4. Disease-related data association with reclassification.

IBD Patients	Reclassified after Carotid Ultrasound	
	OR (95%CI), *p*	
	Unadjusted	Adjusted *
Disease duration since diagnosis, years	1.02 (0.99–1.05), 0.31	
log METs/week	1.28 (0.91–1.80), 0.15	1.20 (0.86–1.97), 0.21
Crohn's Disease related data		
A1 below 16 years	0.45 (0.14–1.45), 0.18	0.99 (0.26–3.75), 0.99
A2 between 17 and 40 years	0.81 (0.39–1.68), 0.57	
A3 above 40 years	**2.70 (1.12–6.48), 0.026**	0.87 (0.27–2.75), 0.81
L1 ileal	0.94 (0.45–1.95), 0.86	
L2 colonic	0.97 (0.38–2.49), 0.95	
L3 ileocolonic	1.10 (0.53–2.30), 0.80	
L4 isolated upper disease	0.66 (0.17–2.63), 0.56	
B1 non-stricturing, non-penetrating	0.96 (0.46–1.98), 0.91	
B2 stricturing	0.87 (0.40–1.86), 0.71	
B3 penetrating	0.70 (0.21–2.38), 0.57	
CDAI score	1.00 (0.99–1.00), 0.40	
Asymptomatic remission	-	
Mildly to moderately active	0.42 (0.09–2.09), 0.29	
Moderately to severely active	-	
Severely active to fulminant disease	-	
log Harvey score	0.78 (0.49–1.25), 0.30	
Clinical remission	-	
Mildly active disease	0.45 (0.12–1.71), 0.24	
Moderately active disease	0.55 (0.11–2.86), 0.48	
Severely active disease	-	
Ulcerative colitis-related data		
Proctosigmoiditis	1.23 (0.20–7.46), 0.82	
Left-sided colitis	1.21 (0.38–3.86), 0.74	
Pancolitis	0.61 (0.19–1.90), 0.39	
log Partial Mayo score	0.94 (0.37–2.39), 0.90	
<2	-	
≥2	1.64 (0.50–5.43), 0.41	
Fecal calprotectin, mcg/g		
Perianal disease	0.93 (0.36–2.40), 0.87	
Previous surgery	1.41 (0.73–2.73), 0.31	
Extraintestinal manifestations	1.27 (0.64–2.51), 0.50	
Arthritis	1.07 (0.49–2.32), 0.86	
Uveitis	1.97 (0.27–14.30), 0.50	
Erythema nodosum	-	
Psoriasis	0.48 (0.05–4.36), 0.51	
Current prednisone	0.39 (0.50–3.41), 0.40	
Prednisone, mg/day	-	
Oral mesalazine	1.12 (0.58–2.14), 0.74	
Methotrexate	**3.07 (1.22–7.74), 0.018**	1.71 (0.55–5.33), 0.36
Azathioprine	0.86 (0.44–1.67), 0.66	
Anti-TNF therapy	0.73 (0.37–1.45), 0.37	
Adalimumab	0.38 (0.12–1.17), 0.093	0.48 (0.14–1.67), 0.25
Infliximab	1.18 (0.54–2.58), 0.68	
Ustekinumab	0.66 (0.13–3.35), 0.61	
Vedolizumab	1.34 (0.22–8.26), 0.75	
Tofacitinib	-	
Certolizumab	-	

Reclassification is considered the dependent variable in the logistic regression analysis. TNF: tumor necrosis factor; CDAI: Crohn's Disease Activity Index. Significant *p*-values are given in bold. * Adjusted for age, sex, hypertension, and dyslipidemia (variables with a *p*-value < 0.20 in their relation with reclassification in Table 3).

4. Discussion

Patients with IBD have a higher risk of atherosclerosis and, consequently, a higher risk of CV events. For this reason, the identification of IBD patients at high-CV risk is crucial. Our work is the first study that includes the use of carotid ultrasound to reclassify the CV risk of IBD patients. According to our results, the effect of the detection of carotid plaques by carotid ultrasound, which allows the reclassification of individuals in the category of very high CV risk, is observed more frequently in patients with IBD than in controls.

Carotid ultrasound has been previously used for the reclassification of the CV risk of patients with chronic inflammatory diseases. In this regard, in an earlier work of our group that included 343 patients diagnosed with ankylosing spondylitis and 177 controls, patients were more likely reclassified into the very high-CV risk category than controls following carotid ultrasound assessment [21]. In addition, patients with psoriatic arthritis were more frequently reclassified into very high-CV risk following carotid ultrasound assessment than controls [22]. In these patients with psoriatic arthritis, the reclassification was independently explained by the disease activity. Likewise, in a cross-sectional study that included 276 patients with systemic lupus erythematosus, following carotid ultrasound assessment, 32% of the patients were reclassified into the very high-CV risk category [10]. In systemic lupus erythematosus patients, disease duration and damage were independently associated with a higher risk of reclassification. The findings shown in the present study go in the same direction and confirm that carotid ultrasound is useful to identify IBD patients at high risk of CV disease.

IBD-related features have been associated with CV risk. For example, in a nationwide population-based cohort of 28,833 individuals diagnosed with IBD compared with IBD-free individuals, a markedly increased risk of ischemic heart disease was seen within the first year after IBD diagnosis [23]. The risk of ischemic heart disease was lower among patients with IBD using 5-aminosalicylic acid than among non-users, in particular in the group of oral glucocorticoid users, which was used as a proxy for disease severity. Likewise, patients treated surgically or with thiopurines and TNF alpha inhibitors tended to have reduced incidence rate ratio for ischemic heart disease. In another report, patients exposed to anti-TNF therapy compared to those not exposed, but not to thiopurines, were associated with a lower risk of acute arterial events [7]. The cross-sectional nature of our study and the fact that most of our patients were in clinical remission at the time of the assessment may explain why IBD-related characteristics did not show an effect on CV risk reclassification. Perhaps, mechanisms related to the disease itself that are not captured by the clinical manifestations that we registered are responsible for this greater reclassification. However, IBD follows a natural course with alternating periods of remission and relapse. This reinforces the claim that the disease itself, characterized by a chronic pro-inflammatory state, even in the latent stages of the disease, may explain a higher risk of CV reclassification, due to a greater risk of severe atherosclerotic disease in these patients. The fact that in our study, some traditional CV risk factors, such as the presence of dyslipidemia and the body mass index, had a greater effect in controls than in patients suggests that chronic inflammation may be the main mechanism that leads to an increased risk of reclassification in patients with IBD. However, we cannot exclude that a genetic component may also contribute to increased risk of CV disease in IBD, as has been described in other chronic inflammatory diseases [24,25].

In a recent report of the European Society of Hypertension Working Group on Vascular Structure and Function and the ARTERY Society (Association for Research into Arterial Structure, Physiology) evidence regarding the vascular consequences of inflammation has been extensively reviewed [8]. In this statement, it has been demonstrated that immune-mediated mechanisms related to inflammation influence arterial physiology and lead to vascular dysfunction such as atherosclerosis and arterial stiffening. Moreover, it is shown that chronic inflammatory diseases such as rheumatoid arthritis, IBD, and psoriasis are accompanied by profound arterial dysfunction, which is proportional to the severity of inflammation. Taking this into account, we believe that our findings regarding a higher

reclassification into the very-high CV risk category after carotid ultrasound assessment in patients with IBD may be driven by the inflammation present in this disease.

As mentioned above, the cross-sectional nature of the present study is a limitation that does not allow us to know if patients with IBD in whom their risk was reclassified will develop CV events. However, it was recently confirmed that reclassification into very high-CV risk by identifying carotid plaques after carotid ultrasound [12,13] is useful as the best predictor of future CV events in prospectively followed-up patients with rheumatoid arthritis. Another possible limitation may be that the patients and controls were not the same age. However, the size effect of this difference was small (3 years). In this regard, it should be noted that SCORE risk values can be compared between populations of different ages because SCORE is already weighted by age. Furthermore, all the multivariable analyses performed in the present study were adjusted for age.

Finally, in our study, only 34 patients (18%) had arthritis involvement in the form of spondyloarthritis or other types of arthritis associated with IBD. This small number of patients with arthritis constituted another limitation that precluded a comparison of CV risk between them and those without arthritis.

In conclusion, the use of carotid ultrasound in patients with IBD allows identifying IBD patients at very high risk of CV disease. Due to this, we propose the use of carotid ultrasound as an additional tool for the identification of subclinical atherosclerosis in these patients.

Author Contributions: Conceptualization, I.F.-A., M.C.-P., A.H.-C., M.Á.G.-G., M.H.-G.; Methodology, I.F.-A., M.Á.G.-G.; Formal Analysis, I.F.-A., A.H.-C., M.C.-P.; Data Curation, N.H.A.-B., I.A.-A., A.H.-P., M.V., L.R., L.A.; Writing—Original Draft Preparation, I.F.-A., M.Á.G.-G.; Writing—Review and Editing, I.F.-A., M.Á.G.-G.; Funding Acquisition, I.F.-A., M.C.-P., A.H.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a grant to I.F-A. from the Spanish Ministry of Health, Subdirección General de Evaluación y Fomento de la Investigación, Plan Estatal de Investigación Científica y Técnica y de Innovación 2013–2016 and by Fondo Europeo de Desarrollo Regional—FEDER—(Fondo de Investigaciones Sanitarias, FIS PI14/00394, PI17/00083).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Hospital Universitario de Canarias and Hospital Nuestra Señora de la Candelaria (protocol code CHUC_2019_103 date of approval 4 February 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest. Nevertheless, Iván Ferraz-Amaro would like to acknowledge that he has received grants/research supports from Abbott, MSD, Jansen and Roche, as well as consultation fees from company sponsored speakers bureaus associated with Abbott, Pfizer, Roche, Sanofi, Celgene and MSD. M.A. González-Gay has received grants/research supports from Abbvie, MSD, Jansen and Roche, as well as consultation fees/participation from company-sponsored speakers bureaus tied to Abbvie, Pfizer, Roche, Sanofi, Lilly, Celgene and MSD.

References

1. Kirchgesner, J.; Beaugerie, L.; Carrat, F.; Andersen, N.N.; Jess, T.; Schwarzinger, M.; Bouvier, A.M.; Buisson, A.; Carbonnel, F.; Cosnes, J.; et al. Increased risk of acute arterial events in young patients and severely active IBD: A nationwide French cohort study. *Gut* **2018**, *67*, 1261–1268. [CrossRef]
2. Singh, S.; Singh, H.; Loftus, E.V.; Pardi, D.S. Risk of cerebrovascular accidents and ischemic heart disease in patients with inflammatory bowel disease: A systematic review and meta-analysis. *Clin. Gastroenterol. Hepatol.* **2014**, *12*, 382–393. [CrossRef]
3. Yarur, A.J.; Deshpande, A.R.; Pechman, D.M.; Tamariz, L.; Abreu, M.T.; Sussman, D.A. Inflammatory bowel disease is associated with an increased incidence of cardiovascular events. *Am. J. Gastroenterol.* **2011**, *106*, 741–747. [CrossRef] [PubMed]
4. Feng, W.; Chen, G.; Cai, D.; Zhao, S.; Cheng, J.; Shen, H. Inflammatory bowel disease and risk of ischemic heart disease: An updated meta-analysis of cohort studies. *J. Am. Heart Assoc.* **2017**, *6*, e005892. [CrossRef] [PubMed]

5. Aniwan, S.; Pardi, D.S.; Tremaine, W.J.; Loftus, E.V. Increased Risk of Acute Myocardial Infarction and Heart Failure in Patients With Inflammatory Bowel Diseases. *Clin. Gastroenterol. Hepatol.* **2018**, *16*, 1607–1615. [CrossRef]
6. Hansson, G.K. Inflammation, Atherosclerosis, and Coronary Artery Disease. *N. Engl. J. Med.* **2005**, *352*, 1685–1695. [CrossRef]
7. Kirchgesner, J.; Nyboe Andersen, N.; Carrat, F.; Jess, T.; Beaugerie, L. Risk of acute arterial events associated with treatment of inflammatory bowel diseases: Nationwide French cohort study. *Gut* **2020**, *69*, 852–858. [CrossRef] [PubMed]
8. Zanoli, L.; Briet, M.; Empana, J.P.; Cunha, P.G.; Mäki-Petäjä, K.M.; Protogerou, A.D.; Tedgui, A.; Touyz, R.M.; Schiffrin, E.L.; Spronck, B.; et al. Vascular consequences of inflammation. *J. Hypertens.* **2020**, *38*, 1682–1698. [CrossRef]
9. Arts, E.E.A.; Popa, C.; Den Broeder, A.A.; Semb, A.G.; Toms, T.; Kitas, G.D.; Van Riel, P.L.; Fransen, J. Performance of four current risk algorithms in predicting cardiovascular events in patients with early rheumatoid arthritis. *Ann. Rheum. Dis.* **2015**, *74*, 668–674. [CrossRef] [PubMed]
10. Quevedo-Abeledo, J.C.; Rúa-Figueroa, Í.; Sánchez-Pérez, H.; Tejera-Segura, B.; De Vera-González, A.; González-Delgado, A.; Llorca, J.; González-Gay, M.A.; Ferraz-Amaro, I. Disease damage influences cardiovascular risk reclassification based on carotid ultrasound in patients with systemic lupus erythematosus. *J. Rheumatol.* **2019**, *46*, 483–491. [CrossRef]
11. Piepoli, M.F.; Hoes, A.W.; Agewall, S.; Albus, C.; Brotons, C.; Catapano, A.L.; Cooney, M.T.; Corrà, U.; Cosyns, B.; Deaton, C.; et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice. *Eur. Heart J.* **2016**, *37*, 2315–2381. [CrossRef]
12. Corrales, A.; González-Juanatey, C.; Peiró, M.E.; Blanco, R.; Llorca, J.; González-Gay, M.A. Carotid ultrasound is useful for the cardiovascular risk stratification of patients with rheumatoid arthritis: Results of a population-based study. *Ann. Rheum. Dis.* **2014**, *73*, 722–727. [CrossRef]
13. Corrales, A.; Parra, J.A.; González-Juanatey, C.; Rueda-Gotor, J.; Blanco, R.; Llorca, J.; González-Gay, M.A. Cardiovascular risk stratification in rheumatic diseases: Carotid ultrasound is more sensitive than Coronary Artery Calcification Score to detect subclinical atherosclerosis in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **2013**, *72*, 1764–1770. [CrossRef]
14. González-Gay, M.A.; González-Juanatey, C. Cardiovascular risk factor assessment: Still an unmet need in chronic inflammatory diseases. *Heart* **2016**, *102*, 1937–1939. [CrossRef]
15. Emanuel, G.; Charlton, J.; Ashworth, M.; Gulliford, M.C.; Dregan, A. Cardiovascular risk assessment and treatment in chronic inflammatory disorders in primary care. *Heart* **2016**, *102*, 1957–1962. [CrossRef] [PubMed]
16. Harvey, R.F.; Bradshaw, J.M. A Simple Index of Crohn's-Disease Activity. *Lancet* **1980**, *315*, 514. [CrossRef]
17. Lichtenstein, G.R.; Loftus, E.V.; Isaacs, K.L.; Regueiro, M.D.; Gerson, L.B.; Sands, B.E. ACG Clinical Guideline: Management of Crohn's Disease in Adults. *Am. J. Gastroenterol.* **2018**, *113*, 481–517. [CrossRef] [PubMed]
18. Schroeder, K.W.; Tremaine, W.J.; Ilstrup, D.M. Coated Oral 5-Aminosalicylic Acid Therapy for Mildly to Moderately Active Ulcerative Colitis. *N. Engl. J. Med.* **1987**, *317*, 1625–1629. [CrossRef]
19. Craig, C.L.; Marshall, A.L.; Sjöström, M.; Bauman, A.E.; Booth, M.L.; Ainsworth, B.E.; Pratt, M.; Ekelund, U.; Yngve, A.; Sallis, J.F.; et al. International physical activity questionnaire: 12-Country reliability and validity. *Med. Sci. Sports Exerc.* **2003**, *35*, 1381–1395. [CrossRef]
20. Touboul, P.-J.; Hennerici, M.G.; Meairs, S.; Adams, H.; Amarenco, P.; Bornstein, N.; Csiba, L.; Desvarieux, M.; Ebrahim, S.; Fatar, M.; et al. Mannheim carotid intima-media thickness consensus (2004–2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium. 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. *Cerebrovasc. Dis.* **2007**, *23*, 75–80. [CrossRef] [PubMed]
21. Rueda-Gotor, J.; Quevedo-Abeledo, J.C.; Corrales, A.; Genre, F.; Hernández-Hernández, V.; Delgado-Frías, E.; González-Gay, M.A.; Ferraz-Amaro, I. Reclassification into very-high cardiovascular risk after carotid ultrasound in patients with axial spondyloarthritis. *Clin. Exp. Rheumatol.* **2020**, *38*, 724–731. [PubMed]
22. Palmou-Fontana, N.; Martínez-Lopez, D.; Corrales, A.; Rueda-Gotor, J.; Genre, F.; Armesto, S.; González-López, M.A.; Quevedo-Abeledo, J.C.; Portilla-González, V.; Blanco, R.; et al. Disease Activity Influences Cardiovascular Risk Reclassification Based on Carotid Ultrasound in Patients with Psoriatic Arthritis. *J. Rheumatol.* **2020**, *47*, 1344–1353. [CrossRef] [PubMed]
23. Rungoe, C.; Basit, S.; Ranthe, M.F.; Wohlfahrt, J.; Langholz, E.; Jess, T. Risk of ischaemic heart disease in patients with inflammatory bowel disease: A nationwide Danish cohort study. *Gut* **2013**, *62*, 689–694. [CrossRef] [PubMed]
24. López-Mejías, R.; Carmona, F.D.; Genre, F.; Remuzgo-Martínez, S.; González-Juanatey, C.; Corrales, A.; Vicente, E.F.; Pulito-Cueto, V.; Miranda-Filloy, J.A.; Ramírez Huaranga, M.A.; et al. Identification of a 3′-Untranslated Genetic Variant of RARB Associated with Carotid Intima-Media Thickness in Rheumatoid Arthritis: A Genome-Wide Association Study. *Arthritis Rheumatol.* **2019**, *71*, 351–360. [CrossRef] [PubMed]
25. López-Mejías, R.; Castañeda, S.; González-Juanatey, C.; Corrales, A.; Ferraz-Amaro, I.; Genre, F.; Remuzgo-Martínez, S.; Rodriguez-Rodriguez, L.; Blanco, R.; Llorca, J.; et al. Cardiovascular risk assessment in patients with rheumatoid arthritis: The relevance of clinical, genetic and serological markers. *Autoimmun. Rev.* **2016**, *15*, 1013–1030. [CrossRef]

Journal of
Clinical Medicine

MDPI

Article

Prevalence and Clinical Characteristics of Dyssynergic Defecation and Slow Transit Constipation in Patients with Chronic Constipation

Samuel Tanner, Ahson Chaudhry, Navneet Goraya, Rohan Badlani, Asad Jehangir, Dariush Shahsavari, Zubair Malik and Henry P. Parkman *

Section of Gastroenterology, Department of Medicine, Lewis Katz School of Medicine at Temple University, Philadelphia, PA 19140, USA; Samuel.Tanner@uhhospitals.org (S.T.); achaudhry@temple.edu (A.C.); tug69854@temple.edu (N.G.); rohan.badlani@temple.edu (R.B.); asadjehangir@gmail.com (A.J.); Dariush.Shahsavari@tuhs.temple.edu (D.S.); zubair.malik@tuhs.temple.edu (Z.M.)
* Correspondence: henry.parkman@temple.edu; Tel.: +1-215-707-7579

Abstract: Patients with chronic constipation who do not respond to initial treatments often need further evaluation for dyssynergic defecation (DD) and slow transit constipation (STC). The aims of this study are to characterize the prevalence of DD and STC in patients referred to a motility center with chronic constipation and correlate diagnoses of DD and STC to patient demographics, medical history, and symptoms. High-resolution ARM (HR-ARM), balloon expulsion testing (BET) and whole gut transit scintigraphy (WGTS) of consecutive patients with chronic constipation were reviewed. Patients completed questionnaires describing their medical history and symptoms at the time of testing. A total of 230 patients completed HR-ARM, BET, and WGTS. Fifty (22%) patients had DD, and 127 (55%) patients had STC. Thirty patients (13%) had both DD and STC. There were no symptoms that were suggestive of STC vs. DD; however, patients with STC and DD reported more severe constipation than patients with normal transit and anorectal function. Patients with chronic constipation often need evaluation for both DD and STC to better understand their pathophysiology of symptoms and help direct treatment.

Keywords: dyssynergic defecation; constipation; slow transit constipation; motility; colonic transit; anorectal manometry; gastrointestinal disorders

Citation: Tanner, S.; Chaudhry, A.; Goraya, N.; Badlani, R.; Jehangir, A.; Shahsavari, D.; Malik, Z.; Parkman, H.P. Prevalence and Clinical Characteristics of Dyssynergic Defecation and Slow Transit Constipation in Patients with Chronic Constipation. *J. Clin. Med.* **2021**, *10*, 2027. https://doi.org/10.3390/jcm10092027

Academic Editor: Marilena Durazzo

Received: 20 April 2021
Accepted: 6 May 2021
Published: 9 May 2021

1. Introduction

Constipation is a common disorder in Americans: primary constipation, e.g., constipation that is not secondary to another underlying disease or medication, is present in 12–17% of the population [1]. These patients may fail medical therapy and are referred to gastroenterology for evaluation and management. History and physical examination have been suggested to be poor predictors of underlying pathophysiology; further assessment of these patients is often suggested for two common etiologies–slow colon transit and dyssynergic defecation [2].

Two techniques commonly used in the evaluation of patients with constipation are anorectal manometry (ARM) to assess pelvic floor function and colonic transit testing [3]. In ARM, rectal pressures are recorded during balloon inflation and deflation as well as during a simulation of defecation with balloon distension in the rectum, which are used to describe defecation patterns in patients. This test is then used in conjunction with other testing such as balloon expulsion testing and/or defecography to identify underlying anorectal floor pathophysiology, such as dyssynergic defecation, and help direct treatment such as biofeedback therapy [4]. Assessment of colonic transit can be performed by radioopaque markers, wireless motility capsule, or scintigraphy. Whole gut transit scintigraphy (WGTS) assesses colonic transit as well as gastric emptying and small bowel transit [5]. The colonic

transit result is often characterized as normal transit or a severe colonic transit abnormality suggesting colonic inertia, generalized colonic transit delay, and functional rectosigmoid obstruction [6].

In general, patients with chronic constipation can have distinct phenotypes, including slow transit constipation (as assessed by WGTS or other colonic transit study), dyssynergic defecation (as assessed by ARM and other tests of anorectal function), a combination of slow transit constipation and dyssynergic defection, no slow transit constipation or dyssynergic defecation (i.e., normal colonic transit and normal anorectal function). However, few studies have examined the prevalence of these phenotypes or the association of these diagnoses with findings of WGTS and HR-ARM. Moreover, the literature has conflicting data on the association between symptoms and underlying pathophysiology of constipation [7,8]. The aims of this study were to: (1) Assess the prevalence of DD and STC in patients referred to a motility center with chronic constipation; (2) Correlate diagnoses of DD and STC to patient demographics, medical history, and symptoms.

2. Materials and Methods

2.1. Patients

We retrospectively analyzed consecutive patients who underwent whole gut transit scintigraphy and high-resolution anorectal manometry for the evaluation of chronic constipation at Temple University Hospital Motility Center between 1 January 2016 and 31 December 2019. Exclusion criteria included history of surgery on the GI tract, pregnancy, age <18 years, inability to complete testing, or a primary indication other than constipation for testing (such as abdominal pain).

2.2. Questionnaires

Patients were asked to fill out several questionnaires at the time of testing. These questionnaires assessed the patient's demographic profile, current medications, medical and surgical history, upper GI symptom severity via the Patient Assessment of Upper Gastrointestinal Disorders-Symptom Severity Index (PAGI-SYM) with four additional domains including constipation, diarrhea, belching, and flatulence [9]. Symptoms over the prior two weeks were graded by the patient at the time of colonic transit scintigraphy from none = 0 to very severe = 5. The 20-item PAGI-SYM questionnaire was used to calculate composite scores of six symptom domains. The Rome III diagnostic questionnaire for lower GI disorders was also used [10]. Additionally, the frequency of habits related to the patient's bowel movements were also assessed.

2.3. Balloon Expulsion Testing

Balloon expulsion testing was performed [11]. A 4 cm balloon was inserted into the rectum and filled with 50 cc of water. The patient was then sent to the bathroom and asked to measure the amount of time with a stopwatch it took to expulse the balloon. Abnormal balloon expulsion was defined as longer than 60 seconds [12].

2.4. High-Resolution Anorectal Manometry

High-resolution anorectal manometry (HR-ARM) was performed [13–15]. A 4.2 mm diameter solid-state catheter consisting of 12 circumferential sensors (10 sensors at 6 mm intervals along the anal canal and 2 sensors in the rectal balloon was used to measure pressure profiles, reflexes, and sensation in the anorectal region (Medtronic, Inc., Shoreview, MN, USA). The patient was placed in the left lateral decubitus position with their knees flexed. The catheter was inserted and advanced until the high-pressure zone of the internal anal sphincter was localized. This was followed by a 2-minute period of stabilization to allow anal tone to return to baseline. Each patient was then asked to squeeze the anus 2 times for 20 seconds at a time to simulate holding in a stool (volitional contraction) to measure volitional external anal sphincter contraction pressure. Graded balloon distension testing was performed by measuring the basal anal sphincter pressure and then inflating the

rectal balloon by 10 mL to first sensation point then intervals of 30 mL to each subsequent sensation point (desire to defecate, urge to defecate, and maximum tolerance). The presence of the rectoanal inhibitory reflex (relaxation of the internal anal sphincter during rectal distension [RAIR]) was also recorded. The patient was then asked to bear down 3 times (20 seconds each time) to simulate defecation. During bear down maneuvers, the intrarectal pressure and internal sphincter percent relaxation were recorded. Internal anal sphincter percent relaxation was defined as the ratio of amount of anal relaxation to anal resting pressure $\times$ 100 [11]. Dyssynergic defecation was defined as an abnormal balloon expulsion test as well as an abnormal pattern of defecation identified on anorectal manometry by a combination of either incomplete relaxation or paradoxical contraction of the anal sphincter with either inadequate or adequate generation of intra-rectal pressure during bear-down maneuver [16].

2.5. Whole Gut Transit Scintigraphy

Whole gut transit scintigraphy was performed [6,17]. Patients stop any constipation medications for 3 days prior to the study and come in fasting after midnight. Patients consumed a dual-isotope test meal consisting of an egg beater meal labeled with 500 uCi of Tc-99m sulfur colloid added to the egg white portion of the meal. The meal also consisted two pieces of white-bread toast and jam. The liquid portion of the meal consisted of 100 uCi of In-111 DTPA in 6 oz of water. Imaging occurred at 0, 0.5, 1, 2, 3, 4 h to evaluate for gastric emptying, and at 5 and 6 h to evaluate for small bowel transit. Images were then obtained at 24, 48, and 72 h after meal ingestion to determine colonic transit by evaluating geometric centers of colonic activity [18].

Gastric emptying was quantified as the percentage of meal remaining in the stomach region of interest, with delayed gastric emptying defined as >10% of meal remaining at 4 h. Small bowel transit was defined as the percentage of meal remaining prior to the ileocecum at 6 h, with >40% defined as normal small bowel transit. For the colonic images, counts were measured in regions of interest corresponding to the cecum/ascending colon (region 1), hepatic flexure (region 2), transverse colon (region 3), splenic flexure (region 4), descending colon (region 5), and rectosigmoid (region 6). Administered radioactivity that was unaccounted for in the images was assumed to have been eliminated by bowel movements and was designated as region 7. The geometric center for colonic activity was calculated as the summation of scintigraphic counts at each region of interest as a fraction of the total counts, weighted by that region's assigned number. Slow transit constipation was defined as a geometric center $\leq$4.6 at 48 h [19].

2.6. Data Analysis

For multi-population comparisons of continuous variables, the ANOVA test followed by pairwise t tests were used for normally distributed data while the Kruskal–Wallis test followed by Dunn method was used for non-normally distributed data. The Bonferroni correction was applied to adjust for multiple comparisons. Categorical variables were compared using Fisher's exact test. For multi-population testing using Fisher's exact test, post hoc adjusted residuals were calculated. Statistical testing was executed using Microsoft Excel (Microsoft Corp, Redmond, WA, USA) and SPSS (IBM Corp, Armonk, NY, USA).

3. Results

A total of 230 patients completed WGTS, BET and HR-ARM and met inclusion criteria for this study. The mean age of our cohort was 47.5 years, comprised of 89% women, and had a mean BMI of 25.9 kg/m^2. The median duration of constipation symptoms was 2.0 years (interquartile range, 1.0 to 5.0 years).

3.1. Pathophysiology Using BET, HR-ARM and Colonic Transit Scintigraphy

Of the 230 patients, 20 patients (9%) had dyssynergic defecation, 97 patients (42%) had slow transit constipation, 30 patients (13%) had both dyssynergic defecation and slow

transit constipation, and 83 patients (36%) had neither dyssynergic defecation nor slow transit constipation (Figure 1 and Table 1). In total, 50 patients had dyssynergic defecation, of whom 30 (60%) also had slow transit constipation. Conversely, 127 patients had slow transit constipation, of whom 30 (24%) also had dyssynergic defecation.

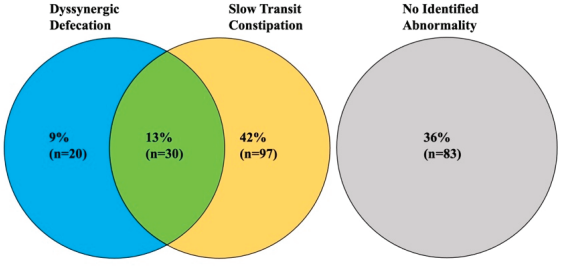


Figure 1. Prevalence of DD and STC in patients presenting to our center for chronic constipation.

Table 1. Comparison of patients with combined vs. singular diagnoses (slow transit constipation and dyssynergic defecation vs. slow transit constipation and normal anorectal function or dyssynergic defecation and normal colonic transit or normal anorectal function and normal colonic transit).

	STC + DD	STC Only	DD Only	No STC or DD	*p*-Value
n	**30**	**97**	**20**	**83**	**–**
Demographics					
Age (mean ± SE)	49.9 ± 3.5	48.0 ± 1.5	47.4 ± 2.3	46.4 ± 1.9	0.88
Gender (% female)	97%	94%	85%	81%	0.02
BMI (mean ± SE)	26.3 ± 1.5	24.9 ± 0.7	25.3 ± 1.0	27.1 ± 0.7	0.10
Race					
White	87%	84%	80%	81	
Black	0%	4%	15%	%1%	0.16
Other	10%	7%	5%	12%	
Unknown	3%	5%	0%	6%	
Past Medical History					
Diabetes	7%	13%	30%	16%	0.17
Anxiety or Depression	20%	23%	45%	29%	0.18
Other psych	10%	12%	10%	7%	0.72
GERD	13%	23%	20%	20%	0.56
Thyroid Disease	13%	14%	10%	6%	0.29
Connective tissue disease	0%	5%	10%	6%	0.39
Whole Gut Transit Scintigraphy					
Gastric Emptying					
2h (%)	41.9 ± 3.2	47.4 ± 2.0	43.2 ± 4.5	38.2 ± 2.2	0.04
4h (%)	13.0 ± 1.9	15.9 ± 1.8	12.5 ± 3.3	9.6 ± 1.3	<0.01
% Delayed	48%	45%	35%	29%	0.10
Small Bowel Transit (% Delayed)	20%	27%	35%	22%	0.55
Colonic Transit (GC)					
24h	3.1 ± 0.1	3.1 ± 0.1	4.8 ± 0.3	4.9 ± 0.1	<0.001
48h	3.6 ± 0.1	3.6 ± 0.1	6.0 ± 0.2	6.1 ± 0.1	<0.001
72h	4.2 ± 0.2	4.0 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	<0.001
Anorectal Manometry					
Mean resting pressure (mmHg)	70.7 ± 3.6	65.2 ± 2.1	78.7 ± 6.5	72.1 ± 2.3	0.03
Maximal squeeze pressure (mmHg)	129.1 ± 11.1	130.5 ± 6.6	135.5 ± 11.4	136.7 ± 7.1	0.82
Intrarectal pressure (mmHg)	51.0 ± 4.1	53.3 ± 2.6	63.5 ± 7.5	61.1 ± 3.7	0.27
% Anal Relaxation	7.6 ± 3.5	23.1 ± 2.3	4.6 ± 5.3	19.3 ± 3.1	<0.001
Rectal Sensitivity Testing					
First sensation (mL)	41.3 ± 8.5	24.0 ± 2.2	25.0 ± 5.5	21.0 ± 1.9	0.19
First desire (mL)	85.5 ± 11.1	64.6 ± 4.0	58.7 ± 8.2	49.9 ± 3.9	<0.01
First urge (mL)	135.0 ± 10.4	109.0 ± 4.7	117.4 ± 12.1	93.5 ± 4.5	<0.01
Maximum tolerance (mL)	162.1 ± 9.6	145.3 ± 4.9	158.9 ± 12.0	134.4 ± 4.9	0.04
RAIR (% not present)	0%	5%	10%	6%	0.44
Abnormal BET	100%	94%	100%	93%	0.99

Table 1. *Cont.*

	STC + DD	STC Only	DD Only	No STC or DD	*p*-Value
n	**30**	**97**	**20**	**83**	**–**
Symptoms					
Duration (median, IQR [years])	1 (1–3)	3 (1–11)	2 (1–10)	2 (1–3)	0.08
BMs per week	1.3 ± 0.2	2.0 ± 0.2	2.4 ± 1.0	4.9 ± 0.6	<0.001
Abdominal Pain (1d/wk or greater)	100%	96%	100%	97%	0.99
Urinary leakage (1d/wk or greater)	27%	33%	18%	17%	0.23
Fecal leakage (1d/wk or greater)	5%	5%	0%	8%	0.90
Fecal urgency (1d/wk or greater)	14%	18%	45%	24%	0.18
Symptom Severity [1]					
Constipation	4.7 ± 0.1	4.4 ± 0.1	4.5 ± 0.2	3.9 ± 0.2	0.02
Diarrhea	0.8 ± 0.3	0.6 ± 0.2	1.8 ± 0.5	1.4 ± 0.2	<0.01
Belching	2.2 ± 0.4	2.2 ± 0.2	3.1 ± 0.5	2.6 ± 0.2	0.27
Flatulence	2.3 ± 0.3	2.6 ± 0.2	2.8 ± 0.4	2.5 ± 0.2	0.68
Regurgitation and heartburn	1.5 ± 0.2	1.5 ± 0.2	2.2 ± 0.4	1.6 ± 0.2	0.42
Fullness and early satiety	3.6 ± 0.2	3.1 ± 0.2	3.4 ± 0.3	3.0 ± 0.2	0.26
Nausea & vomiting	2.0 ± 0.3	1.7 ± 0.2	2.6 ± 0.5	1.6 ± 0.2	0.20
Bloating	3.6 ± 0.3	3.4 ± 0.2	4.2 ± 0.3	3.3 ± 0.2	0.21
Upper abdominal pain	3.1 ± 0.2	2.8 ± 0.2	3.6 ± 0.2	2.8 ± 0.2	0.45
Lower abdominal pain	2.7 ± 0.3	2.8 ± 0.2	3.5 ± 0.3	2.8 ± 0.2	0.42
Bowel Habits [2]					
Hard or lumpy stools	65%	68%	73%	55%	0.43
Straining	78%	81%	92%	79%	0.83
Feeling of incomplete evacuation	83%	81%	92%	79%	0.87
Sensation that stool could not be passed (blocked)	68%	71%	80%	66%	0.81
Press on or around bottom or remove stool to complete BM	36%	31%	27%	27%	0.84
Difficulty "letting go" to allow stool to come out during BM	59%	44%	27%	45%	0.38

[1.] Based on a 5-point scale from 0 (none or absent) to 5 (very severe). Results expressed as mean ± standard error. [2.] Percentage of patients responding often, most of the time, or always on scale of never, rarely, sometimes, often, most of the time, always.

3.2. Findings on Whole Gut Scintigraphy and Anorectal Manometry

There were differences in gastric emptying between the populations at both 2 and 4 h ($p = 0.04$ and $p < 0.01$, respectively) (Table 1). Follow-up testing showed that these differences of gastric emptying at 2 h existed between populations that had a diagnosis of STC vs. no STC (e.g., STC only vs. DD only, STC and DD vs. DD only, STC only vs. no STC or DD, STC and DD vs. no STC or DD, all $p < 0.001$). Similarly, gastric emptying at 4 h was different based on the presence of STC (all $p < 0.001$). No differences were seen in small bowel transit. Colonic transit at 24, 48, and 72 h all differed among the populations ($p < 0.001$).

On HR-ARM, mean resting pressure differed among the populations ($p = 0.03$). Patients with STC only had lower mean resting pressure compared to patients with no STC or DD (65.2 ± 2.1 vs. 72.1 ± 2.3, $p = 0.03$) and there was a trend towards significance in patients with STC only vs. DD only (65.2 ± 2.1 vs. 78.7 ± 6.5, $p = 0.06$). There were also statistically significant differences in anal relaxation on simulated defecation ($p < 0.001$). These differences were seen in populations with DD vs. no DD (e.g., STC only vs. DD only, STC + DD vs. STC only, DD vs. no STC or DD, STC + DD vs. no STC or DD, all $p < 0.001$).

On rectal sensory testing, there were differences on first desire to defecate, first urge to defecate, and maximum tolerance ($p < 0.01$, $p < 0.01$, and $p = 0.04$, respectively). There were differences in first desire to defecate in patients with STC only vs. no STC or DD (64.6 ± 4.0 vs. 49.9 ± 3.9, $p < 0.001$) and STC and DD vs. no STC or DD (85.5 ± 11.1 vs. 49.9 ± 3.9, $p < 0.01$). There are statistically significant differences in first urge to defecate in STC + DD vs. STC only (135.0 ± 10.4 vs. 109.0 ± 4.7, $p = 0.04$), STC + DD vs. no STC or DD (135.0 ± 10.4 vs. 93.5 ± 4.5, $p < 0.001$), and STC only vs. no STC or DD (109.0 ± 4.7 vs. 93.5 ± 4.5, $p = 0.02$). In maximum tolerance, patients with STC + DD had higher thresholds than patients with no STC or DD (162.1 ± 9.6 vs. 134.4 ± 4.9, $p = 0.01$).

3.3. Demographics, Medical History, and Symptoms

There was a difference in genders among the different populations ($p = 0.02$). Patients with STC (with or without DD) were more likely to be female than patients without STC (95% vs. 83%, $p < 0.01$). Otherwise, there was no statistically significant difference in age, BMI, race, or medical history.

Symptomatically, there were differences in the average number of bowel movements per week among the different populations ($p < 0.001$). Patients with either STC + DD or STC only had significantly fewer BMs than patients without STC or DD (both $p < 0.001$). There were also differences in self-reported severity of constipation ($p < 0.02$). Statistically significant differences exist between patients with STC only or STC + DD and patients with no STC or DD (4.4 ± 0.1 vs. 3.9 ± 0.2, $p = 0.03$ and 4.7 ± 0.1 vs. 3.9 ± 0.2, $p < 0.01$, respectively). There were also differences in the severity of intermittent diarrhea ($p < 0.01$), including statistically significant differences between STC + DD and DD only (0.8 ± 0.3 vs. 1.8 ± 0.5, $p = 0.05$), STC + DD and no STC or DD (0.8 ± 0.3 vs. 1.4 ± 0.2, $p = 0.05$), and STC only and no STC or DD (0.6 ± 0.2 vs. 1.4 ± 0.2, $p < 0.01$). There were no statistically significant differences in upper GI symptoms or differences in bowel habits.

4. Discussion

This study describes the prevalence of dyssynergic defecation and slow transit constipation in patients referred to an academic medical center with chronic constipation. Both of these pathophysiological causes were common, with dyssynergic defecation present in 22% of patients and slow transit constipation present in 55% of patients. In this study, only 36% of patients with constipation had normal test results for colonic transit and anorectal coordination (no abnormality in either BET or HR-ARM); most patients (64%) had defined abnormalities of transit and/or defecation explaining their symptoms. Importantly, 13% of all patients had evidence of both dyssynergic defecation and slow transit constipation. This suggests that there may be more than one underlying cause for their constipation—both dyssynergic defecation and slow colonic transit.

Previous studies have had conflicting data regarding the prevalence of slow transit constipation and dyssynergic defecation. We previously reported that 67% of patients presenting with chronic constipation to our center had a colonic transit disorder and 37% had dyssynergic defecation [20]. This contrasts with a study of 1009 patients who underwent both pelvic floor function testing and scintigraphy, where only 7% of patients were found to have slow transit constipation and 27% had pelvic floor dysfunction [21]. A possible explanation for the variance in dyssynergic defecation prevalence in the literature is the differing criteria and diagnostic testing used to define dyssynergic defecation. For example, in our previous study, DD was defined as abnormalities in 2 of 4 of the following tests: ARM, electromyography, BET, and defecography. In contrast, the latter study defined dyssynergic defecation as abnormal BET plus high anal sphincter pressure and/or failure of anorectal angle to open $\geq 15^{\circ}$ between resting and straining. A challenge with applying multiple diagnostic tests to define DD is that there can often be poor agreement between them [22]. This study used the newly proposed London Classification, a consensus agreement among the international anorectal physiology working group (IAPWG), which defines dyssynergic defecation as abnormal BET and anorectal coordination on ARM (although they do concede that additional testing may be needed if a patient has either abnormal BET with normal ARM or normal BET with abnormal ARM if there is clinical suspicion for DD) [16]. However, our study uses the strict definition of abnormal BET and ARM, which may account for the lower prevalence of DD in our population than our previous study (22% vs. 37%) and may underestimate the true number of patients with DD.

Regardless, our study illustrates that some patients can have both dyssynergic defecation and delayed colonic transit. Previous studies have shown that there is an overlap between dyssynergic defecation and slow transit constipation [13,23–25]. Why some patients have both disorders is not clear. One study demonstrated that slow transit constipation improved after the completion of biofeedback therapy, suggesting that an abnormal

colonic transit test may be the result of dyssynergic defecation rather than suggestive of colonic inertia or generalized slow transit constipation [13]. There may be two explanations for this overlap of both findings in the same patient. First, the study used a protocol where colonic transit was measured by the number of retained radioopaque markers at 120 h after ingestion. This does not account for the location of these markers, and many may be accumulated in the rectosigmoid region secondary to poor defecatory mechanics despite normal colonic transit. However, a large multicenter study suggested that the location of markers in the rectosigmoid region is not correlated with dyssynergic defecation [26]. A second explanation is that dyssynergic defecation and slow transit constipation may be linked. A study by *Nullens* et al., showed that dyssynergic defecation is associated with delayed overall colonic transit at 48 h [27]. It has further been suggested that dyssynergic defecation can lead to a reflex inhibition of colonic transit in the proximal colon [28,29]. While this is certainly feasible, this study showed no difference in gastric, small bowel, or colonic transit between patients with dyssynergic defecation and patients without dyssynergic defecation, regardless of whether the patient met diagnostic criteria for slow transit constipation.

This study also examined symptoms and patients' demographics associated with dyssynergic defecation and slow transit constipation. Interestingly, there was no differences in bowel habits between the different populations (STC + DD, STC only, DD only, no STC or DD). This contrasts with previous studies which suggested patients with dyssynergic defecation may be associated with patients using digital maneuvers to complete a bowel movement, excessive straining, a feeling of incomplete evacuation, the passage of hard stools, and infrequent stooling [9,30]. While these symptoms were common in our patient population, they were not unique to patients who had objective evidence on dyssynergic defecation by BET and HR-ARM testing. Conversely, no symptoms were suggestive of slow transit constipation. However, there were differences in the severity of constipation and diarrhea experienced by patients. Patients with STC + DD or STC only had more significant constipation than patients with no abnormalities as assessed by bowel movements per week. The data are also suggestive that patients with DD only had more severe constipation than patients with no abnormalities, although this did not reach statistical significance, perhaps due to small sample size of this population. Self-reported severity of diarrhea was also lower in the STC + DD population compared to DD only or no abnormalities group. However, we do not believe this is clinically significant as most patients only reported none or mild diarrhea.

We also show that slow transit constipation is associated with delayed gastric emptying at 2 and 4 h. This contrasts with a previous study by our group which suggested that both slow transit constipation and dyssynergic defecation was associated with delayed gastric emptying [20]. This study builds on previous studies by assessing upper gastrointestinal symptoms to determine whether upper GI symptoms are prevalent in patients with slow transit constipation. There were no differences in upper GI symptoms, which suggests that findings of delayed gastric emptying in slow transit constipation has unclear clinical significance. Further studies are needed to determine clinical significance of delayed gastric emptying in patients presenting with chronic constipation. Further studies are also needed to investigate the correlation of rectal sensitivity testing to STC and DD as both STC and DD were associated with higher thresholds for rectal distension. This would suggest rectal hyposensitivity, which might also be playing a role in their symptoms of constipation.

An important implication of this study is the diagnostic approach to patients who present with chronic constipation. Currently, both the American Gastroenterology Association (AGA) and American College of Gastroenterology (ACG) guidelines are to start testing with anorectal manometry [31,32]. If ARM reveals a defecatory disorder, then treatment of this, such as biofeedback therapy, should be pursued [32]. However, it is unclear whether biofeedback therapy would also correct slow transit constipation. There have been few studies that have assessed biofeedback therapy in slow transit constipation, with varying reports in effectiveness [28,33,34]. However, all these studies suffer from low or very low quality of evidence as noted in a Cochrane review on biofeedback therapy in

chronic constipation [35]. Symptom profiles among patients with STC + DD compared to singular diagnoses were largely similar. Given that symptom profiles cannot distinguish STC, DD, and STC + DD, it is important that patients undergo testing for both constipations to guide treatment options. The primary treatment modality for dyssynergic defecation is biofeedback therapy [36,37]. In contrast, slow transit constipation that has failed laxative therapy has limited treatment options, and severe cases may require surgery, such as total colectomy [38,39]. The impact of concomitant STC in patients undergoing treatment for DD has not been well-studied. A study of 52 patients found biofeedback therapy more effective in patients with pelvic floor dyssynergia and slow transit constipation compared to patients with slow transit constipation only [28]. However, that study did not compare pelvic floor dyssynergia with slow transit constipation to pelvic floor dyssynergia as all patients included in the study met criteria for slow transit constipation [28]. We did not look at treatment outcomes for treating dyssynergic defecation and/or slow transit constipation. This would be of particular interest in the patients with both disorders to better understand what type of treatment works best, and if the delayed colonic transit normalizes in patients with slow transit constipation and DD with treatment of the DD.

Our study evaluates a large number of patients with chronic constipation, undergoing the state-of-the-art tests–HR-ARM (with BET) and whole gut transit scintigraphy. Validated questionnaires were used to assess their symptoms. The Rome III criteria were used to help characterize the constipation symptoms. However, there are several limitations to this study. First, most patients were referred to our tertiary academic center and, therefore, do not represent the broader community with constipation. Given that patients had failed medical therapy for constipation, there may be a higher proportion of functional disorders such as dyssynergic defecation and slow transit constipation. Second, our symptom survey was limited to symptoms patients had been experiencing over the past several weeks. Other studies have looked at associations between functional constipation and childhood traumas, such as physical or sexual abuse [40]. Another limit of only looking at symptoms in the past several weeks is that many patients were on some sort of laxative therapy, which may have affected constipation symptoms.

5. Conclusions

This study describes the prevalence of dyssynergic defecation and slow transit constipation in patients referred with chronic constipation. In our study, only 36% of patients had normal test results for colonic transit and HR-ARM; most patients (64%) had defined abnormalities of transit and/or defecation explaining their symptoms. Dyssynergic defecation found in 22% of patients and slow transit constipation found in 55% of patients. Importantly, 13% of all patients had evidence of both dyssynergic defecation and slow transit constipation. Symptoms alone were a poor predictor of underlying pathophysiology of constipation. Thus, to get a proper evaluation of the pathophysiology of a patient's constipation from a motility standpoint, both ARM and colonic transit need to be assessed, as both are common, including both disorders in the same patient.

Author Contributions: Conceptualization, S.T., A.J., Z.M., and H.P.P.; methodology, S.T., A.J., Z.M., D.S., and H.P.P.; data curation, S.T., A.C., N.G., and R.B.; formal analysis, S.T., A.J., D.S.; writing—original draft preparation, S.T.; writing—review and editing, S.T., A.C., N.G., R.B., A.J., D.S., Z.M., and H.P.P.; supervision, H.P.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of Temple University (protocol code 27045 and date of approval 4 June 2020).

Informed Consent Statement: Patient consent was waived due to study being a retrospective chart review deleting patient identifiers.

Data Availability Statement: Data supporting reported results are available through reasonable request to the authors.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Suares, N.C.; Ford, A.C. Prevalence of, and Risk Factors for, Chronic Idiopathic Constipation in the Community: Systematic Review and Meta-analysis. *Am. J. Gastroenterol.* **2011**, *106*, 1582–1591. [CrossRef] [PubMed]
2. Patcharatrakul, T.; Rao, S.S. Update on the Pathophysiology and Management of Anorectal Disorders. *Gut Liver* **2018**, *12*, 375–384. [CrossRef] [PubMed]
3. Bharucha, A.E.; Lacy, B.E. Mechanisms, Evaluation, and Management of Chronic Constipation. *Gastroenterology* **2020**, *158*, 1232–1249.e3. [CrossRef] [PubMed]
4. Rao, S.S. Dyssynergic Defecation and Biofeedback Therapy. *Gastroenterol. Clin. North Am.* **2008**, *37*, 569–586. [CrossRef]
5. Bonapace, E.S.; Maurer, A.H.; Davidoff, S.; Krevsky, B.; Fisher, R.S.; Parkman, H.P. Whole Gut Transit Scintigraphy in The Clinical Evaluation of Patients with Upper and Lower Gastrointestinal Symptoms. *Am. J. Gastroenterol.* **2000**, *95*, 2838–2847. [CrossRef]
6. Maurer, A.H. Gastrointestinal Motility, Part 2: Small-Bowel and Colon Transit. *J. Nucl. Med. Technol.* **2015**, *44*, 12–18. [CrossRef]
7. Glia, A.; Lindberg, G.; Nilsson, L.H.; Mihocsa, L.; Åkerlund, J.E. Clinical value of symptom assessment in patients with constipation. *Dis. Colon Rectum* **1999**, *42*, 1401–1408. [CrossRef]
8. Koch, A.; Voderholzer, W.A.; Klauser, A.G.; Müller-Lissner, S. Symptoms in chronic constipation. *Dis. Colon Rectum* **1997**, *40*, 902–906. [CrossRef]
9. Rentz, A.; Kahrilas, P.; Stanghellini, V.; Tack, J.; Talley, N.; De La Loge, C.; Trudeau, E.; Dubois, D.; Revicki, D. Development and psychometric evaluation of the patient assessment of upper gastrointestinal symptom severity index (PAGI-SYM) in patients with upper gastrointestinal disorders. *Qual. Life Res.* **2004**, *13*, 1737–1749. [CrossRef] [PubMed]
10. Drossman, D.; Corazziari, E.; Delvaux, M.; Spiller, R.C.; Talley, N.J.; Thompson, W.G.; Whitehead, W.E. *Rome III: The Functional Gastrointestinal Disorders*; Degnon Associates: McLean, VA, USA, 2006.
11. Rao, S.S.C.; Hatfield, R.; Soffer, E.; Rao, S.; Beaty, J.; Conklin, J.L. Manometric Tests of Anorectal Function in Healthy Adults. *Am. J. Gastroenterol.* **1999**, *94*, 773–783. [CrossRef]
12. Shah, E.D.; Farida, J.D.; Menees, S.; Baker, J.R.; Chey, W.D. Examining Balloon Expulsion Testing as an Office-Based, Screening Test for Dyssynergic Defecation: A Systematic Review and Meta-Analysis. *Am. J. Gastroenterol.* **2018**, *113*, 1613–1620. [CrossRef]
13. Rao, S.S.C.; Mudipalli, R.S.; Stessman, M.; Zimmerman, B. Investigation of the utility of colorectal function tests and Rome II criteria in dyssynergic defecation (Anismus). *Neurogastroenterol. Motil.* **2004**, *16*, 589–596. [CrossRef]
14. Bharucha, A.E.; Wald, A.; Enck, P.; Rao, S. Functional Anorectal Disorders. *Gastroenterol.* **2006**, *130*, 1510–1518. [CrossRef] [PubMed]
15. Lee, T.H. How to Perform and Interpret a High-resolution Anorectal Manometry Test. *J. Neurogastroenterol. Motil.* **2016**, *22*, 46–59. [CrossRef] [PubMed]
16. Carrington, E.V.; Heinrich, H.; Knowles, C.H.; Fox, M.; Rao, S.; Altomare, D.F.; Bharucha, A.E.; Burgell, R.; Chey, W.D.; Chiarioni, G.; et al. The international anorectal physiology working group (IAPWG) recommendations: Standardized testing protocol and the London classification for disorders of anorectal function. *Neurogastroenterol. Motil.* **2020**, *32*, e13679. [CrossRef]
17. Maurer, A.H. Gastrointestinal Motility, Part 1: Esophageal Transit and Gastric Emptying. *J. Nucl. Med. Technol.* **2015**, *56*, 1229–1238. [CrossRef]
18. Maurer, A.H.; Krevsky, B. Whole-gut transit scintigraphy in the evaluation of small-bowel and colon transit disorders. *Semin. Nucl. Med.* **1995**, *25*, 326–338. [CrossRef]
19. Lin, H.C.; Ams Task Force Committee on Gastrointestinal Transit; Prather, C.; Fisher, R.S.; Meyer, J.H.; Summers, R.W.; Pimentel, M.; McCallum, R.W.; Akkermans, L.M.A.; Loening-Baucke, V. Measurement of Gastrointestinal Transit. *Dig. Dis. Sci.* **2005**, *50*, 989–1004. [CrossRef] [PubMed]
20. Shahid, S.; Ramzan, Z.; Maurer, A.H.; Parkman, H.P.; Fisher, R.S. Chronic idiopathic constipation: More than a simple colonic transit disorder. *J. Clin. Gastroenterol.* **2012**, *46*, 150–154. [CrossRef]
21. Nyam, D.C.N.K.; Pemberton, J.H.; Ilstrup, D.M.; Rath, D.M. Long-term results of surgery for chronic constipation. *Dis. Colon Rectum* **1997**, *40*, 273–279. [CrossRef]
22. Palit, S.; Thin, N.; Knowles, C.H.; Lunniss, P.J.; Bharucha, A.E.; Scott, S.M. Diagnostic disagreement between tests of evacuatory function: A prospective study of 100 constipated patients. *Neurogastroenterol. Motil.* **2016**, *28*, 1589–1598. [CrossRef]
23. Glia, G.L.A. Constipation Assessed on the Basis of Colorectal Physiology. *Scand. J. Gastroenterol.* **1998**, *33*, 1273–1279. [CrossRef]
24. Rao, S.S.C.; Welcher, K.D.; Leistikow, J.S. Obstructive Defecation: A Failure of Rectoanal Coordination. *Am. J. Gastroenterol.* **1998**, *93*, 1042–1050. [CrossRef]
25. Singh, S.; Heady, S.; Coss-Adame, E.; Rao, S.S. Clinical utility of colonic manometry in slow transit constipation. *Neurogastroenterol. Motil.* **2013**, *25*, 487-e367. [CrossRef] [PubMed]
26. Staller, K.; Barshop, K.; Ananthakrishnan, A.N.; Kuo, B. Rectosigmoid Localization of Radiopaque Markers Does Not Correlate with Prolonged Balloon Expulsion in Chronic Constipation: Results from a Multicenter Cohort. *Am. J. Gastroenterol.* **2015**, *110*, 1049–1055. [CrossRef] [PubMed]

27. Nullens, S.; Nelsen, T.; Camilleri, M.; Burton, D.; Eckert, D.J.; Iturrino, J.; Vazquezroque, M.I.; Zinsmeister, A.R. Regional colon transit in patients with dys-synergic defaecation or slow transit in patients with constipation. *Gut* **2012**, *61*, 1132–1139. [CrossRef] [PubMed]
28. Chiarioni, G.; Salandini, L.; Whitehead, W.E. Biofeedback Benefits Only Patients With Outlet Dysfunction, Not Patients With Isolated Slow Transit Constipation. *Gastroenterology* **2005**, *129*, 86–97. [CrossRef]
29. Mollen, R.M.H.G.; Salvioli, B.; Camilleri, M.; Burton, D.; Kost, L.J.; Phillips, S.F.; Pemberton, J.H. The effects of biofeedback on rectal sensation and distal colonic motility in patients with disorders of rectal evacuation: Evidence of an inhibitory rectocolonic reflex in humans? *Am. J. Gastroenterol.* **1999**, *94*, 751–756. [CrossRef]
30. Rao, S.S.C.; Tuteja, A.K.; Vellema, T.; Kempf, J.; Stessman, M. Dyssynergic Defecation: Demographics, Symptoms, Stool Patterns, and Quality of Life. *J. Clin. Gastroenterol.* **2004**, *38*, 680–685. [CrossRef]
31. American Gastroenterological Association; Bharucha, A.E.; Dorn, S.D.; Lembo, A.; Pressman, A. American Gastroenterological Association Medical Position Statement on Constipation. *Gastroenterology* **2013**, *144*, 211–217. [CrossRef] [PubMed]
32. Wald, A.; E Bharucha, A.; Cosman, B.C.; Whitehead, W.E. ACG Clinical Guideline: Management of Benign Anorectal Disorders. *Am. J. Gastroenterol.* **2014**, *109*, 1141–1157. [CrossRef] [PubMed]
33. Chiotakakou-Faliakou, E.; Kamm, M.A.; Roy, A.J.; Storrie, J.B.; Turner, I.C. Biofeedback provides long term benefit for patients with intractable, slow and normal transit constipation. *Gut* **1998**, *42*, 517–521. [CrossRef]
34. Emmanuel, A.; Kamm, M. Response to a behavioural treatment, biofeedback, in constipated patients is associated with im-proved gut transit and autonomic innervation. *Gut* **2001**, *49*, 214–219. [CrossRef] [PubMed]
35. Woodward, S.; Norton, C.S.; Chiarelli, P. Biofeedback for treatment of chronic idiopathic constipation in adults. *Cochrane Database Syst. Rev.* **2010**, CD008486. [CrossRef]
36. Narayanan, S.P.; Bharucha, A.E. A Practical Guide to Biofeedback Therapy for Pelvic Floor Disorders. *Curr. Gastroenterol. Rep.* **2019**, *21*, 21. [CrossRef] [PubMed]
37. Rao, S.S.; Patcharatrakul, T. Diagnosis and Treatment of Dyssynergic Defecation. *J. Neurogastroenterol. Motil.* **2016**, *22*, 423–435. [CrossRef] [PubMed]
38. Camilleri, M.; Ford, A.C.; Mawe, G.M.; Dinning, P.G.; Rao, S.S.; Chey, W.D.; Simrén, M.; Lembo, A.; Young-Fadok, T.M.; Chang, L. Chronic constipation. *Nat. Rev. Dis. Prim.* **2017**, *3*, 17095. [CrossRef]
39. Knowles, C.H.; Grossi, U.; Horrocks, E.J.; Pares, D.; Vollebregt, P.F.; Chapman, M.; Brown, S.; Mercer-Jones, M.; Williams, A.B.; Yiannakou, Y.; et al. Surgery for constipation: Systematic review and practice recommendations. *Color. Dis.* **2017**, *19*, 101–113. [CrossRef]
40. Chiarioni, G.; Bassotti, G.; Monsignori, A.; Menegotti, M.; Salandini, L.; Di Matteo, G.; Vantini, I.; Whitehead, W.E. Anorectal Dysfunction in Constipated Women with Anorexia Nervosa. *Mayo Clin. Proc.* **2000**, *75*, 1015–1019. [CrossRef]

Journal of
Clinical Medicine

MDPI

Article

Endoscopic Vacuum Therapy in Patients with Transmural Defects of the Upper Gastrointestinal Tract: A Systematic Review with Meta-Analysis

Da Hyun Jung [1,†], Hae-Ryong Yun [2,†], Se Joon Lee [2], Na Won Kim [3] and Cheal Wung Huh [2,*]

[1] Department of Internal Medicine, Severance Hospital, Yonsei University College of Medicine, Seoul 03722, Korea; JUNGDH@yuhs.ac
[2] Department of Internal Medicine, Yongin Severance Hospital, Yonsei University College of Medicine, Seoul 03722, Korea; siberian82@yuhs.ac (H.-R.Y.); LEESJ@yuhs.ac (S.J.L.)
[3] Medical Library, Yonsei University College of Medicine, Seoul 03722, Korea; NWKIM@yuhs.ac
* Correspondence: huhcw@yuhs.ac
† There are contributed equally to this work.

Abstract: A transmural defect of the upper gastrointestinal (UGI) tract is a life-threatening condition associated with high morbidity and mortality. Recently, endoscopic vacuum therapy (EVT) was used for managing UGI defects and showed promising results. We conducted a systematic review and meta-analysis to synthesize evidence on the efficacy of EVT in patients with transmural defects of the UGI tract. We searched the PubMed, Cochrane Library, and Embase databases for publications on the effect of EVT on successful closure, mortality, complications, and post-EVT strictures. Methodological quality was assessed using the Newcastle–Ottawa quality assessment scale. This meta-analysis included 29 studies involving 498 participants. The pooled estimate rate of successful closure with EVT was 0.85 (95% confidence interval [CI]: 0.81–0.88). The pooled estimate rates for mortality, complications, and post-EVT strictures were 0.11, 0.10, and 0.14, respectively. According to the etiology of the transmural defect (perforation vs. leak and fistula), no significant difference was observed in successful closure (odds ratio [OR]: 1.45, 95% CI: 0.45–4.67, $p = 0.53$), mortality (OR: 0.77, 95% CI: 0.24–2.46, $p = 0.66$), complications (OR: 0.94, 95% CI: 0.17–5.15, $p = 0.94$), or post-EVT stricture rates (OR: 0.70, 95% CI: 0.12–4.24, $p = 0.70$). The successful closure rate was significantly higher with EVT than with self-expanding metal stent (SEMS) placement (OR: 3.14, 95% CI: 1.23–7.98, $p = 0.02$). EVT is an effective and safe treatment for leaks and fistulae, as well as for perforations in the UGI. Moreover, EVT seems to be a better treatment option than SEMS placement for UGI defects.

Keywords: endoscopic vacuum therapy; etiology; transmural defect; upper gastrointestinal tract

Citation: Jung, D.H.; Yun, H.-R.; Lee, S.J.; Kim, N.W.; Huh, C.W. Endoscopic Vacuum Therapy in Patients with Transmural Defects of the Upper Gastrointestinal Tract: A Systematic Review with Meta-Analysis. *J. Clin. Med.* **2021**, *10*, 2346. https://doi.org/10.3390/jcm10112346

Academic Editor: Marilena Durazzo

Received: 16 April 2021
Accepted: 24 May 2021
Published: 27 May 2021

1. Introduction

Transmural defects of the upper gastrointestinal (UGI) tract are categorized as perforations, leaks, or fistulae. A perforation is defined as an acute rupture of the gastrointestinal wall that can occur after an endoscopic procedure or due to underlying pathology, such as massive vomiting (Boerhaave syndrome), foreign bodies, peptic ulcers [1,2]. A leak is a communication between the intraluminal and extraluminal spaces, which occurs because of postsurgical complications, most commonly at the anastomosis site. A fistula that develops owing to prolonged anastomotic leak is defined as an abnormal connection between the gastrointestinal tract and other organs or abscess cavities. Tracheoesophageal fistula is representative. Transmural defects of the UGI are life-threatening and associated with high morbidity and mortality rates [3,4]. The optimal management of UGI transmural defects remains controversial. Though surgery is an important treatment strategy, the associated mortality rate is about 12–50% [3,5,6]. Placement of a self-expanding metal stent (SEMS) was also proven to be an effective treatment strategy for UGI defects [7,8]. However,

SEMS placement can also cause complications such as stent migration, stent ingrowth, perforation, bleeding, epidural abscess, and vascular fistula [9–11].

Recently, endoscopic vacuum therapy (EVT) was used with promising results for managing UGI defects [12–14]. This method involves the application of a continuous negative pressure to drain the infected fluid and accelerates wound healing [15]. EVT is suitable for localized defects for which stent placement is not feasible. Moreover, external drainage is not necessary in most cases [16]; however, the clinical success rate of EVT varies widely from 66.7–100% [17–19]. In addition, corroborating evidence is needed because most studies are limited to case series and retrospective cohort studies with small sample sizes.

We performed a meta-analysis of studies on the clinical outcomes of EVT in patients with transmural defects of the UGI tract. We aimed to assess the effect of EVT on successful closure, mortality, postprocedural complications, and stricture. In addition, we evaluated the efficacy of EVT according to the etiology of the transmural defect (perforation vs. leak and fistula) and treatment method (EVT vs. SEMS placement).

2. Material and Methods

2.1. Literature Search Strategy

We performed a systematic review and meta-analysis following the principles of the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) statement [20]. The PubMed, Cochrane Library, and Embase databases (from inception to April 2020) were independently searched by three authors (DHJ, HRY, and CWH). We used the following search string: anastomotic leak OR anastomotic leakage OR postoperative leak OR postoperative leakage OR esophageal leak OR esophageal leakage OR esophageal fistula OR leakage OR fistula OR leak OR perforation OR upper gastrointestinal tract OR esophagus OR esophageal OR gastric OR stomach OR esophagectomy OR anastomosis AND endoscopic vacuum therapy OR endoscopic vacuum-assisted closure OR endoluminal vacuum therapy OR vacuum therapy OR vacuum-assisted closure OR negative pressure wound therapy OR endoscopic negative pressure therapy OR negative pressure therapy OR endovac therapy OR endo sponge (as illustrated in supplementary Table S1). We manually and repetitively searched the cited references in published studies to identify other studies.

2.2. Study Selection

In the first stage of the study selection, the titles and abstracts of the articles that our keyword search returned were scrutinised to rule out irrelevant articles. Thereafter, the full texts of all selected studies were screened according to our inclusion and exclusion criteria. The inclusion criteria were as follows: (1) a diagnosis of perforation, leak, or fistula of the UGI tract; (2) EVT as a primary or rescue treatment; and (3) investigations of adults aged $\geq$18 years. The exclusion criteria were as follows: (1) article types other than original articles; (2) case reports including fewer than two patients; (3) abstract-only publications; and (4) publications in a language other than English. Only the most recent study was selected if several publications covering the same study population existed.

2.3. Data Extraction

Three authors (DHJ, HRY, and CWH) of this review independently extracted data from the included studies using a predata extraction form. Further, we reviewed the titles and abstracts of all the included studies to exclude irrelevant publications. Any discrepancies in data interpretation were resolved through discussions, rereview of studies, and consultation with another author (SJL). We extracted the following information: year of publication, first author, study design, patient age and sex, sample size, study region, follow-up duration, transmural defect size, time to diagnosis, time to treatment, EVT type, successful closure rate, mortality rate, complication rate, post-EVT stricture rate, hospital length of stay, intensive care unit length of stay, treatment duration, and number of sponge or stent changes.

2.4. Primary and Secondary Outcomes

The primary outcome was the successful closure rate. Successful closure was defined as no evidence of leakage on direct endoscopic visualization and the absence of contrast extravasation on either a computed tomography scan with oral contrast, esophagography, or a UGI study. The secondary outcomes were mortality rate, complication rate (Clavien–Dindo score ≥ 3), and stricture rate after EVT.

2.5. Methodological Quality

The Newcastle–Ottawa quality assessment scale for cohort studies was used to evaluate the risk of bias. This scale rates studies on three sources of bias (selection, comparability, and outcome) based on eight criteria. Each criterion is rated with 1 star except comparability, which is rated a maximum of 2 stars. For this systematic review, the studies scoring 7–9 stars were defined be of low risk of bias, the studies scoring 4–6 stars were defined to be of moderate risk of bias, and the studies scoring 1–3 stars were defined to be of high risk of bias. Three authors (CWH, HRY, and DHJ) independently evaluated the methodological quality of the selected studies. Any disagreement between the three authors was resolved through discussions.

2.6. Statistical Analysis

A meta-analysis was performed using the statistical software R (version 3.3.3; R Foundation for Statistical Computing, Vienna, Austria). The Mantel–Haenszel random-effect model was applied to binary endpoints. The random-effects model was selected because it considers the possibility of heterogeneity. The median difference was used for continuous variables. Pooled medians were estimated using the quantile estimation method. In addition, we performed subgroup analyses according to the following criteria: closure rate, mortality rate, complication rate, post-EVT stricture rate according to the etiology of transmural defect (perforation vs. leak and fistula), closure rate, mortality, treatment duration, hospital stay, and number of sponge/stent changes of EVT and SEMS.

The I^2 test developed by Higgins was used to determine heterogeneity [21]. This test measures the percentage of total variation across studies. In cases of significant heterogeneity ($I^2 > 25\%$), the methodological section of each publication was re-evaluated to determine whether any discrepancy could be checked. We used the Egger test to assess the extent of the publication bias. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Study Selection

A total of 2585 studies were identified. Duplicate articles ($n = 392$) were excluded. Further, 2144 articles were rejected based on the title and abstracts. Forty-nine articles were reviewed. After assessing eligibility, 20 articles were excluded (as illustrated in Figure 1). Finally, a total of 29 articles were included involving 498 participants [13,14,18,22–47]

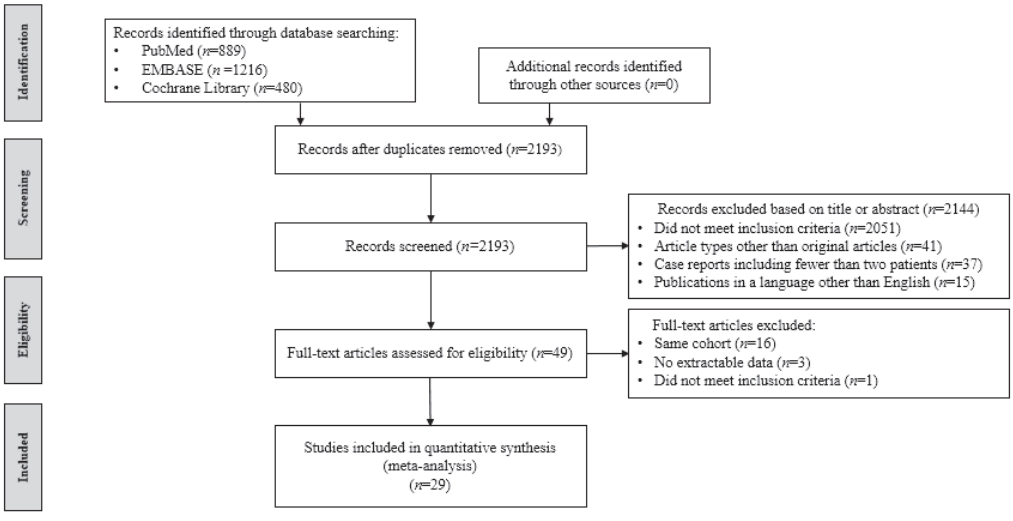


Figure 1. Flowchart of studies included in meta-analysis.

3.2. Study Characteristics and Methodological Quality

The baseline characteristics of the included studies are presented in Table 1. Nineteen articles were retrospective cohort studies, and 10 were case series. Eight studies included only patients with postoperative leaks, [13,23,25,26,33,42,43,46] and two studies included only patients with perforations [24,27]. Eleven studies included patients with both postoperative leaks and perforations [14,18,29,31,32,34,35,38,40,41,44]. Four studies included patients with a fistula [36,37,45,47]. Four studies compared EVT with SEMS placement [22,28,30,39]. A total of 24 studies were conducted in Western countries (Germany 14, United States 4, Switzerland 2, United Kingdom 2, Portugal 1, and Australia 1), whereas five studies were conducted in Asia (Korea 4 and China 1).

The definition of clinical success, detailed indications of treatment, and causes of mortality in the included studies are shown in supplementary Table S2. In addition, four studies that included fistula cases are summarized in supplementary Table S3.

The patient characteristics of studies comparing EVT and SEMS placement are summarized in supplementary Table S4. Brangewitz et al. [22] reported successful closure, mortality, duration of treatment, length of hospital stay, and stricture development in 71 patients with leaks or perforations after esophagectomies, fundoplications, esophageal diverticulotomies, Boerhaave syndrome, and iatrogenic perforations, and compared EVT (n = 32) with SEMS placement (n = 39). Schniewind et al. [23] assessed 47 patients diagnosed with postoperative leaks after esophagectomy. Mortality and length of hospital stay were compared between patients treated with EVT (n = 17) and SEMS placement (n = 12). Mennigen et al. [28] showed that successful closure, mortality, duration of treatment, length of hospital stay, and adverse events were analysed in 45 patients who were diagnosed with postoperative leak following esophagectomy in comparisons between EVT (n = 15) versus SEMS (n = 30). Hwang et al. [30] compared EVT (n = 7) and SEMS placement (n = 11) in South Korea. Although the number of enrolled patients was small, they also showed successful closure, duration of treatment, length of hospital stay, and adverse events. They included eighteen patients who were diagnosed with postoperative leak after esophagectomy or gastrectomy for cancer treatment. Lastly, Berlth et al. [39] reported successful closure, mortality, duration of treatment, length of hospital stay, and adverse events in comparisons between EVT (n = 34) and SEMS (n = 77). One hundred and eleven patients underwent curative surgery to treat malignancies and were diagnosed with postoperative leaks.

Table 1. Characteristics of 29 studies included.

Authors	Study Design	*N*	Male (n)	Age (Median, Years)	BMI (Median)	Region of Study	Follow-Up (Median, Months)	Method of Diagnosis	Defect Size (Median, mm)	Time to Diagnosis (Median, Days)	Time to Treatment (Median, Days)	Intracavitary/ Intraluminary
Palmes 2020	Case series	Fistula: 4	NA	NA	NA	Western (Germany)	MA	Endoscopy CT Esophagogram	NA	NA	NA	NA
Jung 2019	Retrospective	Leak: 23 Perforation: 7	20	65.1 †	NA	Western (Germany)	11.8 †	NA	NA	Leak: 8.5 Perforation: <1	NA	IC: 6, IL: 24
Jeon 2019	Retrospective	Leak: 22	17	68	NA	Eastern (Korea)	29.8	Endoscopy CT Esophagogram	NA	11	NA	IC: 10, IL: 12
Watson 2019	Case series	Leak: 2 Fistula: 1	2	69.6 †	NA	Western (USA)	2.7	Endoscopy	NA	NA	NA	NA
Pinto 2019	Case series	Leak: 2	1	44.0 †	NA	Western (Portugal)	NA	Endoscopy CT	NA	NA	NA	IC: 2, IL: 1
Morell 2019	Case series	Leak: 6	2	49.0 †	44.2	Western (Switzerland)	NA	Endoscopy CT	20†	4.5 †	3.3 †	NA
Min 2019	Retrospective	Leak: 20	20	66.5	NA	Eastern (Korea)	7.1	Endoscopy Esophagogram	17.5	12.5	3	IC: 20
Loske 2019	Case series	Leak: 1 Perforation: 10	4	65.7 †	NA	Western (Germany)	NA	Endoscopy CT	NA	NA	NA	IC: 1 IL: 10
Leeds 2019	Retrospective	Leak: 54 Perforation: 20	NA	NA	NA	Western (USA)	NA	NA	NA	NA	NA	NA
Walsh 2019	Case series	Leak: 1 Perforation: 1	1	69.5	NA	Western (USA)	NA	NA	20	21	NA	NA
Alakkari 2019	Case series	Leak: 1 Perforation: 1	0	67 †	NA	Western (UK)	NA	NA	NA	NA	19 †	IC: 2
Berlth 2018	Retrospective	Leak: 111 EVT: 34 SEMS 77	92	EVT: 65 SEMS: 64	EVT: 26 SEMS: 26	Western (Germany)	NA	Endoscopy CT Esophagogram	NA	EVT: 8 SEMS: 8	EVT: 0 SEMS: 0	NA
Valli 2018	Retrospective	Leak: 11 Fistula: 1	9	Leak: 57.5 Fistula: 80	NA	Western (Switzerland)	NA	Endoscopy CT Esophagogram	NA	NA	Leak: 18.4 Fistula: 52	IC: 7, IL: 5
Still 2018	Retrospective	Leak: 2 Perforation: 9 Fistula: 2	6	63	23	Western (USA)	NA	Endoscopy CT Esophagogram	NA	NA	NA	IC: 3, IL: 7
Pournaras 2018	Retrospective	Leak: 14 Perforation: 7	NA	NA	NA	Western (UK)	NA	NA	NA	NA	NA	NA
Ooi 2018	Retrospective	Leak: 6 Perforation: 4	NA	56.7 †	NA	Western (Australia)	NA	NA	18.3	NA	33.6	NA

Table 1. *Cont.*

Authors	Study Design	*N*	Male (n)	Age (Median, Years)	BMI (Median)	Region of Study	Follow-Up (Median, Months)	Method of Diagnosis	Defect Size (Median, mm)	Time to Diagnosis (Median, Days)	Time to Treatment (Median, Days)	Intracavitary/ Intraluminary
Noh 2018	Retrospective	Leak: 12	12	57.0	NA	Eastern (Korea)	12.9	CT Esophagogram	13	13.5	11	IC: 3, IL: 9
Loske 2018	Case series	Leak: 3 Perforation: 1	NA	NA	NA	Western (Germany)	NA	NA	NA	NA	NA	NA
Laukoetter 2017	Retrospective	Leak: 39 Perforation: 13	31	65	NA	Western (Germany)	5.4	Endoscopy CT Esophagogram	NA	NA	8	NA
Kuehn 2016	Retrospective	Leak: 11 Perforation: 10	15	72	NA	Western (Germany)	17	Endoscopy CT Esophagogram	NA	NA	NA	IC: 10, IL: 11
Hwang 2016	Retrospective	Leak: 18 EVT: 7 SEMS 11	14	EVT: 71.1 SEMS: 67.3	NA	Eastern (Korea)	NA	NA	EVT: 8.1 SEMS: 6.6	NA	NA	NA
Möschler 2015	Retrospective	Leak: 5 Perforation: 5	5	73.9 †	NA	Western (Germany)	4	NA	NA	NA	NA	IC: 6, IL: 4
Mennigen 2015	Retrospective	Leak: 45 EVT: 15 SEMS 30	35	EVT: 56 SEMS: 65.5	NA	Western (Germany)	EVT: 8.3 SEMS: 16.8	Endoscopy CT Esophagogram	NA	EVT: 7 SEMS: 7	NA	IC: 22
Loske 2015	Case series	Perforation: 10	NA	NA	NA	Western (Germany)	2.8	Endoscopy	19	<1	NA	IC: 1, IL: 9
Heits 2014	Retrospective	Perforation: 10	5	66 †	NA	Western (Germany)	9	Endoscopy CT Esophagogram	4.2	NA	NA	NA
Liu 2014	Case series	Leak: 5	NA	61.8 †	NA	Eastern (China)	NA	Endoscopy CT Esophagogram	NA	9.2	NA	NA
Schorsch 2013	Retrospective	Leak: 17	NA	NA	NA	Western (Germany)	NA	NA	14.7	10	NA	IC: 8, IL: 9
Schniewind 2013	Retrospective	Leak: 47	NA	NA	NA	Western (Germany)	NA	NA	NA	NA	NA	NA
Brangewitz 2013	Retrospective	EVT:32 SEMS: 39	58	EVT:63 SEMS: 62	EVT: 25.2 SEMS: 26.4	Western (Germany)	NA	NA	NA	NA	NA	NA

EVT, endoscopic vacuum therapy; CT, computed tomography; IC, intracavitary; IL, intraluminary; NA, not available; SEMS, self-expanding metal stent † Data expressed as mean.

Table 2. Clinical outcomes of 29 studies included.

Authors	N	Successful Closure Rate (*n*, %)	Mortality Rate (*n*, %)	Complication Rate (*n*, %)	Stricture Rate (*n*, %)	Hospital Stay (Median, Days)	ICU Stay (Median, Days)	Duration of Therapy (Median, Days)	Sponge Changes
Palmes 2020	Fistula: 4	2/4 (50)	2/4 (50)	NA	NA	NA	NA	88.5	NA
Jung 2019	Leak: 23 Perforation: 7	20/23 (87.0) 5/7 (71.4)	1/23 (4.3) 1/7 (14.3)	0/23 (0) 0/7 (0)	3/13 (23.1) 1/2 (50.0)	54.4 33.7	NA	15.7 27.0	3.4 6.4
Jeon 2019	Leak: 22	19/22 (86.4)	0/22 (0)	0/22 (0)	3/15 (20.0)	24	NA	13	3
Watson 2019	Leak: 2 Fistula: 1	2/2 (100.0) 1/1 (100.0)	0/2 (0) 0/1 (0)	0/2 (0) 0/1 (0)	0/2 (0) 0/1 (0)	NA	NA	16 40	3 9
Pinto 2019	Leak: 2	1/2 (50.0)	0/2 (0)	0/2 (0)	NA	NA	NA	22	3
Morell 2019	Leak: 6	6/6 (100.0)	0/6 (0)	0/6 (0)	0/6 (0)	39.8 †	10.2 †	32.3 †	4
Min 2019	Leak: 20	19/20	1/20	NA	6/19	49	NA	14.5	5
Loske 2019	Leak: 1 Perforation: 10	1/1 (100.0) 10/10 (100.0)	0/1 (0) 2/10 (20.0)	NA	NA	NA	NA	11	1.8
Leeds 2019	Leak: 54 Perforation: 20	44/54 (81.5) 19/20 (95.0)	NA	NA	NA	NA	NA	NA	NA
Walsh 2019	Leak: 1 Perforation: 1	2/2 (100.0)	1/1 (100.0) 0/1 (0)	0/1 (0) 0/1 (0)	NA	NA	NA	55 42	10 3
Alakkari 2019	Leak: 1 Perforation: 1	1/1 (100.0) 1/1 (100.0)	0/1 (0) 0/1 (0)	0/1 (0) 0/1 (0)	0/1 (0) 0/1 (0)	NA	NA	28 56	6 13
Berlth 2018	Leak: 111 EVT: 34 SEMS 77	24/34 (70.6) 49/77 (63.6)	3/34 (0.9) 11/77 (14.3)	0/27 (0) 13/69 (18.8)	1/27 (3.7) 5/69 (7.2)	37 38	8 7	12 27	3 1
Valli 2018	Leak: 11 Fistula: 1	9/11 (81.8) 0/1 (0)	0/11 (0) 1/1 (100.0)	0/11 (0) 0/1 (0)	2/11 (18.1) 0/1 (0)	NA	NA	20.8 16	5 4
Still 2018	Leak: 2 Perforation: 9 Fistula: 2	NA	1/13 ‡	1/13 ‡	NA	NA	NA	NA	NA
Pournaras 2018	Leak: 14 Perforation: 7	14/14 (100.0) 6/7 (85.7)	0/14 (0) 1/7 (14.3)	1/14 (7.1) 1/7 (14.3)	NA	35	NA	NA	7
Ooi 2018	Leak: 6 Perforation: 4	4/6 (66.7) 2/4 (50.0)	1/6 (16.7) 1/4 (25.0)	2/6 (33.3) 1/4 (25.0)	NA	62	12	25.5 †	8.3 †
Noh 2018	Leak: 12	10/12 (83.3)	1/12 (8.3)	1/12 (8.3)	1/12 (8.3)	NA	NA	25	2.7 †
Loske 2018	Leak: 3 Perforation: 1	3/3 (100.0) 1/1 (100.0)	0/3 (0) 0/1 (0)	0/3 (0) 0/1 (0)	0/3 (0) 0/1 (0)	NA	NA	NA	NA

Table 2. *Cont.*

Authors	N	Successful Closure Rate (*n*, %)	Mortality Rate (*n*, %)	Complication Rate (*n*, %)	Stricture Rate (*n*, %)	Hospital Stay (Median, Days)	ICU Stay (Median, Days)	Duration of Therapy (Median, Days)	Sponge Changes
Laukoetter 2017	Leak: 39 Perforation: 13	36/39 (92.3) 13/13 (100.0)	5/39 (12.8) 0/13 (0)	2/39 (5.1) [§] 0/13 (0)	4/39 (10.2) 0/13 (0)	60 46	NA	20 24	6 6
Kuehn 2016	Leak: 11 Perforation: 10	9/11 (81.8) 10/10 (100.0)	1/11 (18.2) 0/10 (0)	NA	1/11 (18.2) 0/10 (0)	NA	NA	12 15	4 5
Hwang 2016	Leak: 18 EVT: 7	7/7 (100.0)	NA	0/7 (0)	NA	37.1	NA	27	4.3
	SEMS 11	7/11 (63.6)		6/11 (54.5)		87.3		19.2	1.6
Möschler 2015	Leak: 5 Perforation: 5	2/5 (40.0) 5/5 (100.0)	2/5 (40.0) 0/5 (0)	0/5 (0) 0/5 (0)	1/10 (10.0) [§]	38	NA	34.2 13	8.4 2
Mennigen 2015	Leak: 45 EVT: 15	14/15 (93.3)	1/15 (6.6)	0/15 (0)	NA	58	NA	26.5	6.5
	SEMS 30	19/30 (63.3)	8/30 (26.6)	0/30 (0)		53		36	1
Loske 2015	Perforation: 10	10/10 (100.0)	0/10 (0)	0/10 (0)	0/10 (0)	NA	NA	5	2
Heits 2014	Perforation: 10	9/10 (90.0)	1/10 (10.0)	NA	NA	48 [†]	22 [†]	NA	5.4 [†]
Liu 2014	Leak: 5	5/5 (100.0)	0/5 (0)	0/5 (0)	NA	NA	NA	34.2	NA
Schorsch 2013	Leak: 17	16/17 (94.1)	1/17 (5.9)	NA	1/17 (5.9)	NA	NA	12	NA
Schniewind 2013	Leak: 29 EVT: 17	NA	2/17 (11.8)	NA	NA	57 [†]	26 [†]	NA	NA
	SEMS 12		5/12 (41.7)			62 [†]	38 [†]		
Brangewitz 2013	EVT:32 SEMS: 39	27/32 (84.4) 21/39 (53.8)	5/32 (15.6) 11/39 (28.2)	9/32 (28.1) 3/39 (76.9)	3/32 (9.4) 11/39 (28.2)	48.5 41	NA	23 33	7 3

EVT, endoscopic vacuum therapy; ICU, intensive care unit; NA, not available; SEMS, self-expanding metal stent [†] Data expressed as mean. [‡] Only total rate was available. [§] Two patients died because of fatal hemorrhage during EVT.

The methodological quality of the studies is presented in Supplementary Table S5. The quality was poor in 15 studies [24,25,27,29,32,34,36–38,41–45,47] and moderate in 14 studies [13,14,18,22,23,26,28,30,31,33,35,39,40,46].

3.3. Primary and Secondary Outcomes

3.3.1. Primary Outcome—Successful Closure Rate

Twenty-seven studies reported data on successful closure in 456 patients. The pooled estimate rate for successful closure was 0.85 (95% confidence interval [CI]: 0.81–0.88, Figure 2). No heterogeneity was found among the studies ($I^2 = 0\%$, $p = 0.68$). No publication bias was detected by the Egger test ($p = 0.33$).

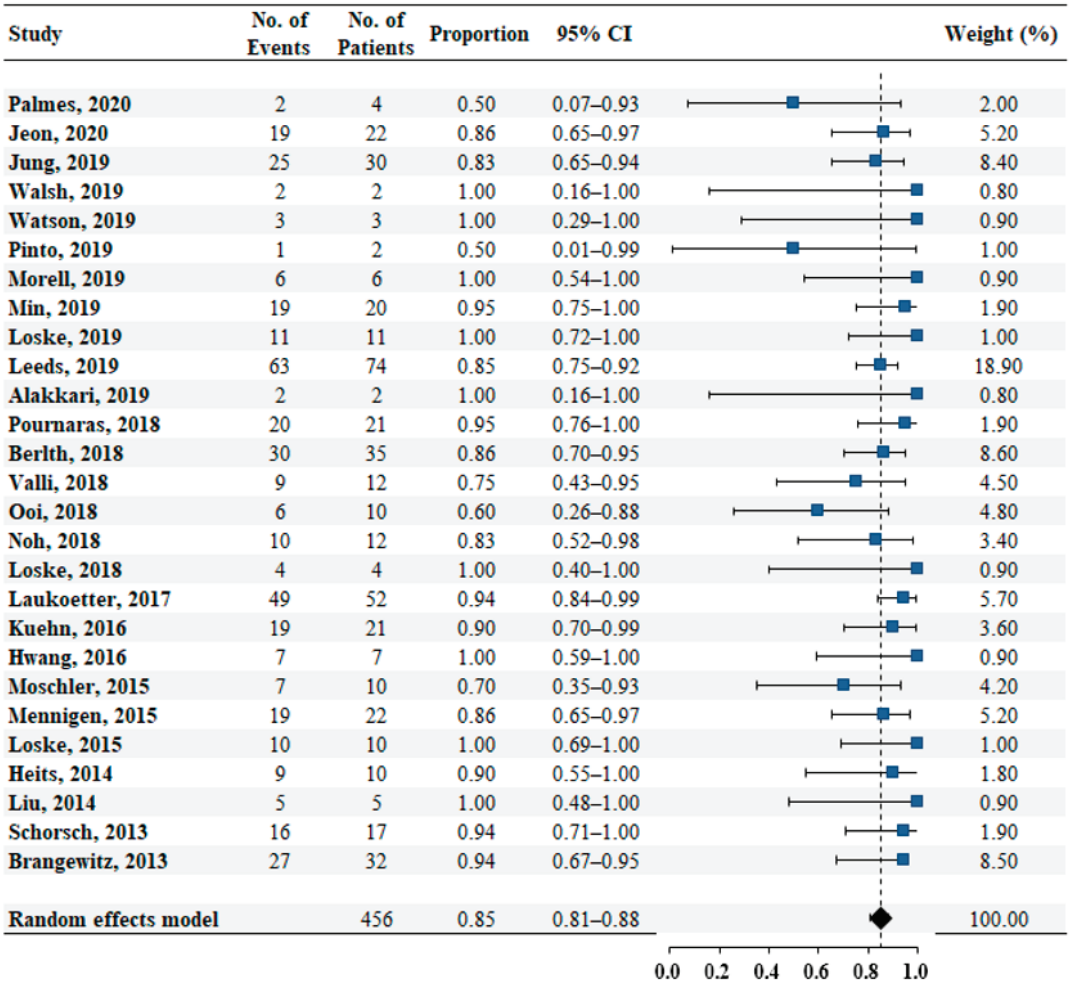


Figure 2. Pooled estimate rate for successful closure in patients with transmural defects of upper gastrointestinal tract.

3.3.2. Secondary Outcomes—Mortality, Complication, and Post-EVT Stricture Rates

Data on mortality were reported in 28 studies comprising a total of 412 patients. The pooled estimated mortality rate was 0.11 (95% CI: 0.09–0.15, as illustrated in Figure 3A). No heterogeneity was found among these studies ($I^2 = 0\%$, $p = 0.96$). No publication

bias was detected by the Egger test (p = 0.38). Twenty-one studies reported data on complications in 304 patients. The pooled estimate rate for complications was 0.10 (95% CI: 0.06–0.15, as illustrated in Figure 3B). Low heterogeneity was found among the studies (I^2 = 13.8%, p = 0.28). Publication bias was detected by the Egger test ($p < 0.05$). Sixteen studies reported data on post-EVT strictures in 240 patients. The pooled estimate rate for post-EVT stricture was 0.14 (95% CI: 0.10–0.20, as illustrated in Figure 3C). No heterogeneity was found among these studies (I^2 = 0%, p = 0.45). The p-value of publication bias by the Egger test was 0.06.

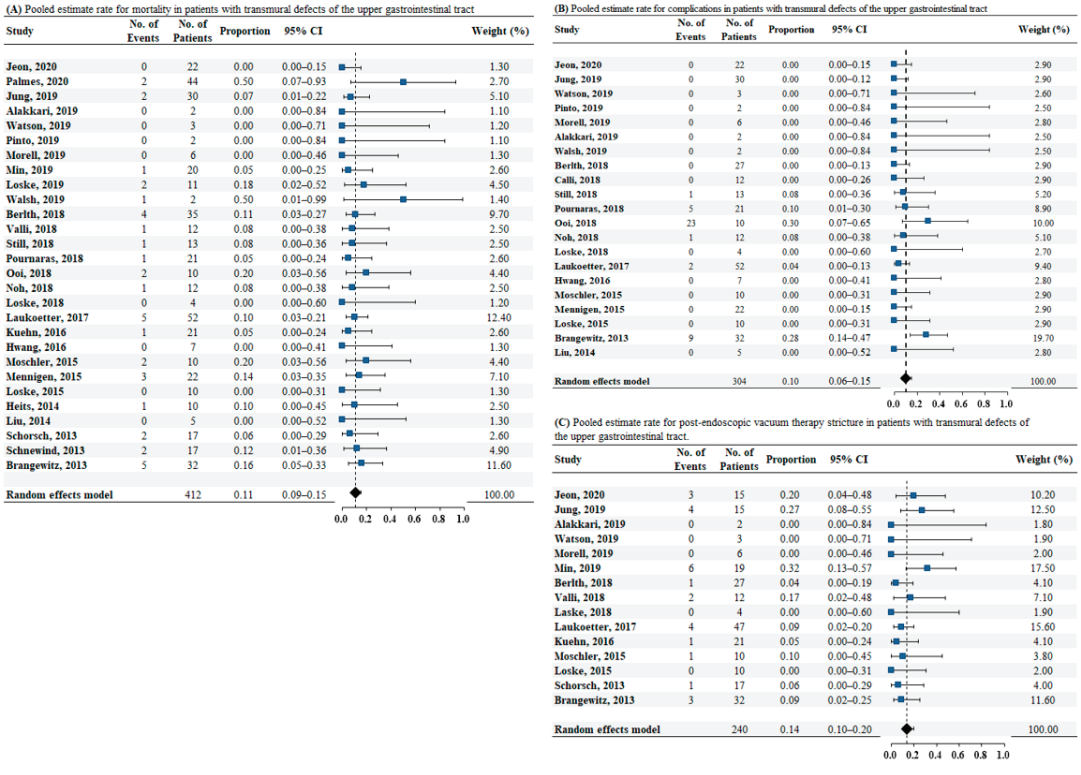


Figure 3. (**A**) Pooled estimate rate for mortality in patients with transmural defects of upper gastrointestinal tract. (**B**) Pooled estimate rate for complications in patients with transmural defects of the upper gastrointestinal tract. (**C**) Pooled estimate rate for postendoscopic vacuum therapy stricture in patients with transmural defects of upper gastrointestinal tract. Abbreviations: No, number; OR, odds ratio; CI, confidence interval.

3.4. Subgroup Analysis

3.4.1. Perforation vs. Leak and Fistula—Successful Closure, Mortality, Complications, and Post-EVT Stricture Rates

According to the etiology of the transmural defect, evaluation of the successful closure rate was performed in 11 studies. The pooled analysis showed that the successful closure rate was similar between the perforation and leak groups (odds ratio [OR]: 1.45, 95% CI: 0.45–4.67, p = 0.53; as illustrated in Figure 4A). We detected low heterogeneity among the studies (I^2 = 24.1%, p = 0.24). Data on mortality according to the etiology of transmural defects were available for 10 studies. The analysis revealed no significant difference between the two groups in terms of mortality rate (OR: 0.77, 95% CI: 0.24–2.46, p = 0.66; as illustrated in Figure 4B), and there was no heterogeneity (I^2 = 0%, p = 0.58). Eight studies reported data on complications according to the etiology of transmural defects. The pooled

analysis showed that the complication rates were similar between the perforation and leak groups (OR: 0.94, 95% CI: 0.17–5.15, $p = 0.94$; as illustrated in Figure 4C). No heterogeneity was detected among the studies ($I^2 = 0\%$, $p = 0.79$). Data on post-EVT stricture rate according to the etiology of transmural defects were available for five studies. No significant difference was observed between the two groups in terms of post-EVT stricture rate (OR: 0.70, 95% CI: 0.12–4.24, $p = 0.70$; as illustrated in Figure 4D), and no heterogeneity was noted ($I^2 = 0\%$, $p = 0.47$).

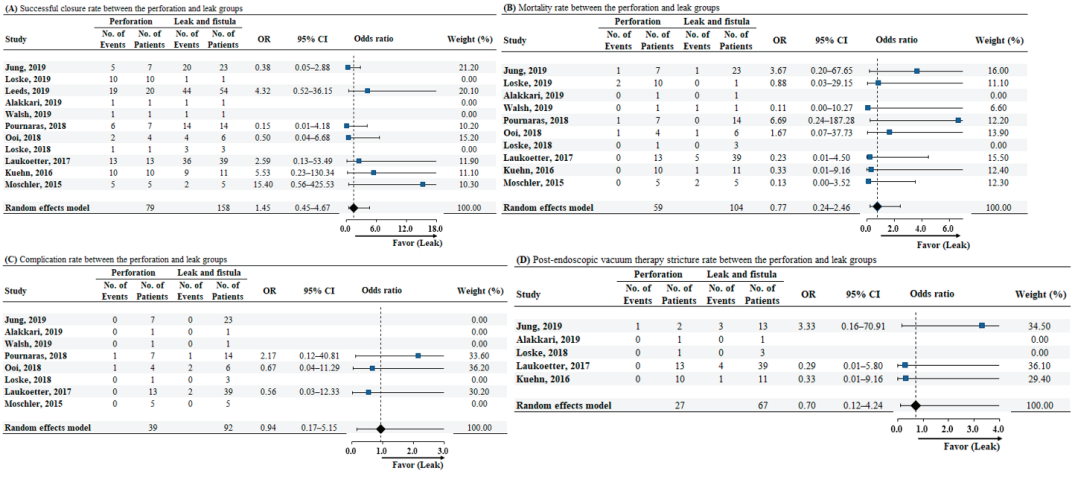


Figure 4. (**A**) Forrest plot of successful closure rate for comparison between the perforation and leak group. (**B**) Forrest plot of mortality rate for comparison between perforation and leak groups. (**C**) Forrest plot of complication rate for comparison between perforation and leak groups. (**D**) Forrest plot of postendoscopic vacuum therapy stricture rate for comparison between perforation and leak groups. Abbreviations: No, number; OR, odds ratio; CI, confidence interval.

3.4.2. EVT vs. SEMS—Successful Closure, Mortality, Treatment Duration, Length of Hospital Stay, and the Number of Endoscopic Stent/Sponge Changes

The length of hospital stay was mentioned in all included studies. Among the four studies that compared EVT and SEMS placement, successful closure rate, mortality rate, duration of treatment, and the number of endoscopic stent/sponge changes were demonstrated. The successful closure rate was significantly higher in the EVT group than in the SEMS group (OR: 3.14, 95% CI: 1.23–7.98, $p = 0.02$) (as illustrated in Figure 5A). The mortality rate was lower in the EVT group than in the SEMS group (OR: 0.39, 95% CI: 0.18–0.83, $p = 0.01$) (as illustrated in Figure 5B). Compared to SEMS placement, EVT showed a shorter treatment duration, with an estimated pooled median difference of 11.90 days (95% CI: −18.59–−5.21, $p < 0.01$), after excluding one study that reported a shorter duration of treatment with SEMS placement (as illustrated in Figure 5C). The length of hospital stay showed similar results between the EVT and SEMS groups with an estimated pooled median difference of 2.81 days (95% CI: 6.20–11.82, $p = 0.27$) (as illustrated in Figure 5D). In addition, the number of endoscopic stent/sponge changes were significantly higher in EVT than with SEMS placement, and an estimated pooled median difference of 3.09 was noted (95% CI 1.54–4.64, $p = 0.03$)) (as illustrated in Figure 5E).

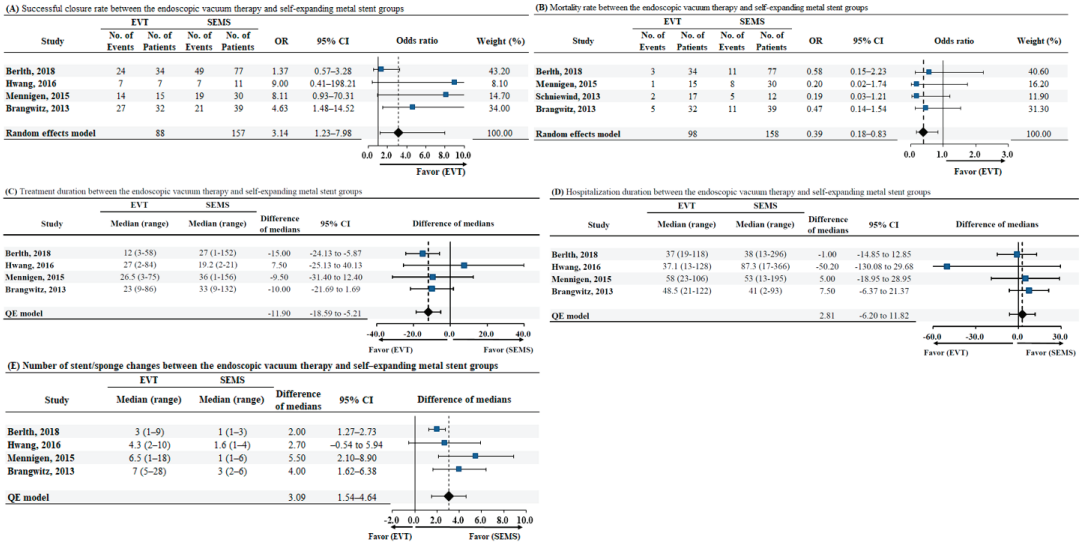


Figure 5. (**A**) Successful closure rate between endoscopic vacuum therapy and self-expanding metal stent groups, (**B**) Mortality rate between endoscopic vacuum therapy and self-expanding metal stent groups, (**C**) Treatment duration between endoscopic vacuum therapy and self-expanding metal stent groups, (**D**) Hospitalization duration between endoscopic vacuum therapy and self-expanding metal stent groups, (**E**) Number of stent/sponge changes between endoscopic vacuum therapy and self-expanding metal stent groups. Abbreviations: EVT, endoscopic vacuum therapy; SEMS self-expanding metal stent; No, number; OR, odds ratio; CI, confidence interval; QE, quantitative estimation.

4. Discussion

To date, many studies reported promising outcomes in patients with transmural defects of the UGI tract with EVT used as a definitive treatment. However, these previous studies included only a limited number of patients. Recently, several systematic reviews reported the usefulness of EVT in transmural defects of the UGI tract. [19,48–50]; however, these reviews were only descriptive and did not conduct statistical analysis with a summary estimate. Therefore, a meta-analysis is needed to compile and analyze the available data on the efficacy of EVT in transmural defects of the UGI tract. Our meta-analysis included case series in which a single group was assessed with no intrastudy comparisons. Nevertheless, this meta-analysis has an advantage over narrative reviews because it assessed effect sizes and integrated them into a single statistical analysis.

In this meta-analysis, the closure rate of transmural UGI defects with EVT was excellent (85%), and EVT was associated with low mortality (11%), complications (10%), and post-EVT stricture rates (14%) rates. Moreover, no significant difference was observed in successful closure (OR: 1.45, 95% CI: 0.45–4.67), mortality (OR: 0.77, 95% CI: 0.24–2.46), complications (OR: 0.94, 95% CI: 0.17–5.15, $p = 0.94$), and post-EVT stricture rates (OR: 0.70, 95% CI: 0.12–4.24, $p = 0.70$) according to the etiology of the transmural defect (perforation vs. leak and fistula). Although the etiology of transmural UGI defects was different, the efficacy of EVT was similar between the groups.

EVT had a significantly higher successful closure rate than with SEMS placement (OR: 3.14, 95% CI: 1.23–7.98). In addition, the mortality rate was lower (OR: 0.39, 95% CI: 0.18–0.83) and the treatment duration was shorter with EVT than with SEMS placement (−11.90, 95% CI: −18.59–−5.21). We believe that this was due to the difference in methodology between EVT and SEMS placement. Generally, SEMS removal or replacement is performed 4–6 weeks after SEMS insertion. Therefore, the successful closure rate with SEMS treatment was determined 4–6 weeks after the previous SEMS insertion. In contrast, because EVT is repeated every 3–5 days, clinicians can also check successful closure every 3–5 days.

Therefore, successful closure could be detected sooner with EVT than with SEMS placement. In addition, EVT treatment could offer the possibility of performing endoscopic lavage and debridement with every change, which was shown to reduce pleural inflammation and leakage-associated mortality.

The principle of EVT is similar to the classical vacuum-assisted closure treatment, which is a well-established therapy for chronic superficial wounds [51]. In EVT, a polyurethane sponge is placed inside the defect to apply negative pressure. Defect healing is achieved through continuous abscess drainage, thus decreasing bacterial colonization, enhancing vascularity, and promoting tissue granulation [51,52]. An internal vacuum sponge (endoSPONGE) device was first successfully used for treating a UGI anastomosis leak in 2008 [15]. Since then, EVT was used to manage UGI defects and showed good short- and long-term clinical outcomes. SEMS placement also showed effective outcomes for UGI defects [7,8]. However, stent therapy is usually accompanied by additional abscess drainage, local pressure necrosis of the mucosa, stent migration, stent ingrowth, bleeding, and perforation. Surgery is also one of the strategies for treating transmural defects of the UGI; however, it is associated with a high mortality rate [5,6]. To date, comparative studies assessing different treatment modalities for UGI defects are rare [53]. Therefore, clinical evidence of efficacy of EVT for treatment of UGI defects is still inadequate for directing treatment modalities. Our meta-analysis showed that EVT is an effective and safe treatment method for treating leaks, fistulae, and perforations.

Usually, transmural defects of the UGI tract are classified as perforations, leaks, or fistulae. Of these, fistulae are the most difficult to close because the epithelial tract is often fibrotic, and these arise in unhealthy tissues, which are inflamed, damaged, or ischemic. Although the included cases were too few (n = 8), this meta-analysis showed a successful closure rate of 50% in patients with a fistula. Given the inadequate response of fistulae to other treatments such as SEMS placement, EVT is a promising option for treating patients with fistula.

The major disadvantages of EVT are the need for repetitive endoscopic procedures, nasogastric tube-related discomfort, and sponge dislocation. The main and most dreadful event associated with EVT is massive bleeding [19,48]. It can occur from a fistula between the cavity and main vessels and from rupture of a pseudoaneurysm from circumjacent vessels or heart chambers. More frequent changes of the sponge may help prevent or reduce the risk of severe bleeding. Moreover, massive bleeding can occur in cases of intracavitary therapy in which direct contact with blood vessels is possible. Therefore, intraluminal EVT may be safer than intracavitary EVT. Additionally, computed tomography scans should be reviewed before initiating intracavitary EVT to exclude vascular complications. In our review, post-EVT strictures occurred in 14% of cases; however, all strictures were easily resolved through endoscopic dilatations (26 cases).

Although the results of this study are promising, it had several limitations. All included studies were retrospective in nature without randomisation. This could have resulted in a selection bias in this study. Typically, the choice of modalities (EVT, SEMS placement, operation, and nonoperative management) were chosen according to the severity of the patients. Patients managed conservatively tend not to be septic and have a contained leak versus those who have apparent mediastinal contamination and warrant endoscopic or surgical intervention. As EVT is a relatively new treatment method, it could be assumed that the first experience of the studies included in this meta-analysis was performed in cases in which a favorable outcome was expected, thus influencing the results. Although randomized controlled trials are considered the best method for evaluating treatment effects, performing such trials would be difficult owing to ethical concerns and methodological difficulties. Second, although the statistical heterogeneity was low, the clinical heterogeneity was high among the included studies. Patient heterogeneity and detailed indication were different among the included studies. Therefore, the complexity and comorbidities of each patient could affect the treatment success. In addition, SEMS placement is a more standard treatment compared to EVT, which may also affect treatment outcomes.

To address this limitation, we have additionally summarized detailed information of the studies included in this meta-analysis (as illustrated in supplementary Tables S1–S4) Third, the included studies had a limited quality. Fourth, the sample size of each study was insufficient to reach definitive conclusions. Therefore, additional data are needed to define the role of EVT in patients with UGI defects. Finally, most of the included studies were from Western countries, especially Germany. Large-scale studies from other regions are required to validate the usefulness of EVT in treating UGI defects in patients of different ethnicities. Despite these limitations, to the best our knowledge, this meta-analysis contains the most comprehensive analysis of the effectiveness of EVT for treating UGI defects.

5. Conclusions

In summary, this meta-analysis revealed that EVT could be an effective and safe treatment method for leaks and fistulae as well as perforations in the UGI. In addition, EVT may be a better treatment option than SEMS placement for UGI defects. However, a definite recommendation cannot be made for the treatment of UGI defects due to the limitations of the included studies mentioned above. We believe that prospective large-scale studies from various regions worldwide are needed to validate the effectiveness of EVT for treating UGI defects.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jcm10112346/s1: Table S1, search terms in PubMed, Embase and Cochrane Library; Table S2, definition of clinical success, detail indication, and cause of mortality in the 29 studies included; Table S3, detailed information of the 4 studies included patients with fistula; Table S4, patients' characteristics of studies comparing endoscopic vacuum therapy and self-expanding metal stent, and Table S5, methodological quality.

Author Contributions: Conceptualization, D.H.J., H.-R.Y., C.W.H.; data curation, N.W.K., C.W.H.; formal analysis, D.H.J., H.-R.Y., C.W.H.; investigation, D.H.J., H.-R.Y., S.J.L., N.W.K., C.W.H.; methodology, D.H.J., H.-R.Y., S.J.L., N.W.K., C.W.H.; project administration, C.W.H.; resources, C.W.H.; software, D.H.J., H.-R.Y., S.J.L., N.W.K., C.W.H.; supervision, S.J.L., C.W.H.; validation, C.W.H.; visualization, H.-R.Y.; writing—original draft, D.H.J., H.-R.Y.; writing—review & editing, C.W.H. All authors read and agreed to the published version of the manuscript, including authorship list.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Freni, F.; Galletti, B.; Bruno, R.; Martines, F.; Abita, P.; Gazia, F.; Sireci, F.; Galletti, F. Multidisciplinary approach in the removal of post-trauma foreign bodies in the head and neck district: Cases report and review of literature. *Acta Med. Mediterr.* **2019**, *35*, 405–410.
2. Canevari, F.R.; Martines, F.; Sorrentino, R.; Nicolotti, M.; Sireci, F. Pseudoaneurysm of Superior Thyroid Artery Following A Transesophageal Echocardiography: A Case Presentation. *Euromediter. Biomed. J.* **2017**, *12*, 010–012.
3. Bufkin, B.L.; Miller, J.I., Jr.; Mansour, K.A. Esophageal perforation: Emphasis on management. *Ann. Thorac. Surg.* **1996**, *61*, 1447–1451. [CrossRef]
4. Altorjay, A.; Kiss, J.; Voros, A.; Bohak, A. Nonoperative management of esophageal perforations. Is it justified? *Ann. Surg.* **1997**, *225*, 415–421. [CrossRef]
5. Brinster, C.J.; Singhal, S.; Lee, L.; Marshall, M.B.; Kaiser, L.R.; Kucharczuk, J.C. Evolving options in the management of esophageal perforation. *Ann. Thorac. Surg.* **2004**, *77*, 1475–1483. [CrossRef]
6. Rohatgi, A.; Papanikitas, J.; Sutcliffe, R.; Forshaw, M.; Mason, R. The role of oesophageal diversion and exclusion in the management of oesophageal perforations. *Int. J. Surg.* **2009**, *7*, 142–144. [CrossRef]
7. Kauer, W.K.; Stein, H.J.; Dittler, H.J.; Siewert, J.R. Stent implantation as a treatment option in patients with thoracic anastomotic leaks after esophagectomy. *Surg. Endosc.* **2008**, *22*, 50–53. [CrossRef] [PubMed]

8. Huh, C.W.; Kim, J.S.; Choi, H.H.; Lee, J.I.; Ji, J.S.; Kim, B.W.; Choi, H. Treatment of benign perforations and leaks of the esophagus: Factors associated with success after stent placement. *Surg. Endosc.* **2018**, *32*, 3646–3651. [CrossRef] [PubMed]
9. Boulis, N.M.; Armstrong, W.S.; Chandler, W.F.; Orringer, M.B. Epidural abscess: A delayed complication of esophageal stenting for benign stricture. *Ann. Thorac. Surg.* **1999**, *68*, 568–570. [CrossRef]
10. Ho, H.S.; Ong, H.S. A rare life-threatening complication of migrated nitinol self-expanding metallic stent (ultraflex). *Surg. Endosc.* **2004**, *18*, 347. [CrossRef]
11. Swinnen, J.; Eisendrath, P.; Rigaux, J.; Kahegeshe, L.; Lemmers, A.; Le Moine, O.; Deviere, J. Self-expandable metal stents for the treatment of benign upper GI leaks and perforations. *Gastrointest. Endosc.* **2011**, *73*, 890–899. [CrossRef] [PubMed]
12. Bludau, M.; Fuchs, H.F.; Herbold, T.; Maus, M.K.H.; Alakus, H.; Popp, F.; Leers, J.M.; Bruns, C.J.; Holscher, A.H.; Schroder, W.; et al. Results of endoscopic vacuum-assisted closure device for treatment of upper gi leaks. *Surg. Endosc.* **2018**, *32*, 1906–1914. [CrossRef] [PubMed]
13. Min, Y.W.; Kim, T.; Lee, H.; Min, B.H.; Kim, H.K.; Choi, Y.S.; Lee, J.H.; Rhee, P.L.; Kim, J.J.; Zo, J.I.; et al. Endoscopic vacuum therapy for postoperative esophageal leak. *BMC Surg.* **2019**, *19*, 37. [CrossRef] [PubMed]
14. Jung, C.F.M.; Muller-Dornieden, A.; Gaedcke, J.; Kunsch, S.; Gromski, M.A.; Biggemann, L.; Hosseini, A.S.A.; Ghadimi, M.; Ellenrieder, V.; Wedi, E. Impact of endoscopic vacuum therapy with low negative pressure for esophageal perforations and postoperative anastomotic esophageal leaks. *Digestion* **2020**, 1–11. [CrossRef] [PubMed]
15. Wedemeyer, J.; Schneider, A.; Manns, M.P.; Jackobs, S. Endoscopic vacuum-assisted closure of upper intestinal anastomotic leaks. *Gastrointest. Endosc.* **2008**, *67*, 708–711. [CrossRef]
16. Loske, G.; Schorsch, T.; Muller, C. Endoscopic vacuum sponge therapy for esophageal defects. *Surg. Endosc.* **2010**, *24*, 2531–2535. [CrossRef] [PubMed]
17. Smallwood, N.R.; Fleshman, J.W.; Leeds, S.G.; Burdick, J.S. The use of endoluminal vacuum (E-vac) therapy in the management of upper gastrointestinal leaks and perforations. *Surg. Endosc.* **2016**, *30*, 2473–2480. [CrossRef]
18. Laukoetter, M.G.; Mennigen, R.; Neumann, P.A.; Dhayat, S.; Horst, G.; Palmes, D.; Senninger, N.; Vowinkel, T. Successful closure of defects in the upper gastrointestinal tract by endoscopic vacuum therapy (EVT): A prospective cohort study. *Surg. Endosc.* **2017**, *31*, 2687–2696. [CrossRef]
19. Virgilio, E.; Ceci, D.; Cavallini, M. Surgical endoscopic vacuum-assisted closure therapy (EVAC) in treating anastomotic leakages after major resective surgery of esophageal and gastric cancer. *Anticancer Res.* **2018**, *38*, 5581–5587. [CrossRef]
20. Liberati, A.; Altman, D.G.; Tetzlaff, J.; Mulrow, C.; Gotzsche, P.C.; Ioannidis, J.P.; Clarke, M.; Devereaux, P.J.; Kleijnen, J.; Moher, D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. *J. Clin. Epidemiol.* **2009**, *62*, e1–e34. [CrossRef] [PubMed]
21. Higgins, J.P.; Thompson, S.G.; Deeks, J.J.; Altman, D.G. Measuring inconsistency in meta-analyses. *BMJ* **2003**, *327*, 557–560. [CrossRef]
22. Brangewitz, M.; Voigtlander, T.; Helfritz, F.A.; Lankisch, T.O.; Winkler, M.; Klempnauer, J.; Manns, M.P.; Schneider, A.S.; Wedemeyer, J. Endoscopic closure of esophageal intrathoracic leaks: Stent versus endoscopic vacuum-assisted closure, a retrospective analysis. *Endoscopy* **2013**, *45*, 433–438. [CrossRef]
23. Schniewind, B.; Schafmayer, C.; Voehrs, G.; Egberts, J.; von Schoenfels, W.; Rose, T.; Kurdow, R.; Arlt, A.; Ellrichmann, M.; Jurgensen, C.; et al. Endoscopic endoluminal vacuum therapy is superior to other regimens in managing anastomotic leakage after esophagectomy: A comparative retrospective study. *Surg. Endosc.* **2013**, *27*, 3883–3890. [CrossRef]
24. Heits, N.; Stapel, L.; Reichert, B.; Schafmayer, C.; Schniewind, B.; Becker, T.; Hampe, J.; Egberts, J.H. Endoscopic endoluminal vacuum therapy in esophageal perforation. *Ann. Thorac. Surg.* **2014**, *97*, 1029–1035. [CrossRef] [PubMed]
25. Liu, Y.N.; Yan, Y.; Li, S.J.; Liu, H.; Wu, Q.; Zhang, L.J.; Yang, Y.; Chen, J.F. Reliable management of post-esophagectomy anastomotic fistula with endoscopic trans-fistula negative pressure drainage. *World J. Surg. Oncol.* **2014**, *12*, 240. [CrossRef] [PubMed]
26. Schorsch, T.; Muller, C.; Loske, G. Endoscopic vacuum therapy of perforations and anastomotic insufficiency of the esophagus. *Chirurg* **2014**, *85*, 1081–1093. [CrossRef]
27. Loske, G.; Schorsch, T.; Dahm, C.; Martens, E.; Muller, C. Iatrogenic perforation of esophagus successfully treated with endoscopic vacuum therapy (EVT). *Endosc. Int. Open* **2015**, *3*, E547–E551. [CrossRef]
28. Mennigen, R.; Harting, C.; Lindner, K.; Vowinkel, T.; Rijcken, E.; Palmes, D.; Senninger, N.; Laukoetter, M.G. Comparison of endoscopic vacuum therapy versus stent for anastomotic leak after esophagectomy. *J. Gastrointest. Surg.* **2015**, *19*, 1229–1235. [CrossRef]
29. Moschler, O.; Nies, C.; Mueller, M.K. Endoscopic vacuum therapy for esophageal perforations and leakages. *Endosc. Int. Open* **2015**, *3*, E554–E558. [CrossRef] [PubMed]
30. Hwang, J.J.; Jeong, Y.S.; Park, Y.S.; Yoon, H.; Shin, C.M.; Kim, N.; Lee, D.H. Comparison of endoscopic vacuum therapy and endoscopic stent implantation with self-expandable metal stent in treating postsurgical gastroesophageal leakage. *Medicine* **2016**, *95*, e3416. [CrossRef]
31. Kuehn, F.; Schiffmann, L.; Janisch, F.; Schwandner, F.; Alsfasser, G.; Gock, M.; Klar, E. Surgical endoscopic vacuum therapy for defects of the upper gastrointestinal tract. *J. Gastrointest. Surg.* **2016**, *20*, 237–243. [CrossRef] [PubMed]
32. Loske, G.; Schorsch, T.; Rucktaeschel, F.; Schulze, W.; Riefel, B.; van Ackeren, V.; Mueller, C.T. Open-pore film drainage (OFD): A new multipurpose tool for endoscopic negative pressure therapy (ENPT). *Endosc. Int. Open* **2018**, *6*, E865–E871. [CrossRef] [PubMed]

33. Noh, S.M.; Ahn, J.Y.; Lee, J.H.; Jung, H.Y.; AlGhamdi, Z.; Kim, H.R.; Kim, Y.H. Endoscopic vacuum-assisted closure therapy in patients with anastomotic leakage after esophagectomy: A single-center experience. *Gastroenterol. Res. Pr.* **2018**, *2018*, 1697968. [CrossRef] [PubMed]
34. Ooi, G.; Burton, P.; Packiyanathan, A.; Loh, D.; Chen, R.; Shaw, K.; Brown, W.; Nottle, P. Indications and efficacy of endoscopic vacuum-assisted closure therapy for upper gastrointestinal perforations. *ANZ J. Surg.* **2018**, *88*, E257–E263. [CrossRef]
35. Pournaras, D.J.; Hardwick, R.H.; Safranek, P.M.; Sujendran, V.; Bennett, J.; Macaulay, G.D.; Hindmarsh, A. Endoluminal vacuum therapy (E-vac): A treatment option in oesophagogastric surgery. *World J. Surg.* **2018**, *42*, 2507–2511. [CrossRef]
36. Still, S.; Mencio, M.; Ontiveros, E.; Burdick, J.; Leeds, S.G. Primary and rescue endoluminal vacuum therapy in the management of esophageal perforations and leaks. *Ann. Thorac. Cardiovasc. Surg.* **2018**, *24*, 173–179. [CrossRef]
37. Valli, P.V.; Mertens, J.C.; Kroger, A.; Gubler, C.; Gutschow, C.; Schneider, P.M.; Bauerfeind, P. Stent-over-sponge (SOS): A novel technique complementing endosponge therapy for foregut leaks and perforations. *Endoscopy* **2018**, *50*, 148–153. [CrossRef]
38. Alakkari, A.; Sood, R.; Everett, S.M.; Rembacken, B.J.; Hayden, J.; Sarela, A.; Mohammed, N. First UK experience of endoscopic vacuum therapy for the management of oesophageal perforations and postoperative leaks. *Frontline Gastroenterol.* **2019**, *10*, 200–203. [CrossRef]
39. Berlth, F.; Bludau, M.; Plum, P.S.; Herbold, T.; Christ, H.; Alakus, H.; Kleinert, R.; Bruns, C.J.; Holscher, A.H.; Chon, S.H. Self-expanding metal stents versus endoscopic vacuum therapy in anastomotic leak treatment after oncologic gastroesophageal surgery. *J. Gastrointest. Surg.* **2019**, *23*, 67–75. [CrossRef]
40. Leeds, S.G.; Mencio, M.; Ontiveros, E.; Ward, M.A. Endoluminal vacuum therapy: How I do it. *J. Gastrointest. Surg.* **2019**, *23*, 1037–1043. [CrossRef]
41. Loske, G.; Rucktaeschel, F.; Schorsch, T.; Moenkemueller, K.; Mueller, C.T. Endoscopic negative pressure therapy (ENPT) for duodenal leakage—novel repair technique using open-pore film (OFD) and polyurethane-foam drainages (OPD). *Endosc. Int. Open* **2019**, *7*, E1424–E1431. [CrossRef] [PubMed]
42. Morell, B.; Murray, F.; Vetter, D.; Bueter, M.; Gubler, C. Endoscopic vacuum therapy (EVT) for early infradiaphragmal leakage after bariatric surgery-outcomes of six consecutive cases in a single institution. *Langenbecks Arch. Surg.* **2019**, *404*, 115–121. [CrossRef]
43. Rodrigues-Pinto, E.; Morais, R.; Vilas-Boas, F.; Pereira, P.; Macedo, G. Role of endoscopic vacuum therapy, internal drainage, and stents for postbariatric leaks. *VideoGIE* **2019**, *4*, 481–485. [CrossRef] [PubMed]
44. Walsh, L.T.; Loloi, J.; Manzo, C.E.; Mathew, A.; Maranki, J.; Dye, C.E.; Levenick, J.M.; Taylor, M.D.; Moyer, M.T. Successful treatment of large cavity esophageal disruptions with transluminal washout and endoscopic vacuum therapy: A report of two cases. *Adv. Gastrointest. Endosc.* **2019**, *12*, 2631774519860300. [CrossRef]
45. Watson, A.; Zuchelli, T. Repair of upper-GI fistulas and anastomotic leakage by the use of endoluminal vacuum-assisted closure. *VideoGIE* **2019**, *4*, 40–44. [CrossRef] [PubMed]
46. Jeon, J.H.; Jang, H.J.; Han, J.E.; Park, Y.S.; Seong, Y.W.; Cho, S.; Jheon, S.; Kim, K. Endoscopic vacuum therapy in the management of postoperative leakage after esophagectomy. *World J. Surg.* **2020**, *44*, 179–185. [CrossRef] [PubMed]
47. Palmes, D.; Kebschull, L.; Bahde, R.; Senninger, N.; Pascher, A.; Laukotter, M.G.; Eichelmann, A.K. Management of nonmalignant tracheo- and bronchoesophageal fistula after esophagectomy. *Thorac. Cardiovasc. Surg.* **2020**, *69*, 216–222. [CrossRef]
48. Kuehn, F.; Loske, G.; Schiffmann, L.; Gock, M.; Klar, E. Endoscopic vacuum therapy for various defects of the upper gastrointestinal tract. *Surg. Endosc.* **2017**, *31*, 3449–3458. [CrossRef]
49. Verstegen, M.H.P.; Bouwense, S.A.W.; van Workum, F.; Ten Broek, R.; Siersema, P.D.; Rovers, M.; Rosman, C. Management of intrathoracic and cervical anastomotic leakage after esophagectomy for esophageal cancer: A systematic review. *World J. Emerg. Surg.* **2019**, *14*, 17. [CrossRef]
50. Newton, N.J.; Sharrock, A.; Rickard, R.; Mughal, M. Systematic review of the use of endo-luminal topical negative pressure in oesophageal leaks and perforations. *Dis. Esophagus* **2017**, *30*, 1–5. [CrossRef]
51. Argenta, L.C.; Morykwas, M.J. Vacuum-assisted closure: A new method for wound control and treatment: Clinical experience. *Ann. Plast. Surg.* **1997**, *38*, 563–576. [CrossRef]
52. Wedemeyer, J.; Brangewitz, M.; Kubicka, S.; Jackobs, S.; Winkler, M.; Neipp, M.; Klempnauer, J.; Manns, M.P.; Schneider, A.S. Management of major postsurgical gastroesophageal intrathoracic leaks with an endoscopic vacuum-assisted closure system. *Gastrointest. Endosc.* **2010**, *71*, 382–386. [CrossRef] [PubMed]
53. Schaheen, L.; Blackmon, S.H.; Nason, K.S. Optimal approach to the management of intrathoracic esophageal leak following esophagectomy: A systematic review. *Am. J. Surg.* **2014**, *208*, 536–543. [CrossRef] [PubMed]

Journal of
Clinical Medicine

MDPI

Article

Cost-Effectiveness, Efficacy, and Safety Analysis of Tailored Therapy in Patients with *Helicobacter pylori* Infection

A Reum Choe, Ki-Nam Shim *, Yehyun Park, Eun-Mi Song, Chung Hyun Tae and Sung-Ae Jung

Department of Internal Medicine, College of Medicine, Ewha Womans University, Seoul 07985, Korea; archoi20@ewha.ac.kr (A.R.C.); splendidyh1029@ewha.ac.kr (Y.P.); rainbowtip@ewha.ac.kr (E.-M.S.); jhtae@ewha.ac.kr (C.H.T.); jassa@ewha.ac.kr (S.-A.J.)
* Correspondence: shimkn@ewha.ac.kr; Tel.: +82-2-6986-3122; Fax: +82-6986-3129

Abstract: Recently in Korea, where triple therapy is accepted as the first-line *Helicobacter pylori* (*H. pylori*) eradication treatment, antibiotic resistance to clarithromycin has increased considerably, resulting in eradication rates of less than 80%. We investigated the efficacy of tailored therapy after a clarithromycin resistance test compared with empirical therapy for *H. pylori* eradication. The cost-effectiveness of *H. pylori* eradication success was evaluated according to the average medical cost per patient. A total of 364 patients were enrolled in the study. The first-line *H. pylori* eradication rate was significantly higher in patients who received tailored therapy than in those who received empirical therapy. The total medical costs for the tailored and empirical groups were 46,374 Won and 53,528 Won. The total treatment period for each ultimately successful eradication in the tailored group was 79.8 $\pm$ 2.8 days, which is shorter than that of the empirical group (99.2 $\pm$ 7.4 days). The rate of eradication-related adverse events for the tailored group and empirical group was 12.9% and 14.8%, respectively. Tailored therapy could be a useful option to achieve a higher successful eradication rate, shorter treatment periods, and lower medical costs than empirical therapy in the era of increasing antibiotic resistance.

Keywords: *Helicobacter pylori*; eradication; antibiotic resistance; tailored; empirical

Citation: Choe, A.R.; Shim, K.-N.; Park, Y.; Song, E.-M.; Tae, C.H.; Jung, S.-A. Cost-Effectiveness, Efficacy, and Safety Analysis of Tailored Therapy in Patients with *Helicobacter pylori* Infection. *J. Clin. Med.* **2021**, *10*, 2619. https://doi.org/10.3390/jcm10122619

Academic Editor: Marilena Durazzo

Received: 8 April 2021
Accepted: 11 June 2021
Published: 14 June 2021

1. Introduction

Helicobacter pylori (*H. pylori*) is associated with peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma, and gastric cancer [1–3], and Korean guidelines recommend triple therapy as the first-line eradication treatment for *H. pylori* infection [4]. However, due to the increase of antibiotic-resistant strains, the eradication rate has gradually decreased [5,6], and several clinical studies have reported that the eradication rate of triple therapy is less than 80% [7–9]. The causes of this overall decrease in eradication rate include antibiotic resistance, age, smoking status, the difference in host immunity, underlying disease, and poor drug compliance [10,11], but antibiotic resistance is known as the most important factor among them [12].

Kuo et al. recently reported on patients with refractory *H. pylori* infection in Taiwan. Dual resistance to both clarithromycin and levofloxacin was found in 73.2%. This study highlighted ways to decide the optimum *H. pylori* eradication strategy according to the results of antibacterial susceptibility analysis [13]. In Italy, current guidelines recommend 10-day bismuth-based or sequential and concomitant regimens for first-line *H. pylori* eradication. Bismuth-based and bismuth-free therapies are equally effective for first-line *H. pylori* eradication [14]. Therefore, in areas with high clarithromycin resistance, opinions have arisen that the eradication treatment should be improved by adding more antibiotics instead of the triple therapy as the primary treatment, or changing to a new antibiotic of another class [15].

An understanding of the mechanism of *H. pylori* resistance is extremely complex. The *H. pylori* virulence factors are involved in the induction of inflammatory responses,

and control and regulate those responses, maintaining chronic inflammation [16]. The *H. pylori* exhibit an expanded complex of mechanisms that alter host cellular responses and signaling pathways. *H. pylori* elicit numerous adaptive mechanisms that enable effective bacterial adherence, colonization, and cellular alterations that provide the induction of further premalignant changes in the gastric microenvironment [17].

In the Maastricht V/Florence guidelines, bismuth-containing quadruple therapy or concomitant therapy is recommended in areas where the clarithromycin resistance rate is higher than 15% [18]. Recently, the clarithromycin resistance rate in Korea has been about 30% [19]. Based on the aforementioned guidelines, it is necessary to establish a treatment plan based on the results of antibiotic susceptibility tests rather than maintaining the existing standard triple therapy that is based on clarithromycin as the primary treatment. Resistance to clarithromycin is mostly caused by a point mutation at position 2142 or 2143 of the 23S ribosomal RNA gene [20], and antibiotic resistance can also be predicted by using a dual-priming oligonucleotide (DPO)-based multiplex polymerase chain reaction (PCR) test [21]. This method is an excellent test that shows a relatively high sensitivity of 82% to 90% and a specificity of 95% or more. Moreover, it has the advantage of being able to confirm the resistance to clarithromycin in addition to other antibiotics [22]. Therefore, it is possible to check the individual's resistance to antibiotics and then perform tailored therapy for *H. pylori*.

Tailored therapy based on the DPO-PCR test could be a useful regimen to increase the eradication rate of *H. pylori* infection. However, the cost-effectiveness of this test has not yet been definitively identified. The DPO-based multiplex PCR is more expensive than the Giemsa stain and rapid urease tests. It is difficult to perform this test routinely in clinical practice before the eradication of *H. pylori*. Therefore, we aimed to evaluate the eradication rate, adverse drug events, and cost-effectiveness of empirical and tailored therapies for the treatment of *H. pylori* infection.

2. Materials and Methods

2.1. Study Design and Subjects

This retrospective study was performed at two university hospitals in South Korea (Ewha Womans University Mokdong Hospital and Ewha Womans University Seoul Hospital) from January 2017 to December 2019. We included 435 subjects who met the following criteria: (1) the presence of *H. pylori* was confirmed by rapid urease test, histology such as Giemsa stain, urea breath test (UBT), or DPO-PCR test; (2) patients receiving empirical therapy or tailored therapy based on the DPO-PCR results. Subjects (n = 71) were excluded in the following conditions: (1) patients aged >80 years (n = 13); (2) history of gastrectomy (n = 18); (3) severe systemic illness, such as severe cardiopulmonary dysfunction, liver cirrhosis, or renal failure (n = 17); (4) history of any allergic reaction to antibiotics (n = 8); (5) loss to follow-up (n = 15).

A total of 364 patients who received *H. pylori* eradication treatment (n = 121 in Ewha Womans University Mokdong Hospital and n = 243 in Ewha Womans University Seoul Hospital) were enrolled in this study. Data for January 2017 and January 2018 were collected through chart review in Ewha Womans University Mokdong Hospital, and data for February 2018 and December 2019 were collected using data extracted from the Clinical Data Warehouse of the Ewha Womans University Seoul Hospital. This study was approved by the Institutional Review Board (IRB approval number: 2020-09-013).

2.2. H. Pylori Diagnosis and DPO-Based Multiplex PCR

Infection with *H. pylori* was regarded as positive when at least one positive result was obtained in UBT (Otsuka®, Tokyo, Japan), rapid urease test (CLOtest®; Delta West, Bentley, Australia), or histologic assessment (Giemsa staining) conducted using gastric biopsy specimens from the antrum and greater curvature of the body. In the tailored therapy group, DNA was extracted from frozen gastric biopsy specimens to detect clarithromycin-resistant *H. pylori* mutants. DPO-based multiplex PCR (Seeplex® *H. pylori*-ClaR ACE

Detection; Seegene, Inc., Seoul, Korea) was performed. Point mutations were identified by PCR amplification of a portion of the 23S ribosomal RNA gene. The amplified DNA products were visualized on a UV transilluminator after electrophoresis on a 2% agarose gel. The amplified DNA products were determined to have point mutations.

2.3. H. Pylori Eradication Therapy Regimen

There were a total of three regimens for empirical therapy, which depended on the doctor's preference: (1) triple therapy (proton-pump inhibitor bid, amoxicillin 1 g bid, clarithromycin 500 mg bid) for 14 days; (2) sequential therapy (proton-pump inhibitor bid and amoxicillin 1 g for 5 days followed by proton-pump inhibitor bid, clarithromycin 500 mg, and metronidazole 500 mg for 5 days) for 10 days; (3) bismuth-containing quadruple therapy (proton-pump inhibitor bid, bismuth subcitrate 300 mg qid, metronidazole 500 mg tid, tetracycline 500 mg qid) for 14 days. In the tailored therapy group, patients received the eradication regimen based on the results of the DPO-PCR test. Six weeks after the end of the treatment, compliance with therapy and side effects were assessed through personal interviews. An adverse event was defined as an unscheduled early visit to the outpatient clinic with symptoms during eradication or when the *H. pylori* eradication treatment was stopped due to adverse drug effects. UBT, rapid urease test, or Giemsa staining of gastric biopsy specimens were performed to confirm successful *H. pylori* eradication.

2.4. Medical Cost

The total medical cost per patient was assessed as the sum of the diagnostic and regimen costs. The cost of the DPO-PCR test was 38,350 Won, while those of the Giemsa stain and rapid urease test were 11,427 Won and 10,504 Won, respectively. The cost of 14-day triple therapy was calculated to be 34,300 Won. The cost of 10 day sequential therapy was 41,800 Won and that of the 14-day quadruple therapy was 25,746 Won.

2.5. Statistical Analysis

Continuous variables were presented as mean ± standard deviation, and categorical variables were presented as the number of subjects and percentage. Group comparisons were performed using independent samples *t*-tests or Mann–Whitney U tests for continuous variables and Pearson's chi-squared tests or Fisher's exact tests for categorical variables. Categorical variables are presented as numbers and proportions. All statistical analyses were 2-sided, and results were considered statistically significant at $p < 0.05$. The Statistical Package for the Social Science (SPSS) software (version 21.0; SPSS Inc., Chicago, IL, U.S.) was used for statistical analysis.

3. Results

3.1. Baseline Characteristics of Study Subjects

During the study period, 435 patients received *H. pylori* eradication treatment. After excluding 71 patients, 364 patients were finally enrolled in the study. Empirical therapy was given to 155 patients, and 209 patients received tailored therapy. There were older and more current smokers in the tailored therapy group. The most common cause of need for *H. pylori* eradication was *H. pylori* associated gastritis in both groups (Table 1).

Table 1. Baseline characteristics of empirical and tailored therapy groups.

	Total (n = 364)	Empirical Therapy (n = 155)	Tailored Therapy (n = 209)	p Value
Age (years), mean ± SD	56.0 ± 12.5	54.8 ± 11.7	56.9 ± 13.0	0.117
Sex, n (%)				
Male	197 (54.1)	79 (51.0)	118 (56.5)	0.298
Female	167 (45.9)	76 (49.0)	91 (43.5)	
Smoking, n (%)	108 (29.7)	37 (23.9)	71 (34.0)	0.037
Alcohol drinking, n (%)	110 (30.2)	56 (36.1)	54 (25.8)	0.034
Disease for *H. pylori* eradication, n (%)				<0.001
Peptic ulcer	109 (29.9)	39 (25.2)	70 (33.5)	
Post-ESD for EGC	24 (6.6)	5 (3.2)	19 (9.1)	
Post-ER for adenoma	35 (9.6)	11 (7.1)	24 (11.5)	
MALT Lymphoma	4 (1.1)	2 (1.3)	2 (1.0)	
H. pylori gastritis	175 (48.1)	93 (60.0)	82 (39.2)	
Lymphoid follicular gastritis	13 (3.6)	4 (2.6)	9 (4.3)	
Family history of gastric cancer	4 (1.1)	1 (0.6)	3 (1.5)	

Abbreviations: SD, standard deviation; ESD, endoscopic submucosal dissection; EGC, early gastric cancer; ER, endoscopic resection; MALT lymphoma, mucosa-associated lymphoid tissue lymphoma.

3.2. H. pylori Eradication Rate of Study Subjects

Of 155 patients in the empirical therapy group, 111 (71.6%) received sequential therapy as the first-line *H. pylori* eradication regimen. Of 209 patients in the tailored therapy group, 133 (66.5%) received 7-day triple therapy as the first-line *H. pylori* eradication regimen. As for the eradication rate according to regimen, the 14-day triple therapy was 81.5%, bismuth-containing quadruple therapy was 88.2%, and sequential therapy was 82.0% in the empirical therapy group. In the tailored therapy group, the eradication rate for 7-day triple therapy was 89.2%, bismuth-containing quadruple therapy was 91.7%, and sequential therapy was 80.0%. The eradication rate was significantly higher in those who received tailored therapy than in those who received empirical therapy (82.6% vs. 91.2%; $p = 0.023$) (Table 2).

Table 2. *H. pylori* eradication rate of study subjects.

		Empirical Therapy (n = 155)	Tailored Therapy (n = 209)	p Value
1st line eradication regimen, n (%)	Triple	27 (17.4)	139 (66.5)	-
	Quadruple	17 (11.0)	60 (28.7)	-
	Sequential	111 (71.6)	10 (4.8)	-
1st line eradication rate according to regimen, n (%)	Triple	22/27 (81.5)	124/139 (89.2)	0.328
	Quadruple	15/17 (88.2)	55/60 (91.7)	0.646
	Sequential	91/111 (82.0)	8/10 (80.0)	1.000
Outcome of *H. pylori* 1st line eradication, % (n/N)				
Eradication rate in analysis		82.6 (128/155)	91.7 (187/204)	0.023

3.3. Medical Cost of Study Subjects

The total medical costs per tailored group were 46,374 Won and those for the empirical group were 53,528 Won ($p < 0.001$). The cost of the diagnostic method was higher in the tailored therapy group (22,914 Won vs. 11,282 Won; $p < 0.001$). The cost of the eradication regimen was higher in the empirical group (38,746 Won vs. 20,704 Won; $p < 0.001$). The total treatment duration for each ultimately successful eradication in the tailored therapy group was 79.8 ± 2.8 days, which is significantly shorter than that of the empirical group's 99.2 ± 7.4 days ($p = 0.013$). (Figure 1)

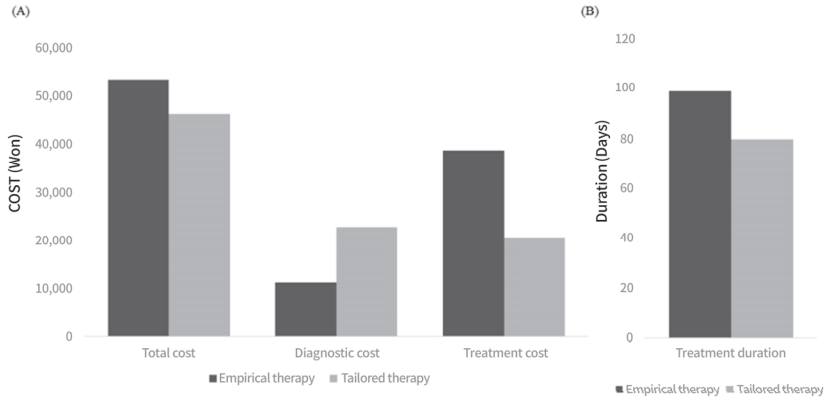


Figure 1. The total medical cost and treatment duration in patients on tailored therapy vs. empirical therapy (**A**) The total medical cost consisting of diagnostic and treatment cost in patients treated with tailored and empirical therapy. (**B**) The duration of treatment for each ultimately successful eradication in patients treated with tailored and empirical therapy.

3.4. Adverse Effects Seen in Study Subjects

Adverse drug events were more common in the empirical therapy group than in the tailored therapy group (14.8% vs. 12.9%, $p = 0.028$). The most common adverse event was nausea or vomiting in both groups (26.1% vs. 29.7%) (Table 3). Five patients in the empirical and three patients in the tailored therapy group discontinued *H. pylori* eradication treatment due to side effects. After *H. pylori* eradication failure, there were 6 patients (3.9%) in the empirical therapy group who did not want the next eradication treatment, but none of the patients in the tailored therapy group denied further treatment.

Table 3. *H. pylori* eradication related adverse effects of study subjects.

	Empirical Therapy	Tailored Therapy	*p* Value
Adverse event for eradication treatment, *n* (%)	23 (14.8)	27 (12.9)	0.028
Abdominal pain	5 (21.8)	2 (7.4)	
Nausea/Vomiting	6 (26.1)	8 (29.7)	
Headache	4 (17.4)	2 (7.4)	
Diarrhea	5 (21.7)	4 (14.8)	
Dyspepsia	0 (0.0)	7 (25.9)	
Metallic taste	3 (13.0)	4 (14.8)	
No further treatment after eradication fail, *n* (%)	6 (3.9)	0 (0.0)	NA

4. Discussion

In this study, the eradication rate of *H. pylori* was significantly higher in those who received tailored therapy as their first-line treatment in comparison to those who received empirical therapy. The *H. pylori* eradication regimen is more cost-effective in tailored therapy than empirical therapy. The total treatment periods for ultimately successful eradication were shorter in the tailored therapy group, and fewer eradication-related adverse drug events were observed compared to the empirical therapy group.

The first-line *H. pylori* eradication rate was significantly higher with tailored therapy than with empirical therapy. Recently, as the clarithromycin resistance rate of *H. pylori* has increased, the eradication rate of the existing triple therapy for seven days has decreased. To overcome this situation, prolonged treatment periods, various regimens such as quadruple therapy with bismuth, sequential and concurrent therapy, or tailored therapy may be used [8,23–25]. It is important to succeed as a first-line eradication treatment because the *H.*

pylori eradication regimen requires a large amount of medicine, including two antibiotics to be taken. In particular, if you are already taking other drugs due to a comorbid disease, the *H. pylori* eradication treatment might a great burden. Moreover, antibiotic resistance is the leading cause of failure of the *H. pylori* eradication treatment, and we should try to reduce antibiotic resistance. Empirical quadruple therapy with bismuth as the first-line therapy raises public health concerns regarding increasing resistance to the constituent antibiotics. Finally, in tailored therapy, the risk of misuse of the antibiotics is lower due to reduced *H. pylori* eradication retreatment.

The *H. pylori* eradication regimen was more cost-effective in the tailored therapy than in the empirical therapy in this study. Different studies that have evaluated *H. pylori* tailored therapy have achieved contradictory results. Liou et al. in Taiwan found that 6920 USD would be required to additionally cure one patient using the genotype resistance guide therapy, compared to empirical therapy, which is clearly not a cost-effective option [26]. Chang et al. in South Korea evaluated the cost-effectiveness of tailored therapy, and compared the results of standard triple therapy with those of empirical bismuth quadruple therapy. Total per capita medical costs were 503.50 USD in the tailored group and 406.50 USD in the empirical group [27]. However, Cosme et al. reported that in Spain, the culture-based approach was more cost-effective than standard first-line therapy given empirically [28]. Gweon et al. showed that the cost for a successful eradication using DPO-based PCR would be similar or superior to the expected cost of a successful eradication with a 14-day empirical treatment when the first-line eradication rate is $\leq$80% [29]. Since *H. pylori* antibiotic resistance varies among different geographic areas, the cost-effectiveness may vary according to the cost of care in a given country, and therefore the same conclusion may not be applicable to other healthcare systems [30].

A novel view of *H. pylori* infections is emerging in microbiological point. The changes of gut microbiota are greatly implicated in the pathogenicity of *H. pylori* Infections. The antimicrobial peptides (AMPs) have great importance in the innate immune reactions to *H. pylori* and participate in conservative co-evolution with an intricate microbiome. During *H. pylori* infections, AMP expression is able to eradicate the bacteria, thereby preventing *H. pylori* infections in the gastrointestinal tract [31]. The β-Defensins which belong to the AMP group expression, are enhanced during *H. pylori* infection [32].

In chronic inflammation induced by *H. pylori* infection, COX-2 is modulated by DNA methylation. The DNA methylation changes at the COX-2 promoter are associated with transcriptional activation and precede histone modifications in gastric cells exposed to *H. pylori* [33]. Woo et al. reported that the genome-wide methylation profiles associated with *H. pylori* infection. The gastric cancer is regulated by methylation mechanism rather than genetic linkage, and *H. pylori* leads DNA methylation [34]. Therefore, methylation-based biomarkers could be used for monitoring the prognosis of treatment, drug response, and recurrence in gastric cancer.

The prevalence of 23S rRNA point mutations was 28.7% in the study population. A total of 139 patients (66.5%) who received 7-day triple therapy were included in the tailored therapy group. This regimen might decrease drug-related adverse events and result in shorter treatment duration and minimal antibiotic overuse. Better compliance and fewer adverse effects were observed in patients in the tailored therapy group, similar to those reported in a previous study [35,36]. Choi et al. showed that the rate of eradication–related side effects for tailored regimens was 12.0%, which differed significantly from that of empirical bismuth quadruple therapy for first-line *H. pylori* treatment [36]. Therefore, tailored therapy with DPO-based multiplex PCR for *H. pylori* eradication may be superior in quality, with fewer adverse events, compared to empirical therapy in Korea, where clarithromycin resistance is high.

There are several limitations in this study. First, patients with a negative *H. pylori* infection were not enrolled in this study, and the total medical cost would be underestimated. Second, as this study was conducted retrospectively, there were limitations in obtaining detailed medical information that could have an influence on eradication failure or diagno-

sis of infection. Third, this study enrolled patients from only two medical centers, and a majority of them resided in Seoul. This study may have been subject to a selection bias. Fourth, DPO-based multiplex PCR could determine only the presence of clarithromycin resistance, and we did not check for resistance to other antibiotics such as A2115G, G2141A, A2142T, and T2182C.

5. Conclusions

In conclusion, this study showed that tailored therapy could be a useful option to achieve a higher successful eradication rate, shorter treatment periods, and lower medical costs than empirical therapy in the era of increasing antibiotic resistance.

Author Contributions: Conceptualization, A.R.C., K.-N.S., and C.H.T.; data curation, A.R.C., Y.P., and E.-M.S.; formal analysis, A.R.C., and K.-N.S.; investigation, K.-N.S., Y.P., E.-M.S., and S.-A.J.; methodology, C.H.T. and S.-A.J.; project administration, K.-N.S.; software, Y.P., E.-M.S., C.H.T., and S.-A.J.; supervision, K.-N.S.; visualization, S.-A.J.; writing—original draft, A.R.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of the Ewha Womans University hospital (SEUMC 2020-09-013).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets generated and/or analyzed during the current study are not publicly available due to our IRB policy but are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Farinha, P.; Gascoyne, R.D.J.G. *Helicobacter pylori* and MALT lymphoma. *Gastroenterology* **2005**, *128*, 1579–1605. [CrossRef] [PubMed]
2. Huang, J.-Q.; Sridhar, S.; Hunt, R.H.J.T.L. Role of *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: A meta-analysis. *Lancet* **2002**, *359*, 14–22. [CrossRef]
3. Wroblewski, L.E.; Peek, R.M.; Wilson, K.T. *Helicobacter pylori* and gastric cancer: Factors that modulate disease risk. *Clin. Microbiol. Rev.* **2010**, *23*, 713–739. [CrossRef] [PubMed]
4. Jung, H.K.; Kang, S.J.; Lee, Y.C.; Yang, H.-J.; Park, S.-Y.; Shin, C.M.; Kim, S.E.; Lim, H.C.; Kim, J.-H.; Nam, S.Y.; et al. Evidence-based Guidelines for the Treatment of *Helicobacter pylori* Infection in Korea: 2020 Revised Edition. *Korean J. Helicobacter Up. Gastrointest. Res.* **2020**, *20*, 261–287. [CrossRef]
5. Kim, J.M.; Kim, J.S.; Jung, H.C.; Kim, N.; Kim, Y.-J.; Song, I.S. Distribution of antibiotic MICs for *Helicobacter pylori* strains over a 16-year period in patients from Seoul, South Korea. *Antimicrob. Agents Chemother.* **2004**, *48*, 4843–4847. [CrossRef] [PubMed]
6. Hwang, T.J.; Kim, N.; Kim, H.B.; Lee, B.H.; Nam, R.H.; Park, J.H.; Lee, M.K.; Park, Y.S.; Lee, D.H.; Jung, H.C.; et al. Change in antibiotic resistance of *Helicobacter pylori* strains and the effect of A2143G point mutation of 23S rRNA on the eradication of *H. pylori* in a single center of Korea. *J. Clin. Gastroenterol.* **2010**, *44*, 536–543. [CrossRef] [PubMed]
7. De Francesco, V.; Giorgio, F.; Hassan, C.; Manes, G.; Vannella, L.; Panella, C.; Ierardi, E.; Zullo, A. Worldwide *H. pylori* antibiotic resistance: A systematic review. *J. Gastrointest. Liver Dis.* **2010**, *19*, 409–414.
8. Kim, B.J.; Lee, H.; Lee, Y.C.; Jeon, S.W.; Kim, G.H.; Kim, H.-S.; Sung, J.K.; Lee, D.H.; Kim, H.U.; Park, M.I.; et al. Ten-Day Concomitant, 10-Day Sequential, and 7-Day Triple Therapy as First-Line Treatment for *Helicobacter pylori* Infection: A Nationwide Randomized Trial in Korea. *Gut Liver* **2019**, *13*, 531–540. [CrossRef] [PubMed]
9. Kim, J.S.; Kim, B.W.; Hong, S.J.; Kim, J.I.; Shim, K.-N.; Kim, J.-H.; Baik, G.H.; Kim, S.W.; Song, H.J.; Kim, J.H. Sequential Therapy versus Triple Therapy for the First Line Treatment of *Helicobacter pylori* in Korea: A Nationwide Randomized Trial. *Gut Liver* **2016**, *10*, 556–561. [CrossRef] [PubMed]
10. Yan, T.L.; Gao, J.G.; Wang, J.H.; Chen, D.; Lu, C.; Xu, C.-F. Current status of *Helicobacter pylori* eradication and risk factors for eradication failure. *World J. Gastroenterol.* **2020**, *26*, 4846–4856. [CrossRef]
11. Tang, Y.; Tang, G.; Pan, L.; Zhu, H.; Zhou, S.; Wei, Z. Clinical factors associated with initial *Helicobacter pylori* eradication therapy: A retrospective study in China. *Sci. Rep.* **2020**, *10*, 1–5. [CrossRef]
12. Liou, J.M.; Chen, P.Y.; Kuo, Y.T.; Wu, M.-S. The Taiwan Gastrointestinal Disease and Helicobacter Consortium. Toward population specific and personalized treatment of *Helicobacter pylori* infection. *J. Biomed. Sci.* **2018**, *25*, 70. [CrossRef]

13. Kuo, C.J.; Lee, C.H.; Chang, M.L.; Lin, C.-Y.; Lin, W.-R.; Su, M.-Y.; Chiu, C.-H.; Tseng, C.-N.; Wu, Y.-S.; Chiu, C.-T.; et al. Multidrug resistance: The clinical dilemma of refractory *Helicobacter pylori* infection. *J. Microbiol. Immunol. Infect.* **2021**. [CrossRef]
14. De Francesco, V.; Pontone, S.; Bellesia, A.; Serviddio, G.; Panetta, C.; Palma, R.; Zullo, A. Quadruple, sequential, and concomitant first-line therapies for *H. pylori* eradication: A prospective, randomized study. *Dig. Liver Dis.* **2018**, *50*, 139–141. [CrossRef]
15. Chey, W.D.; Leontiadis, G.I.; Howden, C.W.; Moss, S.F. ACG Clinical Guideline: Treatment of *Helicobacter pylori* Infection. *Am. J. Gastroenterol.* **2017**, *112*, 212–239. [CrossRef]
16. Baj, J.; Forma, A.; Sitarz, M.; Portincasa, P.; Garruti, G.; Krasowska, D.; Maciejewski, R. *Helicobacter pylori* Virulence Factors-Mechanisms of Bacterial Pathogenicity in the Gastric Microenvironment. *Cells* **2020**, *10*, 27. [CrossRef]
17. Imkamp, F.; Lauener, F.N.; Pohl, D.; Lehours, P.; Vale, F.F.; Jehanne, Q.; Zbinden, R.; Keller, P.M.; Wagner, K. Rapid Characterization of Virulence Determinants in *Helicobacter pylori* Isolated from Non-Atrophic Gastritis Patients by Next-Generation Sequencing. *J. Clin. Med.* **2019**, *8*, 1030. [CrossRef]
18. Malfertheiner, P.; Megraud, F.; O'Morain, C.A.; Gisbert, J.P.; Kuipers, E.J.; Axon, A.T.; Bazzoli, F.; Gasbarrini, A.; Atherton, J.; Graham, D.Y.; et al. Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. *Gut* **2017**, *66*, 6–30. [CrossRef]
19. Lee, J.Y.; Kim, N.; Nam, R.H.; Choi, S.I.; Lee, J.W.; Lee, D.H. Primary and secondary antibiotic resistance of *Helicobacter pylori* in Korea from 2003 to 2018. *Helicobacter* **2019**, *24*, e12660. [CrossRef]
20. Seo, S.I.; Do, B.J.; Kang, J.G.; Kim, H.S.; Jang, M.K.; Kim, H.Y.; Shin, W.G. *Helicobacter pylori* eradication according to sequencing-based 23S ribosomal RNA point mutation associated with clarithromycin resistance. *J. Clin. Med.* **2020**, *9*, 54. [CrossRef]
21. Kim, J.L.; Cho, S.-J.; Chung, S.J.; Lee, A.; Choi, J.; Chung, H.; Kim, S.G. Empiric Versus Clarithromycin Resistance-Guided Therapy for *Helicobacter pylori* Based on Polymerase Chain Reaction Results in Patients with Gastric Neoplasms or Gastric Mucosa-Associated Lymphoid Tissue Lymphoma: A Randomized Controlled Trial. *Clin. Transl. Gastroenterol.* **2020**, *11*, e00194. [CrossRef] [PubMed]
22. Skrebinska, S.; Mégraud, F.; Bessède, E.J.H. Diagnosis of *Helicobacter pylori* infection. *Helicobacter* **2018**, *23*, e12515. [CrossRef] [PubMed]
23. Jung, Y.S.; Park, C.H.; Park, J.H.; Nam, E.; Lee, H.L. Efficacy of *Helicobacter pylori* eradication therapies in Korea: A systematic review and network meta-analysis. *Helicobacter* **2017**, *22*. [CrossRef] [PubMed]
24. Liu, W.Z.; Xie, Y.; Lu, H.; Cheng, H.; Zeng, Z.R.; Zhou, L.Y.; Chen, Y.; Wang, J.B.; Du, Y.Q.; Lu, N.H.; et al. Fifth Chinese National Consensus Report on the management of *Helicobacter pylori* infection. *Helicobacter* **2018**, *23*, e12475. [CrossRef]
25. Huang, Y.K.; Wu, M.C.; Wang, S.S.; Kuo, C.-H.; Lee, Y.-C.; Chang, L.-L.; Wang, T.-H.; Chen, Y.-H.; Wang, W.-M.; Wu, D.-C.; et al. Lansoprazole-based sequential and concomitant therapy for the first-line *Helicobacter pylori* eradication. *J. Dig. Dis.* **2012**, *13*, 232–238. [CrossRef]
26. Liou, J.M.; Chen, P.Y.; Luo, J.C.; Lee, J.-Y.; Chen, C.-C.; Fang, Y.-J.; Yang, T.-H.; Chang, C.-Y.; Bair, M.-J.; Chen, M.-J.; et al. Efficacies of Genotypic Resistance-Guided vs Empirical Therapy for Refractory *Helicobacter pylori* Infection. *Gastroenterology* **2018**, *155*, 1109–1119. [CrossRef] [PubMed]
27. Chang, Y.W.; Shin, G.Y.; Kim, J.W.; Moon, J.-C.; Chang, E.J.; Oh, C.H.; Jang, J.-Y. Cost-Effectiveness of Empirical Bismuth-Based Quadruple Therapy and Tailored Therapy After Clarithromycin Resistance Tests for *Helicobacter pylori* Eradication. *Dig. Dis. Sci.* **2021**. [CrossRef]
28. Cosme, A.; Montes, M.; Martos, M.; Gil, I.; Mendarte, U.; Salicio, Y.; Piñeiro, L.; Recasens, M.T.; Ibarra, B.; Sarasqueta, C.; et al. Usefulness of antimicrobial susceptibility in the eradication of *Helicobacter pylori*. *Clin. Microbiol. Infect.* **2013**, *19*, 379–383. [CrossRef]
29. Gweon, T.G.; Kim, J.S.; Kim, B.W. An Economic Modeling Study of *Helicobacter pylori* Eradication: Comparison of Dual Priming Oligonucleotide-Based Multiplex Polymerase Chain Reaction and Empirical Treatment. *Gut Liver* **2018**, *12*, 648–654. [CrossRef]
30. Gisbert, J.P. Empirical or susceptibility-guided treatment for *Helicobacter pylori* infection? A comprehensive review. *Ther. Adv. Gastroenterol.* **2020**, *13*, 1756284820968736. [CrossRef] [PubMed]
31. Pero, R.; Brancaccio, M.; Laneri, S.; De Biasi, M.G.; Lombardo, B.; Scudiero, O. A Novel View of Human *Helicobacter pylori* Infections: Interplay between Microbiota and Beta-Defensins. *Biomolecules* **2019**, *9*, 237. [CrossRef]
32. Pero, R.; Coretti, L.; Nigro, E.; Lembo, F.; Laneri, S.; Lombardo, B.; Daniele, A.; Scudiero, O. β-Defensins in the Fight against *Helicobacter pylori*. *Molecules* **2017**, *22*, 424. [CrossRef] [PubMed]
33. Angrisano, T.; Pero, R.; Brancaccio, M.; Coretti, L.; Florio, E.; Pezone, A.; Calabrò, V.; Falco, G.; Keller, S.; Lembo, F.; et al. Cyclical DNA Methylation and Histone Changes Are Induced by LPS to Activate COX-2 in Human Intestinal Epithelial Cells. *PLoS ONE* **2016**, *11*, e0156671. [CrossRef]
34. Woo, H.D.; Fernandez-Jimenez, N.; Ghantous, A.; Degli Esposti, D.; Cuenin, C.; Cahais, V.; Choi, I.J.; Kim, Y.-I.; Kim, J.; Herceg, Z. Genome-wide profiling of normal gastric mucosa identifies *Helicobacter pylori*- and cancer-associated DNA methylome changes. *Int. J. Cancer* **2018**, *143*, 597–609. [CrossRef]
35. Zhou, L.; Zhang, J.; Song, Z.; He, L.; Li, Y.; Qian, J.; Bai, P.; Xue, Y.; Wang, Y.; Lin, S. Tailored versus Triple plus Bismuth or Concomitant Therapy as Initial *Helicobacter pylori* Treatment: A Randomized Trial. *Helicobacter* **2016**, *21*, 91–99. [CrossRef]
36. Choi, Y.I.; Chung, J.W.; Park, D.K.; Kim, K.O.; Kwon, K.A.; Kim, Y.J.; Seo, J.Y. Tailored eradication vs empirical bismuth-containing quadruple therapy for first-line *Helicobacter pylori* eradication: A comparative, open trial. *World J. Gastroenterol.* **2019**, *25*, 6743–6751. [CrossRef]

Journal of
Clinical Medicine

MDPI

Article

Characteristics, Location, and Clinical Outcomes of Gastrointestinal Bleeding in Patients Taking New Oral Anticoagulants Compared to Vitamin K Antagonists

A Reum Choe [1], Chang Mo Moon [1,2,*], Chung Hyun Tae [1], Jaeyoung Chun [3,4], Ki Bae Bang [5], Yoo Jin Lee [6], Hyun Seok Lee [7], Yunho Jung [8], Sung Chul Park [9] and Hoon Sup Koo [10]

1 Department of Internal Medicine, College of Medicine, Ewha Womans University, Seoul 07985, Korea; prems0121@naver.com (A.R.C.); jhtae@ewha.ac.kr (C.H.T.)
2 Inflammation-Cancer Microenvironment Research Center, College of Medicine, Ewha Womans University, Seoul 07804, Korea
3 Department of Internal Medicine, Seoul National University College of Medicine, Liver Research Institute, Seoul 03080, Korea; J40479@gmail.com
4 Department of Internal Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul 06273, Korea
5 Department of Internal Medicine, Dankook University College of Medicine, Cheonan 31116, Korea; kibaebang@gmail.com
6 Department of Internal Medicine, Keimyung University School of Medicine, Daegu 42601, Korea; doctorlyj@dsmc.or.kr
7 Department of Internal Medicine, School of Medicine, Kyungpook National University, Kyungpook National University Hospital, Daegu 41404, Korea; lhsworld@nate.com
8 Department of Internal Medicine, Soonchunhyang University College of Medicine, Cheonan 31151, Korea; yoonho7575@naver.com
9 Department of Internal Medicine, Kangwon National University School of Medicine, Chuncheon 24289, Korea; schlp@hanmail.net
10 Department of Internal Medicine, Konyang University College of Medicine, Daejeon 35365, Korea; koo7574@naver.com
* Correspondence: mooncm27@ewha.ac.kr; Tel.: +82-2-2650-2945

Citation: Choe, A.R.; Moon, C.M.; Tae, C.H.; Chun, J.; Bang, K.B.; Lee, Y.J.; Lee, H.S.; Jung, Y.; Park, S.C.; Koo, H.S. Characteristics, Location, and Clinical Outcomes of Gastrointestinal Bleeding in Patients Taking New Oral Anticoagulants Compared to Vitamin K Antagonists. *J. Clin. Med.* **2021**, *10*, 2693. https://doi.org/10.3390/jcm10122693

Academic Editor: Mohammad H. Derakhshan

Received: 6 May 2021
Accepted: 10 June 2021
Published: 18 June 2021

Abstract: New oral anticoagulants (NOACs) are commonly used in clinical practice as alternatives to vitamin K antagonists (VKA). However, the etiology, clinical course, and risk of gastrointestinal (GI) bleeding remain unclear. We aimed to evaluate the clinical characteristics and location of acute GI bleeding associated with NOACs and its severity and outcomes compared to VKA. This retrospective multicenter study included 381 subjects on anticoagulants who underwent appropriate diagnostic examination due to GI bleeding. Regarding the characteristics of acute GI bleeding, the proportion of vascular lesions was significantly lower in the NOACs group than that in the VKA group. Small bowel bleeding occurred less commonly in the NOACs group, but the difference did not reach statistical significance. Regarding severity and clinical outcomes, patients on NOACs received significantly smaller volumes of transfused blood products and had shorter ICU stays than those on VKA. Moreover, the need for surgery and the risk of rebleeding in the NOACs group were significantly lower than those in the VKA group. Patients on NOACs have better clinical outcomes in terms of severity of acute GI bleeding or rebleeding than patients on VKA. Patients on NOACs demonstrate different characteristics and location of acute GI bleeding than those on VKA.

Keywords: gastrointestinal bleeding; new oral anticoagulants; vitamin K antagonist; rebleeding

1. Introduction

Since the Food and Drug Administration (FDA) approved new oral anticoagulants (NOACs) in 2010 [1,2], direct factor Xa inhibitors (rivaroxaban, apixaban, and edoxaban) and direct thrombin inhibitors (dabigatran) are now available in clinical practice [3,4]. The 2016 European Society of Cardiology guidelines recommended NOACs for patients with

non-valvular atrial fibrillation (NVAF) to prevent stroke [5]. The 2016 American College of Chest Physician guideline and expert panel report also suggested a prescription in favor of NOACs to vitamin K antagonist (VKA) for the initial and long-term management of venous thromboembolism in patients without cancer [6].

The VKA inhibits vitamin K epoxide reductase, thereby attenuating the reduction of oxidized vitamin K in the liver. In contrast to VKA, the NOACs directly inhibit a single clotting enzyme; dabigatran inhibits thrombin, whereas rivaroxaban, apixaban, and edoxaban inhibit factor Xa [7,8]. The NOACs have major pharmacologic advantages over VKA, including fast onset/offset of action, few clinically relevant interactions with other drug and food, and predictable pharmacokinetics, simple administration by fixed doses without any monitoring [9–11].

Recently, several randomized clinical trials have shown that NOACs is preferred to VKA, due to its efficacy in preventing stroke and systemic embolisms in patients with NVAF [12–14]. NOACs have been reported to significantly decrease the prevalence of major bleeding, particularly the rates of intracranial hemorrhage and critical bleeding [4,15]. Moreover, several meta-analyses have shown that NOACs have a more favorable safety profile than VKA [16–19]. However, the risk of NOAC-associated bleeding, particularly gastrointestinal (GI) bleeding, is still a concern. The ROCKET AF trial [20], a comparative study of rivaroxaban and warfarin for the prevention of stroke and embolism, showed that patients treated with rivaroxaban had a significantly higher rate of GI bleeding than those treated with VKA. Contrarily, the XANTUS registry [21] investigated the stroke prevention effect of anticoagulants in patients with AF and showed that major GI bleeding occurred less frequently in the rivaroxaban group. To date, it remains unclear whether NOACs increases the risk of GI bleeding compared to warfarin. Moreover, few studies have reported the exact source and location of GI bleeding during NOACs treatment with comprehensive examination methods, including gastrointestinal endoscopy or abdominal pelvis computed tomography (CT).

Therefore, we aimed to assess the clinical and endoscopic features of acute GI bleeding in patients prescribed NOACs and evaluate the severity and clinical outcomes of these events compared to VKA.

2. Materials and Methods

2.1. Study Population

In this retrospective multicenter cohort study, we analyzed the clinical data of study subjects collected at eight tertiary medical institutions between January 2014 and October 2017 in the Republic of Korea. We included subjects who met the following three criteria: (1) patients who visited the hospital with symptoms of overt GI bleeding; (2) patients treated with anticoagulants (dabigatran, rivaroxaban, apixaban, edoxaban, and warfarin) for at least 3 months; (3) patients who underwent diagnostic esophagogastroduodenoscopy (EGD), colonoscopy, sigmoidoscopy, small bowel (SB) enteroscopy, or capsule endoscopy to identify the focus of GI bleeding, according to the diagnostic strategy of each hospital. Subjects were excluded in the following conditions: (1) those diagnosed with GI cancer before overt GI bleeding episode (n = 95); (2) GI ulcers within 6 months before starting anticoagulants (n = 107); (3) inflammatory bowel disease or intestinal Behçet's disease (n = 9); and (4) hematologic diseases with a bleeding tendency (n = 23). Finally, a total of 381 patients were included in this study (Figure 1). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee of all participating hospitals.

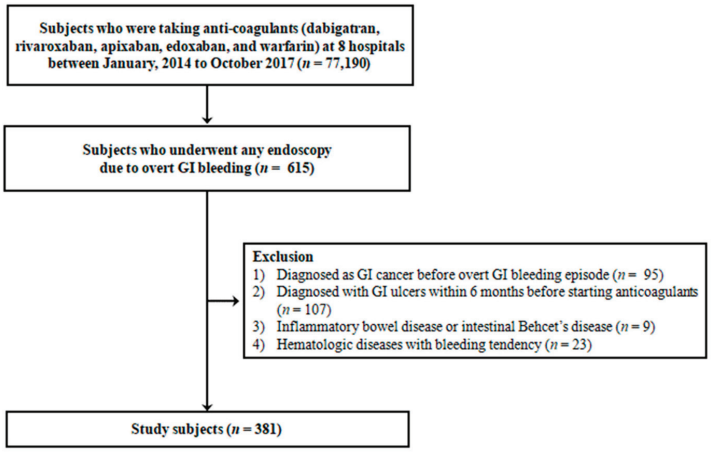


Figure 1. Flow diagram. A total of 615 patients who underwent any endoscopy due to overt GI bleeding were enrolled from eight large-volume university hospitals. Of these, 234 patients were excluded, and 381 patients were enrolled for analysis.

2.2. Data Collection and Definition of Variables

We collected the demographic, clinical, and laboratory data from the patients at the time of presentation. The baseline characteristics included the presence of major GI bleeding, history of prior GI bleeding, indication for anticoagulation, medical comorbidities, and any concomitant drugs associated with GI bleeding. The risk of major bleeding was calculated using the HAS-BLED (old age, drugs/alcohol intake, hypertension, abnormal liver/kidney function, stroke, bleeding predisposition or history, and labile international normalized ratio) scoring system including six comorbid conditions.

GI bleeding was identified from the medical records by the presence of hematemesis, melena, or hematochezia. Major bleeding was defined as fatal or symptomatic bleeding in a critical organ or bleeding that caused a decrease in hemoglobin level of 2 g/dL or more, leading to transfusion of 2 or more units of whole or red blood cells [22]. Location of GI bleeding was identified as upper GI, small bowel, lower GI, or indeterminate by reviewing endoscopic or radiologic records. The diagnostic modalities for identifying the causes of GI bleeding included EGD, colonoscopy/sigmoidoscopy, SB enteroscopy, capsule endoscopy, or abdominal pelvic computerized tomography (CT).

GI bleeding lesions were divided into four types according to the endoscopic characteristics: (1) vascular lesion (angiodysplasia, Dieulafoy's lesion, varices, gastric antral vascular ectasia, hemorrhoid, and ischemic colitis); (2) inflammatory lesion (esophagitis, gastritis, colitis, erosion, ulcer, and inflammatory bowel disease); (3) neoplastic lesion (polyp, tumor); (4) anatomic lesion and others (diverticulum, Mallory–Weiss syndrome, post-procedural bleeding after polypectomy, or endoscopic submucosal dissection).

Clinical outcomes were investigated by hemodynamic instability at the point of admission, need for angiographic or surgical intervention, in-hospital mortality, and rebleeding. Hemodynamic instability was defined as one or more out-of-range vital sign measurements, such as systolic blood pressure < 90 mmHg or heart rate > 100/min. Rebleeding was defined as endoscopic confirmation of newly developed GI bleeding or an explained drop in hemoglobin more than 2 g/dL after 7 days of initial endoscopic hemostasis treatment [23,24].

2.3. Statistical Analysis

Continuous variables were presented as mean ± standard deviation, and categorical variables were presented as the number of subjects and percent. Group comparison was performed by using independent-samples t-tests or Mann–Whitney U-tests for continuous

variables and Pearson's chi-squared tests or Fisher's exact tests for categorical variables. The adjusted odds ratio for clinical outcomes was obtained by multivariable logistic-regression analysis adjusted for sex and HAS-BLED score. Any variable with a p-value < 0.2 in univariate analysis was accepted as a candidate for multivariate analysis along with variables with known clinical importance. Finally, statistical significance was considered as $p < 0.05$ with a two-tailed test. We used the analysis of covariance for the number of red blood cell transfusions, days in the hospital, and ICU days. The analyses were adjusted for sex and HAS-BLED score as continuous variables. All statistical analyses were performed using SPSS for Windows version 21.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Baseline Characteristics of Study Subjects

The baseline characteristics of the patients on NOACs or VKA who experienced acute GI bleeding are shown in Table 1. Among them, 144 patients were prescribed NOACs, and 237 patients used VKA (mean age; 77.9 ± 7.8 vs. 73.3 ± 11.9 years). Regarding indications for anticoagulation, NOACs were used for AF or atrial flutter in 108 cases (75.0%) and pulmonary embolism or deep vein thrombosis in 29 cases (20.1%). VKAs were used for AF or atrial flutter in 117 cases (49.4%) and prosthetic valves in 69 cases (29.1%). Twenty-five of 144 (17.3%) patients on NOACs concomitantly had antiplatelet agents (aspirin, clopidogrel), whereas 36 of 237 (15.2%) on VKA used antiplatelet agents. The concomitant use of proton pump inhibitor did not differ significantly between the two groups, while the use of H2 receptor antagonist showed more common in NOACs group. There was no difference in examination modalities between the two groups.

Table 1. Baseline characteristics of the patients prescribed with NOACs or VKA who experienced GI bleeding.

	NOACs (*n* = 144)	**VKA (*n* = 237)**	***p* Value**
Mean age, years (range) *	77.9 ± 7.8 (54–95)	73.3 ± 11.9 (29–95)	<0.001
Male sex (%)	63 (43.8%)	122 (51.5%)	0.071
Mean body mass index *	23.3 ± 3.8	22.1 ± 4.1	0.005
History of smoking (%)			0.187
No	124 (86.1%)	186 (78.5%)	
Ex-smoker	15 (10.4%)	38 (16.0%)	
Current smoker	5 (3.5%)	13 (5.5%)	
History of alcohol intake (%)			0.368
No	117 (81.3%)	198 (83.5%)	
Social	14 (9.7%)	26 (11.0%)	
Heavy	13 (9.0%)	13 (5.5%)	
History of major bleeding † (%)	17 (11.8%)	26 (11.0%)	0.903
History of prior gastrointestinal bleeding (%)	29 (20.1%)	42 (17.7%)	0.678
Symptom (%)			0.061
Hematemesis	25 (17.4%)	43 (18.1%)	
Melena	60 (41.7%)	124 (52.3%)	
Hematochezia	59 (41.0%)	70 (29.5%)	
Indication for Anticoagulation (%)			
Atrial fibrillation/flutter	108 (75.0%)	117 (49.4%)	<0.001
Pulmonary embolism/DVT	29 (20.1%)	40 (16.9%)	0.329
Prosthetic valve	1 (0.7%)	69 (29.1%)	<0.001
Stroke prevention	6 (4.2%)	11 (4.6%)	0.533
Comorbidities (%)			
Congestive heart failure	49 (34.0%)	77 (32.5%)	0.954
Hypertension	100 (69.4%)	137 (57.8%)	0.071
Arrythmia	108 (75.0%)	144 (60.8%)	0.019

Table 1. *Cont.*

	NOACs (n = 144)	VKA (n = 237)	p Value
Diabetes mellitus	53 (36.8%)	74 (31.2%)	0.362
Dyslipidemia	31 (21.5%)	42 (17.7%)	0.460
Coronary heart disease	29 (20.1%)	38 (16.0%)	0.394
Stroke	52 (36.1%)	58 (24.5%)	0.028
History of transient ischemic attack	4 (2.8%)	3 (1.3%)	0.314
Chronic kidney disease	14 (9.7%)	53 (22.4%)	0.001
Chronic obstructive pulmonary disease	6 (4.2%)	5 (2.1%)	0.273
Chronic hepatitis	1 (0.7%)	8 (3.4%)	0.086
Liver cirrhosis	13 (9.0%)	21 (8.9%)	0.955
Pulmonary embolism/DVT	26 (18.1%)	32 (13.5%)	0.297
Peripheral arterial occlusive disease	3 (2.1%)	13 (5.5%)	0.094
Prosthetic valve	2 (1.4%)	74 (31.2%)	<0.001
Concomitant medications (%)			
Aspirin	13 (9.0%)	27 (11.4%)	0.135
Clopidogrel	12 (8.3%)	9 (3.8%)	0.173
NSAIDs	5 (3.5%)	18 (7.6%)	0.080
Steroid	7 (4.9%)	15 (6.3%)	0.474
Proton pump inhibitor	29 (20.1%)	35 (14.8%)	0.233
H2 receptor antagonist	18 (12.5%)	10 (4.2%)	0.004
Examination Modalities (%)			
Esophagogastroduodenoscopy	43 (21.0%)	52 (16.0%)	0.116
Colonoscopy/Sigmoidfibroscopy	91 (44.4%)	160 (49.2%)	0.269
SB enteroscopy	0 (0.0%)	3 (0.9%)	0.294
Capsule endoscopy	12 (5.9%)	24 (7.4%)	0.591
Abdomen pelvis CT	59 (28.8%)	86 (26.5%)	0.440

NOACs, new oral anticoagulants; VKA, vitamin K antagonist; GI, gastrointestinal; DVT, deep vein thrombosis; NSAIDs, non-steroidal anti-inflammatory drugs; SB, small bowel; CT, computerized tomography; * Mean ± standard deviation; † History of major bleeding defined by International Society on Thrombosis and Hemostasis as fatal bleeding or symptomatic bleeding in a critical organ, or bleeding causing a decrease in hemoglobin level of 2 g/dL or more, leading to transfusion of 2 or more units of whole blood or red blood cells.

3.2. Source, Lesion, and Location of Acute GI Bleeding in Patients on NOACs or VKA

The most common site of acute GI bleeding was the upper GI tract in the NOACs (51/144, 35.4%) and the VKA group (98/237, 41.4%). Small bowel bleeding was observed in 6/144 (4.2%) in the NOACs group and 16/237 (6.8%) in the VKA group. The prevalence of lower GI bleeding was 33/144 (22.9%) in the NOACs group and 43/237 (18.1%) in the VKA group.

Among the 90 patients on NOACs who experienced GI bleeding, the common causes of upper GI bleeding were benign gastric ulcer in 25 (27.8%) patients, duodenal ulcer in 5 (5.6%), gastric varix in 3 (3.3%), and Mallory–Weiss syndrome in 3 (3.3%) patients. The common causes of small bowel bleeding were vascular lesions in 4 (4.4%) and inflammatory lesions in 2 (2.2%) patients. The common causes of lower GI bleeding were rectal ulcer without exposed vessels in 8 (8.9%) patients, diverticuli without current bleeding in 7 (7.8%), and colon polyp bleeding in 5 (5.6%) patients. Among the 157 patients on VKA who experienced GI bleeding, the common causes of upper GI bleeding were benign gastric ulcer in 47 (29.9%) patients, duodenal ulcer in 14 (8.9%), and gastric angiodysplasia in 9 (5.7%) patients. The common causes of small bowel bleeding were inflammatory lesions in 9 (5.7%) and vascular lesions in 6 (3.8%) patients. The common causes of lower GI bleeding were hemorrhoid bleeding in 10 (6.4%) patients, colon polyp bleeding in 10 (6.4%), rectal ulcer without exposed vessels in 4 (2.5%), and diverticuli without current bleeding in 4 (2.5%) patients (Table 2).

Table 2. Sources of GI bleeding in patients with NOACs or VKA.

	NOACs (n = 144)	VKA (n = 237)
Upper GI findings (%)	51 (35.4)	98 (41.4)
Esophagus	8 (5.6)	13 (5.5)
Esophagitis	2 (1.4)	1 (0.4)
Esophageal ulcer	1 (0.7)	1 (0.4)
Mallory-Weiss syndrome	3 (2.1)	7 (3.0)
Esophageal angiodysplasia	0 (0)	1 (0.4)
Esophageal varix	2 (1.4)	3 (1.3)
Stomach	38 (26.4)	69 (29.1)
Gastric varix	3 (2.1)	1 (0.4)
Gastric antral vascular ectasia	1 (0.7)	2 (0.8)
Gastric erosion	2 (1.4)	3 (1.3)
Benign gastric ulcer	25 (17.4)	47 (19.8)
Gastric cancer	2 (1.4)	1 (0.4)
Gastric angiodysplasia	2 (1.4)	9 (3.8)
Gastric dieulafoy	1 (0.7)	6 (2.5)
Gastric polypectomy Or endoscopic submucosal dissection bleeding	2 (1.4)	0 (0)
Duodenum	5 (3.5)	16 (6.8)
Duodenal ulcer	5 (3.5)	14 (5.9)
Duodenal angiodysplasia	0 (0)	1 (0.4)
Duodenal dieulafoy lesion	0 (0)	1 (0.4)
Duodenitis	0 (0)	0 (0)
Small bowel findings (%)	6 (4.2)	16 (6.8)
Inflammatory lesion	2 (1.4)	9 (3.8)
Neoplastic lesion	0 (0)	0 (0)
Vascular lesion	4 (2.8)	6 (2.5)
Others	0 (0)	1 (0.4)
Lower GI findings (%)	33 (22.9)	43 (18.1)
Vascular lesion	5 (3.5)	13 (5.5)
Hemorrhoid	4 (2.8)	10 (4.2)
Ischemic colitis	1 (0.7)	3 (1.3)
Anatomic lesion	8 (5.6)	7 (3.0)
Diverticuli without bleeding	7 (4.9)	4 (1.7)
Diverticuli with current bleeding	1 (0.7)	3 (1.3)
Inflammatory lesion	14 (9.7)	10 (4.2)
Rectal ulcer only	8 (5.6)	4 (1.7)
Rectal ulcer with exposed vessel	1 (0.7)	1 (0.4)
Colon ulcer	3 (2.1)	1 (0.4)
Infectious colitis	1 (0.7)	1 (0.4)
Pseudomembranous colitis	1 (0.7)	2 (0.8)
Inflammatory bowel disease	0 (0)	1 (0.4)
Neoplastic lesion	6 (4.2)	13 (5.5)
Colon polyp	5 (3.5)	10 (4.2)
Colon cancer	1 (0.7)	3 (1.3)
Unidentified lesion (%)	54 (37.5)	80 (33.8)

GI, gastrointestinal; NOACs, new oral anticoagulants; VKA, vitamin K agonist.

Regarding the characteristics of GI bleeding in the two groups, the proportion of vascular lesions in the location of GI bleeding, bleeding in the small bowel occurred less commonly in patients on NOACs, but the difference could not reach statistical significance (6.7% vs. 10.2%, p = patients on NOACs was significantly lower than in those patients on VKA (15.6% vs. 25.5%, p = 0.038). Regarding 0.090) (Table 3).

Table 3. Lesion characteristics and location of GI bleeding in patients with NOACs or VKA.

	NOACs (*N* = 90)	VKA (*N* = 157)	*p* Value
Lesion characteristics (%)			
Vascular lesion	14 (15.6)	40 (25.5)	0.038
Inflammatory lesion	49 (54.4)	81 (51.6)	0.775
Neoplastic lesion	7 (7.8)	14 (8.9)	0.604
Anatomic lesion & Others *	20 (22.2)	22 (14.0)	0.638
Location (%)			
Esophagus	8 (8.9)	13 (8.3)	0.912
Stomach	38 (42.2)	69 (43.9)	0.334
Duodenum	5 (5.6)	16 (10.2)	0.284
Small bowel	6 (6.7)	16 (10.2)	0.090
Colon	33 (36.7)	43 (27.4)	0.460

NOACs, new oral anticoagulants; VKA, vitamin K agonist. * Others category was included diverticular bleeding, Mallory-Weiss syndrome, post polypectomy bleeding, and post endoscopic submucosal dissection bleeding.

3.3. Comparison of Clinical Outcomes in Patients on NOACs vs. VKA

Regarding clinical outcomes, patients treated with NOACs received significantly smaller volumes of blood transfusions with packed red blood cells than those taking VKA (2.1 ± 0.3 vs. 3.1 ± 0.2, $p = 0.009$). Patient treated with NOACs stayed in ICU significantly shorter than those taking VKA (0.5 ± 0.2 vs. 1.0 ± 0.2, $p = 0.049$). However, there was no significant difference in the stay of hospital between patients treated NOACs and VKA (9.0 ± 1.2 vs. 10.4 ± 0.9, $p = 0.344$) (Figure 2).

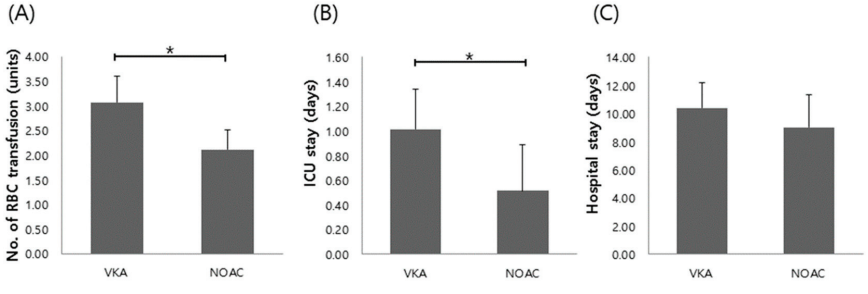


Figure 2. Clinical outcomes related to the severity of GI bleeding in patients on NOACs vs. VKA (**A**) number of red blood cell transfusion, (**B**) duration of ICU stay, (**C**) duration of hospital stay in patients treated with VKA and NOACs. * $p < 0.05$. VKA, vitamin K antagonist; NOAC, new oral anticoagulants; RBC, red blood cell; ICU, intensive care unit.

In multivariate analysis adjusted for sex and HAS-BLED scores, rebleeding was less common in patients on NOACs than in those on VKA (adjusted OR 0.42, 95% CI 0.22–0.79, $p = 0.007$). Regarding the need for surgery, a very low number of patients required a surgical intervention in both group (1 case in NOAC group and 4 cases in VKA group). There was no significant difference in hemodynamic instability at admission, the need for angiography, and mortality during hospitalization between the two groups (Table 4).

Table 4. Comparison of clinical outcomes in the patients with NOACs vs. VKA.

Clinical Outcomes	NOACs (%) (*n* = 59)	VKA (%) (*n* = 123)	Multivariate Logistic Regression Analysis *	
			Adjusted OR (95% CI)	*p* Value
Hemodynamic instability at admission	26 (17.7%)	50 (21.6%)	0.81 (0.47–1.37)	0.167
Rebleeding	15 (10.6%)	46 (20.9%)	0.42 (0.22–0.79)	0.007
Need for angiography	11 (8.1%)	12 (5.6%)	1.47 (0.62–3.45)	0.112
Mortality during Hospital day	6 (4.1%)	11 (4.7%)	0.84 (0.30–2.35)	0.729
Need for surgery †	1 (0.7%)	4 (1.7%)	0.82 (0.69–0.98)	0.045

NOACs, new oral anticoagulants; VKA, vitamin K agonist; OR, odds ratio; CI, confidence interval; * These odds ratios and 95% CIs were adjusted for sex, HAS-BLED score; † The type of surgery in patient with NOACs was distal gastrectomy. The patients with VKA underwent distal gastrectomy (2 cases), small bowel segmental resection (1 case), and right hemicolectomy (1 case).

We analyzed the clinical outcomes in the patients associated with different NOACs such as dabigatran, rivaroxaban, apixaban, and edoxaban. Consequently, unfavorable clinical outcomes such as hemodynamic instability at admission, need for angiography or surgery, mortality during hospital days, and rebleeding were the most frequent in those with rivaroxaban compared with other NOACs (Table 5).

Table 5. The clinical outcomes in the patients associated with different NOACs.

Outcomes \ NOACs	Dabigatran (*n* = 32, 22.2%)	Rivaroxaban (*n* = 72, 50.0%)	Apixaban (*n* = 28, 19.5%)	Edoxaban (*n* = 12, 8.3%)
Hemodynamic instability at admission	5 (19.3%)	13 (50.0%)	7 (26.9%)	1 (3.8%)
Need for angiography	3 (27.3%)	7 (63.7%)	1 (9.0%)	0 (0.0%)
Need for surgery	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)
Mortality during Hospital day	1 (16.7%)	4 (66.6%)	1 (16.7%)	0 (0.0%)
Rebleeding	2 (13.3%)	9 (60.0%)	4 (26.7%)	0 (0.0%)

NOACs, new oral anticoagulants.

4. Discussion

In the present study, patients treated with NOACs who experienced acute GI bleeding had different characteristics and clinical outcomes than those treated with VKA. The proportion of vascular lesions and small bowel bleeding was lower in the NOACs group than that in the VKA group. The clinical outcomes in terms of severity and rebleeding are better in the NOACs group than in the VKA group.

Patients on NOACs who experienced GI bleeding had fewer unfavorable outcomes such as critical bleeding events requiring blood transfusion or rebleeding than those on VKA. Our results suggest that acute GI bleeding associated with NOACs may be less severe than that associated with VKA, which may be explained by the short half-life of NOACs (NOACs around 8–14 h, VKA 36–42 h) [1,25]. Therefore, the cessation of NOACs leads to a return of the coagulant function and recovery in a short period [26]. If GI bleeding is recognized, discontinuation of NOACs can quickly attenuate their anticoagulation effect. Moreover, this difference in the results achieved with NOACs and VKA was due to the potentially dangerous overdosing of VKA, which frequently occurs in clinical settings [27–29]. VKA have a large number of food or drug interactions, which complicate its anticoagulation effect [30]. Especially, acute illness such as infection and organ failure can prolong the international normalized ratios (INRs) in patients on VKA [31]. The intrinsic difficulty in maintaining therapeutic levels in those treated with VKA results in supra-therapeutic INRs and a risk of severe bleeding [32]. Therefore, the difference in severity and outcomes of acute GI bleeding between NOACs and VKA may be explained by their pharmacological properties.

In this study, regarding the location of GI bleeding, bleeding in the small bowel occurred less common in patients on NOACs, but the difference could not reach statistical

significance. Generally, bleeding in the small bowel remains relatively rare, accounting for 5–10% of all patients with GI bleeding [33]. Bleeding originated from the small bowel in 6 (6.7%) patients on NOACs and 16 (10.2%) patients on VKA in our study. Likewise, Diamantopoulou, et al. presented that the site of bleeding was located in the small bowel in 2/43 of NOAC patients and 6/68 of warfarin group [34]. Another cohort study also reported that GI bleeding associated with the use of dabigatran was more common from a source distal to the ligament of Treitz [35]. The pathophysiological explanation may relate to a low bioavailability of dabigatran [36]. Despite the similar mode of action, bioavailability differs according to the NOACs (dabigatran, 3–7%; apixaban, 50–60%; edoxaban, 62%; rivaroxaban 66–100%). The incidence of small bowel bleeding varies depending on the type or dosage of NOACs. This difference in results may be influenced by the type or dosage of NOACs and the characteristics of the study subjects. Therefore, further large-scale prospective studies are warranted to evaluate small bowel bleeding between these four NOACs.

In our cohort, vascular lesions were less common in patients on NOACs than in those on VKA. Pathophysiologically, NOACs is a non-absorbed, active anticoagulant within the GI tract lumen and promotes GI bleeding from vulnerable mucosal erosions [37]. Considering this characteristic, the use of NOACs may have no significant effect on intact mucosal lesions such as hemorrhoids, but can trigger bleeding in vulnerable mucosal lesions such as erosions or ulcers. These results may help to predict and prevent acute GI bleeding and evaluate the patients' existing GI conditions before prescribing anticoagulants. In a recent network meta-analysis, apixaban had the highest probability to be the safest option with regard to the risk of GI bleeding, followed by edoxaban, warfarin, dabigatran, and rivaroxaban [38].

Our study has limitations. First, this study was conducted in an observational and retrospective manner, which may limit the generalization of its results and cause potential bias. It is impossible to completely control confounding factors such as comorbidities and medications that can affect acute GI bleeding. However, we tried to reduce this effect by adjusting for sex and HAS-BLED scores as confounding variables in our multivariate analysis. Second, diagnostic tests for GI bleeding such as EGD, colonoscopy, sigmoidoscopy, capsule endoscopy, SB enteroscopy, and abdomen pelvis CT were not equally performed in all patients. Also, some diagnostic modalities were not conducted in some subjects. However, as the eight institutions participating in this study were tertiary referral hospitals, the diagnostic strategy for acute overt GI bleeding was relatively similar. Third, due to the retrospective study design, there was a limitation in analyzing the acute changes just before GI bleeding, which could affect events.

Despite these limitations, our study had the following advantages. It showed the source of acute GI bleeding in NOACs, examined by endoscopic and imaging modalities. Moreover, we compared the clinical severity and outcomes of acute GI bleeding between NOACs and VKA by analyzing a relatively large amount of patient data.

5. Conclusions

Acute GI bleeding in patients on NOACs showed favorable clinical outcomes, such as the need for transfusion or surgery and rebleeding than in patients on VKA. Further, the characteristics and location of acute GI bleeding lesions differed between the NOACs and VKA group. Our results may help to determine the diagnostic and therapeutic approaches when physicians encounter acute GI bleeding events in patients on anticoagulants.

Author Contributions: Conceptualization, C.M.M. and A.R.C.; Funding acquisition, C.M.M.; Investigation, A.R.C., C.H.T., J.C., Y.J. and H.S.K.; Methodology, C.M.M.; Project administration, A.R.C. and C.M.M.; Resources, Y.J.L. and H.S.L.; Software, C.H.T., J.C., K.B.B., Y.J.L., H.S.L., S.C.P. and H.S.K.; Supervision, C.M.M.; Visualization, Y.J. and S.C.P.; Writing—original draft, A.R.C.; Writing—review & editing, C.M.M., C.H.T., J.C. and K.B.B. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2020R1A2C1010786 and 2020R1A5A2019210; Chang Mo Moon).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of Ewha Womans University Mokdong Hospital (EUMC 2017-11-026 and 2017-12-07), Seoul National University Hospital (H-2012-171-1185 and 2021-01-07), Dankook University Hospital (DKUH 2018-07-002 and 2018-07-19), Keimyung University Dongsan Medical Center (DSMC 2020-12-066 and 2020-12-28), Kyungpook National University Chilgok Hospital (KNUCH 2018-08-013 and 2018-08-10), Soonchunhyang University Hospital (SCHCA 2018-03-004 and 2018-03-28), Kangwon National University Hospital (KNUH 2018-08-009-001 and 2018-10-29), and Konyang University Hospital (KYUH 2018-07-019 and 2018-07-25).

Informed Consent Statement: Patient consent was waived due to the study's retrospective nature.

Data Availability Statement: The datasets generated and/or analyzed during the current study are not publicly available due to our IRB policy.

Acknowledgments: I would like to express my special thanks to Jung Min Moon and Hosim Soh at the Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Korea, for their assistance and effort.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Mekaj, Y.H.; Mekaj, A.Y.; Duci, S.B.; Miftari, E.I. New oral anticoagulants: Their advantages and disadvantages compared with vitamin K antagonists in the prevention and treatment of patients with thromboembolic events. *Ther. Clin. Risk. Manag.* **2015**, *11*, 967–977. [CrossRef]
2. Greety, T.; Kumar, K.K.; Jayapraksah, K. A review on novel oral anticoagulants. *Hygeia J. D Med.* **2015**, *7*, 51–56.
3. Kuznetsov, S.; Barcelona, R.; Josephson, R.A.; Mohan, S.K.M. The Role of Nonvitamin K Antagonist Oral Anticoagulants (NOACs) in Stroke Prevention in Patients with Atrial Fibrillation. *Curr. Neurol. Neurosci. Rep.* **2016**, *16*. [CrossRef]
4. Yao, X.; Abraham, N.S.; Sangaralingham, L.R.; Bellolio, M.F.; McBane, R.; Shah, N.D.; Noseworthy, P.A. Effectiveness and Safety of Dabigatran, Rivaroxaban, and Apixaban Versus Warfarin in Nonvalvular Atrial Fibrillation. *J. Am. Hearth Assoc.* **2016**, *5*. [CrossRef] [PubMed]
5. Kirchhof, P.; Benussi, S.; Kotecha, D.; Ahlsson, A.; Atar, D.; Casadei, B.; Castella, M.; Diener, H.C.; Heidbuchel, H.; Hendriks, J.; et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. *Eur. Heart J.* **2016**, *37*, 2893–2962. [CrossRef]
6. Kearon, C.; Akl, E.A.; Ornelas, J.; Blaivas, A.; Jimenez, D.; Bounameaux, H.; Huisman, M.; King, C.S.; Morris, T.A.; Sood, N.; et al. Antithrombotic therapy for VTE disease: CHEST guideline and expert panel report. *Chest* **2016**, *149*, 315–352. [CrossRef] [PubMed]
7. Ingrasciotta, Y.; Crisafulli, S.; Pizzimenti, V.; Marcianò, I.; Mancuso, A.; Andò, G.; Corrao, S.; Capranzano, P.; Trifirò, G. Pharmacokinetics of new oral anticoagulants: Implications for use in routine care. *Expert Opin. Drug Metab. Toxicol.* **2018**, *14*, 1057–1069. [CrossRef] [PubMed]
8. Yeh, C.H.; Hogg, K.; Weitz, J.I. Overview of the new oral anticoagulants: Opportunities and challenges. *Arterioscler. Thromb. Vasc. Biol.* **2015**, *35*, 1056–1065. [CrossRef]
9. Ciurus, T.; Sobczak, S.; Cichocka-Radwan, A. New oral anticoagulants—A practical guide. *Kardiochir. Torakochirurgia Pol.* **2015**, *12*, 111–118.
10. Helms, T.M.; Silber, S.; Schäfer, A.; Masuhr, F.; Palm, F.; Darius, H.; Schrör, K.; Bänsch, D.; Bramlage, P.; Hankowitz, J.; et al. Consensus statement: Management of oral anticoagulation for stroke prevention in patients with nonvalvular atrial fibrillation. *Herzschrittmacherther Elektrophysiol.* **2016**, *27*, 295–306. [CrossRef]
11. Senoo, K.; Lip, G. Comparative Efficacy and Safety of the Non–Vitamin K Antagonist Oral Anticoagulants for Patients with Nonvalvular Atrial Fibrillation. *Semin. Thromb. Hemost.* **2015**, *41*, 146–153. [CrossRef]
12. Connolly, S.J.; Ezekowitz, M.D.; Yusuf, S.; Eikelboom, J.; Oldgren, J.; Parekh, A.; Pogue, J.; Reilly, P.A.; Themeles, E.; Varrone, J.; et al. Dabigatran versus Warfarin in Patients with Atrial Fibrillation. *N. Engl. J. Med.* **2009**, *361*, 1139–1151. [CrossRef]
13. Granger, C.B.; Alexander, J.H.; McMurray, J.J.; Lopes, R.D.; Hylek, E.M.; Hanna, M.; Al-Khalidi, H.R.; Ansell, J.; Atar, D.; Avezum, A.; et al. Apixaban versus Warfarin in Patients with Atrial Fibrillation. *N. Engl. J. Med.* **2011**, *365*, 981–992. [CrossRef]
14. Koretsune, Y.; Yamashita, T.; Yang, Y.; Chen, S.A.; Chung, N.; Giugliano, R.P. Edoxaban versus warfarin in east-asian (including Japanese) patients with atrial fibrillation—An engage AF-TIMI 48 sub-analysis. *Circ. J.* **2016**, *80*, 860–869.

15. Caldeira, D.; Barra, M.; Pinto, F.J.; Ferreira, J.J.; Costa, J. Intracranial hemorrhage risk with the new oral anticoagulants: A systematic review and meta-analysis. *J. Neurol.* **2015**, *262*, 516–522. [CrossRef]
16. Skaistis, J.; Tagami, T. Risk of Fatal Bleeding in Episodes of Major Bleeding with New Oral Anticoagulants and Vitamin K Antagonists: A Systematic Review and Meta-Analysis. *PLoS ONE* **2015**, *10*, e0137444. [CrossRef]
17. Katsanos, A.H.; Schellinger, P.D.; Köhrmann, M.; Filippatou, A.; Gurol, M.E.; Caso, V.; Paciaroni, M.; Perren, F.; Alexandrov, A.V.; Tsivgoulis, G. Fatal oral anticoagulant-related intracranial hemorrhage: A systematic review and meta-analysis. *Eur. J. Neurol.* **2018**, *25*, 1299–1302. [CrossRef]
18. Almutairi, A.R.; Zhou, L.; Gellad, W.F.; Lee, J.K.; Slack, M.K.; Martin, J.R.; Lo-Ciganic, W.H. Effectiveness and safety of non–vitamin K antagonist oral anticoagulants for atrial fibrillation and venous thromboembolism: A systematic review and meta-analyses. *Clin. Ther.* **2017**, *39*, 1456–1478. [CrossRef]
19. Caldeira, D.; Rodrigues, F.B.; Barra, M.; Santos, A.T.; de Abreu, D.; Gonçalves, N.; Pinto, F.J.; Ferreira, J.J.; Costa, J. Non-vitamin K antagonist oral anticoagulants and major bleeding-related fatality in patients with atrial fibrillation and venous thromboembolism: A systematic review and meta-analysis. *Heart* **2015**, *101*, 1204–1211. [CrossRef]
20. Patel, M.R.; Mahaffey, K.W.; Garg, J.; Pan, G.; Singer, D.E.; Hacke, W.; Breithardt, G.; Halperin, J.L.; Hankey, G.; Piccini, J.P.; et al. Rivaroxaban versus Warfarin in Nonvalvular Atrial Fibrillation. *N. Engl. J. Med.* **2011**, *365*, 883–891. [CrossRef]
21. Camm, A.J.; Amarenco, P.; Haas, S.; Hess, S.; Kirchhof, P.; Kuhls, S.; Van Eickels, M.; Turpie, A.G. XANTUS: A real-world, prospective, observational study of patients treated with rivaroxaban for stroke prevention in atrial fibrillation. *Eur. Hearth J.* **2016**, *37*, 1145–1153. [CrossRef]
22. Schulman, S.; Kearon, C. Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. *J. Thromb. Haemost* **2005**, *3*, 692–694.
23. Chahal, D.; Lee, J.G.; Ali-Mohamad, N.; Donnellan, F. High rate of re-bleeding after application of Hemospray for upper and lower gastrointestinal bleeds. *Dig. Liver Dis.* **2020**, *52*, 768–772. [CrossRef]
24. Wetwittayakhlang, P.; Wonglhow, J.; Netinatsunton, N.; Chamroonkul, N.; Piratvisuth, T. Re-bleeding and its predictors after capsule en-doscopy in patients with obscure gastrointestinal bleeding in long-term follow-up. *BMC Gastroenterol.* **2019**, *19*, 216. [CrossRef] [PubMed]
25. Beyer-Westendorf, J.; Förster, K.; Pannach, S.; Ebertz, F.; Gelbricht, V.; Thieme, C.; Michalski, F.; Köhler, C.; Werth, S.; Sahin, K.; et al. Rates, management, and outcome of rivaroxaban bleeding in daily care: Results from the Dresden NOAC registry. *Blood* **2014**, *124*, 955–962. [CrossRef] [PubMed]
26. Lee, J.H.; Lim, H.E.; Lim, W.-H.; Ahn, J.; Cha, M.-J.; Park, J.; Lee, K.H.; Park, H.-C.; Choi, E.-K.; Joung, B. The 2018 Korean Heart Rhythm Society Practical Guidelines on the use of Non-Vitamin K-Antagonist Oral Anticoagulants: Bleeding Control and Perioperative Management. *Korean J. Med.* **2019**, *94*, 40–56. [CrossRef]
27. Suh, D.-C.; Nelson, W.W.; Choi, J.C.; Choi, I. Risk of Hemorrhage and Treatment Costs Associated with Warfarin Drug Interactions in Patients with Atrial Fibrillation. *Clin. Ther.* **2012**, *34*, 1569–1582. [CrossRef] [PubMed]
28. Snipelisky, D.; Kusumoto, F. Current strategies to minimize the bleeding risk of warfarin. *J. Blood Med.* **2013**, *4*, 89–99. [CrossRef]
29. Pannach, S.; Goetze, J.; Marten, S.; Schreier, T.; Tittl, L.; Beyer-Westendorf, J. Management and outcome of gastrointestinal bleeding in patients taking oral anticoagulants or antiplatelet drugs. *J. Gastroenterol.* **2017**, *52*, 1211–1220. [CrossRef]
30. Vranckx, P.; Valgimigli, M.; Heidbuchel, H. The Significance of Drug–Drug and Drug–Food Interactions of Oral Anticoagulation. *Arrhythmia Electrophysiol. Rev.* **2018**, *7*, 55–61. [CrossRef]
31. Walborn, A.; Williams, M.; Fareed, J.; Hoppensteadt, D. International Normalized Ratio Relevance to the Observed Coagulation Abnormalities in Warfarin Treatment and Disseminated Intravascular Coagulation. *Clin. Appl. Thromb.* **2018**, *24*, 1033–1041. [CrossRef]
32. Brodie, M.M.; Newman, J.C.; Smith, T.; Rockey, D.C. Severity of Gastrointestinal Bleeding in Patients Treated with Direct-Acting Oral Anticoagulants. *Am. J. Med.* **2018**, *131*, 573. [CrossRef]
33. Gerson, L.B.; Fidler, J.L.; Cave, D.R.; Leighton, J.A. ACG Clinical Guideline: Diagnosis and Management of Small Bowel Bleeding. *Am. J. Gastroenterol.* **2015**, *110*, 1265–1287. [CrossRef]
34. Diamantopoulou, G.; Konstantakis, C.; Skroubis, G.; Theocharis, G.; Theopistos, V.; Triantos, C.; Thomopoulosa, K. Acute Lower Gastrointestinal Bleeding in Patients Treated with Non-Vitamin K Antagonist Oral Anticoagulants Compared With Warfarin in Clinical Practice: Characteristics and Clinical Outcome. *Gastroenterol. Res.* **2019**, *12*, 21–26. [CrossRef]
35. Desai, J.; Kolb, J.M.; Weitz, J.I.; Aisenberg, J. Gastrointestinal bleeding with the new oral anticoagulants–defining the issues and the management strategies. *Thromb. Haemost.* **2013**, *110*, 205–212. [CrossRef]
36. Black, S.A.; Cohen, A.T. Anticoagulation strategies for venous thromboembolism: Moving towards a personalised approach. *Thromb. Haemost.* **2015**, *114*, 660–669.
37. Hakeam, H.A.; Al-Sanea, N. Effect of major gastrointestinal tract surgery on the absorption and efficacy of direct acting oral anticoagulants (DOACs). *J. Thromb. Thrombolysis* **2017**, *43*, 343–351. [CrossRef]
38. Guo, W.Q.; Chen, X.H.; Tian, X.Y.; Li, L. Differences in Gastrointestinal Safety Profiles Among Novel Oral Anticoagulants: Evidence from a Network Meta-Analysis. *Clin. Epidemiol.* **2019**, *11*, 911–921. [CrossRef]

Journal of
Clinical Medicine

MDPI

Article

Effect of DA-9701 on the Gastrointestinal Motility in the Streptozotocin-Induced Diabetic Mice

Changyoon Ha [1,2,3], Heejin Kim [1,3,4], Rari Cha [1,3,4], Jaemin Lee [1,3,4], Sangsoo Lee [1,3,4], Jung-Hwa Ryu [5], Hyunjin Kim [1,3,4,*] and Ok-Jae Lee [1,2,3,*]

1 Department of Internal Medicine, Gyeongsang National University College of Medicine, Jinju 52727, Korea; cyha@gnu.ac.kr (C.H.); heejin1231@hanmail.net (H.K.); rari83@naver.com (R.C.); 01179jm@naver.com (J.L.); 3939lee@naver.com (S.L.)
2 Division of Gastroenterology, Gyeongsang National University Hospital, Jinju 52727, Korea
3 Institute of Health Sciences, Gyeongsang National University, Jinju 52727, Korea
4 Division of Gastroenterology, Gyeongsang National University Changwon Hospital, Changwon 51472, Korea
5 Department of Internal Medicine, Ewha Womans University College of Medicine, Seoul 07804, Korea; singrum@gmail.com
* Correspondence: imdrkim@naver.com (H.K.); ojlee@gnu.ac.kr (O.-J.L.)

Abstract: Background: Compared to the general population, diabetic patients experience more frequent episodes of gastrointestinal (GI) motility dysfunction, owing to the disruption of functional innervations. DA-9701 is a new prokinetic agent formulated from the extracts of *Pharbitidis* semen and *Corydalis* tuber. Aim: To investigate the effect of DA-9701 on GI motility in an animal model of streptozotocin (STZ)-induced diabetes. Methods: Diabetes was induced in mice by intraperitoneal injection of STZ (40 mg/kg of body weight in 0.1 M citrate buffer) for 3 days. Diabetic mice were divided into four groups and administered DA-9701 in different doses (1, 3, and 10 mg/kg) or placebo for 2 weeks. Intestinal transit was assessed using charcoal meal movement. GI isometric contraction was measured by applying an isometric force transducer on a circular muscle strip of the antrum, ileum, and proximal colon of sacrificed mice. Gastric emptying rate was evaluated by measuring the dye percentage remaining in the stomach relative to the total dye amount recovered in a standardization group of mice. Results: Body weight and antral and small intestinal motility were less in diabetic mice than in control mice, and colonic motility was similar in both. DA-9701 showed a dose-dependent increase in the amplitude of spontaneous phasic contractions in the antrum, ileum, and colon in diabetic mice without influencing body weight or blood glucose levels. The degree of improvement was comparable between diabetic and control mice. Intestinal transit was significantly more delayed in diabetic mice than in controls ($43 \pm 7\%$ vs. $67 \pm 8\%$, $p < 0.05$); however, DA-9701 restored the delayed intestinal transit more effectively compared to placebo (75% vs. 50%). The gastric emptying rate was significantly more delayed in diabetic mice than in controls ($43 \pm 10\%$ vs. $62 \pm 12\%$, $p < 0.05$), and was improved by DA-9701 in a dose-dependent manner (50%, 55%, and 60% in mice treated with 1, 3, and 10 mg/kg of DA-9701, respectively, vs. 43% in placebo-treated and 60% in control mice). Conclusions: DA-9701 improved GI contractility without affecting blood sugar and body weight in diabetic mice. DA-9701 could improve the decreased GI motility and clinical symptoms in progressive diabetic patients.

Keywords: DA-9701; diabetic mouse model; functional dyspepsia; diabetic gastroparesis; STZ; gastrointestinal motility

Citation: Ha, C.; Kim, H.; Cha, R.; Lee, J.; Lee, S.; Ryu, J.-H.; Kim, H.; Lee, O.-J. Effect of DA-9701 on the Gastrointestinal Motility in the Streptozotocin-Induced Diabetic Mice. *J. Clin. Med.* **2021**, *10*, 5282. https://doi.org/10.3390/jcm10225282

Academic Editor: Marilena Durazzo

Received: 29 September 2021
Accepted: 8 November 2021
Published: 13 November 2021

1. Introduction

Functional gastrointestinal (GI) disorders occur more frequently in patients with diabetes than in the general population. Longstanding diabetes induces GI motility dysfunction via the disruption of nerve functions regulating the motility of the gut, which causes incomplete emptying of the different sections of the gastrointestinal tract. This

process finally leads to gastroenteropathy, a composite disorder of the esophagus, stomach, small intestine, and colon [1]. Thus, diabetic patients experience delayed gastric emptying (GE) and various GI symptoms, such as nausea, vomiting, early satiety, bloating, postprandial discomfort, anorexia, weight loss, and abdominal pain. Recently, more concise pathophysiologic mechanisms of diabetic gastroparesis have been elucidated; these include extrinsic denervation of the stomach, causing delayed gastric emptying, and loss of nitric oxide synthase (NOS) in the enteric nerve, causing impaired inhibitory input, which induces decreased gastric accommodation and decreased gastric emptying, pylorospasm, and altered function of immune cells, such as type 2 macrophages, which can trigger damage to the interstitial cells of Cajal (ICC) and smooth muscle atrophy [2,3].

DA-9701 is a new prokinetic agent formulated from the extracts of *Pharbitidis* semen and *Corydalis* tuber [4]. This medicine, which is known to function as a 5-HT_{1A} agonist, 5-HT_4 agonist, and 5-HT_3 partial antagonist, was developed for the treatment of functional dyspepsia [4–6]. *Pharbitidis* semen is known to have natural ingredients that control edema, fullness, fecal and urinary retention, phlegm, fluid retention, and abdominal pain due to parasitic infestations. *Corydalis* tuber is known to be effective in adjusting mild depression, severe nerve damage, tremors, and intestinal spasm [7].

In this study, we investigated the effect of DA-9701 on gastrointestinal motility, the intestinal transit, and gastric emptying rate in a streptozotocin (STZ)-induced diabetic mouse model.

2. Materials and Methods

2.1. Streptozotocin (STZ)-Induced Diabetic Mice Model

All animal care and experimental procedures were approved by the Ethics of Animal Experiments Committee of Kyungpook National University. Six-week-old male Institute of Cancer Research (ICR) mice weighing 25–30 g were used as experimental animals. They were housed at 24 °C, were allowed free access to water and feed, and lighting was repeated in a darkness and lighting cycle every 12 h. After acclimatization for 1 week, the mice were randomly divided into two groups: the STZ-induced diabetic group and the normal control group.

STZ was dissolved in 0.1 mM citric acid buffer solution (STZ 40 mg/kg, pH 4.0) every day and injected via the intraperitoneal (IP) route for 3 consecutive days. In the normal control group, the same volume of citric acid buffer was injected intraperitoneally (Figure 1). Body weights were measured weekly during the experimental period. Two weeks after the first day of STZ injection, blood was collected from the tail vein and blood glucose levels were measured in each mouse. Individuals with a level of ≥300 mg/dL were considered to have diabetes. Blood glucose levels were measured at random times without overnight fasting using a GlucoDr™ Plus (Allmedicus, Anyang, Gyeonggi-do, Republic of Korea) test strip and a measuring instrument.

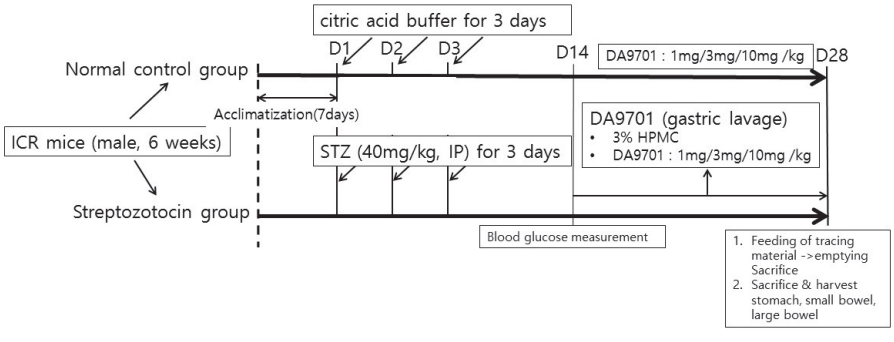


Figure 1. Schematic diagram of the experimental protocol.

After the acclimatization phase of 7 days, streptozotocin was injected via intraperitoneal at a dose of 40 mg/kg for 3 days. The same volume of citric acid buffer was injected into normal control mice. Blood glucose level was measured after 14 days. Normal control and streptozotocin-induced mice were treated with DA-9701 in different doses (1, 3, and 10 mg/kg) for 14 days. On day 28, all mice were sacrificed, and gastrointestinal motility was assessed.

2.2. DA-9701 Treatment in Experimental Mice

The STZ-induced diabetic mice were divided into four groups, each with 10 mice: STZ-induced diabetic mice treated with different doses of DA-9701 at 1, 3, and 10 mg/kg, and STZ-induced diabetic mice with the placebo treatment. DA-9701 was suspended and administered orally at different doses of 1, 3, and 10 mg/kg for 2 weeks, and the placebo group was orally administered 3% hydroxypropyl methylcellulose (HPMC) for 2 weeks (Figure 1). To show the maximum changes in GI muscular contractility according to the DA-9701 dose escalation, the normal control or STZ-induced diabetic mice were also treated at higher doses (>10 mg/kg; 30, 100, and 300 mg/kg). In this experiment, the mice were exposed to 1, 3, 10, 30, 100, and 300 mg/kg of DA-9701.

2.3. Assessment of GI Motility Using Isometric Contraction Measurement

All studies were performed on normal or STZ mice after 2 weeks of treatment with DA-9701 or placebo. DA-9701 was given to the mice in different doses of 1, 3, 10, 30, 100, and 300 mg/kg. After 16 h of fasting, the mice were sacrificed. GI motility was evaluated by measuring isometric contractions in each segment of the bowel using circular muscle strips from the antrum, ileum, and proximal colon. From the sacrificed mice, 1–1.5 cm circular muscle strips of the antrum, ileum, and proximal colon were isolated and flushed with Krebs solution. The muscle strips were immediately placed in a 10 mL organ bath containing oxygenated (95% O_2 + 5% CO_2) Krebs solution at 37 °C. The distal end of the muscle segment was tied to a fixed mount and the tied proximal end was fixed to an isometric force-displacement transducer (FT-03, Grass-telefactor, Providence, RI, USA). Tension was monitored using an isometric force transducer and an index of the longitudinal muscle response was recorded. The signal was analyzed using a digital recording system. The signals from the transducers were processed using Powerlab 4/30 and Chart 7.2 (AD Instruments, Bella Vista, Australia).

The composition of Krebs solution was 10.1 mM glucose, 115.5 mM NaCl, 21.9 mM $NaHCO_3$, 4.61 mM KCl, 1.14 mM NaH_2PO_4, 2.5 mM $CaCl_2$, and 1.16 mM $MgSO_4$.

2.4. Measurement of Intestinal Transit

All experiments were performed after 2 weeks of treatment with DA-9701 or 3% HPMC (placebo). After 16 h of fasting, the mice were administered a single dose of liquid charcoal meal (10% *w*/*v* charcoal suspension in 5% *w*/*v* suspension of acacia). Each subject was treated with a charcoal meal of 0.1 mL/10 g (body weight) via the oral route. All mice were euthanized via cervical dislocation 30 min after administration of the charcoal meal. To measure intestinal transit, the stomach and small intestine were isolated. They were then extended to a clean surface. The distance moved by the charcoal meal from the pylorus and the total length of the small intestine were measured. Intestinal transit was expressed as a percentage of the distance traveled by charcoal over the total length from the pylorus to the cecum.

2.5. Measurement of GE Rate with Phenol Red Marker

GE was determined by the phenol red method. All studies were performed on mice after 2 weeks of treatment with DA-9701 or 3% HPMC. After 16 h of fasting, the mice were treated orally with 1.5% carboxymethylcellulose (Sigma-Aldrich, Millipore, St. Louis, MI, USA) containing 0.05% phenol red (Sigma-Aldrich, Millipore, St. Louis, MI, USA) at a dose of 0.1 mL/10 g, 1 h after treatment with 3% HPMC or DA-9701. After another

30 min, the mice were sacrificed by cervical dislocation. The abdomen was opened carefully and the gastroesophageal junction and pylorus were tied to prevent the contents from flowing out; the stomach was then separated. The extracted stomach was ground using a homogenizer in 0.1 M NaOH, and the suspension was allowed to stabilize for 1 h at room temperature. Then, trichloroacetic acid (2% final concentration) was added and samples were centrifuged at 2500× *g* for 10 min. The supernatant was mixed with 0.05 M NaOH, and the amount of phenol red was measured colorimetrically at 560 nm using a microplate reader (Multiskan GO, Thermo Fisher Scientific, USA). The standard sample (zero-time control) was determined by the amount of phenol red recovered from mice sacrificed immediately after oral administration of 1.5% carboxymethylcellulose containing 0.05% phenol red. The gastric emptying rate was calculated using the following Equation:

$$\text{Gastric Emptying rate } (\%) = [1 - \frac{\text{ABS560nm of test stomach}}{\text{ABS560nm of 0 time control stomach}}] \times 100 \quad (1)$$

2.6. Statistical Analysis

Statistical analysis of the experimental results was performed with Student's t-test, using the Prism program (GraphPad Prism quickCalcs, San Diego, CA, USA). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Blood Glucose and Body Weight in the STZ-Induced Diabetic Mice Model

Two weeks after completion of the 3-day treatment with STZ or citric acid buffer, STZ-induced diabetic mice showed significantly higher blood glucose levels and lower body weight compared to normal controls ($p < 0.05$, Figure 2). There was no significant difference in the blood glucose concentration and the degree of body-weight loss in the diabetic group.

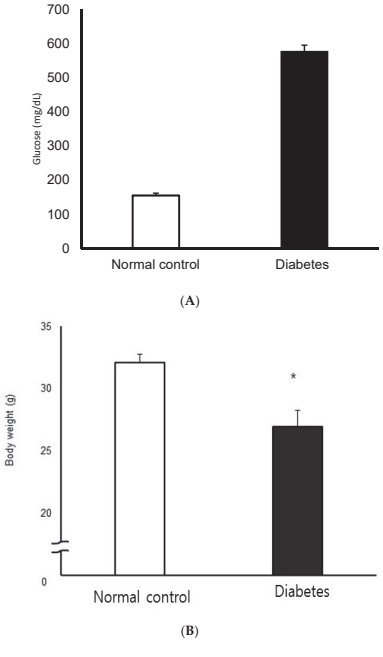


Figure 2. Blood glucose concentration (**A**) and body weight (**B**) in normal control and streptozotocin-induced diabetic mice. STZ-induced diabetic mice showed significantly higher blood glucose levels and lower body weight compared to normal control (n = 10 in each group) * $p < 0.05$.

DA-9701 had no effect on either blood glucose level (Figure 3) or body weight (Figure 4) in STZ-induced diabetic mice.

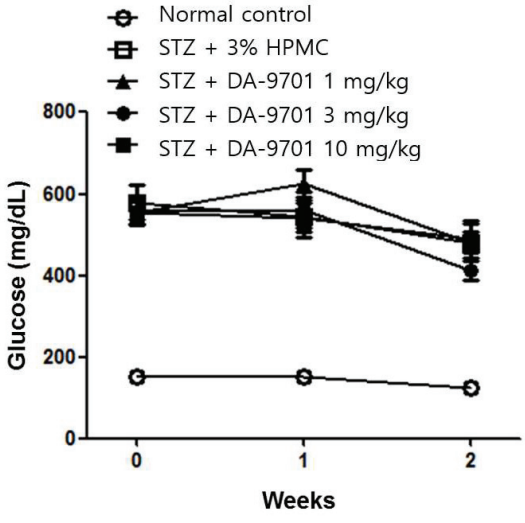


Figure 3. Effect of DA-9701 on blood glucose level in normal control and streptozotocin-induced diabetic mice. Streptozotocin-induced diabetic mice showed significantly higher blood glucose levels than control. DA-9701 did not affect blood glucose level in the streptozotocin-induced diabetic mice. (n = 10 in each group).

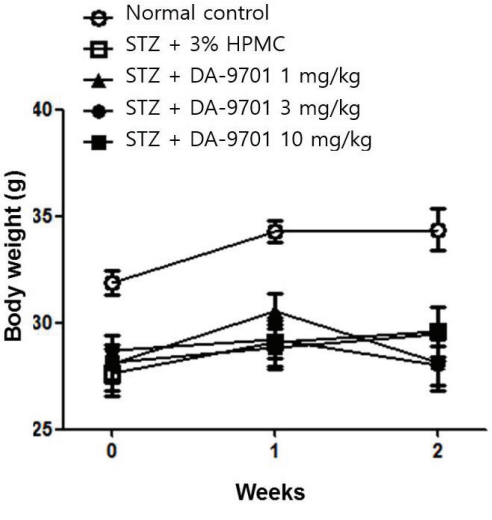


Figure 4. Effect of DA-9701 on body weight in normal control and streptozotocin-induced diabetic mice. STZ-induced diabetic mice had significantly lower body weight compared to control. DA-9701 had no effect on the body weight of the streptozotocin-induced diabetic mice. (n = 10 in each group).

3.2. GI Motility in the STZ-Induced Diabetic Mice Model

The isometric contractions of the antrum and small bowel were significantly decreased in the STZ-induced diabetic mice group compared to the normal control group (100% vs. 87.4%, Figure 5A, and 100% vs. 87.4%, Figure 5B). However, colonic motility was not significantly different between the groups (Figure 5C).

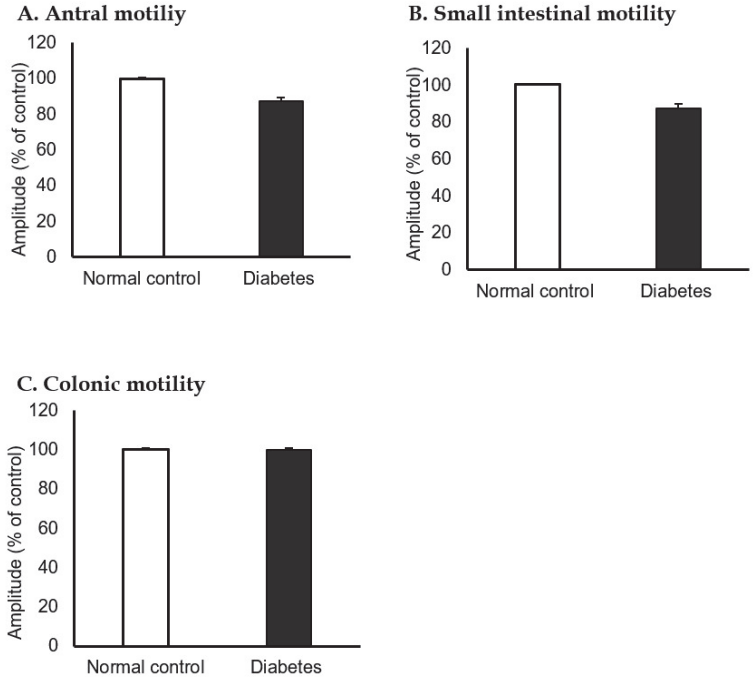


Figure 5. Antral, small intestinal, and colonic motility in normal control and streptozotocin-induced diabetic mice. The isometric contractions of each (**A**) antrum and (**B**) small bowel were significantly decreased in the streptozotocin-induced diabetic group compared to the normal control group (100% vs. 87.4%, 100% vs. 87.4%). (**C**) Colonic motility was not different between the normal control and diabetic group (100% vs. 100%). (n = 10 in each group).

3.3. Effects of DA-9701 on GI Motility

The antral motility decreased in STZ-induced diabetic mice before any treatment and was increased after DA-9701 treatment in a dose-dependent manner (Figure 6). Although the antral motility in the diabetic group was improved according to the increased dosage of DA-9701, the amplitude was higher in the normal control group than in the diabetic group. However, the amplitude of antral motility was even higher in the diabetic group than in the normal control group at a dose of >100 mg/kg of DA-9701.

In both the normal control and diabetic groups, DA-9701 increased spontaneous movement of the small intestinal muscle in a dose-dependent manner, without significant differences between the two groups. Motility showed no significant improvement in either diabetic or normal control groups under 30 mg/kg of DA-9701. However, the ileal muscular amplitude was significantly improved at over 30 mg/kg of DA-9701, with an increasing linear pattern ($p < 0.05$). This improvement was more prominent in the diabetic group (Figure 7).

Baseline colonic motility showed no definite difference between the diabetic and normal control groups. Despite the similar initial contractility between the two groups, colonic motility was dramatically improved after DA-9701 treatment in the diabetic group in a dose-dependent manner. In the control group, colonic motility did not respond to treatment with 30 mg/kg of DA-9701 and improved at over 30 mg/kg of DA-9701. However, the improvement was less than that in the diabetic group (Figure 8).

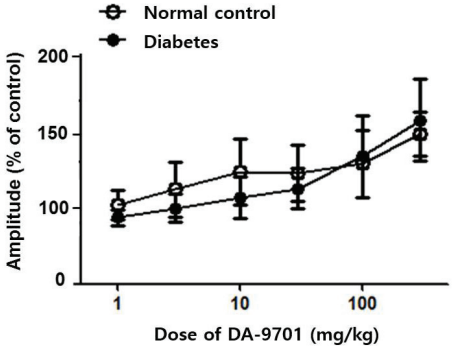


Figure 6. Effect of DA-9701 on antral motility in normal control and streptozotocin-induced diabetic mice. DA-9701 increased the spontaneous movement of the pyloric sinus ciliary muscle in a dose-dependent manner in both normal control and diabetic groups, without significant difference between two groups. (n = 10 in each group).

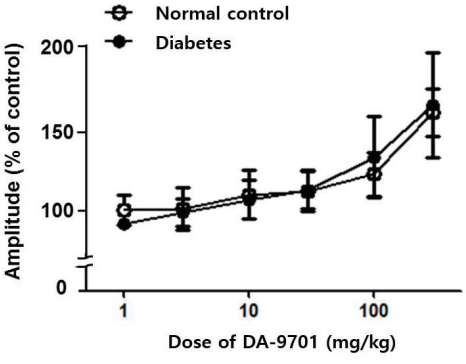


Figure 7. Effect of DA-9701 on small intestinal motility in normal control and streptozotocin-induced diabetic mice. In both the normal control and diabetic groups, DA-9701 increased the spontaneous movement of the small intestinal muscle in a dose-dependent manner, without significant difference between two groups. (n = 10 in each group).

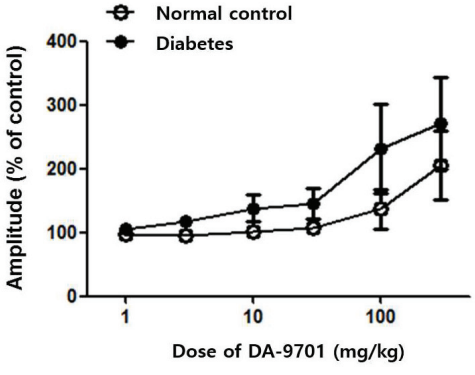


Figure 8. Effect of DA-9701 on colonic motility in normal control and streptozotocin-induced diabetic mice. DA-9701 administration showed a dose-dependent increase in the amplitude of spontaneous phasic contractions in the colon in normal controls and streptozotocin-induced diabetic mice, without significant difference between the two groups. (n = 10 in each group).

3.4. Small Intestinal Transit and GE Rate in the STZ-Induced Diabetic Mice

The intestinal transit decreased significantly in STZ-induced diabetic mice compared to normal controls (43 ± 7% vs. 67 ± 8%, $p < 0.05$, Figure 9).

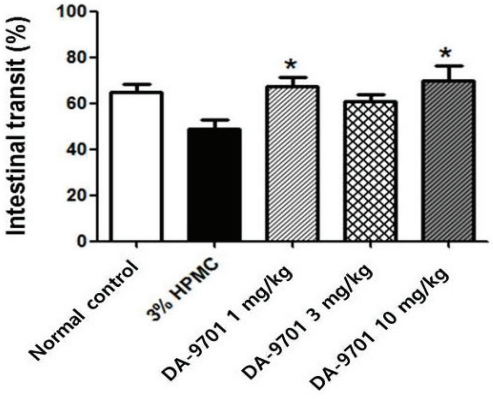


Figure 9. Effect of DA-9701 on intestinal transit in streptozotocin-induced diabetic mice. Intestinal transit was significantly decreased in streptozotocin-induced diabetic mice compared to normal control and improved in DA-9701 treatment group compared to placebo (3% HPMC-treated) group. The intestinal transit was 67 ± 4.0%, 60 ± 4.2%, 70 ± 6.6% in the treatment with 1 mg/kg, 3 mg/kg, and 10 mg/kg of DA-9701, and 49 ± 4.0% placebo groups, respectively. In particular, the intestinal transit improved significantly with 1 mg/Kg and 10 mg/kg of DA-9701 treatment compared to placebo (n = 10 in each group) * $p < 0.05$.

The GE rate was also significantly delayed in the diabetic group compared to that in the normal control group (43 ± 10% vs. 62 ± 12%, $p < 0.05$, Figure 10).

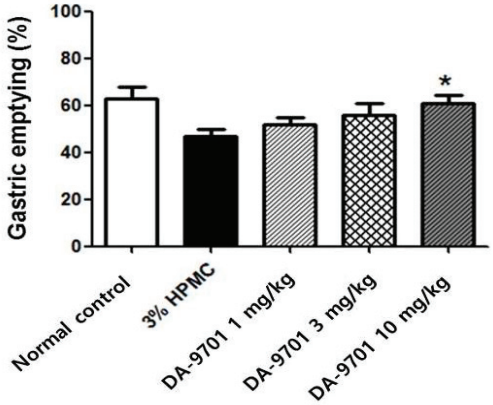


Figure 10. Effect of DA-9701 on gastric emptying in streptozotocin-induced diabetic mice. Gastric emptying was significantly delayed in streptozotocin-induced diabetic mice and improved with DA-9701 in a dose-dependent manner (50 ± 11 %, 55 ± 12%, and 60 ± 11% in 1 mg/kg, 3 mg/kg, and 10 mg/kg of DA-9701 treatment, respectively), compared to the 3% HPMC-treated placebo group. In particular, treatment with 10 mg/kg of DA-9701 significantly improved gastric emptying rate compared to placebo (60 ± 11% vs. 43 ± 10%, * $p < 0.05$) (n = 10 in each group).

3.5. Effects of DA-9701 on Intestinal Transit

Significantly decreased intestinal transit in STZ-induced diabetic mice was improved with DA-9701 treatment, compared to placebo (3% HPMC). The intestinal transit was

67 ± 4.0%, 60 ± 4.2%, and 70 ± 6.6% in the treatment with 1 mg/kg, 3 mg/kg, and 10 mg/kg of DA-9701, and 49 ± 4.0% placebo (3% HMC) groups, respectively. In particular, the intestinal transit improved significantly with 1 mg/kg and 10 mg/kg of DA-9701 treatment compared to placebo (Figure 9).

3.6. Effects of DA-9701 on GE Rate

Delayed GE in STZ-induced diabetic mice improved with administration of DA-9701 in a dose-dependent manner (50 ± 11 %, 55 ± 12%, and 60 ± 11% at 1 mg/kg, 3 mg/kg, and 10 mg/kg of DA-9701, respectively). In particular, treatment with 10 mg/kg of DA-9701 significantly improved the GE rate compared to the placebo-treated diabetic group (60 ± 11% vs. 43 ± 10%, $p < 0.05$, Figure 10).

4. Discussion

Diabetic gastroparesis affects 20–50% of people with type 1 diabetes and 5% of patients who have been diagnosed with type 2 diabetes for more than 10 years [8]. The stomach is more susceptible to diabetic complications than the small intestine; approximately 75% of patients with diabetes have gastrointestinal symptoms, one-third of whom have stomach symptoms [9,10].

It is considered that this disease spectrum can be caused by autonomic neuropathy [3,11]. Diabetes causes loss of myelinated sympathetic trunk fibers and enlarged dystrophic axons and nerve terminals in the prevertebral sympathetic ganglia [12]. ICC loss in diabetes has been observed in human and animal models [13,14]. Reduced insulin and IGF-1 signaling may cause ICC loss, smooth muscle atrophy, and reduced stem cell factor production [15]. Another mechanism is that oxidative stress results in the loss of heme oxygenase 1 (HO1)-containing macrophages. HO1 is an enzyme expressed in macrophages and has a protective effect against oxidative stress. The main source of HO1 is the stomach muscle wall [10]. Pathological findings associated with gastric sensorimotor dysfunction can occur; these include delayed GE, gastric dysrhythmia, fundic accommodation, weakened antral pump, antroduodenal discoordination, duodenal neuromuscular dysfunction, and abnormal duodenal feedback [16,17].

This study presented several important findings associated with diabetic gastroparesis and gastroenteropathy. First, GI motility significantly decreased with site selectivity in diabetic conditions. The bowel motility power was found to be weak from the stomach to the small intestine in a diabetic model; however, colonic motility showed no significant decline. Although various factors may affect the colonic symptoms, this finding is one explanation for why diabetic patients present with various colonic symptoms, such as diarrhea, abdominal pain, and constipation. Second, GI transit and GE were appreciably delayed in STZ-induced diabetic mice compared to normal controls. Finally, DA-9701, which has been developed as a prokinetic drug, restored the decreased GI motility and delayed intestinal transit and GE in STZ-induced diabetic mice.

The present study confirmed that gastric and small intestinal motility decreased in STZ-induced diabetic mice compared to that in normal mice. However, colonic motility was not significantly decreased in STZ-induced diabetic mice. There is a lack of well-designed studies on abnormal colonic motility in patients with diabetes [18]. However, there is a pathological change in large bowel motility because lower GI symptoms, such as diarrhea or constipation, are easily noted in longstanding diabetic patients. There is some evidence of delayed colonic transit, colonic myenteric neuronal loss, [19] anorectal dysfunction due to impaired external anal sphincter function, diminished rectal sensation to distension [18], and increased oxidative stress in colonic tissues [19]. Although muscular strength in the colon was preserved in our model, there could be other structural abnormalities or early changes in another colonic site. The definite mechanism in diabetic gastroparesis should be revealed in future studies using multiple samples from the GI tract.

This study showed that DA-9701 was effective in improving GI motility, small bowel transit, and gastric emptying in STZ-induced diabetic mice. Interestingly, the changes in

motility amplitude were greater in the colon than in the antral or small intestine after DA-9701 treatment. Although the baseline colonic motility in STZ-induced diabetic mice was not different from that in control mice, the response to the drug was more remarkable in the colon than in other organs. DA-9701 improved functional constipation in non-diabetic patients by accelerating colonic transit in a single-center experience [20]. However, this drug did not improve constipation symptoms in patients with Parkinson's disease [21]. Further studies are required to determine whether DA-9701 would improve lower GI symptoms more than upper GI symptoms in diabetic patients, and furthermore, how DA-9701 increases colonic motility more than other organs in a diabetic model.

In this study, DA-9701 improved both small intestinal transit and GE in a dose-dependent manner. Many investigators have reported the effect of DA-9701 on the restoration of reduced GI transit and GE. Ramsbottom et al. [22] compared the efficacy of DA-9701 in GE with cisapride, clonidine, and apomorphine in a delayed GE model. The prokinetic effects of DA-9701 were comparable to those of cisapride (10 mg/kg) in normal animals and a delayed GE model at doses of 0.3–3 mg/kg [6]. In an in vitro study using an ICC, it was suggested that DA-9701 might affect GI motility by modulating pacemaker activity in the ICC [23,24]. DA-9701 accelerated GE, which was confirmed by a 13C-octanoic acid breath test with repeated measurements in normal mice [25]. DA-9701 improved stress-induced delayed GE, and this effect might be associated with the inhibition of stress-induced increases in plasma levels of adrenocorticotropic hormone (ACTH) and ghrelin [26]. The first multicenter, double-blind, randomized clinical trial was performed with concealed allocation, comparing the safety and efficacy of DA-9701 and itopride hydrochloride in Korea [24]. DA-9701 significantly improved both functional dyspepsia symptoms and quality of life in patients with functional dyspepsia. The efficacy of DA-9701 was not inferior to that of itopride [24]. Although DA-9701 was effective in treating functional gastric diseases, the effects of this drug in diabetic patients have not been proven yet. Thus, our results provide evidence for the use of DA-9701 in the treatment of diabetic gastroparesis and gastroenteropathy. Because of the heterogeneous symptom pattern and various causes of functional gastroparesis in diabetic patients, effective and safe doses of DA-9701 according to symptoms should be confirmed in a future study.

This study has several limitations. First, colonic motility was not significantly decreased in patients with diabetes. This might be explained by insufficient diabetic stimulation of the colon or the short duration of diabetes. Dysregulated colonic motility occurs in the advanced diabetic stage. Second, a colonic transit study of diabetes patients was not included in this study. To demonstrate the mechanism of colonic motility improvement, colonic transit or neuronal studies in colonic tissue should be performed. Further studies on the pathological findings and colonic motility function using DA-9701 will be required in diabetic animal models and clinical settings. Finally, the clinical application of DA-9701 should be added to prove its positive effect on diabetic gastroparesis. Although DA-9701 was proven to be effective in functional gastroparesis, diabetic gastroparesis is different from general functional disorders.

5. Conclusions

In conclusion, DA-9701 improved GI contractility without affecting blood sugar and body weight in STZ-induced diabetic mice, similar to the normal controls; in particular, it had a good effect on increasing colon motility in normal and diabetic mice. The efficacy of DA-9701 in intestinal transit and gastric emptying was similar in STZ-induced diabetic mice and normal mice. DA-9701 might be helpful in improving decreased upper and lower GI motility and various clinical symptoms in patients with progressive diabetes. Further studies are required to evaluate the effect of DA-9701 on various bowel motility dysfunctions, even in advanced diabetic patients.

Author Contributions: C.H. experimented, analyzed and interpreted the data, and wrote the manuscript; O.-J.L. and H.K. (Heejin Kim) designed, organized and supervised the manuscript; H.K. (Hyunjin Kim), R.C., J.L., S.L. and J.-H.R. supported the statistical analysis and helped to prepare manuscript including tables and the figures, and mediated the present study. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: All animal care and experimental procedures were approved by the Ethics of Animal Experiments Committee of Gyeongsang National University (GNU-210928-M0081).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Parkman, H.P.; Hasler, W.L.; Fisher, R.S. American Gastroenterological Association technical review on the diagnosis and treatment of gastroparesis. *Gastroenterology* **2004**, *127*, 1592–1622. [CrossRef]
2. Krishnasamy, S.; Abell, T.L. Diabetic Gastroparesis: Principles and Current Trends in Management. *Diabetes Ther. Res. Treat. Educ. Diabetes Relat. Disord.* **2018**, *9*, 1–42. [CrossRef]
3. Yagihashi, S.; Sima, A.A. Diabetic autonomic neuropathy in BB rat. Ultrastructural and morphometric changes in parasympathetic nerves. *Diabetes* **1986**, *35*, 733–743. [CrossRef]
4. Kwon, Y.S.; Son, M. DA-9701: A New Multi-Acting Drug for the Treatment of Functional Dyspepsia. *Biomol. Ther.* **2013**, *21*, 181–189. [CrossRef]
5. Kim, E.R.; Min, B.H.; Lee, S.O.; Lee, T.H.; Son, M.; Rhee, P.L. Effects of DA-9701, a novel prokinetic agent, on gastric accommodation in conscious dogs. *J. Gastroenterol. Hepatol.* **2012**, *27*, 766–772. [CrossRef]
6. Lee, T.H.; Choi, J.J.; Kim, D.H.; Choi, S.; Lee, K.R.; Son, M.; Jin, M. Gastroprokinetic effects of DA-9701, a new prokinetic agent formulated with Pharbitis Semen and Corydalis Tuber. *Phytomed. Int. J. Phytother. Phytopharm.* **2008**, *15*, 836–843. [CrossRef]
7. Lee, S.P.; Lee, K.N.; Lee, O.Y.; Lee, H.L.; Jun, D.W.; Yoon, B.C.; Choi, H.S.; Hwang, S.J.; Lee, S.E. Effects of DA-9701, a novel prokinetic agent, on phosphorylated extracellular signal-regulated kinase expression in the dorsal root ganglion and spinal cord induced by colorectal distension in rats. *Gut Liver* **2014**, *8*, 140–147. [CrossRef] [PubMed]
8. Sanders, K.M.; Ordog, T.; Koh, S.D.; Ward, S.M. A Novel Pacemaker Mechanism Drives Gastrointestinal Rhythmicity. *News Physiol. Sci. Int. J. Physiol. Prod. Jt. By Int. Union Physiol. Sci. Am. Physiol. Soc.* **2000**, *15*, 291–298. [CrossRef]
9. Waseem, S.; Moshiree, B.; Draganov, P.V. Gastroparesis: Current diagnostic challenges and management considerations. *World J. Gastroenterol.* **2009**, *15*, 25–37. [CrossRef]
10. Choi, K.M.; Gibbons, S.J.; Nguyen, T.V.; Stoltz, G.J.; Lurken, M.S.; Ordog, T.; Szurszewski, J.H.; Farrugia, G. Heme Oxygenase-1 Protects Interstitial Cells of Cajal From Oxidative Stress and Reverses Diabetic Gastroparesis. *Gastroenterology* **2008**, *135*, 2055–2064.e2. [CrossRef]
11. Kniel, P.C.; Junker, U.; Perrin, I.V.; Bestetti, G.E.; Rossi, G.L. Varied effects of experimental diabetes on the autonomic nervous system of the rat. *Lab. Investig. A J. Tech. Methods Pathol.* **1986**, *54*, 523–530.
12. Duchen, L.W.; Anjorin, A.; Watkins, P.J.; Mackay, J.D. Pathology of autonomic neuropathy in diabetes mellitus. *Ann. Intern. Med.* **1980**, *92*, 301–303. [CrossRef]
13. Iwasaki, H.; Kajimura, M.; Osawa, S.; Kanaoka, S.; Furuta, T.; Ikuma, M.; Hishida, A. A deficiency of gastric interstitial cells of Cajal accompanied by decreased expression of neuronal nitric oxide synthase and substance P in patients with type 2 diabetes mellitus. *J. Gastroenterol.* **2006**, *41*, 1076–1087. [CrossRef] [PubMed]
14. Lin, Z.; Sarosiek, I.; Forster, J.; Damjanov, I.; Hou, Q.; McCallum, R.W. Association of the status of interstitial cells of Cajal and electrogastrogram parameters, gastric emptying and symptoms in patients with gastroparesis. *Neurogastroenterol. Motil.* **2010**, *22*, 56–61.e10. [PubMed]
15. Horváth, V.J.; Vittal, H.; Lörincz, A.; Chen, H.; Almeida–Porada, G.; Redelman, D.; Ördög, T. Reduced Stem Cell Factor Links Smooth Myopathy and Loss of Interstitial Cells of Cajal in Murine Diabetic Gastroparesis. *Gastroenterology* **2006**, *130*, 759–770. [CrossRef]
16. Kindt, S.; Dubois, D.; Van Oudenhove, L.; Caenepeel, P.; Arts, J.; Bisschops, R.; Tack, J. Relationship between symptom pattern, assessed by the PAGI-SYM questionnaire, and gastric sensorimotor dysfunction in functional dyspepsia. *Neurogastroenterol. Motil.* **2009**, *21*, 1183-e1105. [CrossRef]
17. Kim, B.J.; Kuo, B. Gastroparesis and Functional Dyspepsia: A Blurring Distinction of Pathophysiology and Treatment. *J. Neurogastroenterol. Motil.* **2019**, *25*, 27–35. [CrossRef] [PubMed]
18. Phillips, L.K.; Rayner, C.K.; Jones, K.L.; Horowitz, M. An update on autonomic neuropathy affecting the gastrointestinal tract. *Curr. Diabetes Rep.* **2006**, *6*, 417–423. [CrossRef] [PubMed]

19. Chandrasekharan, B.; Anitha, M.; Blatt, R.; Shahnavaz, N.; Kooby, D.; Staley, C.; Mwangi, S.; Jones, D.P.; Sitaraman, S.V.; Srinivasan, S. Colonic motor dysfunction in human diabetes is associated with enteric neuronal loss and increased oxidative stress. *Neurogastroenterol. Motil.* **2011**, *23*, 131–138. [CrossRef]
20. Kim, S.Y.; Woo, H.S.; Kim, K.O.; Choi, S.H.; Kwon, K.A.; Chung, J.; Kim, Y.J.; Kim, J.H.; Park, D.K. DA-9701 improves colonic transit time and symptoms in patients with functional constipation: A prospective study. *J. Gastroenterol. Hepatol.* **2017**, *32*, 1943–1948. [CrossRef] [PubMed]
21. Choi, J.H.; Lee, J.Y.; Cho, J.W.; Koh, S.B.; Yang, Y.S.; Yoo, D.; Shin, C.M.; Kim, H.T. Double-Blind, Randomized, Placebo-Controlled Trial of DA-9701 in Parkinson's Disease: PASS-GI Study. *Mov. Disord. Off. J. Mov. Disord. Soc.* **2020**, *35*, 1966–1976. [CrossRef] [PubMed]
22. Ramsbottom, N.; Hunt, J.N. Studies of the effect of metoclopramide and apomorphine on gastric emptying and secretion in man. *Gut* **1970**, *11*, 989–993. [CrossRef]
23. Choi, S.; Choi, J.J.; Jun, J.Y.; Koh, J.W.; Kim, S.H.; Kim, D.H.; Pyo, M.Y.; Choi, S.; Son, J.P.; Lee, I.; et al. Induction of pacemaker currents by DA-9701, a prokinetic agent, in interstitial cells of Cajal from murine small intestine. *Mol. Cells* **2009**, *27*, 307–312. [CrossRef]
24. Choi, M.-G.; Rhee, P.-L.; Park, H.; Lee, O.Y.; Lee, K.J.; Choi, S.C.; Seol, S.Y.; Chun, H.J.; Rew, J.-S.; Lee, N.H.; et al. Randomized, Controlled, Multi-center Trial: Comparing the Safety and Efficacy of DA-9701 and Itopride Hydrochloride in Patients With Functional Dyspepsia. *J. Neurogastroenterol. Motil.* **2015**, *21*, 414–422. [CrossRef] [PubMed]
25. Lim, C.-H.; Choi, M.-G.; Park, H.; Baeg, M.K.; Park, J.M. Effect of DA-9701 on gastric emptying in a mouse model: Assessment by 13C-octanoic acid breath test. *World J. Gastroenterol.* **2013**, *19*, 4380–4385. [CrossRef]
26. Jung, Y.S.; Kim, M.Y.; Lee, H.S.; Park, S.L.; Lee, K.J. Effect of DA-9701, a novel prokinetic agent, on stress-induced delayed gastric emptying and hormonal changes in rats. *Neurogastroenterol. Motil.* **2013**, *25*, 254–259.e166. [CrossRef] [PubMed]

MDPI
St. Alban-Anlage 66
4052 Basel
Switzerland
Tel. +41 61 683 77 34
Fax +41 61 302 89 18
www.mdpi.com

Journal of Clinical Medicine Editorial Office
E-mail: jcm@mdpi.com
www.mdpi.com/journal/jcm

MDPI
St. Alban-Anlage 66
4052 Basel
Switzerland

Tel: +41 61 683 77 34
Fax: +41 61 302 89 18

www.mdpi.com

ISBN 978-3-0365-3686-6