ISSN 2150-5330 (online)

World Journal of *Gastrointestinal Pathophysiology*

World J Gastrointest Pathophysiol 2022 May 22; 13(3): 59-113

Published by Baishideng Publishing Group Inc

World Journal of Gastrointestinal Pathophysiology

Contents

Bimonthly Volume 13 Number 3 May 22, 2022

REVIEW

ORIGINAL ARTICLE

Basic Study

Retrospective Study

Observational Study

CASE REPORT

World Journal of Gastrointestinal Pathophysiology

Bimonthly Volume 13 Number 3 May 22, 2022

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Gastrointestinal Pathophysiology*, João Batista M Tajra, MD, MsC, PhD, Chief Doctor, Full Professor, Surgeon Coloproctology Department, Hospital de Base do Distrito Federal, Brasilia 71215770, Distrito Federal, Brazil. joao003905@unieuro.com.br

AIMS AND SCOPE

The primary aim of the *World Journal of Gastrointestinal Pathophysiology* (*WJGP*, *World J Gastrointest Pathophysiol*) is to provide scholars and readers from various fields of gastrointestinal pathophysiology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJGP mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal pathophysiology and covering a wide range of topics including disorders of the esophagus, stomach and duodenum, small intestines, pancreas, biliary system, and liver.

INDEXING/ABSTRACTING

The *WJGP* is now abstracted and indexed in Emerging Sources Citation Index (Web of Science), PubMed, PubMed Central, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Gastrointestinal Pathophysiology

ISSN
ISSN 2150-5330 (online)

LAUNCH DATE
April 15, 2010

FREQUENCY
Bimonthly

PUBLICATION DATE
May 22, 2022

World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Pathophysiol 2022 May 22; 13(3): 59-72

DOI: 10.4291/wjgp.v13.i3.59

ISSN 2150-5330 (online)

REVIEW

Gut microbiome: Linking together obesity, bariatric surgery and associated clinical outcomes under a single focus

Konstantinos Georgiou, Nikolay A Belev, Tilemachos Koutouratsas, Hector Katifelis, Maria Gazouli

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Dilek ON, Turkey; Salimi M, Iran; Yang M, United States

Received: November 10, 2021
Peer-review started: November 10, 2021
First decision: December 12, 2021
Revised: December 21, 2021
Accepted: March 25, 2022
Article in press: March 25, 2022
Published online: May 22, 2022

Konstantinos Georgiou, The First Propaedeutic Surgical Unit, Hippocrateion Athens General Hospital, Medical School, National and Kapodistrian University of Athens, Athens 11527, Greece

Nikolay A Belev, Medical Simulation Training Center, Research Institute of Medical University of Plovdiv, and UMPHAT "Eurohospital", Medical University of Plovdiv, Plovdiv 4002, Bulgaria

Tilemachos Koutouratsas, Hector Katifelis, Maria Gazouli, Basic Medical Sciences, Medical School, National and Kapodistrian University of Athens, Athens 11527, Greece

Corresponding author: Maria Gazouli, PhD, Professor, Basic Medical Sciences, Medical School, National and Kapodistrian University of Athens, Michalakopoulou 176, Athens 11527, Greece. mgazouli@med.uoa.gr

Abstract

Obesity is increasingly prevalent in the post-industrial era, with increased mortality rates. The gut microbiota has a central role in immunological, nutritional and metabolism mediated functions, and due to its multiplexity, it is considered an independent organ. Modern high-throughput sequencing techniques have allowed phylogenetic exploration and quantitative analyses of gut microbiome and improved our current understanding of the gut microbiota in health and disease. Its role in obesity and its changes following bariatric surgery have been highlighted in several studies. According to current literature, obesity is linked to a particular microbiota profile that grants the host an augmented potential for calorie release, while limited diversity of gut microbiome has also been observed. Moreover, bariatric surgery procedures represent effective interventions for sustained weight loss and restore a healthier microbiota, contributing to the observed fat mass reduction and lean mass increase. However, newer evidence has shown that gut microbiota is only partially recovered following bariatric surgery. Moreover, several targets including FGF15/19 (a gut-derived peptide), could be responsible for the favorable metabolic changes of bariatric surgery. More randomized controlled trials and larger prospective studies that include well-defined cohorts are required to better identify associations between gut microbiota, obesity, and bariatric surgery.

Key Words: Bariatric surgery; Obesity; Gut microbiota; Micronutrient deficiency; Probiotics

Core Tip: Obesity represents a major cause of morbidity and mortality globally. Current knowledge suggests a connection between gut microbiota characteristics and obesity, while bariatric surgery has been shown to promote a healthier microbiota composition. However, the exact effects of these procedures remain unclear. In general, an increase in members of the phylum Bacteroidetes and Proteobacteria, and a decrease in members of the phylum Firmicutes is a common finding. This field of research can also inform clinicians' predictions of outcomes before and after bariatric surgery through analysis of patterns in gut microbiota.

Citation: Georgiou K, Belev NA, Koutouratsas T, Katifelis H, Gazouli M. Gut microbiome: Linking together obesity, bariatric surgery and associated clinical outcomes under a single focus. *World J Gastrointest Pathophysiol* 2022; 13(3): 59-72
URL: https://www.wjgnet.com/2150-5330/full/v13/i3/59.htm
DOI: https://dx.doi.org/10.4291/wjgp.v13.i3.59

INTRODUCTION

Obesity represents a huge health burden in society, and is linked with an increase in mortality rates[1]. Recent data suggest a crosstalk between gut microbiota (GM) and obesity, while obesity itself seems to be both a cause and a result of GM alterations[2]. In health, the GM is involved in energy intake, adjustment of glucose and lipid homeostasis, and micronutrient and vitamin composition[3]. This GM balance is disturbed in obesity presenting a series of pathological manifestations, including chronic inflammation, insulin resistance, and metabolic disturbance[2,3]. Moreover, obesity is linked with vitamin and mineral deficiencies, that aggravate GM synthesis and function[4,5].

Bariatric surgery (BS) is currently the sole long-term effective therapeutic option for morbid obesity [6]. A number of studies have identified important qualitative and quantitative changes in the GM after BS. Such treated patients have micronutrient deficiencies that may lead to deficiency-related syndromes [7,8], that include anemia (10%-74%) and neurological disorders (5%-9%)[7,9].

Given the presence of other coexisting factors that impair the postoperative nutritional status of these patients [energy-restricted higher protein intake and adequate nutritional supplementation diet, anatomical and physiological impairment of the gastrointestinal tract (GIT)][7,10], a consistent follow-up is essential.

The complicated interaction between obesity and GM phyla that includes gut microbiome modulations (and of their by-products) in obese subjects who undergo BS as treatment, are the aim of this review.

OBESITY

Obesity represents the discrepancy between caloric intake and energy expenditure and is affected by genetic and environmental factors[11]. Obesity has been associated with type 2 diabetes mellitus (T2DM), increased arterial pressure, hypercholesterolemia, cardiovascular disease, apnea, musculo-skeletal disorders, cancer, impaired fertility, anxiety, and psychiatric disorders[12]. Currently, obesity results in more deaths than undernourishment and starvation together[13].

Worldwide, the term body mass index (BMI) is a tool for estimating obesity severity and is calculated by dividing the body weight (kg) by the square of height (m^2) of the individual. In adult subjects, a BMI between 18.5 to 25 kg m^{-2} is considered normal; overweight is BMI 25 to 30, while obesity is defined as BMI over 30 kg m^{-2}. Obesity is classified by the World Health Organization into three categories; class I corresponds to a BMI of 30.00 to 34.99; class II between 35.00 and 39.99 and class III is a BMI that exceeds 40[14]. Additionally, a BMI > 50 kg m^{-2} is termed superobesity. Regarding the treatment of obesity, it has been shown that in a time period of 2 years, most subjects reach or even exceed their initial weight[15].

GUT MICROBIOTA IN HEALTHY SUBJECTS

Glossary of microbiota-related terms

Microorganisms are present in the skin, respiratory system, the GIT, and the male and female genitourinary tracts[16].

The ecological community of symbiotic and pathogenic microbes composes the microbiota[17]. The term microbiota includes all species which form microbial communities, such as eubacteria, archeobacteria, fungi, and protists[18].

The term 'microbiome' refers to the microorganisms themselves. The study of all microbial DNA directly recovered from a sample such as from the gut is called metagenomics. The metagenome, refers to the complete genome of the microbiota[17], while the term 'shotgun metagenomics' describes the process of a sample's next-generation sequencing. This process produces primer-independent data that can then be analyzed with various reference-based and/or reference-free methods[16].

Gut microbiota under normal conditions

In health, the microbial composition remains constant[19]. The largest microbe concentrations are found in the intestine, the skin, and the oral cavity[20]. Of these sites, the GIT is the most intensively colonized organ. In the past, it was widely shown that a healthy gut contains 1-1.5 kg of microbes a number that exceeds by about 10 times the number of the host's (human) cells[21]. However, more recent estimates suggest that the number of gut bacteria is of the same order as the number of human cells, weighing a total of 0.2 kg[22]. Approximately 1000 species colonize the gut, with microbial density increasing along the GIT from 10^1 to 10^4 microbes in the stomach to 10^{10} to 10^{12} cells per gram in the colon[17].

Due to the antimicrobial effects of hydrochloric acid and nitric oxide, microbes in the stomach and the small intestine are few[23,24]. However, the large intestine presents a better milieu for microbes, with better conditions to extract energy as well as essential nutrients[25,26]. The largest number of living microbes is located in the colon but due to the impermeable adherent mucus layer, there is no direct contact with the epithelium[27]. It is believed these bacterial species collectively yield 2 million genes (100 times the number of human genes. The number above agrees with the actual extent of microbial gene catalogs found in MetaHIT and the Human Microbiota Project[28].

Gut microbiota in obese subjects

The GM along with the host's genotype and lifestyle, affect the pathophysiology of the disease and thus research interest in these associations has increased[2,29].

An important increase in adipose tissue of germ-free (GF) mice implanted with microbiota harvested from the cecum of ob/ob mice has been found, when compared to mice transplanted with a GM from lean rodents[30]. Transferring GM from genetically obese mice resulted in a 47% increase in fat mass, while the inoculation from lean mice increased adipose tissue mass by 26%[31].

Several factors contribute to how GM affects obesity, such as nutrient metabolism. For instance, hippurate, a microbial metabolite of dietary polyphenols, is reported to be associated with *Eubacterium dolichum* and visceral fat mass[32]. Additionally, it has been postulated that the circadian clock, which regulates diurnal oscillations of different biological processes such as feeding, can be influenced by the GM and therefore act as a contributor to diet-induced obesity[33].

Obesity also triggers low-grade chronic inflammation. A high-fat diet for 28 d, increased more than twice the systemic lipopolysaccharide (LPS) levels and the LPS-containing GM, thus presenting what is known as "metabolic endotoxemia". The increased LPS levels could trigger inflammation thus contributing to obesity and T2DM[34,35].

BARIATRIC SURGERY

Bariatric surgery modalities

When lifestyle and/or medication-based approaches are ineffective, BS is an option, as it is a highly effective therapeutic procedure for the treatment of obesity[36]. BS can be either restrictive or malabsorptive, by reducing food intake and promoting weight loss[37]. The available metabolic surgery procedures includes laparoscopic adjustable gastric band, vertical sleeve gastrectomy (VSG), Roux-en-Y gastric bypass (RYGB), biliopancreatic diversion (BPD), and BPD with duodenal switch (BPD/DS)[7,37].

Vertical banded gastroplasty

This is a restrictive procedure. An incision is made on the lesser curvature of the stomach 6 cm from the esophagogastric junction. The lesser omentum is dissected followed by a 2 cm opening of the lesser sac. Dissection continues downward to 1 cm above the uppermost portion of the short gastric vessels. A calibrated transgastric window is created using a circular stapler creating a 20 mL gastric pouch volume. A polypropylene band is placed around the distal part of the gastric pouch[36,38,39].

Laparoscopic adjustable gastric band

This is a restrictive procedure, more widely performed in the past, but its use has declined in popularity in the last 5 years[38]. A synthetic band is placed around the upper portion of the stomach, immediately after the gastroesophageal junction, thus creating a small gastric pouch of 20-30 mL. The band is inflated or deflated with saline to alter the level of constriction and to maintain a feeling of fullness with a smaller volume of food. At first, the early and prolonged satiety was attributed to the physically restricted meal volume and the delayed emptying of food from the pouch[40]. Today, it has been proved that most of the procedure's efficiency is due to the pressure applied on the intraganglionic laminar endings which convey afferent signals resulting in hunger reduction[41]. The average weight loss is about 45%-47% of the excess weight by 4-5 years postoperatively[42].

RYGB

RYGB represents both a restrictive and malabsorptive procedure. Of note, apart from the mechanical restriction of caloric intake, RYGB impairs the absorption of nutrients. Of note, 15%-30% of the weight loss is maintained for at least 20 years after RYGB[43]. Moreover, after RYGB glycemic control improves in 90% of recipients[44].

VSG

This is a restrictive procedure. VSG has increased in popularity as it is relatively easy to perform and a good clinical outcome is achieved[45]. In VSG, a vertical excision of approximately 75% of the stomach lengthwise with preservation of the pylorus is performed. It aims to make a small gastric pouch ("sleeve"), with a volume of approximately 100 mL, and to create a high-pressure chamber that easily produces sufficient pressure to overcome the tone of the pyloric sphincter, thus resulting in rapid gastric emptying[46]. This decreased gastric reservoir does not permit any distention and therefore provokes premature satiety, resulting in substantially reduced portion sizes.

Sleeve creation has an impact on hormone regulation, decreasing blood ghrelin levels and enhancing a state of satiety. The average weight loss is 60% excess body weight after two years postoperatively, along with an improvement in associated comorbidities[42]. Both short- and medium-term research reports showed that VSG is almost as effective as RYGB in reducing body weight and improving glycemic control[10,47].

BPD and BPD with duodenal switch (BPD and BPD/DS)

This is a malabsorptive procedure. Being a quite radical procedure, it is only used occasionally. The BPD procedure involves a sleeve gastrectomy with the creation of a 200-500 mL gastric pouch. A Roux-en-Y gastroileostomy of 200 cm is formed with a common channel 50 cm from the ileocecal valve joining biliary and digestive enzymes. The weight loss achieved *via* BPD and/or BPD/DS is the greatest among any of the other bariatric procedures with excess weight loss of 70%-80% postoperatively[42,48].

Of all the aforementioned procedures, half of the bariatric procedures are VSG and approximately 40% are RYGB[49]. RYGB has been the primary choice for decades and thus millions of RYGB patients are present in the general population[13]. Table 1 shows the comparison between these bariatric approaches.

Today, BS is regarded as the only effective treatment for a pronounced and permanent weight loss [13]. The Swedish Obese Subject trial reported a weight loss following RYGB of 27% in 15 years, while non-operative approaches (lifestyle changes or pharmacological treatment) had no effect over this period. Controlled long-term studies (> 5 years) on the effects of VSG on weight loss are still scarce, but weight loss up to 5 years is similar to that of RYGB[13].

Lastly, branched-chain amino acids were significantly reduced after BS, a finding associated with alleviation of the "metabolic overload" observed in some tissues[50]. Trimethylamine-n-oxide, a metabolite proposed as a cardiovascular marker, was found to increase following BS. This increase was probably related to the GM changes observed after BS[50].

THE MECHANISMS OF GASTRIC BYPASS

The gastric bypass procedure is an artificial condition in which the intestinal mucosal energy outflow is variable and capable of altering BMI and glucose levels.

The main reason behind weight reduction is a modified eating behavior that reduces energy intake. According to the foregut theory, food bypasses both the stomach and the duodenum, and the release of gut-derived hormones originating from these areas is altered, *e.g.*, the release of glucose-dependent insulinotropic peptide from the duodenum. A second theory known as the hindgut theory states that since the more distal parts of the intestine are now (following the procedure) exposed to nutrients and contact food sooner than normal, this provokes faster humoral responses.

RYGB also changes the circulating bile acid levels and those of the intestinal microbiota: Bile acids regulate glucose metabolism causing the release of GLP-1, provoking the synthesis and release of

Table 1 Comparison of the two main bariatric surgery procedures

	Roux-en-Y gastric bypass	Vertical sleeve gastrectomy
Technique	(1) 15-30 mL gastric pouch; (2) Gastrojejunostomy (GJ); (3) Jejunojejunal anastomosis (Roux-en-Y); (4) 30-50 cm distal to the ligament of Treitz; and (5) Remnant disconnected but left *in situ*	(1) Excision of lateral 70%-80% of stomach along the greater curvature; and (3) Approximately 100 mL gastric reservoir (sleeve)
Mechanism of action	(1) Instantaneous food transfer to small intestine, altering: Gut hormones; Bile acids; Neural signaling; Gut microbiota; Gut-brain-endocrine; Adipocyte-brain axes; and (2) Results in reduced food intake, increased satiety and altered food preferences	(1) Alterations in: Gut hormones; Bile acids; Neural signaling; Gut microbiota; Gut-brain-endocrine; Adipocyte-brain axes; and (2) Results in reduced food intake, hunger, increased satiety and altered food preferences
Advantages	(1) Significant long-term weight loss; (2) Glycemic control improvement in 90% of cases; (3) Maintain percent EWL in the long term; (4) Hunger reduction and satiety; (5) Food preferences changes; and (6) Increases energy expenditure	(1) Significant long-term weight loss (approximately 10% less than RYGB); (2) Glycemic control as effective as RYBG; (3) Maintain percent EWL in the long-term; (4) Hunger reduction and satiety; (5) Food preferences changes; (6) No anatomical rerouting of food; (7) Short length of stay (< 2 d); (8) Technically simpler than RYGB; and (9) Lower complication rate than RYGB
Disadvantages	(1) Technically complex (two anastomoses) compared with AGB or VSG; (2) Higher complication rate than AGB or LSG; for example, anastomotic leak or dumping syndrome can occur; (3) Longer length of stay; (4) Long-term vitamin and/or mineral deficiencies (for example, vitamin B12, iron, calcium or folate); (5) Requires lifelong vitamin and/or mineral supplementation; (6) Lifelong dietary changes; (7) Increases alcohol addiction and suicide rates; and (8) postprandial hypoglycemia	(1) Anastomotic leak can be difficult to manage; (2) Susceptible to long-term vitamin and/or mineral deficiencies (less common than with RYGB); (3) Precautionary lifelong vitamin and/or mineral supplementation; (4) Lifelong dietary changes; (5) Irreversible; and (6) potential risk of Barrett esophagus

EWL: excess weight loss; RYGB: Roux-en-Y gastric bypass.

fibroblast growth factor 19 which improves insulin sensitivity and glycemic control[51].

Circulating exosome microRNAs (miRNAs) constitute another mechanism that could explain bariatric surgery–associated outcomes[6]. Several studies have identified miRNAs that tend to increase or decrease in expression after bariatric surgery[52,53]. Of these, miRNA MiR-7, which has shown the most concrete post-surgical increase in studies, plays a role in the regulation of pancreatic beta-cell function in humans[53].

SIDE EFFECTS OF BARIATRIC SURGERY

The 1-year mortality rate after BS is 1% and the 5-year mortality rate is 6%[54]. 4% of patients after BS experience surgical complications during the first month[55,56]. These include anastomotic leakage, hemorrhage, perforation, infection and inner herniation[55]. However, the latter is considerably decreased when the closure of any mesenteric defect became routine practice during the BS approach [57].

Chronic abdominal pain is a common side effect seen in patients after RYGB; half of RYGB patients experience abdominal pain and in a 5-year follow-up, a third of them still experienced pain[58]. It is important to clarify the underlying pathology following BS but its etiology remains obscure[59]. Furthermore, it is believed that 4% of patients who were not on opioids, became chronic users after BS [60] and therefore the attending physician of such patients who develops nausea and pain, must bear in mind the risk of iatrogenic opioid addiction.

Hypoglycemia in non-diabetic subjects appears in more than 64% of patients during the first 5 years after BS[61]. Several theories related to this have been proposed including enhanced B cell mass and function, lowered ghrelin levels, improved insulin sensitivity, and inadequate counter regulation[62]. Unfortunately, the side effects of hypoglycemia often persist for years and can decrease the patient's quality of life.

GUT MICROBIOTA AFTER BARIATRIC SURGERY

A plethora of diseases are connected to GM changes including, atherosclerosis, non-alcoholic fatty liver disease, inflammatory bowel disease, and colorectal cancer[16]. BS plays a central role by affecting the abundance of many microbial species of the GM.

Most often, a decrease in *Firmicutes* and an increase in *Bacteroidetes* and *Proteobacteria*, abundance is observed after BS[63]. Both RYGB and vertical banded gastroplasty, have comparable long-term effects on GM function and composition. Moreover, feces from BS patients were transplanted in germ-free mice, and the mice gained less fat when compared to reciprocal mice transplanted with GM from obese subjects. These findings show a causal relationship between GM and BS-induced weight reduction[64].

Another study employed GM transplantation from mice that underwent RYGB to sham-surgery germ-free mice, which provoked weight loss compared to recipients of GM from non-operated mice[65].

The increase in pH (following BS) in the lumen and high levels of dissolved oxygen, affect the growth of aerobic microorganisms (such as *Proteobacteria*) and inhibit the growth of anaerobic bacteria[66].

In a recent systematic review, Davies *et al*[67] summarized 14 clinical studies involving 222 subjects (RYGB = 146, VSG = 25, biliointestinal bypass = 30, vertical banded gastroplasty = 7, and adjustable gastric band = 14). Major changes included a reduction in the abundance of *Faecalibacterium prausnitzii* and an increase in *E. coli*. Following VSG, a decrease in the abundance of *Firmicutes* was observed, while after RYBG an increase in *Bacteroidetes* and *Proteobacteria* was observed.

Their findings are summarized in Table 2. It was found that different types of BS result in dramatic changes in GM.

A systematic meta-analysis of 22 articles investigated the effect of BS on metabolic and GM profiles. Only two studies were randomized, while the rest were prospective studies[64,68,69]. The total sample size was 562; 411 patients underwent RYGB, and 97 underwent VSG[70].

As shown in Table 3, several microbes are affected by BS: some authors found increased *Bacteroides* while *Firmicutes* and *Bifidobacterium* had lower abundance in post-RYGB subjects[70,71].

In summary, it appears that BS reestablishes a healthier microbiota together with a slimmer metabolic profile, and possibly this microbiota readjustment contributes to a diminished fat mass and an increased lean mass. Nevertheless, the pathways through which the gut microbiota and their metabolites affect obesity are still obscure, and robust microbe manipulations that interfere with the host-bacteria interactions for the management of obesity still need to be developed[16].

EFFECT OF BARIATRIC SURGERY ON SMALL INTESTINE BACTERIA

Obese subjects after BS can develop small intestine bacterial overgrowth (SIBO), which is defined as greater than 10^5 colony-forming units per mL of proximal jejunal aspiration[72]. SIBO is a manifestation of obesity and a prospective study including 378 subjects with morbid obesity, reported that 15% of patients before undergoing RYGB had SIBO, and that this figure increased to 40% following the procedure[72].

SIBO diagnosis is made following a small intestine aspirate test. However, due to the invasive nature of this process the most acceptable detection technique is the "therapeutic trial", by empirically administering antibiotics due to the clinical complications associated with SIBO[73].

The malabsorption of vitamins A, D, E, and K (fat-soluble vitamins) is due to the bacterial deconjugation of bile acids by small intestine bacteria, while the formation of a toxic compound (lithocholic acid) further aggravates intestinal epithelial cell dysfunction and aggravates carbohydrate and protein malabsorption[74]. In contrast, in subjects with SIBO, vitamin K levels are within normal levels or increased as bacteria are capable of synthesizing menaquinone[75].

EFFECT OF BARIATRIC SURGERY ON GUT HORMONES

Typically, food intake suppresses the hunger hormone ghrelin; however, in obese subjects, this mechanism might be disrupted. Thus, it has been reported that within days after BS, as a more quick release of nutrients to the distal small intestine starts to occur, increased production of gut satiety hormones such as PYY and GLP-1, and a reduced increase in ghrelin takes place[76].

After a meal, both PYY and GLP-1 are, proportional to the consumed calories, released from the L cells of the distal small intestine[77]. Following BS, the postprandial PYY levels are increased and the new levels are correlated with postoperative weight loss[78]. Also, the role of PYY in the regulation of feeding after RYGB has been assessed using octreotide, which blocks the secretion of most gut hormones and therefore increases food consumption[76].

Although the effects of PYY and GLP-1 on gastric emptying, glucagon secretion, and insulin release from the pancreas are well understood, the appetite change after BS seems to be a synergistic response of more than one gut hormone[79].

Gut microbiota signatures as predictors of long-term outcomes in bariatric surgery

In a study by Gutiérrez-Repiso *et al*[80], fecal samples from 24 patients who had undergone bypass surgery at least two years previously were studied. The authors reported that patients who would go on to show greater rates of weight loss and low weight maintenance in the long-term tended to have a higher diversity of core microbiota in the mid-term. Furthermore, the bacterial genera *Sarcina*, *Butyrivibrio*, *Alkaliphilus*, *Lachnospira*, *Pseudoalteromonas*, and *Cetobacterium* were more abundant in stool samples in patients for whom gastric bypass surgery was more successful in the long-term[80]. Nevertheless, another study by Fouladi *et al*[81] failed to prove a significant difference in the microbiota between subjects with successful and poor BMI reduction after RYGB surgery[81]. In the same study,

Table 2 Changes in human gut microbiota following bariatric surgery

↑/↓	RYGB	VSG
↑	*Akkermansia* (Verrucomicrobia)	*Bulleidia* (Firmicutes)
↑	*Escherichia* (Protobacteria)	*Roseburia intestinalis* (Firmicutes)
↑	*Klebsiella* (Protobacteria)	*Faecalibacterium prausnitzii* (Firmicutes)
↓	*Lactobacillus* (Firmicutes)	*Coprococcus comes* (Firmicutes)
↓	*Bifidobacterium* (Actinobacteria)	
↓	*Faecalibacterium prausnitzii* (Firmicutes)	
↓	*Coprococcus comes* (Firmicutes)	

RYGB: Roux-en-Y gastric bypass; VSG: Vertical sleeve gastrectomy.

Table 3 Literature findings on the postoperative changes of gut microbiota

Ref.	Postoperative GM changes		
	Increased abundance	Decreased abundance	Comments
Graessler *et al*[71], 2013	*Enterobacter, Citrobacter, Neurospora, Veillonella, Salmonella, Shigella, E. coli* tended to increase	*Faecalibacterium, Coprococcus, Helicobacter, Dictyostelium, Epidinium, Anaerostipes, Nakamurella, Methanospirillum, Thermomicrobium*	-
Kong *et al* [68], 2013	*Bacteroides, Alistipes, Escherichia*	Firmicutes (*Lactobacillus, Dorea, Blautia*) *Bifidobacterium*	Increased richness of GM after RYGB
Palleja *et al* [50], 2016	*Escherichia coli*, *Klebsiella pneumoniae*, 10 species belonging to the genus *Streptococcus*, 4 from *Veillonella*, 2 from *Alistipes*, *Bifidobacterium dentium*, *Enterococcus faecalis*, *F. nucleatum*, and *Akkermansia muciniphila*	*E. prausnitzii*	-
Tremaroli *et al*[64], 2015	Gammaproteobacteria; Several Proteobacteria *(Escherichia, Klebsiella, Pseudomonas); E. coli* tended to increase but was not statistically significant	3 species of Firmicutes; (*Clostridium difficile, Clostridium hiranonis, Gemella sanguinis*)	-

GM: Gut microbiota; RYGB: Roux-en-Y gastric bypass.

Fouladi *et al*[81] transplanted fecal samples from patients with poor weight loss (PWL) and successful weight loss in antibiotic-treated mice, and reported that mice transplanted with PWL feces tended to gain more weight despite exhibiting similar feeding behaviors. Steinert *et al*[82] reported decreased mycobiotic diversity in fecal samples from patients before and after RYGB surgery.

MICRONUTRIENT DEFICIENCIES AFTER BARIATRIC SURGERY

After BS, the micronutrient status of patients further deteriorates, which, in turn, affects the structure and composition of the GM[83]. Thus, after BS, more than 30% of patients develop nutritional deficiencies that may result in edema, hypoalbuminemia, anemia, and even peripheral neuropathy and Wernicke encephalopathy[83].

Unfortunately, these deficiencies persist despite vitamin and mineral supplementation. The deficiencies observed after BS are affected by eating behavior, decreased absorption, SIBO, or poor compliance to the suggested optimization of diet[84].

There is strong evidence that after RYGB and VSG, food intake restriction, reduced appetite, and gastrointestinal hormones changes are mechanisms for the observed weight loss[85]. VSG promotes gastric emptying, reduces gastroduodenal transit time, and decreases the release of hydrochloric acid and intrinsic factor. These effects, due to gastric fundus resection, affect gastrointestinal motility and therefore, the release and dissolution of several vitamins and minerals are diminished[86].

Vitamin B_{12}

The anatomic alterations of the GIT due to BS lead to impaired release of both HCl and pepsin from the functional part of the remnant. In turn, this leads to diminished vitamin B_{12} absorption, as well as to less interaction of gastric content with parietal cells, which produce the intrinsic factor, causing

malabsorption and deficiency of cobalamin[87,88]. It has also been shown that the deficiency of intrinsic factor is the main driver of post-surgical B_{12} deficiency, although other molecules such as transcobalamin-1 may participate[89]. As expected, RYGB patients display a higher frequency of vitamin B_{12} deficiency (37%-50%) than VSG patients (10%-20%)[90]. It has been reported that, despite adequate supplementation with physiological doses, B_{12} levels are found to decrease within a few months following BS, and therefore, administration of high doses of B_{12} is recommended right after BS[91].

Folic acid

It is expected that after BS, folate absorption should be impaired due to hypochlorhydria and altered pH in the proximal jejunum[92]. However, it has been reported that folic acid may also be synthesized by bacteria in the colon. It seems that it is absorbed throughout the small intestine and even the colon, with a lowered rate of absorption. Therefore, following RYGB, the administration of usual doses of folate supplement is sufficient to prevent or correct folate deficiency, because a compensatory mechanism of intestinal absorptive capacity may be present[93].

Vitamin B_1 (thiamine)

Thiamine deficiency symptoms rapidly develop after only 20 d of insufficient oral intake, faster than for any other vitamins[94]. Hyperemesis, a symptom rather common after BS surgery, impairs B_1 absorption and thus its deficiency can appear despite any oral supplementation. A large variety of pathologies are associated with thiamine deficiency, including beriberi, neuropathy, and Wernicke encephalopathy[95], which may present a medical emergency.

Bariatric patients may develop vitamin B_1 deficiency within six months following surgery. A study reported that in 118 cases of Wernicke encephalopathy detected postoperatively after either RYGB or VSG, almost 90% had hyperemesis[96]. A study reported that two years after RYGB, thiamine levels were deficient in 18% of patients[96]. In a recent retrospective study of VSG patients, 25.7% of subjects showed decreased thiamine levels within one year after VSG [97].

Vitamin D and calcium

Following BS, bariatric patients have an increased risk of developing metabolic bone disease at any time during the rest of their lives. Furthermore, after BS, SIBO can also aggravate vitamin D deficiency[98]. As diminished acid secretion occurs after both RYGB and VSG, impaired dissolution and solubilization of nutrients can develop. Chronic vitamin D deficiency which subsequently leads to decreased bone mineral density has been observed three years after RYGB and VSG[99].

Following VSG, vitamin D malabsorption might be the effect of diminished exposure of nutrients to the digestive mucosa[100]. Although VSG does not involve intestinal anatomy, calcium uptake might be hampered through several possible mechanisms such as reduced calorie intake, hypochlorhydria, or the use of proton pump inhibitors[100]. In a large cohort study including 999 subjects, the prevalence of hypocalcemia postoperatively was 3.6%, with 15 patients (1.9%) undergoing RYGB, and 13 patients (9.3%) undergoing VSG. In the same study, the lowest calcium concentrations were found after approximately 3 years in the RYGB group, and after 239 d in the VSG group, respectively. The daily calcium intake administered was approximately 1750 mg[101].

Iron

Following RYGB, 18%-53% of patients develop iron deficiency compared to 1%-53% of patients after VSG[102]. This is rather expected after RYGB, as the duodenum, which is the most efficient area for iron absorption, is bypassed. A study including 72 post-RYGB patients reported red meat intolerance in 49.2%, 42.2%, 46.4%, and 39% of subjects after 1, 2, 3, and 4 postoperative years, respectively[103]. Following VSG, iron deficiency is dominant and defined by malabsorption secondary to the amount of gastric resection which prevents reduction of Fe^{3+} to Fe^{2+}.

Several mechanisms underlie the pathogenesis of postsurgical iron deficiency: After ingestion, the gastric acidic environment enhances iron absorption by favoring its ferrous form (2+), the only form of iron that can be absorbed[104]. Reduced HCl release in the gastric pouch and administration of H_2 blockers significantly impair iron absorption[105]. Also, iron-rich alimentation after BS is largely decreased due to caloric restriction and food aversions, especially to red meat[87].

OTHER MICRONUTRIENT DEFICIENCIES

Fat-soluble vitamins

After BS, some deficiencies of fat-soluble vitamin (vitamin A, E, and K) levels in plasma are observed due to malabsorption[7], but the frequency of these deficiencies is low with rarely reported clinical manifestations[106,107].

Vitamin A deficiency can be induced by diminished retinol and carotenoid intake due to calorie restriction. Additionally, the recommended low-fat diet following BS, contributes to poor absorption.

Interestingly, cirrhosis observed in BS subjects may impede vitamin A storage and synthesis[107]. Thus, the prevalence of vitamin A deficiency following RYGB is approximately 10%[108]. However, no changes in serum vitamin A concentration or optical function following RYGB or VSG were reported in a recent study[109].

Zinc, copper, and selenium

A study analyzing micronutrient deficiencies after both RYGB and VSG during a follow-up of five years found reduced serum zinc concentrations in 25.7% and 12.5% of patients, respectively[110].

The prevalence of copper deficiency after RYGB is 10%. The development of symptomatic hypocupremia after BS is uncommon among subjects who adhere to the prescribed supplementation[111].

Selenium is a trace element and an important antioxidant (selenocysteine)[112]. Serum levels of zinc, selenium, and copper were stable following RYGB and VSG in subjects receiving supplementation[113].

PROBIOTICS AND GUT MICROBIOTA: IMPLICATIONS FOR BARIATRIC PATIENTS

Probiotics are beneficial to the host even without inhabiting the gut or making major changes to GM [29]. The most common administered probiotics are *Lactobacillus, Bifidobacterium,* and *Sacharomyces genera*[114].

Although probiotic use is common postoperatively, studies on their efficacy after BS are scarce[115]. It is been reported that the high pH setting after RYGB, allows higher survival of probiotic bacteria during transition through the acidic milieu of the GI, thus making BS patients suitable candidates for probiotic therapy. Administration of probiotics appears to offer many beneficial effects to BS patients such as greater weight loss, decreased SIBO, improved vitamin synthesis and availability, and optimized micronutrient status[116].

CONCLUSION

BS, the most effective operation for severe obesity, is continuously expanding its applications. However, the role of GM on the host's metabolism and digestion is also widely recognized. Nevertheless, current understanding of the mechanisms that link obesity and concurrent changes in GM remains unclear and current data suggest that BS can only partially restore the microbial imbalance.

The exact mechanisms that induce GM changes after BS remain unclear as different factors including diet, weight loss, and surgery are involved. Moreover, side effects that are triggered by the SIBO effect may also affect the weight loss process in patients who undergo BS.

The impact of BS is not well described, as microbiota alterations are not consistent, and they should be considered in the context of energy intake restriction and altered dietary quality. At the same time, no differences regarding GM modulation were observed among the two most common weight loss surgery techniques (RYGB and VSG). In general, an increase in members of the phylum *Bacteroidetes* and *Proteobacteria*, and a decrease in members of the phylum *Firmicutes* are the most consistently reported findings.

In brief, BS attempts to restore a healthier GM with a leaner metabolic profile, and this microbiota realignment could contribute to the observed reduced adipose tissue reduction, the increase in lean mass, and the reduction in obesity-related morbidity. However, the mechanisms by which microorganisms and their by-products restore the GM are poorly understood. Finally, the prognostic significance of microbiota patterns on long-term outcomes after BS require further elucidation.

FOOTNOTES

Author contributions: Georgiou K, Koutouratsas K, and Katifelis H wrote the original draft, Gazouli M, and Belev NA edited and reviewed the final version of the manuscript; all authors have read and agreed to the published version of the manuscript.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Country/Territory of origin: Greece

ORCID number: Konstantinos Georgiou 0000-0003-3615-2500; Nikolay V Belev 0000-0001-9248-8194; Tilemachos Koutouratsas 0000-0001-5161-7383; Hector Katifelis 0000-0001-5741-4288; Maria Gazouli 0000-0002-3295-6811.

S-Editor: Liu JH
L-Editor: Webster JR
P-Editor: Liu JH

REFERENCES

1 **Blüher M**. Obesity: global epidemiology and pathogenesis. *Nat Rev Endocrinol* 2019; **15**: 288-298 [PMID: 30814686 DOI: 10.1038/s41574-019-0176-8]
2 **Cătoi AF**, Vodnar DC, Corina A, Nikolic D, Citarrella R, Pérez-Martínez P, Rizzo M. Gut Microbiota, Obesity and Bariatric Surgery: Current Knowledge and Future Perspectives. *Curr Pharm Des* 2019; **25**: 2038-2050 [PMID: 31298152 DOI: 10.2174/1381612825666190708190437]
3 **Pascale A**, Marchesi N, Marelli C, Coppola A, Luzi L, Govoni S, Giustina A, Gazzaruso C. Microbiota and metabolic diseases. *Endocrine* 2018; **61**: 357-371 [PMID: 29721802 DOI: 10.1007/s12020-018-1605-5]
4 **Astrup A**, Bügel S. Overfed but undernourished: recognizing nutritional inadequacies/deficiencies in patients with overweight or obesity. *Int J Obes (Lond)* 2019; **43**: 219-232 [PMID: 29980762 DOI: 10.1038/s41366-018-0143-9]
5 **Mohajeri MH**, Brummer RJM, Rastall RA, Weersma RK, Harmsen HJM, Faas M, Eggersdorfer M. The role of the microbiome for human health: from basic science to clinical applications. *Eur J Nutr* 2018; **57**: 1-14 [PMID: 29748817 DOI: 10.1007/s00394-018-1703-4]
6 **Xu G**, Song M. Recent advances in the mechanisms underlying the beneficial effects of bariatric and metabolic surgery. *Surg Obes Relat Dis* 2021; **17**: 231-238 [PMID: 33036939 DOI: 10.1016/j.soard.2020.08.028]
7 **Lupoli R**, Lembo E, Saldalamacchia G, Avola CK, Angrisani L, Capaldo B. Bariatric surgery and long-term nutritional issues. *World J Diabetes* 2017; **8**: 464-474 [PMID: 29204255 DOI: 10.4239/wjd.v8.i11.464]
8 **Neylan CJ**, Kannan U, Dempsey DT, Williams NN, Dumon KR. The Surgical Management of Obesity. *Gastroenterol Clin North Am* 2016; **45**: 689-703 [PMID: 27837782 DOI: 10.1016/j.gtc.2016.07.006]
9 **Xanthakos SA**. Nutritional deficiencies in obesity and after bariatric surgery. *Pediatr Clin North Am* 2009; **56**: 1105-1121 [PMID: 19931066 DOI: 10.1016/j.pcl.2009.07.002]
10 **Al-Najim W**, Docherty NG, le Roux CW. Food Intake and Eating Behavior After Bariatric Surgery. *Physiol Rev* 2018; **98**: 1113-1141 [PMID: 29717927 DOI: 10.1152/physrev.00021.2017]
11 **Gadde KM**, Martin CK, Berthoud HR, Heymsfield SB. Obesity: Pathophysiology and Management. *J Am Coll Cardiol* 2018; **71**: 69-84 [PMID: 29301630 DOI: 10.1016/j.jacc.2017.11.011]
12 **Upadhyay J**, Farr O, Perakakis N, Ghaly W, Mantzoros C. Obesity as a Disease. *Med Clin North Am* 2018; **102**: 13-33 [PMID: 29156181 DOI: 10.1016/j.mcna.2017.08.004]
13 **Björklund P**, Fändriks L. The pros and cons of gastric bypass surgery - The role of the Roux-limb. *Best Pract Res Clin Gastroenterol* 2019; **40-41**: 101638 [PMID: 31594646 DOI: 10.1016/j.bpg.2019.101638]
14 **Di Angelantonio E**, Bhupathiraju SN, Wormser D, Gao P, Kaptoge S, de Gonzalez AB, Cairns BJ, Huxley R, Jackson CL, Joshy G, Lewington S, Manson JE, Murphy N, Patel AV, Samet JM, Woodward M, Zheng W, Zhou M, Bansal N, Barricarte A, Carter B, Cerhan JR, Collins R, Smith GD, Fang X, Franco OH, Green J, Halsey J, Hildebrand JS, Jung KJ, Korda RJ, McLerran DF, Moore SC, O'Keeffe LM, Paige E, Ramond A, Reeves GK, Rolland B, Sacerdote C, Sattar N, Sofianopoulou E, Stevens J, Thun M, Ueshima H, Yang L, Yun YD, Willeit P, Banks E, Beral V, Chen Z, Gapstur SM, Gunter MJ, Hartge P, Jee SH, Lam T-H, Peto R, Potter JD, Willett WC, Thompson SG, Danesh J, Hu FB. Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. *The Lancet* 2016; **388**: 776-786 [DOI: 10.1016/s0140-6736(16)30175-1]
15 **Stefan N**, Häring HU, Schulze MB. Metabolically healthy obesity: the low-hanging fruit in obesity treatment? *Lancet Diabetes Endocrinol* 2018; **6**: 249-258 [PMID: 28919065 DOI: 10.1016/S2213-8587(17)30292-9]
16 **Chen EB**, Cason C, Gilbert JA, Ho KJ. Current State of Knowledge on Implications of Gut Microbiome for Surgical Conditions. *J Gastrointest Surg* 2018; **22**: 1112-1123 [PMID: 29623674 DOI: 10.1007/s11605-018-3755-4]
17 **Thomas S**, Izard J, Walsh E, Batich K, Chongsathidkiet P, Clarke G, Sela DA, Muller AJ, Mullin JM, Albert K, Gilligan JP, DiGuilio K, Dilbarova R, Alexander W, Prendergast GC. The Host Microbiome Regulates and Maintains Human Health: A Primer and Perspective for Non-Microbiologists. *Cancer Res* 2017; **77**: 1783-1812 [PMID: 28292977 DOI: 10.1158/0008-5472.CAN-16-2929]
18 **Knight R**, Callewaert C, Marotz C, Hyde ER, Debelius JW, McDonald D, Sogin ML. The Microbiome and Human Biology. *Annu Rev Genomics Hum Genet* 2017; **18**: 65-86 [PMID: 28375652 DOI: 10.1146/annurev-genom-083115-022438]
19 **Li SS**, Zhu A, Benes V, Costea PI, Hercog R, Hildebrand F, Huerta-Cepas J, Nieuwdorp M, Salojärvi J, Voigt AY, Zeller G, Sunagawa S, de Vos WM, Bork P. Durable coexistence of donor and recipient strains after fecal microbiota transplantation. *Science* 2016; **352**: 586-589 [PMID: 27126044 DOI: 10.1126/science.aad8852]
20 **Sender R**, Fuchs S, Milo R. Are We Really Vastly Outnumbered? *Cell* 2016; **164**: 337-340 [PMID: 26824647 DOI: 10.1016/j.cell.2016.01.013]
21 **Fändriks L**. Roles of the gut in the metabolic syndrome: an overview. *J Intern Med* 2017; **281**: 319-336 [PMID: 27991713 DOI: 10.1111/joim.12584]
22 **O'Brien PE**, Hindle A, Brennan L, Skinner S, Burton P, Smith A, Crosthwaite G, Brown W. Long-Term Outcomes After Bariatric Surgery: a Systematic Review and Meta-analysis of Weight Loss at 10 or More Years for All Bariatric Procedures and a Single-Centre Review of 20-Year Outcomes After Adjustable Gastric Banding. *Obes Surg* 2019; **29**: 3-

14 [PMID: 30293134 DOI: 10.1007/s11695-018-3525-0]
23 **Lundberg JO**, Weitzberg E. Biology of nitrogen oxides in the gastrointestinal tract. *Gut* 2013; **62**: 616-629 [PMID: 22267589 DOI: 10.1136/gutjnl-2011-301649]
24 **Nardone G**, Compare D. The human gastric microbiota: Is it time to rethink the pathogenesis of stomach diseases? *United European Gastroenterol J* 2015; **3**: 255-260 [PMID: 26137299 DOI: 10.1177/2050640614566846]
25 **Mowat AM**, Agace WW. Regional specialization within the intestinal immune system. *Nat Rev Immunol* 2014; **14**: 667-685 [PMID: 25234148 DOI: 10.1038/nri3738]
26 **Woting A**, Blaut M. The Intestinal Microbiota in Metabolic Disease. *Nutrients* 2016; **8**: 202 [PMID: 27058556 DOI: 10.3390/nu8040202]
27 **Johansson ME**, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc Natl Acad Sci U S A* 2008; **105**: 15064-15069 [PMID: 18806221 DOI: 10.1073/pnas.0803124105]
28 **Gilbert JA**, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R. Current understanding of the human microbiome. *Nat Med* 2018; **24**: 392-400 [PMID: 29634682 DOI: 10.1038/nm.4517]
29 **Cani PD**, Van Hul M, Lefort C, Depommier C, Rastelli M, Everard A. Microbial regulation of organismal energy homeostasis. *Nat Metab* 2019; **1**: 34-46 [PMID: 32694818 DOI: 10.1038/s42255-018-0017-4]
30 **Maruvada P**, Leone V, Kaplan LM, Chang EB. The Human Microbiome and Obesity: Moving beyond Associations. *Cell Host Microbe* 2017; **22**: 589-599 [PMID: 29120742 DOI: 10.1016/j.chom.2017.10.005]
31 **Castaner O**, Goday A, Park YM, Lee SH, Magkos F, Shiow STE, Schröder H. The Gut Microbiome Profile in Obesity: A Systematic Review. *Int J Endocrinol* 2018; **2018**: 4095789 [PMID: 29849617 DOI: 10.1155/2018/4095789]
32 **Fleissner CK**, Huebel N, Abd El-Bary MM, Loh G, Klaus S, Blaut M. Absence of intestinal microbiota does not protect mice from diet-induced obesity. *Br J Nutr* 2010; **104**: 919-929 [PMID: 20441670 DOI: 10.1017/S0007114510001303]
33 **Le Chatelier E**, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, Leonard P, Li J, Burgdorf K, Grarup N, Jørgensen T, Brandslund I, Nielsen HB, Juncker AS, Bertalan M, Levenez F, Pons N, Rasmussen S, Sunagawa S, Tap J, Tims S, Zoetendal EG, Brunak S, Clément K, Doré J, Kleerebezem M, Kristiansen K, Renault P, Sicheritz-Ponten T, de Vos WM, Zucker JD, Raes J, Hansen T; MetaHIT consortium, Bork P, Wang J, Ehrlich SD, Pedersen O. Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013; **500**: 541-546 [PMID: 23985870 DOI: 10.1038/nature12506]
34 **Gravina G**, Ferrari F, Nebbiai G. The obesity paradox and diabetes. *Eat Weight Disord* 2021; **26**: 1057-1068 [PMID: 32954485 DOI: 10.1007/s40519-020-01015-1]
35 **Gomes JMG**, Costa JA, Alfenas RCG. Metabolic endotoxemia and diabetes mellitus: A systematic review. *Metabolism* 2017; **68**: 133-144 [PMID: 28183445 DOI: 10.1016/j.metabol.2016.12.009]
36 **Tuomi K**, Logomarsino JV. Bacterial Lipopolysaccharide, Lipopolysaccharide-Binding Protein, and Other Inflammatory Markers in Obesity and After Bariatric Surgery. *Metab Syndr Relat Disord* 2016; **14**: 279-288 [PMID: 27228236 DOI: 10.1089/met.2015.0170]
37 **Ciobârcă D**, Cătoi AF, Copăescu C, Miere D, Crișan G. Bariatric Surgery in Obesity: Effects on Gut Microbiota and Micronutrient Status. *Nutrients* 2020; **12** [PMID: 31963247 DOI: 10.3390/nu12010235]
38 **Morino M**, Toppino M, Bonnet G, Rosa R, Garrone C. Laparoscopic vertical banded gastroplasty for morbid obesity. Assessment of efficacy. *Surg Endosc* 2002; **16**: 1566-1572 [PMID: 12063579 DOI: 10.1007/s00464-001-9196-1]
39 **Goergen M**, Arapis K, Limgba A, Schiltz M, Lens V, Azagra JS. Laparoscopic Roux-en-Y gastric bypass versus laparoscopic vertical banded gastroplasty: results of a 2-year follow-up study. *Surg Endosc* 2007; **21**: 659-664 [PMID: 17180269 DOI: 10.1007/s00464-006-9081-z]
40 **Belachew M**, Legrand M, Vincent V, Lismonde M, Le Docte N, Deschamps V. Laparoscopic adjustable gastric banding. *World J Surg* 1998; **22**: 955-963 [PMID: 9717421 DOI: 10.1007/s002689900499]
41 **Stefanidis A**, Forrest N, Brown WA, Dixon JB, O'Brien PB, Juliane Kampe, Oldfield BJ. An investigation of the neural mechanisms underlying the efficacy of the adjustable gastric band. *Surg Obes Relat Dis* 2016; **12**: 828-838 [PMID: 27090808 DOI: 10.1016/j.soard.2015.11.020]
42 **Sawaya RA**, Jaffe J, Friedenberg L, Friedenberg FK. Vitamin, mineral, and drug absorption following bariatric surgery. *Curr Drug Metab* 2012; **13**: 1345-1355 [PMID: 22746302 DOI: 10.2174/138920012803341339]
43 **Holzer P**, Reichmann F, Farzi A. Neuropeptide Y, peptide YY and pancreatic polypeptide in the gut-brain axis. *Neuropeptides* 2012; **46**: 261-274 [PMID: 22979996 DOI: 10.1016/j.npep.2012.08.005]
44 **Sjöström L**, Peltonen M, Jacobson P, Sjöström CD, Karason K, Wedel H, Ahlin S, Anveden Å, Bengtsson C, Bergmark G, Bouchard C, Carlsson B, Dahlgren S, Karlsson J, Lindroos AK, Lönroth H, Narbro K, Näslund I, Olbers T, Svensson PA, Carlsson LM. Bariatric surgery and long-term cardiovascular events. *JAMA* 2012; **307**: 56-65 [PMID: 22215166 DOI: 10.1001/jama.2011.1914]
45 **Carlin AM**, Zeni TM, English WJ, Hawasli AA, Genaw JA, Krause KR, Schram JL, Kole KL, Finks JF, Birkmeyer JD, Share D, Birkmeyer NJ; Michigan Bariatric Surgery Collaborative. The comparative effectiveness of sleeve gastrectomy, gastric bypass, and adjustable gastric banding procedures for the treatment of morbid obesity. *Ann Surg* 2013; **257**: 791-797 [PMID: 23470577 DOI: 10.1097/SLA.0b013e3182879ded]
46 **Jacobs DM**, Gaudier E, van Duynhoven J, Vaughan EE. Non-digestible food ingredients, colonic microbiota and the impact on gut health and immunity: a role for metabolomics. *Curr Drug Metab* 2009; **10**: 41-54 [PMID: 19149512 DOI: 10.2174/138920009787048383]
47 **Stefater MA**, Pérez-Tilve D, Chambers AP, Wilson-Pérez HE, Sandoval DA, Berger J, Toure M, Tschöp M, Woods SC, Seeley RJ. Sleeve gastrectomy induces loss of weight and fat mass in obese rats, but does not affect leptin sensitivity. *Gastroenterology* 2010; **138**: 2426-2436, 2436.e1 [PMID: 20226189 DOI: 10.1053/j.gastro.2010.02.059]
48 **Fontana MA**, Wohlgemuth SD. The surgical treatment of metabolic disease and morbid obesity. *Gastroenterol Clin North Am* 2010; **39**: 125-133 [PMID: 20202585 DOI: 10.1016/j.gtc.2009.12.010]
49 **Pucci A**, Batterham RL. Mechanisms underlying the weight loss effects of RYGB and SG: similar, yet different. *J Endocrinol Invest* 2019; **42**: 117-128 [PMID: 29730732 DOI: 10.1007/s40618-018-0892-2]

50 **Palleja A**, Kashani A, Allin KH, Nielsen T, Zhang C, Li Y, Brach T, Liang S, Feng Q, Jørgensen NB, Bojsen-Møller KN, Dirksen C, Burgdorf KS, Holst JJ, Madsbad S, Wang J, Pedersen O, Hansen T, Arumugam M. Roux-en-Y gastric bypass surgery of morbidly obese patients induces swift and persistent changes of the individual gut microbiota. *Genome Med* 2016; **8**: 67 [PMID: 27306058 DOI: 10.1186/s13073-016-0312-1]

51 **Madsbad S**, Dirksen C, Holst JJ. Mechanisms of changes in glucose metabolism and bodyweight after bariatric surgery. *Lancet Diabetes Endocrinol* 2014; **2**: 152-164 [PMID: 24622719 DOI: 10.1016/S2213-8587(13)70218-3]

52 **Bae YU**, Kim Y, Lee H, Kim H, Jeon JS, Noh H, Han DC, Ryu S, Kwon SH. Bariatric Surgery Alters microRNA Content of Circulating Exosomes in Patients with Obesity. *Obesity (Silver Spring)* 2019; **27**: 264-271 [PMID: 30624857 DOI: 10.1002/oby.22379]

53 **Atkin SL**, Ramachandran V, Yousri NA, Benurwar M, Simper SC, McKinlay R, Adams TD, Najafi-Shoushtari SH, Hunt SC. Changes in Blood microRNA Expression and Early Metabolic Responsiveness 21 Days Following Bariatric Surgery. *Front Endocrinol (Lausanne)* 2018; **9**: 773 [PMID: 30687230 DOI: 10.3389/fendo.2018.00773]

54 **Omalu BI**, Ives DG, Buhari AM, Lindner JL, Schauer PR, Wecht CH, Kuller LH. Death rates and causes of death after bariatric surgery for Pennsylvania residents, 1995 to 2004. *Arch Surg* 2007; **142**: 923-8; discussion 929 [PMID: 17938303 DOI: 10.1001/archsurg.142.10.923]

55 **Schulman AR**, Thompson CC. Complications of Bariatric Surgery: What You Can Expect to See in Your GI Practice. *Am J Gastroenterol* 2017; **112**: 1640-1655 [PMID: 28809386 DOI: 10.1038/ajg.2017.241]

56 **Sjöström L**, Lindroos AK, Peltonen M, Torgerson J, Bouchard C, Carlsson B, Dahlgren S, Larsson B, Narbro K, Sjöström CD, Sullivan M, Wedel H; Swedish Obese Subjects Study Scientific Group. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N Engl J Med* 2004; **351**: 2683-2693 [PMID: 15616203 DOI: 10.1056/NEJMoa035622]

57 **Stenberg E**, Szabo E, Ågren G, Ottosson J, Marsk R, Lönroth H, Boman L, Magnuson A, Thorell A, Näslund I. Closure of mesenteric defects in laparoscopic gastric bypass: a multicentre, randomised, parallel, open-label trial. *Lancet* 2016; **387**: 1397-1404 [PMID: 26895675 DOI: 10.1016/S0140-6736(15)01126-5]

58 **Gribsholt SB**, Pedersen AM, Svensson E, Thomsen RW, Richelsen B. Prevalence of Self-reported Symptoms After Gastric Bypass Surgery for Obesity. *JAMA Surg* 2016; **151**: 504-511 [PMID: 26747510 DOI: 10.1001/jamasurg.2015.5110]

59 **Greenstein AJ**, O'Rourke RW. Abdominal pain after gastric bypass: suspects and solutions. *Am J Surg* 2011; **201**: 819-827 [PMID: 21333269 DOI: 10.1016/j.amjsurg.2010.05.007]

60 **Raebel MA**, Newcomer SR, Bayliss EA, Boudreau D, DeBar L, Elliott TE, Ahmed AT, Pawloski PA, Fisher D, Toh S, Donahoo WT. Chronic opioid use emerging after bariatric surgery. *Pharmacoepidemiol Drug Saf* 2014; **23**: 1247-1257 [PMID: 24733580 DOI: 10.1002/pds.3625]

61 **Schauer PR**, Bhatt DL, Kirwan JP, Wolski K, Aminian A, Brethauer SA, Navaneethan SD, Singh RP, Pothier CE, Nissen SE, Kashyap SR; STAMPEDE Investigators. Bariatric Surgery versus Intensive Medical Therapy for Diabetes - 5-Year Outcomes. *N Engl J Med* 2017; **376**: 641-651 [PMID: 28199805 DOI: 10.1056/NEJMoa1600869]

62 **Abdeen G**, le Roux CW. Mechanism Underlying the Weight Loss and Complications of Roux-en-Y Gastric Bypass. Review. *Obes Surg* 2016; **26**: 410-421 [PMID: 26530712 DOI: 10.1007/s11695-015-1945-7]

63 **David LA**, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014; **505**: 559-563 [PMID: 24336217 DOI: 10.1038/nature12820]

64 **Tremaroli V**, Karlsson F, Werling M, Ståhlman M, Kovatcheva-Datchary P, Olbers T, Fändriks L, le Roux CW, Nielsen J, Bäckhed F. Roux-en-Y Gastric Bypass and Vertical Banded Gastroplasty Induce Long-Term Changes on the Human Gut Microbiome Contributing to Fat Mass Regulation. *Cell Metab* 2015; **22**: 228-238 [PMID: 26244932 DOI: 10.1016/j.cmet.2015.07.009]

65 **Liou AP**, Paziuk M, Luevano JM Jr, Machineni S, Turnbaugh PJ, Kaplan LM. Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. *Sci Transl Med* 2013; **5**: 178ra41 [PMID: 23536013 DOI: 10.1126/scitranslmed.3005687]

66 **Medina DA**, Pedreros JP, Turiel D, Quezada N, Pimentel F, Escalona A, Garrido D. Distinct patterns in the gut microbiota after surgical or medical therapy in obese patients. *PeerJ* 2017; **5**: e3443 [PMID: 28649469 DOI: 10.7717/peerj.3443]

67 **Davies NK**, O'Sullivan JM, Plank LD, Murphy R. Altered gut microbiome after bariatric surgery and its association with metabolic benefits: A systematic review. *Surg Obes Relat Dis* 2019; **15**: 656-665 [PMID: 30824335 DOI: 10.1016/j.soard.2019.01.033]

68 **Kong LC**, Tap J, Aron-Wisnewsky J, Pelloux V, Basdevant A, Bouillot JL, Zucker JD, Doré J, Clément K. Gut microbiota after gastric bypass in human obesity: increased richness and associations of bacterial genera with adipose tissue genes. *Am J Clin Nutr* 2013; **98**: 16-24 [PMID: 23719559 DOI: 10.3945/ajcn.113.058743]

69 **Liu R**, Hong J, Xu X, Feng Q, Zhang D, Gu Y, Shi J, Zhao S, Liu W, Wang X, Xia H, Liu Z, Cui B, Liang P, Xi L, Jin J, Ying X, Zhao X, Li W, Jia H, Lan Z, Li F, Wang R, Sun Y, Yang M, Shen Y, Jie Z, Li J, Chen X, Zhong H, Xie H, Zhang Y, Gu W, Deng X, Shen B, Yang H, Xu G, Bi Y, Lai S, Wang J, Qi L, Madsen L, Ning G, Kristiansen K, Wang W. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat Med* 2017; **23**: 859-868 [PMID: 28628112 DOI: 10.1038/nm.4358]

70 **Lips MA**, Van Klinken JB, van Harmelen V, Dharuri HK, 't Hoen PA, Laros JF, van Ommen GJ, Janssen IM, Van Ramshorst B, Van Wagensveld BA, Swank DJ, Van Dielen F, Dane A, Harms A, Vreeken R, Hankemeier T, Smit JW, Pijl H, Willems van Dijk K. Roux-en-Y gastric bypass surgery, but not calorie restriction, reduces plasma branched-chain amino acids in obese women independent of weight loss or the presence of type 2 diabetes. *Diabetes Care* 2014; **37**: 3150-3156 [PMID: 25315204 DOI: 10.2337/dc14-0195]

71 **Graessler J**, Qin Y, Zhong H, Zhang J, Licinio J, Wong ML, Xu A, Chavakis T, Bornstein AB, Ehrhart-Bornstein M, Lamounier-Zepter V, Lohmann T, Wolf T, Bornstein SR. Metagenomic sequencing of the human gut microbiome before and after bariatric surgery in obese patients with type 2 diabetes: correlation with inflammatory and metabolic parameters.

Pharmacogenomics J 2013; **13**: 514-522 [PMID: 23032991 DOI: 10.1038/tpj.2012.43]
72 **DiBaise JK**. Nutritional consequences of small intestinal bacterial overgrowth. *Practical Gastroenterology* 2008; **32**: 15-28
73 **Adike A**, DiBaise JK. Small Intestinal Bacterial Overgrowth: Nutritional Implications, Diagnosis, and Management. *Gastroenterol Clin North Am* 2018; **47**: 193-208 [PMID: 29413012 DOI: 10.1016/j.gtc.2017.09.008]
74 **Dukowicz AC**, Lacy BE, Levine GM. Small intestinal bacterial overgrowth: a comprehensive review. *Gastroenterol Hepatol (N Y)* 2007; **3**: 112-122 [PMID: 21960820]
75 **Grace E**, Shaw C, Whelan K, Andreyev HJ. Review article: small intestinal bacterial overgrowth--prevalence, clinical features, current and developing diagnostic tests, and treatment. *Aliment Pharmacol Ther* 2013; **38**: 674-688 [PMID: 23957651 DOI: 10.1111/apt.12456]
76 **Goldstone AP**, Miras AD, Scholtz S, Jackson S, Neff KJ, Pénicaud L, Geoghegan J, Chhina N, Durighel G, Bell JD, Meillon S, le Roux CW. Link Between Increased Satiety Gut Hormones and Reduced Food Reward After Gastric Bypass Surgery for Obesity. *J Clin Endocrinol Metab* 2016; **101**: 599-609 [PMID: 26580235 DOI: 10.1210/jc.2015-2665]
77 **Batterham RL**, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, Bloom SR. Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature* 2002; **418**: 650-654 [PMID: 12167864 DOI: 10.1038/nature00887]
78 **Meguid MM**, Glade MJ, Middleton FA. Weight regain after Roux-en-Y: a significant 20% complication related to PYY. *Nutrition* 2008; **24**: 832-842 [PMID: 18725080 DOI: 10.1016/j.nut.2008.06.027]
79 **Russell-Jones D**, Gough S. Recent advances in incretin-based therapies. *Clin Endocrinol (Oxf)* 2012; **77**: 489-499 [PMID: 22804841 DOI: 10.1111/j.1365-2265.2012.04483.x]
80 **Gutiérrez-Repiso CaM-I**, Isabel and de Hollanda, Ana and Martin-Nuñez, Gracia and Vidal, Josep and Tinahones, Francisco. Gut microbiota specific signatures are related to the successful rate of bariatric surgery. American journal of translational research 2019; 11: 942-952
81 **Fouladi F**, Brooks AE, Fodor AA, Carroll IM, Bulik-Sullivan EC, Tsilimigras MCB, Sioda M, Steffen KJ. The Role of the Gut Microbiota in Sustained Weight Loss Following Roux-en-Y Gastric Bypass Surgery. *Obes Surg* 2019; **29**: 1259-1267 [PMID: 30604078 DOI: 10.1007/s11695-018-03653-y]
82 **Steinert RE**, Rehman A, Souto Lima EJ, Agamennone V, Schuren FHJ, Gero D, Schreiner P, Vonlanthen R, Ismaeil A, Tzafos S, Hosa H, Vetter D, Misselwitz B, Bueter M. Roux-en-Y gastric bypass surgery changes fungal and bacterial microbiota in morbidly obese patients-A pilot study. *PLoS One* 2020; **15**: e0236936 [PMID: 32735609 DOI: 10.1371/journal.pone.0236936]
83 **Bal BS**, Finelli FC, Shope TR, Koch TR. Nutritional deficiencies after bariatric surgery. *Nat Rev Endocrinol* 2012; **8**: 544-556 [PMID: 22525731 DOI: 10.1038/nrendo.2012.48]
84 **Ogata H**, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res* 1999; **27**: 29-34 [PMID: 9847135 DOI: 10.1093/nar/27.1.29]
85 **Patel JJ**, Mundi MS, Hurt RT, Wolfe B, Martindale RG. Micronutrient Deficiencies After Bariatric Surgery: An Emphasis on Vitamins and Trace Minerals [Formula: see text]. *Nutr Clin Pract* 2017; **32**: 471-480 [PMID: 28609642 DOI: 10.1177/0884533617712226]
86 **Ferraz ÁAB**, Carvalho MRC, Siqueira LT, Santa-Cruz F, Campos JM. Micronutrient deficiencies following bariatric surgery: a comparative analysis between sleeve gastrectomy and Roux-en-Y gastric bypass. *Rev Col Bras Cir* 2018; **45**: e2016 [PMID: 30540099 DOI: 10.1590/0100-6991e-20182016]
87 **Nielsen MJ**, Rasmussen MR, Andersen CB, Nexø E, Moestrup SK. Vitamin B12 transport from food to the body's cells--a sophisticated, multistep pathway. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 345-354 [PMID: 22547309 DOI: 10.1038/nrgastro.2012.76]
88 **Decker GA**, Swain JM, Crowell MD, Scolapio JS. Gastrointestinal and nutritional complications after bariatric surgery. *Am J Gastroenterol* 2007; **102**: 2571-80; quiz 2581 [PMID: 17640325 DOI: 10.1111/j.1572-0241.2007.01421.x]
89 **Sala P**, Belarmino G, Torrinhas RS, Machado NM, Fonseca DC, Ravacci GR, Ishida RK, Guarda IF, de Moura EG, Sakai P, Santo MA, da Silva ID, Pereira CC, Logullo AF, Heymsfield S, Giannella-Neto D, Waitzberg DL. Gastrointestinal Transcriptomic Response of Metabolic Vitamin B12 Pathways in Roux-en-Y Gastric Bypass. *Clin Transl Gastroenterol* 2017; **8**: e212 [PMID: 28055029 DOI: 10.1038/ctg.2016.67]
90 **Ley RE**, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A* 2005; **102**: 11070-11075 [PMID: 16033867 DOI: 10.1073/pnas.0504978102]
91 **Kornerup LS**, Hvas CL, Abild CB, Richelsen B, Nexo E. Early changes in vitamin B12 uptake and biomarker status following Roux-en-Y gastric bypass and sleeve gastrectomy. *Clin Nutr* 2019; **38**: 906-911 [PMID: 29506877 DOI: 10.1016/j.clnu.2018.02.007]
92 **Visentin M**, Diop-Bove N, Zhao R, Goldman ID. The intestinal absorption of folates. *Annu Rev Physiol* 2014; **76**: 251-274 [PMID: 24512081 DOI: 10.1146/annurev-physiol-020911-153251]
93 **Milman N**. Intestinal absorption of folic acid - new physiologic & molecular aspects. *Indian J Med Res* 2012; **136**: 725-728 [PMID: 23287118]
94 **Stroh C**, Meyer F, Manger T. Beriberi, a severe complication after metabolic surgery - review of the literature. *Obes Facts* 2014; **7**: 246-252 [PMID: 25095897 DOI: 10.1159/000366012]
95 **Aasheim ET**. Wernicke encephalopathy after bariatric surgery: a systematic review. *Ann Surg* 2008; **248**: 714-720 [PMID: 18948797 DOI: 10.1097/SLA.0b013e3181884308]
96 **Becker DA**, Balcer LJ, Galetta SL. The Neurological Complications of Nutritional Deficiency following Bariatric Surgery. *J Obes* 2012; **2012**: 608534 [PMID: 22970351 DOI: 10.1155/2012/608534]
97 **Clements RH**, Katasani VG, Palepu R, Leeth RR, Leath TD, Roy BP, Vickers SM. Incidence of vitamin deficiency after laparoscopic Roux-en-Y gastric bypass in a university hospital setting. *Am Surg* 2006; **72**: 1196-202; discussion 1203 [PMID: 17216818 DOI: 10.1177/000313480607201209]
98 **Williams SE**. Metabolic bone disease in the bariatric surgery patient. *J Obes* 2011; **2011**: 634614 [PMID: 21274274 DOI: 10.1155/2011/634614]

99 **Diaz de Barboza G**, Guizzardi S, Tolosa de Talamoni N. Molecular aspects of intestinal calcium absorption. *World J Gastroenterol* 2015; **21**: 7142-7154 [PMID: 26109800 DOI: 10.3748/wjg.v21.i23.7142]

100 **Schafer AL**, Weaver CM, Black DM, Wheeler AL, Chang H, Szefc GV, Stewart L, Rogers SJ, Carter JT, Posselt AM, Shoback DM, Sellmeyer DE. Intestinal Calcium Absorption Decreases Dramatically After Gastric Bypass Surgery Despite Optimization of Vitamin D Status. *J Bone Miner Res* 2015; **30**: 1377-1385 [PMID: 25640580 DOI: 10.1002/jbmr.2467]

101 **Schafer AL**. Vitamin D and intestinal calcium transport after bariatric surgery. *J Steroid Biochem Mol Biol* 2017; **173**: 202-210 [PMID: 28027914 DOI: 10.1016/j.jsbmb.2016.12.012]

102 **Gletsu-Miller N**, Wright BN. Mineral malnutrition following bariatric surgery. *Adv Nutr* 2013; **4**: 506-517 [PMID: 24038242 DOI: 10.3945/an.113.004341]

103 **Jericó C**, Bretón I, García Ruiz de Gordejuela A, de Oliveira AC, Rubio MÁ, Tinahones FJ, Vidal J, Vilarrasa N. [Diagnosis and treatment of iron deficiency, with or without anemia, before and after bariatric surgery]. *Endocrinol Nutr* 2016; **63**: 32-42 [PMID: 26611153 DOI: 10.1016/j.endonu.2015.09.003]

104 **Santiago P**. Ferrous versus ferric oral iron formulations for the treatment of iron deficiency: a clinical overview. *ScientificWorldJournal* 2012; **2012**: 846824 [PMID: 22654638 DOI: 10.1100/2012/846824]

105 **Engebretsen KV**, Blom-Høgestøl IK, Hewitt S, Risstad H, Moum B, Kristinsson JA, Mala T. Anemia following Roux-en-Y gastric bypass for morbid obesity; a 5-year follow-up study. *Scand J Gastroenterol* 2018; **53**: 917-922 [PMID: 30231804 DOI: 10.1080/00365521.2018.1489892]

106 **Poitou Bernert C**, Ciangura C, Coupaye M, Czernichow S, Bouillot JL, Basdevant A. Nutritional deficiency after gastric bypass: diagnosis, prevention and treatment. *Diabetes Metab* 2007; **33**: 13-24 [PMID: 17258928 DOI: 10.1016/j.diabet.2006.11.004]

107 **Zalesin KC**, Miller WM, Franklin B, Mudugal D, Rao Buragadda A, Boura J, Nori-Janosz K, Chengelis DL, Krause KR, McCullough PA. Vitamin a deficiency after gastric bypass surgery: an underreported postoperative complication. *J Obes* 2011; **2011** [PMID: 20871833 DOI: 10.1155/2011/760695]

108 **Stein J**, Stier C, Raab H, Weiner R. Review article: The nutritional and pharmacological consequences of obesity surgery. *Aliment Pharmacol Ther* 2014; **40**: 582-609 [PMID: 25078533 DOI: 10.1111/apt.12872]

109 **Cuesta M**, Pelaz L, Pérez C, Torrejón MJ, Cabrerizo L, Matía P, Pérez-Ferre N, Sánchez-Pernaute A, Torres A, Rubio MA. Fat-soluble vitamin deficiencies after bariatric surgery could be misleading if they are not appropriately adjusted. *Nutr Hosp* 2014; **30**: 118-123 [PMID: 25137270 DOI: 10.3305/nh.2014.30.1.7471]

110 **Moizé V**, Andreu A, Flores L, Torres F, Ibarzabal A, Delgado S, Lacy A, Rodriguez L, Vidal J. Long-term dietary intake and nutritional deficiencies following sleeve gastrectomy or Roux-En-Y gastric bypass in a mediterranean population. *J Acad Nutr Diet* 2013; **113**: 400-410 [PMID: 23438491 DOI: 10.1016/j.jand.2012.11.013]

111 **Myint ZW**, Oo TH, Thein KZ, Tun AM, Saeed H. Copper deficiency anemia: review article. *Ann Hematol* 2018; **97**: 1527-1534 [PMID: 29959467 DOI: 10.1007/s00277-018-3407-5]

112 **Roman M**, Jitaru P, Barbante C. Selenium biochemistry and its role for human health. *Metallomics* 2014; **6**: 25-54 [PMID: 24185753 DOI: 10.1039/c3mt00185g]

113 **Papamargaritis D**, Aasheim ET, Sampson B, le Roux CW. Copper, selenium and zinc levels after bariatric surgery in patients recommended to take multivitamin-mineral supplementation. *J Trace Elem Med Biol* 2015; **31**: 167-172 [PMID: 25271186 DOI: 10.1016/j.jtemb.2014.09.005]

114 **Azad MAK**, Sarker M, Li T, Yin J. Probiotic Species in the Modulation of Gut Microbiota: An Overview. *Biomed Res Int* 2018; **2018**: 9478630 [PMID: 29854813 DOI: 10.1155/2018/9478630]

115 **Sherf-Dagan S**, Zelber-Sagi S, Zilberman-Schapira G, Webb M, Buch A, Keidar A, Raziel A, Sakran N, Goitein D, Goldenberg N, Mahdi JA, Pevsner-Fischer M, Zmora N, Dori-Bachash M, Segal E, Elinav E, Shibolet O. Probiotics administration following sleeve gastrectomy surgery: a randomized double-blind trial. *Int J Obes (Lond)* 2018; **42**: 147-155 [PMID: 28852205 DOI: 10.1038/ijo.2017.210]

116 **Woodard GA**, Encarnacion B, Downey JR, Peraza J, Chong K, Hernandez-Boussard T, Morton JM. Probiotics improve outcomes after Roux-en-Y gastric bypass surgery: a prospective randomized trial. *J Gastrointest Surg* 2009; **13**: 1198-1204 [PMID: 19381735 DOI: 10.1007/s11605-009-0891-x]

World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Pathophysiol 2022 May 22; 13(3): 73-84

DOI: 10.4291/wjgp.v13.i3.73

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Basic Study

Evaluating the regulation of transporter proteins and P-glycoprotein in rats with cholestasis and its implication for digoxin clearance

Parker Giroux, Patrick B Kyle, Chalet Tan, Joseph D Edwards, Michael J Nowicki, Hua Liu

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): E

P-Reviewer: Chen L, China; Kreisel W, Germany; Tajiri K, Japan

Received: August 9, 2021
Peer-review started: August 9, 2021
First decision: October 16, 2021
Revised: October 26, 2021
Accepted: April 21, 2022
Article in press: April 21, 2022
Published online: May 22, 2022

Parker Giroux, Joseph D Edwards, Michael J Nowicki, Hua Liu, Division of Pediatric Gastroenterology, Department of Pediatrics, University of Mississippi Medical Center, Jackson, MS 39216, United States

Patrick B Kyle, Department of Pathology, University of Mississippi Medical Center, Jackson, MS 39216, United States

Chalet Tan, Department of Pharmaceutics and Drug Delivery, University of Mississippi, Oxford, MS 38677, United States

Corresponding author: Hua Liu, MD, Assistant Professor, Division of Pediatric Gastroenterology, Department of Pediatrics, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216, United States. hliu@umc.edu

Abstract

BACKGROUND

Cardiac and hepatic functionality are intertwined in a multifaceted relationship. Pathologic processes involving one may affect the other through a variety of mechanisms, including hemodynamic and membrane transport effects.

AIM

To better understand the effect of extrahepatic cholestasis on regulations of membrane transporters involving digoxin and its implication for digoxin clearance.

METHODS

Twelve adult rats were included in this study; baseline hepatic and renal laboratory values and digoxin pharmacokinetic (PK) studies were established before evenly dividing them into two groups to undergo bile duct ligation (BDL) or a sham procedure. After 7 d repeat digoxin PK studies were completed and tissue samples were taken to determine the expressions of cell membrane transport proteins by quantitative western blot and real-time polymerase chain reaction. Data were analyzed using SigmaStat 3.5. Means between pre-surgery and post-surgery in the same experimental group were compared by paired *t*-test, while independent *t*-test was employed to compare the means between sham and BDL groups.

RESULTS

Digoxin clearance was decreased and liver function, but not renal function, was impaired in BDL rats. BDL resulted in significant up-regulation of multidrug resistance 1 expression in the liver and kidney and its down-regulation in the small intestine. Organic anion transporting polypeptides (OATP)1A4 was up-regulated in the liver but down-regulated in intestine after BDL. OATP4C1 expression was markedly increased in the kidney following BDL.

CONCLUSION

The results suggest that cell membrane transporters of digoxin are regulated during extrahepatic cholestasis. These regulations are favorable for increasing digoxin excretion in the kidney and decreasing its absorption from the intestine to compensate for reduced digoxin clearance due to cholestasis.

Key Words: Cholestasis; Digoxin clearance; Organic anion transporting polypeptides; P-glycoproteins/multidrug resistance 1; Bile duct ligation

Core Tip: The heart, kidney and liver are inextricably linked by virtue of blood flow and metabolism of medications. Cholestasis induced by bile duct ligation resulted in liver functional injury and a decrease in digoxin clearance. Quantitative western blot and real-time polymerase chain reaction demonstrated the up or down regulation of membrane transporters multidrug resistance 1, organic anion transporting polypeptides (OATP)1A4, and OATP4C1 in the liver, kidney, and intestine. Cell digoxin transporters are regulated during cholestasis which is favorable for increasing digoxin excretion.

Citation: Giroux P, Kyle PB, Tan C, Edwards JD, Nowicki MJ, Liu H. Evaluating the regulation of transporter proteins and P-glycoprotein in rats with cholestasis and its implication for digoxin clearance. *World J Gastrointest Pathophysiol* 2022; 13(3): 73-84

URL: https://www.wjgnet.com/2150-5330/full/v13/i3/73.htm

DOI: https://dx.doi.org/10.4291/wjgp.v13.i3.73

INTRODUCTION

The heart and liver are inextricably linked by virtue of blood flow and metabolism of medications, respectively. Chronic cardiac failure is characterized by cholestatic liver disease, manifested as elevation of gamma-glutamyl transferase and bilirubin[1]. Conversely, cholestatic liver disease can lead to cardiac dysfunction. Drugs with biliary elimination may have a decreased clearance in patients with cholestasis [2]. In an experimental model of cholestasis, bile duct ligation (BDL) in rats results in cardiomyopathy characterized by impaired basal cardiac contractility and reduced left ventricular pressure[3]. Furthermore, obstructive cholestasis results in impaired excretion of digoxin[4,5].

The identification of a number of organic anion transporting polypeptides (OATP) and P-glycoproteins also known as multidrug resistance 1 (MDR1) has revolutionized our understanding of the transport of biologic compounds and medications. To date, three transporters have been identified which are integral in digoxin clearance - MDR1, OATP1A4, and OATP4C1.

The main route of elimination of digoxin is renal excretion, which is closely correlated with the glomerular filtration rate and combined with tubular secretion and reabsorption. Smaller portion of digoxin is eliminated by bile duct with certain degree of enterohepatic recycling[6]. The movement of digoxin in to and out of cells is mediated by different cell membrane transporters. In the rat, OATP1A4 (also known as OATP2) is found on the basolateral membrane of hepatocytes and the membrane of enterocytes serving as an influx transporter[7-9]. Administration of the OATP1A4 inhibitor, amiodarone, resulted in increased plasma levels of intravenously administered digoxin secondary to decreased biliary excretion, liver distribution, and intestinal distribution of digoxin[10]. Administration of phenobarbital increased expression of *OATP1A4* mRNA and protein, resulting in a 4-fold increase in digoxin uptake[11].

The MDR1 transporter is found in the canaliculus of the liver, the apical membrane of mucosal cells in the intestine, and the apical membrane of proximal tubule epithelial cells in the kidney, and it has been shown as an efflux pump for digoxin[12,13]. In rodents MDR1 is coded for by 2 genes, *MDR1A* and *MDR1B*. *MDR1A* is highly expressed in the intestine, intermediately expressed in the brain, low expression in the kidney, and minimally expressed in the liver[14]. *MDR1B* is intermediately expressed in the kidney and has low expression in the brain and liver[14]. The ontogeny of *MDR1A* and *MDR1B*

expression in the kidney correlates with digoxin clearance[15]. MDR1 is important in the elimination of digoxin. It is located on the canalicular membrane of hepatocytes, where it transports digoxin into the canaliculus. In the intestine, MDR1 is found on the apical membrane of enterocytes, where it serves an effluxer role to inhibit absorption of digoxin. In the kidney, MDR1 is found on the apical membrane of the proximal tubule, where it transports digoxin into the urine[16]. OATP4C1 is found in the kidney, located on the basolateral membrane of proximal tubule epithelia cells[17]. The physiological role of OATP4C1 in the kidney has been shown to be coupled with MDR1 to promote the renal clearance of digoxin[17].

The distributions of cell membrane transporters vary in different tissues, and a transporter may function differently among the tissues[18]. This makes it difficult to explain the body's response to increased blood digoxin during cholestasis. Cholestasis results in increased expression of OATP1A4 and MDR1 in the liver which favors improved hepatobiliary excretion of digoxin[19-21]. The effect of cholestasis on OATP4C1 has not been studied to date.

We performed this study to determine the effect of cholestasis on the expression of transporters responsible for the uptake and excretion of digoxin in the liver, kidney, and intestine. The implications of the changes in the transporters for digoxin pharmacokinetics (PKs) are discussed.

MATERIALS AND METHODS

Chemicals

Unless otherwise stated, all chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, United States). Digoxin injection solution was purchased from Baxter Healthcare Corporation (Deerfield, IL, United States). Antibodies for western blot were purchased as follows: Anti-MDR1 (Cat: ab170904; Lot: GR21757-38) and anti-OATP1A4 antibody (Cat: ab224610; Lot: GR319515-7) were purchased from abcam (Cambridge, MA, Unite States). Anti-OATP4C1 (Cat: 24584-1-AP) was purchased from Proteintech (Rosemont, IL, United States).

Animals and treatment

Adult male Sprague Dawley rats (225-250 g, Harlan Sprague Dawley, Inc. Indianapolis, IN, United States) were used for the study. They were kept in plastic cages with free access to food and water with alternating 12-h periods of light and darkness. Rats were randomly divided into a sham group (n = 6) and a BDL group (n = 6).

BDL was performed as described in previous publications[22,23]. In brief, rats were anaesthetized with isoflurane, and a midline ventral incision was made through the linea alba and the bile duct was isolated. A ligature was placed to the proximal portion and another ligature to the distal portion of the bile duct and then the ligatures were tightened. The bile duct was divided between the ligatures. The abdomen was closed by double-layer running suture, and the animal was allowed to wake up on a heating pad. Sham-operated control rats underwent similar surgical procedures except the ligatures were withdrawn, leaving the bile duct intact. The animals were sacrificed post-surgery day 7 after a post-surgery PK study. Tissue samples (liver, small intestine, and kidney) were collected and saved at -80 °C and RNAlater solution (Ambion, Foster City, CA, United States). The study was approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center.

PK Study for digoxin clearance

Digoxin clearance was examined by PK studies two days prior to BDL/sham surgery and seven days following the surgeries. In brief, digoxin 0.02 mg/kg was injected through penile vein. Blood samples were obtained *via* tail vein at 0, 2, 5, 10, 30, 60, 120, 240, and 360 min following administration of digoxin for the measurement of digoxin. A separate blood sample (250 μL) was collected from tail vein for the measurement of liver function and bilirubin. Biochemical measurements were performed using a Roche-cobas® c501 analyzer (Roche Diagnostics, Indianapolis, IN, United States) for serum digoxin, total protein, albumin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), bilirubin, blood urea nitrogen (BUN), and creatinine.

Real time-polymerase chain reaction for MDR1, OATP1A4, AND OATP4C1

RNA was isolated from the tissues (liver, small intestine, and kidney) using a PureLink RNA Mini Kit (Invitrogen, Waltham, MA, United States) following the manufacturer's protocol. First-strand cDNA was synthesized through reverse transcription of 0.5 μg of total RNA using iScript cDNA Synthesis system (Bio-Rad Hercules, CA, United States). Controls without reverse transcriptase were performed for each sample to ensure absence of genomic DNA. Real time polymerase chain reaction (RT-PCR) was carried out in a real time thermal cycler (iCycler, Bio-Rad) using iQ SYBR Green Supermix (Bio-Rad). Cycling conditions were 3 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 20 s at 60 °C, then 30 s at 72 °C. PCR specificity was tested *via* analysis of the melting curve and agarose gel electrophoresis. To semi-quantify input amounts of templates, standard curves were constructed with serial dilutions of

cDNA sample from a positive control (kidney cDNA for *MDR1* and *OATP4C1*, liver cDNA for *OATP1A4*). To standardize results, interpolated values for each sample were divided by the value of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase. Primers were designed with Primer 3 software[24] and checked for absence of cross-reactivity by BLAST search. The primer pairs used, product size, and positive controls are shown in Table 1.

Quantitative western blotting for MDR1, OATP1A4 and OATP4C1

Cell membrane proteins were extracted from liver, intestine, and kidney tissues by using a Mem-PER Plus kit (Thermo Scientific, Rockford, IL, United States) following the manufacturer's protocol. Halt Protease & Phosphatase inhibitor cocktail (Thermo Scientific, Rockford, IL, United States) was added to the extracting buffer to avoid protein degradation during procedures. Sample protein concentration was determined by using a BCA Protein Assay kit (Thermo Scientific). The protein sample was prepared for western blot by a Pierce SDS-PAGE Sample Prep Kit (Thermo Scientific) for concentrating samples while removing interfering substances. After sample buffer treatment proteins were loaded and separated on a pre-casted 4%-20% gradient SDS-PAGE gel (Bio-Red, Hercules, CA, United States) and transferred to an Immobilon-FL PVDF membrane (Merck KGaA, Darmstadt, Germany). After transfer, membrane was stained with REVERT™ Total Protein Stain (LI-COR Biosciences, Lincoln, NE, United States) for 5 min at room temperature, and then the blot image was analyzed with the Odyssey CLx® infrared imaging system (LI-COR Biosciences, Lincoln, NE, United States). Following total protein stain, the membranes were incubated with Odyssey Blocking Buffer (Li-cor, Lincoln, NE, United States) for 1 h at room temperature for blocking nonspecific binding sites. Then membranes were incubated overnight at 4 °C with primary antibodies against MDR1 (1:1600, Cat: ab170904; Lot: GR21757-38, abcam Cambridge, MA, United States), anti-OATP1A4 antibody (1:1000, Cat: ab224610; Lot: GR319515-7, abcam)[25], and anti-OATP4C1 (1:600, Cat: 24584-1-AP, Proteintech, Rosemont, IL, United States). Following the primary antibody treatments, the membranes were incubated with secondary IR dye-800 conjugated anti-rabbit antibody (1:10000, IRDy 800CW, Li-cor, Lincoln, NE, United States) for 1 h at room temperature. Western blot images were captured with the Odyssey CLx® infrared imaging system (LI-COR Biosciences, Lincoln, NE, United States) and analyzed for fluorescence density using Odyssey 2.0 software. Validation tests for sample loading sizes of each tissue, primary antibodies and secondary antibody were performed before the measurements. MDR1, OATP1A4 and OATP4C1 signals were normalized to total protein of each sample.

Statistical analysis

Data were analyzed by SigmaStat 3.5. The paired *t*-test was used to compare the means between pre-surgery and post-surgery in the same experimental group sham or BDL. The independent *t*-test was employed to compare the means between sham and BDL groups. The values from 6 rats in each group showed normal distributions. All tests were two-sided. The PKs of digoxin was analyzed by non-compartmental techniques. The area under the plasma area under the curve (AUC) was calculated. Values are expressed as mean ± SD. Statistical significance was considered at $P < 0.05$. The statistical methods of this study were reviewed by Dr. Lei Zhang, a biostatistician, at University of Mississippi Medical Center, Jackson, MS, United States.

RESULTS

Effect of BDL on PKs of digoxin in rats

Digoxin PK studies were performed 2 d prior to BDL or sham surgery; the results were compared with digoxin PK studies performed 7 d following surgery. As shown in Figure 1, there was no difference in digoxin PKs between BDL and sham group prior surgery (Figure 1A). Following surgery, digoxin clearance was reduced in the BDL group as compared to the sham group (Figure 1B).

AUC of the post-BDL rats was significantly increased compared to the AUC of the pre-BDL and the post-surgery sham group (Figure 1C). AUC of the post-surgery sham group was slightly higher than that of the pre-surgery sham group but did not reach statistical significance. The change of AUC in the sham group following surgery may result from stress, change of gastrointestinal motility, or other factors induced by the sham surgery.

Biochemical parameters

Biochemical parameters including serum total protein, albumin, ALT, AST, ALP, total bilirubin, direct bilirubin, BUN, and creatinine are represented in Table 2. There was significant liver functional injury in BDL rats as indicated by decreased serum albumin and increased ALT, AST and ALP. Obstructive jaundice developed in the post-BDL group as shown by increased total and direct bilirubin. Sham surgery did not affect liver function or bilirubin levels as compared to pre-surgery sham rats. Kidney function as measured by BUN and creatinine was not altered by BDL or sham surgery.

Table 1 Real time polymerase chain reaction primer sequences, product size and positive controls

Target gene	Primer sequences (5'-3')	Size (bp)	Positive control
MDR1	ATCAACTCGCAAAAGCATCC (F)	116	Kidney
	AATTCAACTTCAGGATCCGC (R)		
OATP1A4	TGTGATGACCTGTGATAATTTTCCA (F)	81	Liver
	TTCTCCACATATAGTTGGTGCTGAA (R)		
OATP4C1	TCAAGCTGGCAAAACTTCCC (F)	239	Kidney
	CCGCAAAGCTCGATGTCAAT (R)		
GAPDH	AAGATGGTGAAGGTCGGTGT (F)	98	Liver
	GTTGATGGCAACAATGTCCACT (R)		

OATP: Organic anion transporting polypeptides; MDR1: Multidrug resistance 1; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

Table 2 Liver panel, bilirubin, blood urea nitrogen and creatinine

	Sham		BDL	
	Pre-surgery	Post-surgery	Pre-surgery	Post-surgery
Tot protein	6.63 ± 0.27	6.53 ± 0.35	6.53 ± 0.42	6.75 ± 0.23
Albumin	4.08 ± 0.17	3.85 ± 0.34	4.05 ± 0.14	3.40 ± 0.13[a]
ALP	137.8 ± 19.78	122.3 ± 14.45	141.5 ± 12.74	467.2 ± 59.79[a]
Bilirubin, D	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	6.62 ± 1.72[a]
Bilirubin, T	0.04 ± 0.02	0.06 ± 0.01	0.05 ± 0.02	11.67 ± 1.82[a]
ALT	36.00 ± 12.02	57.00 ± 10.47	24.83 ± 8.28	191.8 ± 42.29[a]
AST	71.83 ± 11.53	82.17 ± 4.92	64.17 ± 7.57	525.8 ± 107.11[a]
BUN	17.54 ± 2.71	16.17 ± 3.13	18.23 ± 4.21	19.00 ± 5.57
Creatinine	0.27 ± 0.03	0.25 ± 0.02	0.29 ± 0.03	0.28 ± 0.04

[a]$P < 0.05$ *vs* pre-surgery.

Values are expressed as means ± SD of 6 rats per group. BDL: Bile duct ligation; ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; BUN: Blood urea nitrogen.

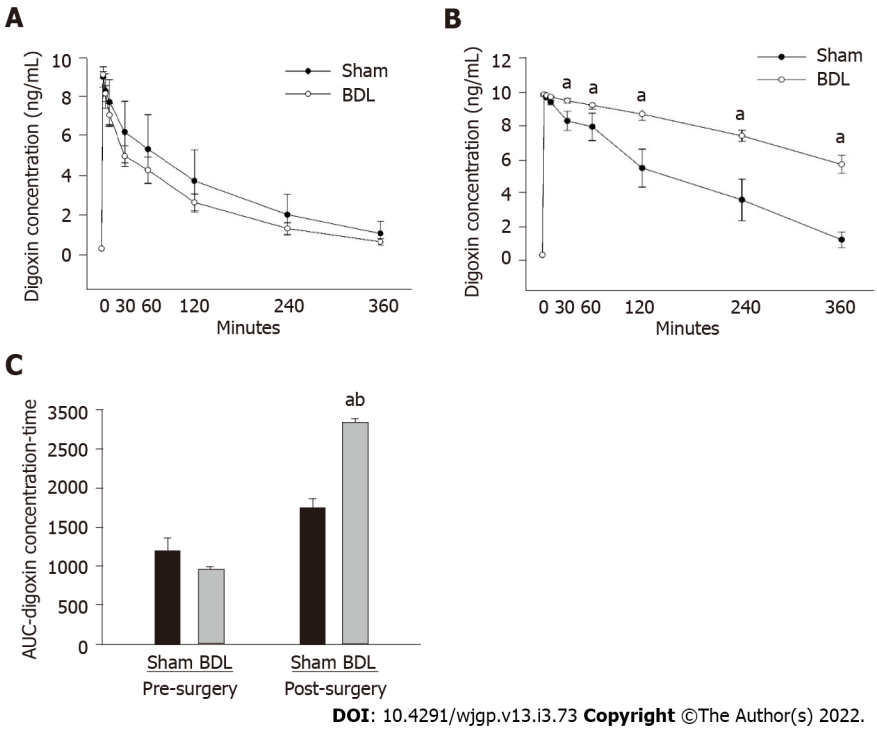
Effect of BDL on protein expressions of MDR1, OATP1A4, and OATP4C1

The expression of the organic anion transporters was analyzed by quantitative western blot as described in the methods. MDR1 was expressed in all the tissues examined: Liver, kidney, and small intestine (Figures 2A and 2B). BDL resulted in significant up-regulation of MDR1 expression in the liver and kidney and its down-regulation in the small intestine.

OATP1A4 protein was expressed in the liver and small intestine but it was not detectable in the kidney. OATP1A4 was significantly up-regulated by BDL in the liver and down-regulated in the small intestine (Figures 3A and 3B). The expression of the organic anion transporter OATP4C1 was tested in the kidney. BDL led to a significantly increased expression of OATP4C1 as compared with sham surgery rats (Figures 4A and 4B).

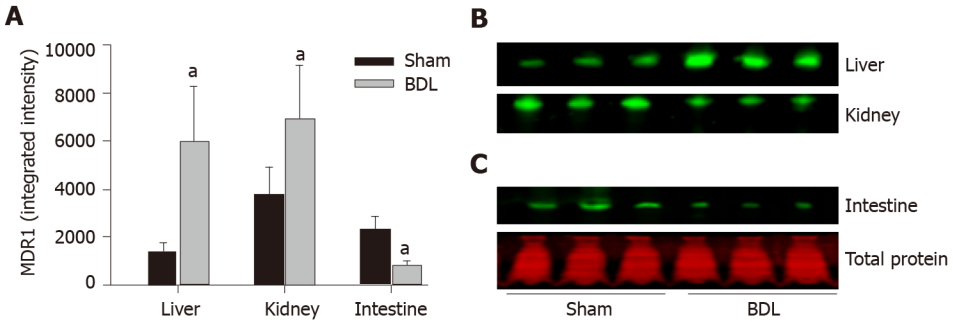
Effect of BDL on mRNA expressions of MDR1, OATP1A4, and OATP4C1

Transcription levels of *MDR1*, *OATP1A4* and *OATP4C1* were examined by mRNA expressions *via* RT-PCR. *MDR1* mRNA was presented in all the tissues examined (Figure 5A). BDL markedly up-regulated *MDR1* expression in the liver and kidney, down-regulated it in the small intestine as compared with sham surgery rats. *OATP1A4* mRNA was expressed in the liver and small intestine (Figure 5B). A trace amount of *OATP1A4* mRNA was tested in the kidney tissue. *OATP1A4* mRNA was significantly up-regulated by BDL in the liver and down-regulated in the small intestine as compared with sham surgery rats. BDL did not alter *OATP1A4* mRNA expression in the kidney (Figure 5B). *OATP4C1* mRNA was expressed in the kidney and was significantly elevated after BDL surgery as compared with sham surgery rats (Figure 5C). A summary of the regulations of cell membrane transporters in kidney,



DOI: 10.4291/wjgp.v13.i3.73 **Copyright** ©The Author(s) 2022.

Figure 1 Effect of bile duct ligation on pharmacokinetics of digoxin in rats. A: Pre-surgery digoxin pharmacokinetic studies was compared and presented as digoxin concentration-*versus*-time line curves; B: Post-surgery digoxin pharmacokinetic studies were compared and presented as digoxin concentration-*versus*-time line curves, C: Area under the curve, the area under the digoxin plasma concentration-*versus*-time. Values are expressed as means ± SD, n = 6; ^{a}P < 0.05 *vs* pre-surgery bile duct ligation group, ^{b}P < 0.05 *vs* post-surgery sham. BDL: Bile duct ligation; AUC: Area under the curve.



DOI: 10.4291/wjgp.v13.i3.73 **Copyright** ©The Author(s) 2022.

Figure 2 Effect of bile duct ligation on protein expressions of multidrug resistance 1. Multidrug resistance 1 (MDR1) protein assay was performed by quantitative western blot. A: Fluorescence densities of the protein bands were measured and normalized to the relative total protein amount of each sample; B: The representative western blot images for the expression of MDR1 in the liver, kidney, small intestine; C: Representative western blot image for the total protein stain by REVERT™ Total Protein Stain kit. Values are depicted as means ± SD; *n* = 6; ^{a}P < 0.05 compared with sham surgery rats. BDL: Bile duct ligation; MDR1: Multidrug resistance 1.

intestine and liver, and potential effects on digoxin clearance are shown in Table 3.

DISCUSSION

Digoxin remains an important medication for treatment of cardiac dysfunction, a condition known to predispose to hepatic injury resulting in cholestasis. Cholestasis predisposes to elevated serum levels of digoxin with increased risk of toxicity. Clearance of digoxin is a complex process with differences between humans and rodents. In the rat about 60%-70% of digoxin is metabolized and the remainder excreted by the kidney (about 20%-30%) and liver (about 10%)[26,27]. In normal conditions, renal excretion of digoxin is closely correlated with the glomerular filtration rate with certain degree of tubular secretion and reabsorption. A small portion of digoxin eliminated by the bile duct goes through enterohepatic cycling[6]. The trafficking of digoxin in and out of cells is mediated by different cell

Table 3 Summary of the regulations of cell membrane transporters and potential effects on digoxin clearance

	Efflux	Influx	Effects
Kidney	MDR1: Up-regulated	OATP4C1: Up-regulated	Increase tubule exclusion
Intestine	MDR1: Down-regulated	OATP1A4: Down-regulated	Decrease intestinal absorption
Liver	MDR1: Up-regulated	OATP4C1: Up-regulated	Increase exclusion into bile duct

OATP: Organic anion transporting polypeptides; MDR1: Multidrug resistance 1.

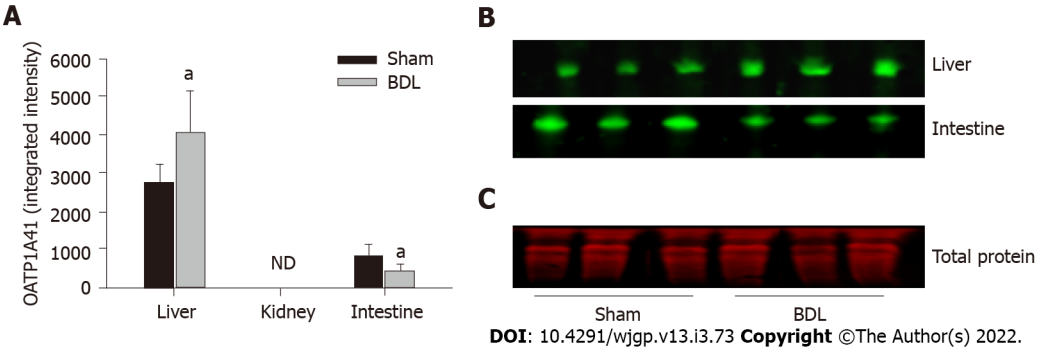


Figure 3 Effect of bile duct ligation on protein expressions of organic anion transporting polypeptides 1A4. Organic anion transporting polypeptides (OATP)1A4 protein assay was performed by quantitative western blot. A: Fluorescence densities of the protein bands were measured and normalized to the relative total protein amount of each sample; B: The representative western blot images for the expression of OATP1A4 protein in the liver, small intestine. OATP1A4 was not detected in the kidney by western blot; C: Representative western blot image for the total protein stain by REVERT™ Total Protein Stain kit. Values are expressed as means ± SD; $n = 6$; $^aP < 0.05$ compared with sham surgery rats. ND: Not detected; BDL: Bile duct ligation; OATP: Organic anion transporting polypeptides.

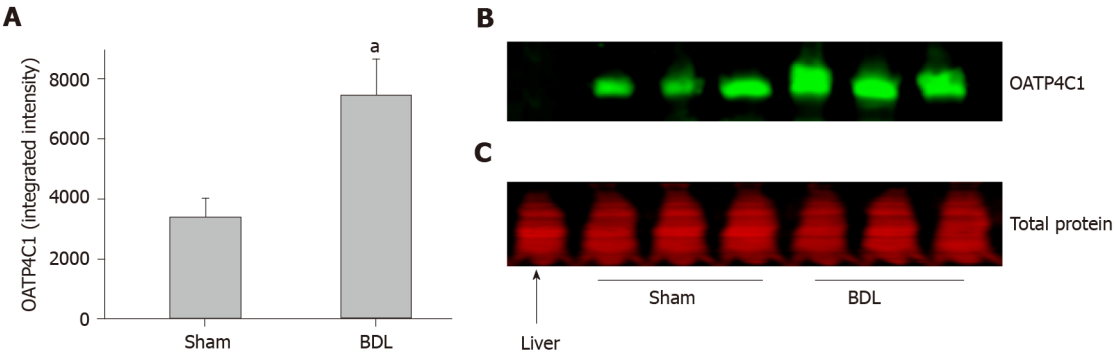
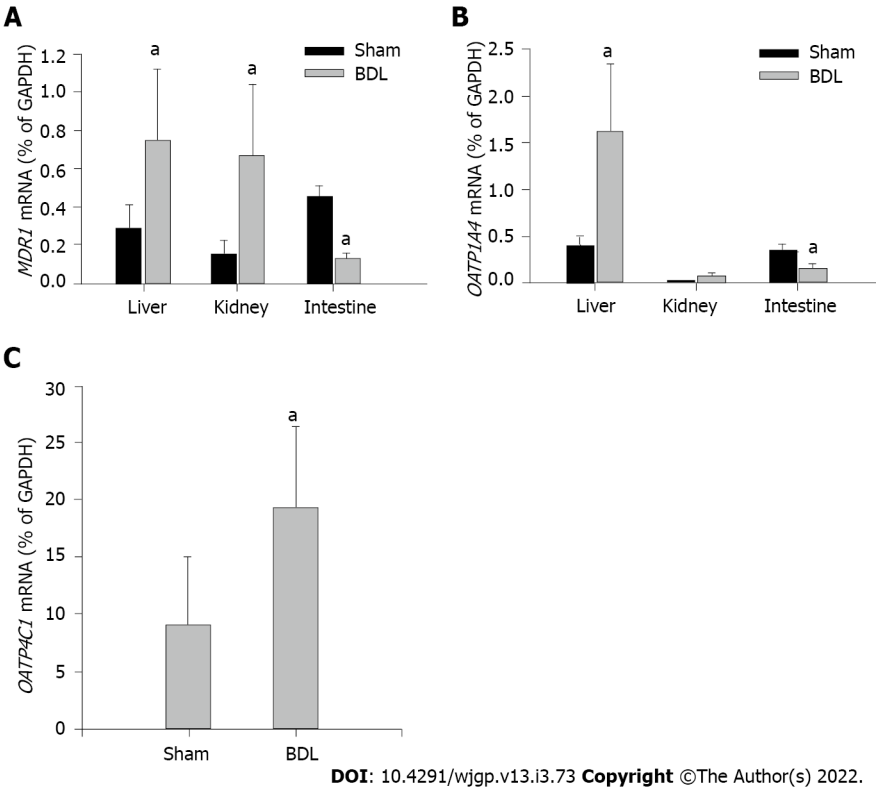


Figure 4 Effect of bile duct ligation on protein expressions of organic anion transporting polypeptides 4C1 in the kidney. Organic anion transporting polypeptides (OATP)4C1 protein assay was performed by quantitative western blot. A: Fluorescence densities of the protein bands were measured and normalized to the relative total protein amount of each sample; B: The representative western blot images for the expression of OATP4C1 protein in the kidney. Liver sample was loaded with kidney samples as negative control for OATP4C1; C: Representative western blot image for the total protein stain by REVERT™ Total Protein Stain kit. Values are expressed as means ± SD; $n = 6$; $^aP < 0.05$ compared with sham surgery rats. BDL: Bile duct ligation; OATP: Organic anion transporting polypeptides.

membrane transporters. Previous studies have demonstrated that uptake and efflux of digoxin are mediated by OATP1A4 and MDR1, respectively, in the liver and intestine[7-9], and by OATP4C1 and MDR1 in the kidney[17]. Cholestasis alters expression of MDR1 and OATP1A4 in a manner favorable for an increase in excretion of digoxin[19-21], while the effect of cholestasis on OATP4C1 in the kidney has not been studied to date. We undertook this study to determine changes in these digoxin transporters in a model of cholestasis and their implications for digoxin clearance.

Cholestasis was induced by BDL as evidenced by elevated serum transaminase and bilirubin levels. Digoxin clearance was decreased in the BDL group in keeping with prior studies in a rabbit model[4,5]. In the earliest study, BDL also resulted in elevation of serum creatinine prompting the authors to propose decreased renal excretion of orally administered digoxin as the major mechanism for decreased



DOI: 10.4291/wjgp.v13.i3.73

Figure 5 Effect of bile duct ligation on mRNA expressions of *multidrug resistance 1, organic anion transporting polypeptides 1a4* and *4C1*. mRNA expression in each sample was standardized to its glyceraldehyde-3-phosphate dehydrogenase level. A: Expressions of *multidrug resistance 1* in the liver, kidney, small intestine, and the effect of bile duct ligation (BDL) on the mRNA expressions in each tissue; B: Expression of *organic anion transporting polypeptides* (*OATP*)*1A4* mRNA in the liver, kidney and small intestine, and the effect of BDL on *OATP1A4* mRNA expressions; C: Expression of *OATP4C1* mRNA in the kidney and the effect of BDL on its expression. Values are depicted as means ± SD; $n = 6$; $^{a}P < 0.05$ compared with sham surgery rats. BDL: Bile duct ligation; OATP: Organic anion transporting polypeptides; MDR1: Multidrug resistance 1; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

clearance with disruption of the enterohepatic circulation as a potential complicating factor[4]. In a follow-up study, BDL led to decreased clearance of intravenously administered digoxin, but with absence of elevated serum creatinine. The authors concluded that impaired hepatic function and interruption of the enterohepatic circulation impaired digoxin elimination[5]. Discovery of MDR1, OATP1A4, and OATP4C1 has allowed more in-depth investigation into the mechanisms of digoxin absorption and clearance.

MDR1 is found on the apical membranes of proximal tubule cells, enterocytes, and hepatocytes where it is responsible for efflux of digoxin. In rodents MDR1 is the product of the *MDR1* gene, which is made up of two forms, *MDR1A* and *MDR1B*[28]. Initial studies assessing the role of MDR1 in digoxin clearance focused on inhibiting the protein with quinidine, which inhibits intestinal excretion of digoxin [29]. To further study the role of MDR1 in digoxin clearance a knock-out model for MDR1A was created. In this model, fecal excretion of digoxin decreased and renal excretion increased compared to wild type animals, while there was no significant change in biliary excretion[30]. The authors concluded that the lower fecal excretion of digoxin was secondary to a decrease in drug excretion by the intestinal epithelium, rather than a decrease in biliary excretion. Increased renal excretion was surprising in the absence of MDR1A expression in the kidneys. The authors surmised that the increased renal clearance may be explained by other transporters (MDR1B) or increased glomerular filtration. They concluded that MDR1 contributes substantially to digoxin excretion *via* the intestinal epithelium and decreased re-uptake after biliary excretion[30].

Transport of digoxin in the liver is mediated by OATP1A4, responsible for uptake at the hepatocyte basolateral membrane, and MDR1, responsible for excretion into the bile at the apical membrane[7,14]. In the present study cholestasis/BDL led to increased expression of OATP1A4, increasing hepatic uptake of digoxin from the blood, and increased expression of MDR1, increasing biliary excretion of digoxin. Although these changes would predict increased clearance of digoxin through bile, ligation of the bile duct precludes this mode of clearance.

A carrier-mediated uptake of digoxin is responsible for its reabsorption of digoxin in intestine[31]. The carrier-mediated uptake was found to be sensitive to the OATP inhibitors BSP and apple juice, suggesting an OATP transporter as a likely candidate. Further support for an OATP transporter came from experiments using rat intestinal brush-border membrane vesicles which showed that an increased

digoxin uptake in the presence of proton and bicarbonate gradients and outwardly directed glutathione gradient[31]. Recent studies demonstrated that intestinal OATP1A4 is a carrier protein that transports drugs from gut into the portal circulation[8], and digoxin has been shown as a substrate of OATP1A4 [10]. Our result showed that BDL led to decreased expression of OATP1A4 in the intestine. Decreased expression of OATP1A4 in the intestine favors decreased absorption predicting improved drug clearance in the feces.

Although cholestasis results in changes in MDR1 and OATP1A4 favoring increased digoxin clearance, in the BDL model of cholestasis clearance of intravenously administered digoxin is limited to renal excretion. Although BDL led to changes that would predict increased clearance of digoxin through bile, ligation of the bile duct precludes this mode of clearance. Similarly, changes in the intestine following BDL favoring digoxin clearance in the feces are minimized by the study design. Digoxin administered intravenously would limit to amount of drug in the intestinal lumen. Further, BDL inhibits hepatic excretion of digoxin into the intestine.

In the kidney MDR1 is responsible for excretion of digoxin across the apical membrane of renal cells into urine[16]. Our result showed that OATP1A4 is not expressed in the kidney suggesting another transporter is responsible for transport of digoxin across the basolateral membrane into renal cells[17]. Mikkaichi *et al*[17] isolated an organic acid transporting peptide denoted OATP4C1 both in humans and rats. It is localized on the basolateral membrane of the proximal tubules of the kidney where it has been shown to be the primary transporter of digoxin into renal cells. MDR1 is co-localized with OATP4C1 in the proximal tubule. Renal failure leads to decreased expression in OATP4C1 but has no effect on expression of MDR1 suggesting that decreased digoxin clearance in renal failure is due to loss of OATP4C1 activity[17,32]. We have shown that cholestasis due to BDL results in increased expression in both MDR1 and OATP4C1 in the kidney favoring enhanced vectorial transport of digoxin from blood to urine by proximal tubule cells. To the best of our knowledge, the current report is the first study to investigate the regulation of OATP4C1 in kidney in a pathological model *in vivo*.

It is interesting that MDR1 and OATP1A4 participate in transport of both bile acids and digoxin[33]. Also, there is marked similarity in the method of excretion for bile acids and digoxin in obstructive cholestasis. OATP4C1 may also participate in the excretion of bile acids by the kidney through increased uptake at the basolateral membrane, although the data is conflicting. To date, two studies assessed the transport of bile acids in Madin-Darby canine kidney cells transfected with a plasmid containing OATP4C1, one showed no transport of taurocholate[17], while the other showed transport of both chenodeoxycholate and glycocholate[34]. Our study showed upregulation of OATP4C1 in cholestasis which would increase uptake of bile acids by proximal tubule cells with subsequent excretion at the apical membrane by MDR1.

Bile acids activate the nuclear hormone receptors farnesoid-X-receptor and pregnane-X-receptor (PXR) and in cholestasis there were increased activations of these receptors[35,36]. MDR1 and OATP1A4 are both PXR-responsive and their expression increased in cholestasis. OATP4C1 expression is induced through transitional factor Aryl hydrocarbon receptor (AhR) through binding of the xenobiotic responsive element[37]. Previous studies have shown that AhR is activated in cholestasis[38] through the action of PXR[39]. We propose that the increased expression of OATP4C1 in cholestasis is best explained by this mechanism.

This is an exploratory research to study how the body responds to increased digoxin during cholestasis. Further studies are needed to confirm the implications by measuring digoxin tissue distributions and digoxin concentrations in urine and along the intestinal tract from the duodenum to the ileum. We believe that the findings from the current study will serve as a base for future study of digoxin clearance mediated by renal-expressed OATP4C1 during cholestasis.

CONCLUSION

In conclusion, under physiological conditions, the main route of elimination of digoxin is renal excretion which is closely correlated with glomerular filtration rate. Biliary excretion is the major non-renal route. Enterohepatic cycle has minor importance[6]. Our finding demonstrated that under pathological condition, cholestasis in the current study, cell membrane digoxin transporters are regulated which is in favor of an increase in digoxin excretion in renal tubules and a decrease in its absorption from the tubules of intestine. These changes compensate the reduced digoxin clearance due to cholestasis. This finding could have clinical application by modifying transporters' activities through pharmaceutical approaches for improving digoxin clearance during cholestasis.

ARTICLE HIGHLIGHTS

Research background

The heart and liver are inextricably linked by virtue of blood flow and metabolism of medications.

Drugs with biliary elimination, such as digoxin, decrease clearance with cholestasis.

Research motivation

We performed this study to better understand the effect of extrahepatic cholestasis on regulations of membrane transporters involving digoxin and its implication for digoxin clearance.

Research objectives

The efflux transporter, multidrug resistance 1 (MDR1), and influx transporters, organic anion transporting polypeptides (OATP)1A4 and OATP4C1 in kidney, intestine and liver were examined.

Research methods

Twelve adult Sprague Dawley rats were included in this study; baseline hepatic and renal laboratory values and digoxin pharmacokinetic (PK) studies were established before evenly dividing them into two groups to undergo bile duct ligation (BDL) or a sham procedure. After 7 d repeat digoxin PK studies were completed and tissue samples were taken to determine the expressions of MDR1, OATP1A4 and OATP4C1 by quantitative western blot and real-time polymerase chain reaction.

Research results

Digoxin clearance was decreased and liver function, but not renal function, was impaired in BDL rats. BDL resulted in significant up-regulation of MDR1 expression in the liver and kidney and its down-regulation in the small intestine. OATP1A4 was up-regulated in the liver but down-regulated in intestine after BDL. OATP4C1 expression was markedly increased in the kidney following BDL.

Research conclusions

The results suggest that cell membrane transporters of digoxin are regulated during cholestasis. These regulations are favorable for increasing digoxin excretion in kidney and decreasing its absorption from intestine in order to compensate the reduced digoxin clearance due to cholestasis.

Research perspectives

The current study was designed as an exploratory research for providing clues for future study in this field. Previous studies on the transporters in kidney and intestine were done only by *in vitro* experiments. To the best of our knowledge, the current report is the first study to investigate the regulation of the digoxin transporters in kidney and intestine in animal model of cholestasis. Our results does demonstrate that the cell membrane transporters were regulated which is in favor of digoxin excretion during cholestasis. To confirm our finding, more detailed PK studies need to be done, for example, tissue distributions of digoxin and digoxin concentrations in urine and in intestine. Knock-out (KO) animal lacking the transporters, especially tissue-specific KO, will be a powerful tool in further study.

ACKNOWLEDGEMENTS

We thank Dr. Lei Zhang, a bio-statistician at University of Mississippi Medical Center, for review of the statistical methods.

FOOTNOTES

Author contributions: Giroux P, Nowicki MJ, Tan C and Liu H participated in conception and research design; Giroux P, Kyle PB, and Liu H conducted experiments; Giroux P, Nowicki MJ, Tan C, Edwards JD, and Liu H performed data analysis and interpretation; Giroux P, Kyle PB, Nowicki MJ, Tan C, Edwards JD, and Liu H wrote or contributed to the reversion of the manuscript; and all authors have read and approved the final manuscript.

Institutional review board statement: The study was approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center.

Institutional animal care and use committee statement: The study was approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center.

Conflict-of-interest statement: The authors declare that there is no conflict of interest.

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Country/Territory of origin: United States

ORCID number: Parker Giroux 0000-0002-5644-7720; Patrick B Kyle 0000-0003-0154-7078; Chalet Tan 0000-0001-7489-7699; Joseph D Edwards 0000-0003-4979-2937; Michael J Nowicki 0000-0001-9395-3027; Hua Liu 0000-0002-7936-5330.

Corresponding Author's Membership in Professional Societies: American Heart Association; American Society of Nephrology.

S-Editor: Wang JJ
L-Editor: A
P-Editor: Wang JJ

REFERENCES

1 **Naschitz JE**, Slobodin G, Lewis RJ, Zuckerman E, Yeshurun D. Heart diseases affecting the liver and liver diseases affecting the heart. *Am Heart J* 2000; **140**: 111-120 [PMID: 10874271 DOI: 10.1067/mhj.2000.107177]
2 **Delcò F**, Tchambaz L, Schlienger R, Drewe J, Krähenbühl S. Dose adjustment in patients with liver disease. *Drug Saf* 2005; **28**: 529-545 [PMID: 15924505 DOI: 10.2165/00002018-200528060-00005]
3 **Yu W**, Huang C, Wang Q, Zhao E, Ding Y, Huang T, Ma C, Meng B. Plasma BNP level combined with surgical Apgar score to predict operative major cardiac adverse events in malignant obstructive jaundice patients. *Pak J Med Sci* 2016; **32**: 1188-1193 [PMID: 27882019 DOI: 10.12669/pjms.325.10302]
4 **Wójcicki M**, Drozdzik M, Sulikowski T, Gawronska-Szklarz B, Wójcicki J, Rózewicka L, Skowron J, Zielinski S, Musial HD, Zakrzewski J. Pharmacokinetics of intragastrically administered digoxin in rabbits with experimental bile duct obstruction. *J Pharm Pharmacol* 1997; **49**: 1082-1085 [PMID: 9401942 DOI: 10.1111/j.2042-7158.1997.tb06046.x]
5 **Wójcicki M**, Drozdzik M, Sulikowski T, Wójcicki J, Gawrońska-Szklarz B, Zieliński S, Rózewicka L. Pharmacokinetics of intravenously administered digoxin and histopathological picture in rabbits with experimental bile duct obstruction. *Eur J Pharm Sci* 2000; **11**: 215-222 [PMID: 11042227 DOI: 10.1016/s0928-0987(00)00096-8]
6 **Iisalo E**. Clinical pharmacokinetics of digoxin. *Clin Pharmacokinet* 1977; **2**: 1-16 [PMID: 322907 DOI: 10.2165/00003088-197702010-00001]
7 **Reichel C**, Gao B, Van Montfoort J, Cattori V, Rahner C, Hagenbuch B, Stieger B, Kamisako T, Meier PJ. Localization and function of the organic anion-transporting polypeptide Oatp2 in rat liver. *Gastroenterology* 1999; **117**: 688-695 [PMID: 10464146 DOI: 10.1016/s0016-5085(99)70463-4]
8 **Koitabashi Y**, Kumai T, Matsumoto N, Watanabe M, Sekine S, Yanagida Y, Kobayashi S. Orange juice increased the bioavailability of pravastatin, 3-hydroxy-3-methylglutaryl CoA reductase inhibitor, in rats and healthy human subjects. *Life Sci* 2006; **78**: 2852-2859 [PMID: 16412477 DOI: 10.1016/j.lfs.2005.11.006]
9 **Naud J**, Michaud J, Boisvert C, Desbiens K, Leblond FA, Mitchell A, Jones C, Bonnardeaux A, Pichette V. Down-regulation of intestinal drug transporters in chronic renal failure in rats. *J Pharmacol Exp Ther* 2007; **320**: 978-985 [PMID: 17135344 DOI: 10.1124/jpet.106.112631]
10 **Funakoshi S**, Murakami T, Yumoto R, Kiribayashi Y, Takano M. Role of organic anion transporting polypeptide 2 in pharmacokinetics of digoxin and beta-methyldigoxin in rats. *J Pharm Sci* 2005; **94**: 1196-1203 [PMID: 15858844 DOI: 10.1002/jps.20346]
11 **Hagenbuch N**, Reichel C, Stieger B, Cattori V, Fattinger KE, Landmann L, Meier PJ, Kullak-Ublick GA. Effect of phenobarbital on the expression of bile salt and organic anion transporters of rat liver. *J Hepatol* 2001; **34**: 881-887 [PMID: 11451172 DOI: 10.1016/s0168-8278(01)00097-6]
12 **Bendayan R**. Renal drug transport: a review. *Pharmacotherapy* 1996; **16**: 971-985 [PMID: 8947968]
13 **Schinkel AH**, Wagenaar E, van Deemter L, Mol CA, Borst P. Absence of the mdr1a P-Glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J Clin Invest* 1995; **96**: 1698-1705 [PMID: 7560060 DOI: 10.1172/JCI118214]
14 **Croop JM**, Raymond M, Haber D, Devault A, Arceci RJ, Gros P, Housman DE. The three mouse multidrug resistance (mdr) genes are expressed in a tissue-specific manner in normal mouse tissues. *Mol Cell Biol* 1989; **9**: 1346-1350 [PMID: 2471060 DOI: 10.1128/mcb.9.3.1346-1350.1989]
15 **Pinto N**, Halachmi N, Verjee Z, Woodland C, Klein J, Koren G. Ontogeny of renal P-glycoprotein expression in mice: correlation with digoxin renal clearance. *Pediatr Res* 2005; **58**: 1284-1289 [PMID: 16306209 DOI: 10.1203/01.pdr.0000188697.99079.27]
16 **Thiebaut F**, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci U S A* 1987; **84**: 7735-7738 [PMID: 2444983 DOI: 10.1073/pnas.84.21.7735]
17 **Mikkaichi T**, Suzuki T, Onogawa T, Tanemoto M, Mizutamari H, Okada M, Chaki T, Masuda S, Tokui T, Eto N, Abe M, Satoh F, Unno M, Hishinuma T, Inui K, Ito S, Goto J, Abe T. Isolation and characterization of a digoxin transporter and its

rat homologue expressed in the kidney. *Proc Natl Acad Sci U S A* 2004; **101**: 3569-3574 [PMID: 14993604 DOI: 10.1073/pnas.0304987101]

18 **Klaassen CD**, Aleksunes LM. Xenobiotic, bile acid, and cholesterol transporters: function and regulation. *Pharmacol Rev* 2010; **62**: 1-96 [PMID: 20103563 DOI: 10.1124/pr.109.002014]

19 **Schrenk D**, Gant TW, Preisegger KH, Silverman JA, Marino PA, Thorgeirsson SS. Induction of multidrug resistance gene expression during cholestasis in rats and nonhuman primates. *Hepatology* 1993; **17**: 854-860 [PMID: 8098315]

20 **Slitt AL**, Allen K, Morrone J, Aleksunes LM, Chen C, Maher JM, Manautou JE, Cherrington NJ, Klaassen CD. Regulation of transporter expression in mouse liver, kidney, and intestine during extrahepatic cholestasis. *Biochim Biophys Acta* 2007; **1768**: 637-647 [PMID: 17141734 DOI: 10.1016/j.bbamem.2006.10.008]

21 **Donner MG**, Schumacher S, Warskulat U, Heinemann J, Häussinger D. Obstructive cholestasis induces TNF-alpha- and IL-1 -mediated periportal downregulation of Bsep and zonal regulation of Ntcp, Oatp1a4, and Oatp1b2. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**: G1134-G1146 [PMID: 17916651 DOI: 10.1152/ajpgi.00079.2007]

22 **Kountouras J**, Billing BH, Scheuer PJ. Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rat. *Br J Exp Pathol* 1984; **65**: 305-311 [PMID: 6743531]

23 **Holmberg JT**, Hederström E, Ihse I. A method to prevent recanalization of the transected bile duct in the rat. *Scand J Gastroenterol* 1985; **20**: 428-432 [PMID: 4023608 DOI: 10.3109/00365528509089675]

24 **Rozen S**, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol* 2000; **132**: 365-386 [PMID: 10547847 DOI: 10.1385/1-59259-192-2:365]

25 **Dolezelova E**, Sa ICI, Prasnicka A, Hroch M, Hyspler R, Ticha A, Lastuvkova H, Cermanova J, Pericacho M, Visek J, Lasticova M, Micuda S, Nachtigal P. High soluble endoglin levels regulate cholesterol homeostasis and bile acids turnover in the liver of transgenic mice. *Life Sci* 2019; **232**: 116643 [PMID: 31299237 DOI: 10.1016/j.lfs.2019.116643]

26 **Harrison LI**, Gibaldi M. Pharmacokinetics of digoxin in the rat. *Drug Metab Dispos* 1976; **4**: 88-93 [PMID: 3407]

27 **Wirth KE**, Frölich JC. Effect of spironolactone on excretion of 3H-digoxin and its metabolites in rats. *Eur J Pharmacol* 1974; **29**: 43-51 [PMID: 4435043 DOI: 10.1016/0014-2999(74)90168-x]

28 **Sukhai M**, Yong A, Kalitsky J, Piquette-Miller M. Inflammation and interleukin-6 mediate reductions in the hepatic expression and transcription of the mdr1a and mdr1b Genes. *Mol Cell Biol Res Commun* 2000; **4**: 248-256 [PMID: 11409920 DOI: 10.1006/mcbr.2001.0288]

29 **Su SF**, Huang JD. Inhibition of the intestinal digoxin absorption and exsorption by quinidine. *Drug Metab Dispos* 1996; **24**: 142-147 [PMID: 8742224]

30 **Mayer U**, Wagenaar E, Beijnen JH, Smit JW, Meijer DK, van Asperen J, Borst P, Schinkel AH. Substantial excretion of digoxin *via* the intestinal mucosa and prevention of long-term digoxin accumulation in the brain by the mdr 1a P-glycoprotein. *Br J Pharmacol* 1996; **119**: 1038-1044 [PMID: 8922756 DOI: 10.1111/j.1476-5381.1996.tb15775.x]

31 **Yao HM**, Chiou WL. The complexity of intestinal absorption and exsorption of digoxin in rats. *Int J Pharm* 2006; **322**: 79-86 [PMID: 16781832 DOI: 10.1016/j.ijpharm.2006.05.030]

32 **Laouari D**, Yang R, Veau C, Blanke I, Friedlander G. Two apical multidrug transporters, P-gp and MRP2, are differently altered in chronic renal failure. *Am J Physiol Renal Physiol* 2001; **280**: F636-F645 [PMID: 11249855 DOI: 10.1152/ajprenal.2001.280.4.F636]

33 **Kullak-Ublick GA**, Becker MB. Regulation of drug and bile salt transporters in liver and intestine. *Drug Metab Rev* 2003; **35**: 305-317 [PMID: 14705863 DOI: 10.1081/dmr-120026398]

34 **Yamaguchi H**, Sugie M, Okada M, Mikkaichi T, Toyohara T, Abe T, Goto J, Hishinuma T, Shimada M, Mano N. Transport of estrone 3-sulfate mediated by organic anion transporter OATP4C1: estrone 3-sulfate binds to the different recognition site for digoxin in OATP4C1. *Drug Metab Pharmacokinet* 2010; **25**: 314-317 [PMID: 20610891 DOI: 10.2133/dmpk.25.314]

35 **Sinal CJ**, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell* 2000; **102**: 731-744 [PMID: 11030617 DOI: 10.1016/s0092-8674(00)00062-3]

36 **Staudinger JL**, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, Liu Y, Klaassen CD, Brown KK, Reinhard J, Willson TM, Koller BH, Kliewer SA. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc Natl Acad Sci U S A* 2001; **98**: 3369-3374 [PMID: 11248085 DOI: 10.1073/pnas.051551698]

37 **Suzuki T**, Toyohara T, Akiyama Y, Takeuchi Y, Mishima E, Suzuki C, Ito S, Soga T, Abe T. Transcriptional regulation of organic anion transporting polypeptide SLCO4C1 as a new therapeutic modality to prevent chronic kidney disease. *J Pharm Sci* 2011; **100**: 3696-3707 [PMID: 21656517 DOI: 10.1002/jps.22641]

38 **Volz DC**, Kullman SW, Howarth DL, Hardman RC, Hinton DE. Protective response of the Ah receptor to ANIT-induced biliary epithelial cell toxicity in see-through medaka. *Toxicol Sci* 2008; **102**: 262-277 [PMID: 18187559 DOI: 10.1093/toxsci/kfm308]

39 **Xie W**, Evans RM. Orphan nuclear receptors: the exotics of xenobiotics. *J Biol Chem* 2001; **276**: 37739-37742 [PMID: 11459851 DOI: 10.1074/jbc.R100033200]

World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Pathophysiol 2022 May 22; 13(3): 85-95

DOI: 10.4291/wjgp.v13.i3.85

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Retrospective Study

Increasing thirty-day readmissions of Crohn's disease and ulcerative colitis in the United States: A national dilemma

Dushyant Singh Dahiya, Abhilash Perisetti, Asim Kichloo, Amandeep Singh, Hemant Goyal, Laura Rotundo, Madhu Vennikandam, Hafeez Shaka, Gurdeep Singh, Jagmeet Singh, Sailaja Pisipati, Mohammad Al-Haddad, Madhusudhan R Sanaka, Sumant Inamdar

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Khayat AA, Saudi Arabia; Sato Y, Japan

Received: December 10, 2021
Peer-review started: December 10, 2021
First decision: February 15, 2022
Revised: February 20, 2022
Accepted: March 27, 2022
Article in press: March 27, 2022
Published online: May 22, 2022

Dushyant Singh Dahiya, Asim Kichloo, Department of Internal Medicine, Central Michigan University College of Medicine, Saginaw, MI 48601, United States

Abhilash Perisetti, Division of Gastroenterology, Parkview Cancer Institute, Fort Wayne, IN 46845, United States

Amandeep Singh, Madhusudhan R Sanaka, Division of Gastroenterology and Hepatology, Cleveland Clinic Foundation, Cleveland, OH 44195, United States

Hemant Goyal, Department of Internal Medicine, The Wright Center for Graduate Medical Education, Scranton, PA 18505, United States

Hemant Goyal, Department of Internal Medicine, Mercer University School of Medicine, Macon, GA 31207, United States

Laura Rotundo, Section of Digestive Diseases, Yale New Haven Hospital, New Haven, CT 06510, United States

Madhu Vennikandam, Department of Gastroenterology and Hepatology, Sparrow Hospital/Michigan State University College of Human Medicine, Lansing, MI 48912, United States

Hafeez Shaka, Department of Internal Medicine, John H. Stroger, Jr. Hospital of Cook County, Chicago, IL 60612, United States

Gurdeep Singh, Department of Internal Medicine, Our Lady of Lourdes Memorial Hospital, Binghamton, NY 13905, United States

Jagmeet Singh, Department of Internal Medicine, Guthrie Robert Packer Hospital, Sayre, PA 18840, United States

Sailaja Pisipati, Division of Gastroenterology and Hepatology, Mayo Clinic, Scottsdale, AZ 85259, United States

Mohammad Al-Haddad, Division of Gastroenterology and Hepatology, Indiana University School of Medicine, Indianapolis, IN 46202, United States

Sumant Inamdar, Division of Gastroenterology and Hepatology, University of Arkansas for Medical Sciences, Little Rock, AR 72205, United States

Corresponding author: Dushyant Singh Dahiya, MD, Doctor, Department of Internal Medicine, Central Michigan University College of Medicine, 1015 S Washington Ave, Saginaw, MI 48601, United States.
dush.dahiya@gmail.com

Abstract

BACKGROUND

The prevalence of Crohn's disease (CD) and ulcerative colitis (UC) is on the rise worldwide. This rising prevalence is concerning as patients with CD and UC may frequently relapse leading to recurrent hospitalizations and increased healthcare utilization.

AIM

To identify trends and adverse outcomes for 30 d readmissions for CD and UC.

METHODS

This was a retrospective, interrupted trends study involving all adult (≥ 18 years) 30 d readmissions of CD and UC from the National Readmission Database (NRD) between 2008 and 2018. Patients < 18 years, elective, and traumatic hospitalizations were excluded from this study. We identified hospitalization characteristics and readmission rates for each calendar year. Trends of inpatient mortality, mean length of hospital stay (LOS) and mean total hospital cost (THC) were calculated using a multivariate logistic trend analysis adjusting for age, gender, insurance status, comorbidity burden and hospital factors. Furthermore, trends between CD and UC readmissions were compared using regression of the interaction coefficient after adjusting for age and gender to determine relative trends between the two populations. Stata® Version 16 software (StataCorp, TX, United States) was used for statistical analysis and P value ≤ 0.05 were considered statistically significant.

RESULTS

Total number of 30 d readmissions increased from 6202 in 2010 to 7672 in 2018 for CD and from 3272 in 2010 to 4234 in 2018 for UC. We noted increasing trends for 30-day all-cause readmission rate of CD from 14.9% in 2010 to 17.6% in 2018 (P-trend < 0.001), CD specific readmission rate from 7.1% in 2010 to 8.2% in 2018 (P-trend < 0.001), 30-day all-cause readmission rate of UC from 14.1% in 2010 to 15.7% in 2018 (P-trend = 0.003), and UC specific readmission rate from 5.2% in 2010 to 5.6% in 2018 (P-trend = 0.029). There was no change in the risk adjusted trends of inpatient mortality and mean LOS for CD and UC readmissions. However, we found an increasing trend of mean THC for UC readmissions. After comparison, there was no statistical difference in the trends for 30 d all-cause readmission rate, inpatient mortality, and mean LOS between CD and UC readmissions.

CONCLUSION

There was an increase in total number of 30 d readmissions for CD and UC with a trend towards increasing 30 d all-cause readmission rates.

Key Words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Readmissions; Trends

Core Tip: This retrospective interrupted trend study analyzed 30 d readmissions of Crohn's disease (CD) and ulcerative colitis (UC) in the United States from 2010–2018. There was a rising trend for 30 d all-cause readmission rate of CD and UC, and CD- and UC-specific readmission rate throughout the study period. However, we noted no change in the risk adjusted trends of inpatient mortality and mean length of hospital stay (LOS) for 30 d readmissions of CD and UC. Furthermore, there was no statistical difference in the trends for 30 d all-cause readmission rate, inpatient mortality, and mean LOS between CD and UC readmissions.

Citation: Dahiya DS, Perisetti A, Kichloo A, Singh A, Goyal H, Rotundo L, Vennikandam M, Shaka H, Singh G, Singh J, Pisipati S, Al-Haddad M, Sanaka MR, Inamdar S. Increasing thirty-day readmissions of Crohn's disease and ulcerative colitis in the United States: A national dilemma. *World J Gastrointest Pathophysiol* 2022; 13(3): 85-95
URL: https://www.wjgnet.com/2150-5330/full/v13/i3/85.htm
DOI: https://dx.doi.org/10.4291/wjgp.v13.i3.85

INTRODUCTION

Inflammatory bowel disease (IBD) is characterized by chronic inflammation of the gastrointestinal tract with a propensity of remission and relapse over time[1]. It consists of Crohn's disease (CD) and ulcerative colitis (UC)[2]. The exact pathogenesis of IBD is relatively unknown, but researchers believe that factors such as immune response dysregulation, gut microbiota dysbiosis, environmental changes and genetic variants play a key role[3]. In 2017, there were 6.8 million patients with IBD worldwide with studies reporting continuously rising incidence and prevalence, particularly in North America[4]. The rising rates of IBD are concerning as it is associated with a poor quality of life and places significant social and economic burden on individuals and the United States healthcare system[5,6].

Despite outpatient management by gastroenterologists, patients with IBD are at increased risk of readmission due to relapse, complications of the disease or for additional interventions after index hospitalization. This further exacerbates the impact of the disease on individuals and the healthcare system. Additionally, studies have demonstrated that about 9%–50% of IBD readmissions are preventable and may be directly linked to the quality of hospital care and inadequate post-discharge care[7]. Hence, hospital systems have developed scoring systems to identify individuals at the highest risk of readmission and implemented strategies to reduce readmissions and improve the overall quality of care[8].

In current literature, a majority of the studies investigating readmissions of IBD have been single-center experiences or primarily focused on surgical patients[9,10]. There continues to be relative paucity of data on early (30 d) readmissions of CD and UC in the United States. Hence, this national, retrospective, interrupted trends study was designed to identify the hospitalization characteristics and estimate readmission rates of CD and UC in the United States between 2010–2018. We also identified the trends of inpatient mortality to determine improvements in therapeutic management of the disease. Furthermore, we calculated the burden of the disease on the United States healthcare system in terms of healthcare utilization and hospitalization costs.

MATERIALS AND METHODS

Design and data source

This was a retrospective interrupted trends study involving all adult readmissions of IBD (UC and CD) in the United States between 2010–2018. Data for analysis was extracted from the Nationwide Readmissions Database (NRD) which is a part of the Agency for Healthcare Research and Quality (AHRQ) Healthcare Cost and Utilization Project (HCUP) State Inpatient Databases (SID)[11]. It allows for weighted analysis to obtain 100% of the United States hospitalizations within a given calendar year [11]. The data for NRD is collected using the International Classification of Diseases, Ninth/Tenth Revision, Clinical Modification (ICD-9/10-CM/PCS) codes.

Study population

The study involved all adult (≥ 18 years) 30 d readmissions of CD and UC from the NRD for the years 2010, 2012, 2014, 2016 and 2018. We used all available ICD-9-CM/PCS codes for CD (555X) and UC (556X) along with the equivalent ICD-10-CM/PCS codes K50X and K51X for CD and UC, respectively. The precedence for the utilization of these codes has been established in prior published studies[12]. Individuals < 18 years of age, elective and traumatic hospitalizations were excluded from the analysis. Using unique hospitalization identifiers, index hospitalizations of CD and UC were identified and one subsequent hospitalization within 30 d was tagged as a readmission.

Statistical analysis and outcome measures

The data was analyzed using Stata® Version 16 software (StataCorp, TX, United States). All analyses were conducted using weighted samples for national estimates. *P* value ≤ 0.05 was set as the threshold for statistical significance. We highlighted hospitalization trends and obtained the 30 d all-cause readmission rate, disease specific readmission rate and readmission proportion for specific calendar years. The comorbidity burden was assessed using Sundararajan's adaptation of the modified Deyo's

Charlson comorbidity index[13]. Trends of inpatient mortality, mean length of stay (LOS) and mean hospital cost (THC) for CD and UC readmissions were calculated using a multivariate logistic trend analysis adjusting for age, gender, insurance status, comorbidity burden and hospital factors. The total hospital cost was obtained using the HCUP Cost-to-Charge Ratio files and adjusted for inflation using the Medical Expenditure Panel Survey index for hospital care, with 2018 as the reference point[14,15]. Additionally, trends between CD and UC readmissions were compared using regression of the interaction coefficient after adjusting for age and gender to determine relative trends between the two populations. Furthermore, we report no missing data in this study.

Ethical considerations

The NRD database lacks patient and hospital-specific identifiers. Hence, this study was exempt from Institutional Review Board (IRB) approval for analysis as per guidelines put forth by our institutional IRB for research on database studies.

Data availability statement

The NRD is a large publicly available, multi-ethic, all-payer inpatient care database in the United States, containing data on more than 18 million hospital stays/year. The database can be accessed at: https://www.hcup-us.ahrq.gov/nrdoverview.jsp.

RESULTS

CD: Hospitalization characteristics and outcomes for 30 d readmissions

The total number of 30 d readmissions of CD increased from 6202 in 2010 to 7672 in 2018 (Figure 1). The mean age increased from 41.8 ± 0.9 in 2010 to 43.9 ± 0.7 years in 2018. A female predominance was noted throughout the study period (Table 1); however, a statistically significant trend for gender was absent. Additionally, 30 d readmissions of CD were noted to have an increasing comorbidity burden with time (Table 1). Furthermore, metropolitan teaching hospitals had the majority of the readmissions with a statistically significant trend towards increasing readmissions from 52.1% in 2010 to 77% in 2018 (Table 1).

There was a statistically significant trend towards increasing 30 d all-cause readmission rate of CD from 14.9% in 2010 to 17.6% in 2018 (P-trend < 0.001) (Figure 2). The CD specific readmission rate also had a statistically significant increasing trend with an increase from 7.1% in 2010 to 8.2% in 2018 (P-trend < 0.001). However, we did not observe a significant change in the risk adjusted trends of inpatient mortality, mean LOS, and mean THC for these readmissions.

UC: Hospitalization characteristics and outcomes for 30 d readmissions

Similar to CD, the total number of 30 d readmissions of UC increased from 3272 in 2010 to 4234 in 2018 (Figure 1). The mean age for these readmissions increased from 49.8 ± 1.6 in 2010 to 51.2 ± 0.8 years in 2018. A female predominance without a statistical trend for gender and increasing comorbidity burden with time was also noted. Furthermore, metropolitan teaching hospitals had an increasing trend of readmissions from 53.6% in 2010 to 76.3% in 2018 (Table 2), similar to that for CD.

A rising trend was noted for 30 d all cause readmission rate of UC from 14.1% in 2010 to 15.7% in 2018 (P-trend = 0.003) (Figure 2) and for UC specific readmission rate from 5.2% in 2010 to 5.6% in 2018 (P-trend = 0.029). Additionally, the mean THC increased from $13783 in 2010 to $15929 in 2018 ($P$-trend = 0.009) with a rising trend unlike CD. However, similar to CD, a significant change in the risk adjusted trends was absent for of inpatient mortality and mean LOS (Table 3).

Comparison of trends for 30 d readmissions of CD and UC

Although CD had higher number of 30 d readmissions every year, we did not observe a statistically significant difference in the in the trends for 30 d all-cause readmission rate (interaction P-trend = 0.087), inpatient mortality (interaction P-trend = 0.231), and mean LOS (interaction P-trend = 0.388). However, there was a statistically significant trend towards increasing mean THC for 30 d readmissions of UC relative to 30 d readmissions of CD (interaction P-trend < 0.001).

DISCUSSION

It is essential to identify early (30 d) readmissions of IBD as they may be associated with quality of inpatient care, increased risk of adverse outcomes and place significant burden on the United States healthcare system in terms of healthcare costs and resource utilization. Additionally, as providers become aware of the magnitude of these readmissions and the patient demographics most effected, efforts could be directed at index admissions to further optimize medical therapy before discharge, promote patient education and encourage a greater degree of involvement in their care, and increase

Table 1 Biodemographic characteristics and hospitalization trends for 30 d readmissions of Crohn's disease

Variable	Year				
	2010	2012	2014	2016	2018
Number of readmissions	6202	6580	6475	8278	7672
Age (mean ± SE, yr)	41.8 ± 0.9	41.6 ± 1.1	41.2 ± 0.8	42.5 ± 0.7	43.9 ± 0.7
Gender (%)					
Males	45.5	44.0	45.7	46.7	46.5
Females	54.5	56.0	54.3	53.3	53.5
Charlson comorbidity index score (%)					
0	69.7	72.0	69.9	64.9	61.3
1	19.2	15.5	17.3	19.5	20.0
2	5.9	6.1	6.7	7.5	9.0
≥ 3	5.2	6.4	6.1	8.1	9.7
Insurance type (%)					
Medicare	20.5	29.1	29.3	28.9	30.6
Medicaid	21.5	24.9	26.4	25.5	24.7
Private	41.2	37.1	37.0	40.8	39.0
Uninsured	8.8	8.9	7.3	4.8	5.7
Household income quartile (%)					
1st	27.8	29.2	27.9	29.0	28.6
2nd	23.4	25.6	28.5	26.8	30.0
3rd	24.9	25.1	22.5	24.5	23.7
4th	23.9	20.1	21.1	19.7	17.7
Hospital characteristics					
Hospital bed size (%)					
Small	9.9	9.9	14.2	13.3	15.0
Medium	22.4	22.4	27.3	26.9	26.3
Large	67.7	67.7	58.5	59.8	58.7
Teaching status (%)					
Metropolitan non-teaching	39.2	34.4	25.2	21.8	17.3
Metropolitan teaching	52.1	56.8	68.4	72.3	77.0
Non-metropolitan	8.7	8.8	6.4	5.9	5.7
Hospital volume quintiles (%)					
Q1	1.8	1.9	1.5	1.7	1.3
Q2	4.3	5.4	5.1	4.2	4.5
Q3	10.3	10.0	10.2	8.4	10.4
Q4	19.4	18.1	18.1	18.6	19.1
Q5	64.2	64.6	65.1	67.1	64.7

outpatient follow-up, thereby decreasing early readmissions. A single center retrospective study from 2007–2010 revealed that about 5% patients with IBD were readmitted within 1 wk of hospital discharge, 14% within 1 mo, 23% within 3 mo and about 39% within the year[16]. Another study in the United States reported similar findings with a readmission rate of 18% within 1 mo of hospital discharge[17]. In 2013, an NRD-based study estimated 3037 (7%) readmissions of IBD at 30 d[7].

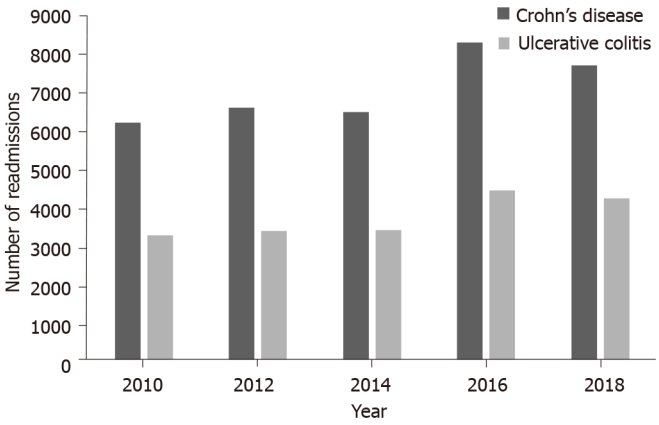
Table 2 Biodemographic characteristics and hospitalization trends for 30 d readmissions of ulcerative colitis

Variable	Year				
	2010	2012	2014	2016	2018
Number of readmissions	3272	3399	3426	4449	4234
Age (mean ± SE, yr)	49.8 ± 1.6	49.6 ± 1.5	48.4 ± 1.1	49.9 ± 1.0	51.2 ± 0.8
Gender (%)					
Males	48.1	45.6	47.5	46.7	49.4
Females	51.9	54.4	52.5	53.3	50.6
Charlson comorbidity index score (%)					
0	57.8	59.6	60.6	55.6	50.9
1	20.3	20.0	18.6	19.4	20.7
2	9.4	9.0	8.5	10.6	10.3
≥ 3	12.5	11.4	12.3	14.4	18.1
Insurance type (%)					
Medicare	36.3	36.6	32.4	35.1	34.8
Medicaid	17.8	17.0	22.3	17.5	19.5
Private	39.4	37.0	40.1	42.2	40.4
Uninsured	6.5	9.4	5.2	5.2	5.3
Household income quartile (%)					
1st	25.5	29.2	26.5	27.2	25.0
2nd	22.5	23.1	25.9	27.5	26.7
3rd	26.4	24.6	22.9	25.0	26.1
4th	25.6	23.1	24.7	20.3	22.2
Hospital characteristics					
Hospital bed size (%)					
Small	10.2	9.8	13.2	13.5	16.8
Medium	19.8	22.4	26.8	25.7	24.3
Large	70.0	67.8	60.0	60.8	58.9
Teaching status (%)					
Metropolitan non-teaching	37.3	38.2	26.1	24.5	19.3
Metropolitan teaching	53.6	53.5	67.7	70.3	76.3
Non-metropolitan	9.1	8.3	6.2	5.2	4.4
Hospital volume quintiles (%)					
Q1	2.4	2.4	2.5	2.1	2.0
Q2	6.0	7.4	5.9	5.8	5.5
Q3	11.8	10.5	11.7	10.3	12.3
Q4	20.2	20.1	19.0	20.4	21.4
Q5	59.6	59.6	60.9	61.4	58.8

In our study, the total number of 30 d readmissions of CD increased from 6202 in 2010 to 7672 in 2018 and for UC from 3,272 in 2010 to 4,234 in 2018, both with a female predominance (Tables 1 and 2). This coincides with rising prevalence of CD and UC in the general population[18]. We also noted an increasing trend for 30 d all-cause readmission rates and disease specific readmission rates for 30 d readmissions of CD and UC (Table 3). These findings may, in part, be due to a rising prevalence of IBD in the general population which increased significantly from 0.9% (2 million adults) in 1999 to 1.3% (3 million adults) in 2015, an increase in the flare-ups of IBD which may account for about 50% of the

Table 3 Readmission rates, inpatient mortality, and healthcare burden for 30 d readmissions of Crohn's disease and ulcerative colitis

Outcomes	Year					*P* trend
	2010	2012	2014	2016	2018	
Crohn's disease						
All-cause readmission rate (%)	14.9	15.5	15.2	18.9	17.6	< 0.001
Crohn's disease specific readmission rate (%)	7.1	6.9	7.0	8.9	8.2	< 0.001
Crohn's disease readmission proportion (%)	54.9	51.8	53.0	55.8	54.6	0.002
Inpatient mortality (%)	0.9	1.4	0.7	0.7	1.0	0.059
Mean length of stay (d)	5.9	5.9	5.3	6.0	6.2	0.927
Mean total hospital cost (USD)	12327	13068	10988	13421	14260	0.210
Ulcerative colitis						
All-cause readmission rate (%)	14.1	14.2	13.5	16.6	15.7	0.003
Ulcerative colitis specific readmission rate (%)	5.2	5.3	5.2	6.1	5.6	0.029
Ulcerative colitis readmission proportion (%)	42.6	42.4	43.4	43.0	41.0	0.566
Inpatient mortality (%)	2.5	1.8	2.2	2.0	2.3	0.912
Mean length of stay (d)	6.8	6.8	6.3	6.8	6.9	0.452
Mean total hospital cost (USD)	13783	13568	13790	15358	15929	0.009

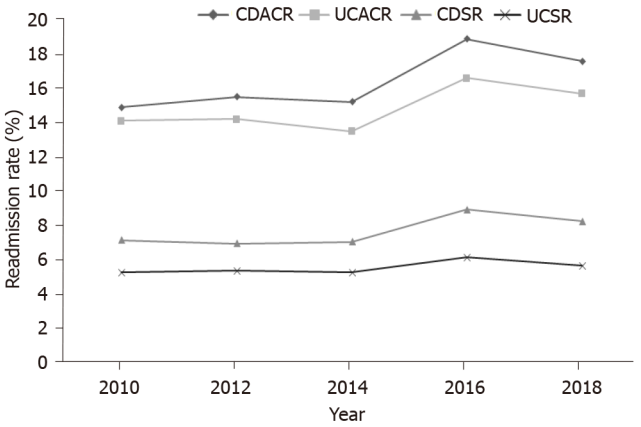


DOI: 10.4291/wjgp.v13.i3.85

Figure 1 Total number of 30 d readmissions of Crohn's disease and ulcerative colitis.

readmissions or due to non-IBD related causes such as infections secondary to the widespread use of biological agents or immunosuppressants[16,18,19]. We performed a trend comparison between 30 d all-cause readmission rate of CD and UC. It was not statistically significant and signified that all-cause readmissions for both CD and UC were increasing proportionately in the United States.

The mean age for 30 d readmissions increased for both CD and UC without a statistically significant trend. The difference in the mean age between the two groups is approximately 7 years. These finding align with current literature which reports that patients with CD tend to be younger and the mean age at the time of diagnosis of CD is usually 5–10 years earlier than that of UC[20]. From a gender standpoint, there is a lower risk of CD until puberty for females when compared to males, after which there is a reversal of this risk[21]. For UC, males and females have a similar incidence until the age of 45 after which males exhibit higher risk of incident UC than females[21]. However, for readmissions of CD and UC, a slight female predominance has been noted in literature[22]. Similarly in our study, a slight female predominance was noted for CD and UC readmissions. Furthermore, we did not find a statistically significant readmission trend for gender over time which implied that the readmission rates for both genders have remained relatively stable. Moreover, we noted an increase in the overall comorbidity burden for 30 d readmissions of CD and UC. This was expected as readmissions for individuals with multiple concurrent co-morbidities have been increasing.



DOI: 10.4291/wjgp.v13.i3.85 **Copyright** ©The Author(s) 2022.

Figure 2 Trends of 30 d readmission following Crohn's disease and ulcerative colitis hospitalizations. CDACR: Crohn's disease all-cause readmission; UCACR: Ulcerative colitis all-cause readmission; CDSR: Crohn's disease specific readmission; UCSR: Ulcerative colitis specific readmission.

From a hospital perspective, large bed-sized hospitals had the highest proportions of 30 d readmissions of CD and UC. This may be due to the fact that larger hospitals have a higher capacity of in-patient admissions. Additionally, metropolitan teaching hospitals consistently had the highest readmission rates with an increasing trend. This may be because these hospitals are usually tertiary care referral center accepting complex patients from large geographical areas and hence, are well equipped with the necessary resources and specialists to manage these readmissions and their complications. Moreover, an urban location, consisting of a greater population density which may be attributed to a demographic shift of non-urban/rural population to urban locations between 2010 and 2018, is more likely to yield higher readmissions[23].

Furthermore, IBD readmissions have been associated with significant inpatient mortality and healthcare burden. As per literature, frailty and length of intensive care unit stay is independently associated with higher rates of inpatient mortality for IBD readmissions[16,24]. From 2010–2014, a study reported that the inpatient mortality for 30 d readmissions of CD was 2.85% per year, the LOS was 6 d, and cost of hospitalization was $11402[25]. In 2017, for 30 d readmissions of UC, literature reported an inpatient mortality of 1.99% along with longer LOS and higher hospitalization costs compared to index admission[26]. In our study, despite an increasing co-morbidity burden (CCI) for the study period, inpatient mortality, and mean LOS for 30 d readmissions of CD and UC did not have a significant change in the risk adjusted trend (Table 3) over time. These stable mortality and LOS trends may reflect optimal guideline driven therapeutic management for the study period. However, the mean THC for 30 d readmission of UC increased from $13783 in 2010 to $15929 ($P$-trend = 0.009) with an increasing trend, while no trend in THC was identified for CD readmissions. Furthermore, a trend comparison of mean THC between CD and UC yielded a statistically significant trend towards increasing mean THC for 30 d readmissions of UC relative to 30 d readmissions of CD. The exact reason for these THC findings is unclear but may be attributed to an increased complexity and complications of UC readmission requiring immediate higher level of care, additional endoscopic interventions, and a multi-disciplinary team approach for management.

Directing our focus to individual calendar years, we noted a decrease in the total number of readmissions for both CD and UC from 2016 to 2018 (Tables 1 and 2). Similarly, the 30 d all-cause readmissions rate and disease specific readmission rate also decreased from 2016 to 2018 (Table 3). These findings may be due to an overall decrease in the readmissions for one particular calendar year and do not reflect an overall trend. In fact, as discussed earlier, when trended from 2010 to 2018, we noted an increasing trend for all-cause readmissions rate and disease specific readmission rate, and with respect to 2010, there was an overall increase in the total number of 30 d readmissions of CD and UC. Hence, future larger studies are needed to assess rate of readmissions from 2018 to evaluate the trends further.

Strength and limitations

The key strengths of this study include the study population, unique study design, and methodology which allowed for a comprehensive analysis. As the data was collected from one of the largest databases containing information on readmissions from hospitals across the United States, the results are applicable to hospitals throughout the United States. Additionally, we studied a 9-year time frame which helped us establish meaningful trends. However, important limitations exist with this study. The NRD does not contain data on the severity of the disease and therefore, we were unable to further stratify the readmissions based on the severity of CD or UC. The NRD also lacks data on the total duration of the illness and the exact duration after discharge to readmissions, limiting our ability to

assess index admissions more prone to earlier readmissions. Furthermore, it does not contain information on the pharmacological treatment, hospital course and management of IBD readmissions. Hence, we could not comment on the treatment aspects of these readmissions. Moreover, this study is amenable to all biases associated with retrospective studies. Finally, the NRD is an administrative database and therefore, susceptible to coding errors. Despite these limitations, this study helps us better understand the hospitalizations characteristics and trends of 30 d readmissions for CD and UC which is critical for management of these patients.

CONCLUSION

In conclusion, the total number of 30 d readmission for CD and UC increased. UC readmissions were older than CD readmissions. We noted an increasing trend for 30 d all-cause readmission rate for CD and UC. However, there was no statistical change in the risk adjusted trends of inpatient mortality and mean LOS for these readmissions. The mean total healthcare cost for 30 d readmissions of UC had a rising trend while no trend was observed for CD readmissions. Future prospective studies are needed to further study these findings.

ARTICLE HIGHLIGHTS

Research background

The prevalence of inflammatory bowel disease (IBD) continues to be on the rise around the globe. Despite outpatient management, these patients are at increased risk of relapse leading to hospitalizations and subsequent readmissions.

Research motivation

Through this study, we attempted to outline the magnitude, characteristics and outcomes of early (30 d) readmissions of IBD in the United States.

Research objectives

This national, retrospective, interrupted trends study aimed to identify hospitalization characteristics, readmission rates, adverse outcomes, and healthcare burden for 30 d readmissions of Crohn's disease (CD) and ulcerative colitis (UC) in the United States between 2010-2018.

Research methods

This was a retrospective, interrupted trends which analyzed data from the National Readmission Database (NRD) on all adult 30 d readmissions of CD and UC in the United States between 2010-2018. Patients < 18 years of age, elective and traumatic hospitalizations were excluded from the analysis. Hospitalization characteristics, readmission rates, adverse outcomes and the healthcare burden was identified. *P*-values ≤ 0.05 were considered statistically significant.

Research results

Total number of 30 d readmissions increased from 6202 in 2010 to 7672 in 2018 for CD and from 3272 in 2010 to 4234 in 2018 for UC. There was an increase in the 30 d all-cause readmission rate of CD and UC for the study period. We did not observe a change in the risk adjusted trends of inpatient mortality and mean length of hospital stay (LOS) for CD and UC readmissions. However, there was a rising trend of mean THC for UC readmissions. After comparison, there was no statistical difference in the trends for 30 d all-cause readmission rate, inpatient mortality, and mean LOS between CD and UC readmissions.

Research conclusions

From 2010 to 2018, there was an increase in the total number of 30 d readmissions with a trend towards increasing 30 d all-cause readmission rates for CD and UC. However, there was no change in the risk adjusted trends of inpatient mortality.

Research perspectives

This study helps clinicians better understand the magnitude and characteristics of 30 d readmissions of CD and UC in the United States. Through this study, we also aim to encourage and promote future research on readmissions of IBD.

FOOTNOTES

Author contributions: Dahiya DS, Kichloo A and Sumant Inamdar S contributed to the conception and design; Dahiya DS, Kichloo A, Al-Haddad M contributed to the administrative support; Kichloo A and Shaka H contributed to the provision, collection, and assembly of data; Dahiya DS, Perisetti A, Singh A, Al-Haddad M, Sanaka MR and Sumant Inamdar S revised the key components of manuscript; and All authors reviewed the literature, drafted the manuscript, finally approved the manuscript, and agreement to be accountable for all aspects of the work.

Institutional review board statement: As the National Readmission Database does not contain patient-specific and hospital-specific identifiers, this study was exempt from the Institutional Review Boards (IRB) as per guidelines put forth by the IRB for research on database studies.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that was obtained after analysis of a national database.

Conflict-of-interest statement: All authors have no financial relationships to disclose.

Data sharing statement: The NIS database can be accessed at https://www.hcup-us.ahrq.gov. No additional data is available.

Country/Territory of origin: United States

ORCID number: Dushyant Singh Dahiya 0000-0002-8544-9039; Abhilash Perisetti 0000-0003-4074-6395; Asim Kichloo 0000-0003-4788-8572; Amandeep Singh 0000-0001-8581-1408; Hemant Goyal 0000-0002-9433-9042; Laura Rotundo 0000-0002-4094-3682; Hafeez Shaka 0000-0002-9456-4581; Gurdeep Singh 0000-0001-6044-7419; Jagmeet Singh 0000-0001-7179-1020; Sailaja Pisipati 0000-0000-0000-0001; Mohammad Al-Haddad 0000-0003-1641-9976; Madhusudhan R Sanaka 0000-0003-2506-8602; Sumant Inamdar 0000-0002-1002-2823.

Corresponding Author's Membership in Professional Societies: American College of Gastroenterology, No. 59498; American Gastroenterological Association, No. 1550524.

S-Editor: Ma YJ
L-Editor: A
P-Editor: Ma YJ

REFERENCES

1 **Ponder A**, Long MD. A clinical review of recent findings in the epidemiology of inflammatory bowel disease. *Clin Epidemiol* 2013; **5**: 237-247 [PMID: 23922506 DOI: 10.2147/CLEP.S33961]
2 **Lamb CA**, Kennedy NA, Raine T, Hendy PA, Smith PJ, Limdi JK, Hayee B, Lomer MCE, Parkes GC, Selinger C, Barrett KJ, Davies RJ, Bennett C, Gittens S, Dunlop MG, Faiz O, Fraser A, Garrick V, Johnston PD, Parkes M, Sanderson J, Terry H; IBD guidelines eDelphi consensus group, Gaya DR, Iqbal TH, Taylor SA, Smith M, Brookes M, Hansen R, Hawthorne AB. British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. *Gut* 2019; **68**: s1-s106 [PMID: 31562236 DOI: 10.1136/gutjnl-2019-318484]
3 **Lee SH**, Kwon JE, Cho ML. Immunological pathogenesis of inflammatory bowel disease. *Intest Res* 2018; **16**: 26-42 [PMID: 29422795 DOI: 10.5217/ir.2018.16.1.26]
4 **GBD 2017 Inflammatory Bowel Disease Collaborators**. The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2020; **5**: 17-30 [PMID: 31648971 DOI: 10.1016/S2468-1253(19)30333-4]
5 **Knowles SR**, Graff LA, Wilding H, Hewitt C, Keefer L, Mikocka-Walus A. Quality of Life in Inflammatory Bowel Disease: A Systematic Review and Meta-analyses-Part I. *Inflamm Bowel Dis* 2018; **24**: 742-751 [PMID: 29562277 DOI: 10.1093/ibd/izx100]
6 **Park KT**, Ehrlich OG, Allen JI, Meadows P, Szigethy EM, Henrichsen K, Kim SC, Lawton RC, Murphy SM, Regueiro M, Rubin DT, Engel-Nitz NM, Heller CA. The Cost of Inflammatory Bowel Disease: An Initiative From the Crohn's & Colitis Foundation. *Inflamm Bowel Dis* 2020; **26**: 1-10 [PMID: 31112238 DOI: 10.1093/ibd/izz104]
7 **Micic D**, Gaetano JN, Rubin JN, Cohen RD, Sakuraba A, Rubin DT, Pekow J. Factors associated with readmission to the hospital within 30 days in patients with inflammatory bowel disease. *PLoS One* 2017; **12**: e0182900 [PMID: 28837634 DOI: 10.1371/journal.pone.0182900]
8 **George LA**, Martin B, Gupta N, Nikhil S, Mukund V, Naik AS. Predicting 30-Day Readmission Rate in Inflammatory Bowel Disease Patients: Performance of LACE Index. *Crohns Colitis 360* 2019; **1**: otz007 [DOI: 10.1093/crocol/otz007]

9 **Kulaylat AN**, Hollenbeak CS, Sangster W, Stewart DB Sr. Impact of smoking on the surgical outcome of Crohn's disease: a propensity-score matched National Surgical Quality Improvement Program analysis. *Colorectal Dis* 2015; **17**: 891-902 [PMID: 25808234 DOI: 10.1111/codi.12958]

10 **Damle RN**, Alavi K. Risk factors for 30-d readmission after colorectal surgery: a systematic review. *J Surg Res* 2016; **200**: 200-207 [PMID: 26216748 DOI: 10.1016/j.jss.2015.06.052]

11 **Overview of the Nationwide Readmissions Database (NRD)**. Overview of the Nationwide Readmissions Database (NRD), Rockville, MD: Healthcare Cost and Utilization Project; 2018. Available from: https://www.hcup-us.ahrq.gov/nrdoverview.jsp

12 **Barnes EL**, Kochar B, Long MD, Kappelman MD, Martin CF, Korzenik JR, Crockett SD. Modifiable Risk Factors for Hospital Readmission Among Patients with Inflammatory Bowel Disease in a Nationwide Database. *Inflamm Bowel Dis* 2017; **23**: 875-881 [PMID: 28426473 DOI: 10.1097/MIB.0000000000001121]

13 **Sundararajan V**, Quan H, Halfon P, Fushimi K, Luthi JC, Burnand B, Ghali WA; International Methodology Consortium for Coded Health Information (IMECCHI). Cross-national comparative performance of three versions of the ICD-10 Charlson index. *Med Care* 2007; **45**: 1210-1215 [PMID: 18007172 DOI: 10.1097/MLR.0b013e3181484347]

14 **Cost-to-Charge Ratio Files**. Cost-to-Charge Ratio Files, Rockville, MD: Healthcare Cost and Utilization Project; 2020. Available from: https://www.hcup-us.ahrq.gov/db/ccr/costtocharge.jsp

15 **Dunn A**, Grosse SD, Zuvekas SH. Adjusting Health Expenditures for Inflation: A Review of Measures for Health Services Research in the United States. *Health Serv Res* 2018; **53**: 175-196 [PMID: 27873305 DOI: 10.1111/1475-6773.12612]

16 **Mudireddy P**, Scott F, Feathers A, Lichtenstein GR. Inflammatory Bowel Disease: Predictors and Causes of Early and Late Hospital Readmissions. *Inflamm Bowel Dis* 2017; **23**: 1832-1839 [PMID: 28858068 DOI: 10.1097/MIB.0000000000001242]

17 **Hazratjee N**, Agito M, Lopez R, Lashner B, Rizk MK. Hospital readmissions in patients with inflammatory bowel disease. *Am J Gastroenterol* 2013; **108**: 1024-1032 [PMID: 23820989 DOI: 10.1038/ajg.2012.343]

18 **Dahlhamer JM**, Zammitti EP, Ward BW, Wheaton AG, Croft JB. Prevalence of Inflammatory Bowel Disease Among Adults Aged ≥18 Years - United States, 2015. *MMWR Morb Mortal Wkly Rep* 2016; **65**: 1166-1169 [PMID: 27787492 DOI: 10.15585/mmwr.mm6542a3]

19 **Malhotra A**, Mandip KC, Shaukat A, Rector T. All-cause hospitalizations for inflammatory bowel diseases: Can the reason for admission provide information on inpatient resource use? *Mil Med Res* 2016; **3**: 28 [PMID: 27602233 DOI: 10.1186/s40779-016-0098-x]

20 **Duricova D**, Burisch J, Jess T, Gower-Rousseau C, Lakatos PL; ECCO-EpiCom. Age-related differences in presentation and course of inflammatory bowel disease: an update on the population-based literature. *J Crohns Colitis* 2014; **8**: 1351-1361 [PMID: 24951261 DOI: 10.1016/j.crohns.2014.05.006]

21 **Rustgi SD**, Kayal M, Shah SC. Sex-based differences in inflammatory bowel diseases: a review. *Therap Adv Gastroenterol* 2020; **13**: 1756284820915043 [PMID: 32523620 DOI: 10.1177/1756284820915043]

22 **Cohen-Mekelburg S**, Rosenblatt R, Wallace B, Shen N, Fortune B, Waljee AK, Saini S, Scherl E, Burakoff R, Unruh M. Inflammatory bowel disease readmissions are associated with utilization and comorbidity. *Am J Manag Care* 2019; **25**: 474-481 [PMID: 31622063]

23 **United States Census Bureau**. 2020 Census Statistics Highlight Local Population Changes and Nation's Racial and Ethnic Diversity; 2020. Available from: https://www.census.gov/newsroom/press-releases/2021/population-changes-nations-diversity.html

24 **Qian AS**, Nguyen NH, Elia J, Ohno-Machado L, Sandborn WJ, Singh S. Frailty Is Independently Associated with Mortality and Readmission in Hospitalized Patients with Inflammatory Bowel Diseases. *Clin Gastroenterol Hepatol* 2021; **19**: 2054-2063.e14 [PMID: 32801013 DOI: 10.1016/j.cgh.2020.08.010]

25 **Kruger AJ**, Hinton A, Afzali A. To the Editors: Index Severity Score and Early Readmission Predicts Increased Mortality in Crohn's Disease Patients. *Inflamm Bowel Dis* 2019; **25**: e74-e76 [PMID: 30597020 DOI: 10.1093/ibd/izy391]

26 **Weissman S**, Sharma S, Fung BM, Aziz M, Feuerstein JD. Increased Mortality and Healthcare Costs upon Hospital Readmissions of Ulcerative Colitis Flares: A Large Population-based Cohort Study. *Crohns Colitis 360* 2021; **3**: otab029 [DOI: 10.1093/crocol/otab029]

World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Pathophysiol 2022 May 22; 13(3): 96-106

DOI: 10.4291/wjgp.v13.i3.96

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Observational Study

Utility of FibroScan-based scoring systems to narrow the risk group of nonalcoholic fatty liver disease with comorbidities

Kouichi Miura, Hiroshi Maeda, Naoki Morimoto, Shunji Watanabe, Mamiko Tsukui, Yoshinari Takaoka, Hiroaki Nomoto, Rie Goka, Kazuhiko Kotani, Hironori Yamamoto

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): E

P-Reviewer: Cao X, China; Portius D, Germany

Received: January 8, 2022
Peer-review started: January 8, 2022
First decision: March 9, 2022
Revised: March 21, 2022
Accepted: May 14, 2022
Article in press: May 14, 2022
Published online: May 22, 2022

Kouichi Miura, Hiroshi Maeda, Naoki Morimoto, Shunji Watanabe, Mamiko Tsukui, Yoshinari Takaoka, Hiroaki Nomoto, Rie Goka, Hironori Yamamoto, Department of Medicine, Division of Gastroenterology, Jichi Medical University School of Medicine, 3311-1 Yakushiji, Shimotsuke 329-0498, Tochigi, Japan

Kazuhiko Kotani, Division of Community and Family Medicine, Center for Community Medicine, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke 329-0498, Japan

Corresponding author: Kouichi Miura, Doctor, Associate Professor, Department of Medicine, Division of Gastroenterology, Jichi Medical University School of Medicine, 3311-1 Yakushiji, Shimotsuke 329-0498, Tochigi, Japan. miura385@jichi.ac.jp

Abstract

BACKGROUND

Vibration-controlled transient elastography (VCTE) is proposed as a second step of examination to assess liver fibrosis in patients with nonalcoholic fatty liver disease (NAFLD) after triaging by the fibrosis-4 (FIB-4) index. Recently, VCTE-based scoring systems, including FibroScan-AST (FAST), Agile 3+, and Agile 4, emerged to determine the status of NAFLD. However, the significance of these scoring systems remains unknown in narrowing the high-risk group of NAFLD patients with comorbidities, including hepatocellular carcinoma (HCC) and esophagogastric varices (EGV).

AIM

To clarify the significance of VCTE-based scoring systems to narrow the high-risk group of NAFLD patients with comorbidities.

METHODS

We performed a cross-sectional study to investigate the usefulness of VCTE-based scoring systems and other fibrosis markers to narrow the high-risk group of patients with NAFLD. FIB-4 index was used for the first triage. Risk groups of FAST, Agile 3+, and Agile 4 were stratified according to the published data. Among the 191 patients with NAFLD, there were 26 (14%) and 25 patients (13%) with HCC and EGV, respectively.

RESULTS

When 1.3 was used as a cutoff value, the FIB-4 index narrowed the risk group to

120 patients, in which all patients with HCC and/or EGV were included. High risk group of Agile 3+ could subsequently narrow the risk group. The prevalence of HCC and EGV at this step were 33% (26/80) and 31% (25/80), respectively. In further narrowing of EGV, Agile 4 aggregated the patients with EGV into 43 patients, of whom 23 (53%) had EGV. FAST failed to narrow the risk group of patients with comorbidities. When 2.6 was used as a cutoff value of the FIB-4 index, three patients with HCC and two patients with EGV were missed at the first triage.

CONCLUSION

Agile 3+ and Agile 4 are useful to narrow the NAFLD patient group, in which patients may have HCC and/or EGV.

Key Words: Nonalcoholic fatty liver disease; Vibration controlled transient elastography; Non-invasive test; Hepatocellular carcinoma; Varix

Core Tip: It is necessary to narrow the high-risk group of nonalcoholic fatty liver disease (NAFLD) patients with comorbidities, including hepatocellular carcinoma (HCC) and esophagogastric varices (EGV). Although the fibrosis-4 index is an excellent formula to narrow the high-risk group, there remain many patients to be ruled out. Vibration controlled transient elastography (VCTE) is proposed as a second step examination. FibroScan-AST, Agile 3+, and Agile 4 emerged as VCTE-based scoring systems to determine the status of patients with NAFLD. Here, we demonstrated that Agile 3+ and Agile 4 are good tools to narrow the high-risk group of patients with HCC and/or EGV.

Citation: Miura K, Maeda H, Morimoto N, Watanabe S, Tsukui M, Takaoka Y, Nomoto H, Goka R, Kotani K, Yamamoto H. Utility of FibroScan-based scoring systems to narrow the risk group of nonalcoholic fatty liver disease with comorbidities. *World J Gastrointest Pathophysiol* 2022; 13(3): 96-106
URL: https://www.wjgnet.com/2150-5330/full/v13/i3/96.htm
DOI: https://dx.doi.org/10.4291/wjgp.v13.i3.96

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. A subset of patients with NAFLD can progress to liver cirrhosis, in which patients may have comorbidities, including hepatocellular carcinoma (HCC) and esophagogastric varices (EGV). Current studies have demonstrated that liver fibrosis is a prognostic factor of patients with NAFLD because comorbidities of NAFLD are noted in patients with liver fibrosis[1,2]. Thus, the assessment of liver fibrosis is essential to identifying patients with comorbidities.

Although liver biopsy remains the gold standard to assess liver fibrosis, it is costly and has a risk of complications, including bleeding. In addition, it is difficult to perform liver biopsy in all patients with NAFLD because the global prevalence of patients with NAFLD is approximately 25%[3]. Thus, the demand for noninvasive tests (NITs) to assess liver fibrosis is expanding. Currently, there are several markers and formulae to assess liver fibrosis using clinical parameters without liver biopsy[4]. In addition, imaging studies, including elastography and magnetic resonance imaging (MRI), are used as NITs for the assessment of liver fibrosis. Each method has both advantages and disadvantages. Among NITs, the fibrosis-4 (FIB-4) index is a widely used formula because this formula uses only 4 components, including age, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and platelet count[5], which are easily available not only for hepatologists but also for general physicians. The merits of using the FIB-4 index are high accuracy and low cost[6]. In addition, many validation studies have been performed in chronic liver diseases, including NAFLD. Furthermore, the FIB-4 index is useful for identifying NAFLD patients with extrahepatic comorbidities, including cardiovascular diseases[7]. However, elderly patients tend to show a high score. In addition, there are many patients who show an intermediate risk for liver fibrosis. As a result, the FIB-4 index is used in the first step to narrow the high-risk group of patients who may have comorbidities of NAFLD.

FibroScan, a vibration-controlled transient elastography (VCTE), is proposed as the second step of NIT that can identify such patients[8]. Liver stiffness measurement (LSM) ≥ 11.9 KPa by FibroScan is highly suspected of liver fibrosis over F4[9]. Although FibroScan shows high sensitivity and specificity in the diagnosis of liver fibrosis, some patients have unexpectedly high LSM, probably due to the presence of obesity and the examiners' skill. Thus, a combination of LSM and laboratory data may reflect a more accurate status of patients with NAFLD. To this end, FibroScan-based scoring systems,

including FibroScan-AST (FAST)[10], Agile 3+[11] and Agile 4[12], have been developed. These scoring systems use data obtained from FibroScan and some clinical parameters, including age, sex, AST, ALT, platelet count, and diabetes status. Among these scoring systems, FAST was designed to identify NAFLD patients with liver fibrosis F ≥ 2. Agile 3+ and Agile 4 were designed to identify NAFLD patients with liver fibrosis at F3-F4 and F4, respectively. Although these FibroScan-based scoring systems are correlated with liver fibrosis, little data are available on the significance of identifying NAFLD patients with comorbidities. Thus, the aim of the present cross-sectional study was to investigate the utility of these FibroScan-based scoring systems to narrow the high-risk group of NAFLD patients with comorbidities after triaging by the FIB-4 index.

MATERIALS AND METHODS

Patients

We investigated 191 patients with NAFLD who visited our hospital between April 2019 and March 2022. The diagnosis of NAFLD was made as follows: Steatosis was determined by an ultrasonographic examination conducted by well-experienced gastroenterologists. Steatosis pointing out past examinations was included. Men who used alcohol > 30 g/d and women who used > 20 g/d were excluded. Patients with HBV infection (positive for HBs antigen), HCV infection (positive for HCV antibody) and other liver diseases, including autoimmune hepatitis and primary biliary cholangitis, were also excluded. In addition, we used data obtained from FibroScan as well as blood tests, including the FIB-4 index and *Wisteria floribunda* agglutinin-positive Mac2-binding protein glycosylation isomer (M2BPGi). Diagnosis of diabetes was defined as a fasting blood glucose ≥ 126 mg/dL, hemoglobin A1c ≥ 6.5% and/or antidiabetic drug use. All patients in the present study had FibroScan examination as well as blood tests. This study was approved by the Institutional Review Board of Jichi Medical University (20-175). The study was performed according to the ethical guidelines of the Declaration of Helsinki.

FibroScan-based scoring systems

Transient elastography was performed with FibroScan (Echosens, Paris, France), using an M probe. The FIB-4 index, FAST score, Agile 3+, and Agile 4 were calculated according to published formulae using age, controlled attenuation parameter (CAP), LSM, AST, ALT, platelet count, and presence of diabetes (Supplementary Figure 1). The impact of these parameters on the scoring systems were shown in Supplementary Table 1. Blood data obtained on the same day of FibroScan examination or within 1 mo from the examination were used (Supplementary Figure 2). CAP and LSM were the mean data of 10 consecutive examinations.

Risk assessments for each formula and factor are shown in Supplementary Table 2. In addition, Baveno VI criteria[13], expanded Baveno VI criteria[14], and New NFLD-cirrhosis criteria[15] were also assessed in narrowing the risk group of patients with EGV.

Diagnosis of HCC and EGV

The diagnosis of HCC was made by hepatologists and radiologists using contrast-enhanced computed tomography and/or contrast-enhanced MRI and/or contrast-enhanced ultrasonography. Histologically proven HCC were also added. Form 1 ≤ were defined as having EGV in patients who underwent esophagogastroduodenal endoscopy (EGD)[16,17]. Patients with histories of HCC and/or endoscopic variceal treatment were included as shown in Supplementary Figure 2. If patients did not have EGD examination within 1 year, we interviewed a history of gastrointestinal bleeding from gastrointestinal varices. If patients reported no history of variceal bleeding, the patient was defined as having no EGV.

Statistical analysis

Statistical analyses were performed using Stata 17 (STATA Corporation, College Station, United States). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. For patient background evaluation, analyses were performed by the chi-square test or Fisher's test as appropriate. In addition, the Mann-Whitney U test was used in a comparison of two groups. In a comparison of three groups, one-way analysis of variance was used. All P values < 0.05 were considered statistically significant.

RESULTS

The characteristics of NAFLD patients with HCC and/or EGV

Table 1 shows the characteristics of patients. The median age was 62 years old, 81 (42.4%) were male, and 75 (39.3%) had diabetes. There were 26 patients with HCC and 25 patients with EGV. Among these patients with HCC and/or EGV, 17 had HCC alone, 16 had EGV alone, and 9 had both HCC and EGV.

Table 1 Characteristics of patients with nonalcoholic fatty liver disease

Patients (*n*)	191
Age (years old)	62 (20-90)
Men (%)	81 (42.4)
diabetes (%)	75 (39.3)
HCC	17
EGV	16
Both HCC and EGV	9
AST (U/L)	36 (13-208)
ALT (U/L)	40 (10-214)
Platelet count ($\times 10^9$/L)	207 (45-445)

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; EGV: Esophagogastric varices; HCC: Hepatocellular carcinoma.

Then, we investigated the scores and values of each formula and marker in patients with HCC and/or EGV (Figure 1). In FIB-4 and FAST, the maximum and the minimum of scores were similar among patients with HCC and/or EGV. In Agile 3+, patients with HCC and/or EGV aggregated into a zone of high score. In Agile 4, LSM, and M2BPGi, the score and values tended to show a stepwise increase from HCC, EGV, and both HCC and EVG.

The high to intermediate-risk group of FIB-4 index includes all patients with HCC and/or EGV

In a stratification of the FIB-4 index, there were 71, 51, and 69 patients in the low-, intermediate-, and high-risk groups, respectively. No patients with HCC and/or EGV were noted in the low-risk group of the FIB-4 index, while three patients with HCC and two patients with EGV were in the intermediate stage. The remining patients with HCC and/or EGV were in the high-risk group (Tables 2 and 3). Thus, the high to intermediate-risk group of FIB-4 index is suitable for the first triage.

The high-risk group of Agile 3+ includes all patients with HCC and/or HGV

Then, we investigated the prevalence of patients with HCC and/or EGV (Tables 2 and 3). When the patients were divided into two groups, including low-risk and high to intermediate-risk, there were no patients with HCC and/or EGV in the low-risk group of Agile 3+ (Table 2). In addition, Agile 3+ was the only examination that included all patients with HCC and/or EGV in the high-risk group (Table 3). As a result, Agile 3+ showed extremely high sensitivity and NPV. In contrast, there were patients with HCC in the low-risk group of FAST, Agile 4, LSM, and M2BPGi and patients with EGV in the low-risk group of FAST, Agile 4, and M2BPGi (Table 2), suggesting that FAST, Agile 4, LSM, and M2BPGi are unsuitable for screening of patients with HCC and/or EGV. Thus, Agile 3+ is a good tool to narrow the high-risk group of patients with HCC and/or EGV.

Agile 4 is a potential tool to narrow the patients with EGV

Although the Agile 3+ could narrow the patients with EGV, we further attempted to narrow the patients with EGV. Patients with EGV tended to have a more advanced stage of fibrosis based on Agile 4, LSM, and M2BPGi (Figure 1). Although there were no patients with EGV in the low-risk group of LSM, the PPV was 21% (Table 2). In contrast, the high-risk groups of Agile 4 and M2BPGi missed one patient with EGV, their PPVs were higher than that of LSM. In addition, the PPV of the high-risk group of Agile 4 was 56%, the highest among tests (Table 3). Despite the high-risk group of Agile 4 missed two patients with EGV, Agile 4 is a potential tool to narrow the risk group of patients with EGV.

Baveno VI and its derivatives did not work in our patient group

Baveno VI criteria, expanded Baveno VI criteria, and new NAFLD-cirrhosis criteria, using LSM and platelet count, are simple tools to rule out patients with varices needing treatment. There were 13 (52%), 17 (68%), and 19 patients (76%) with EGV who were defined as "rule out "of the Baveno VI criteria, expanded Baveno VI criteria, and new NAFLD-cirrhosis criteria, respectively (Table 4). Thus, it was difficult to narrow the patients with EGV using a combination of LSM and platelet count.

Agile 3+ and Agile 4 are good tools to narrow the patients with HCC and/or EGV

We applied our patient group to determine whether VCTE-based scoring systems and other fibrosis markers can narrow the risk group of patients with HCC and/or EGV after triaging by the FIB-4 index (Figure 2A). There were 26 patients with HCC (14%) and 25 patients with EGV (13%) among 191

Table 2 Sensitivity, specificity, positive predictive value, and negative predictive value of each score and marker (L *vs* I-H)

	FIB-4		FAST		Agile 3+		Agile 4		LSM		M2BPGi	
Risk	L	I-H	L	I-H	L	I-H	L	I-H	L	I-H	L	I-H
n	71	120	87	104	96	95	131	60	73	118	102	89
HCC	0	26	10	16	0	26	7	19	4	22	5	21
P value	< 0.01		0.44		< 0.01		< 0.01		< 0.01		< 0.01	
Sensitivity		1		0.62		1		0.73		0.85		0.81
Specificity		0.43		0.53		0.58		0.79		0.44		0.62
PPV		0.22		0.17		0.27		0.36		0.19		0.25
NPV		1		0.90		1		0.95		0.95		0.95
EGV	0	25	6	19	0	25	1	24	0	25	1	24
P value	< 0.01		0.02		< 0.01		< 0.01		< 0.01		< 0.01	
Sensitivity		1		0.76		1		0.96		1		0.96
Specificity		0.43		0.52		0.58		0.79		0.44		0.61
PPV		0.21		0.19		0.26		0.41		0.21		0.27
NPV		1		0.94		1		0.99		1		0.99

L: Low-risk; I: Intermediate-risk; H: High-risk; HCC: Hepatocellular carcinoma; EGV: Esophagogastric varix; HCC: Hepatocellular carcinoma; EGV: Esophagogastric varices; PPV: Positive predictive value, NPV: Negative predictive value; M2BPGi: Mac2-binding protein glycosylation isomer; FAST: FibroScan-AST; FIB-4: Fibrosis-4; LSM: Liver stiffness measurement.

Table 3 Sensitivity, specificity, positive predictive value, and negative predictive value of each score and marker (L-I *vs* H)

	FIB-4		FAST		Agile 3+		Agile 4		LSM		M2BPGi	
Risk	L-I	H	L-I	H	L-I	H	L-I	H	L-I	H	L-I	H
n	122	69	146	45	111	80	148	43	136	55	148	43
HCC	3	23	18	8	0	26	12	14	10	16	11	15
P value	< 0.01		0.35		< 0.01		< 0.01		< 0.01		< 0.01	
Sensitivity		0.89		0.31		1		0.54		0.62		0.58
Specificity		0.74		0.89		0.67		0.90		0.82		0.90
PPV		0.35		0.30		0.33		0.45		0.36		0.47
NPV		0.98		0.89		1		0.93		0.93		0.93
EGV	2	23	14	11	0	25	2	23	3	22	4	21
P value	< 0.01		0.01		< 0.01		< 0.01		< 0.01		< 0.01	
Sensitivity		0.92		0.44		1		0.92		0.88		0.84
Specificity		0.74		0.88		0.67		0.89		0.82		0.89
PPV		0.34		0.36		0.31		0.56		0.42		0.54
NPV		0.98		0.91		1		0.99		0.98		0.97

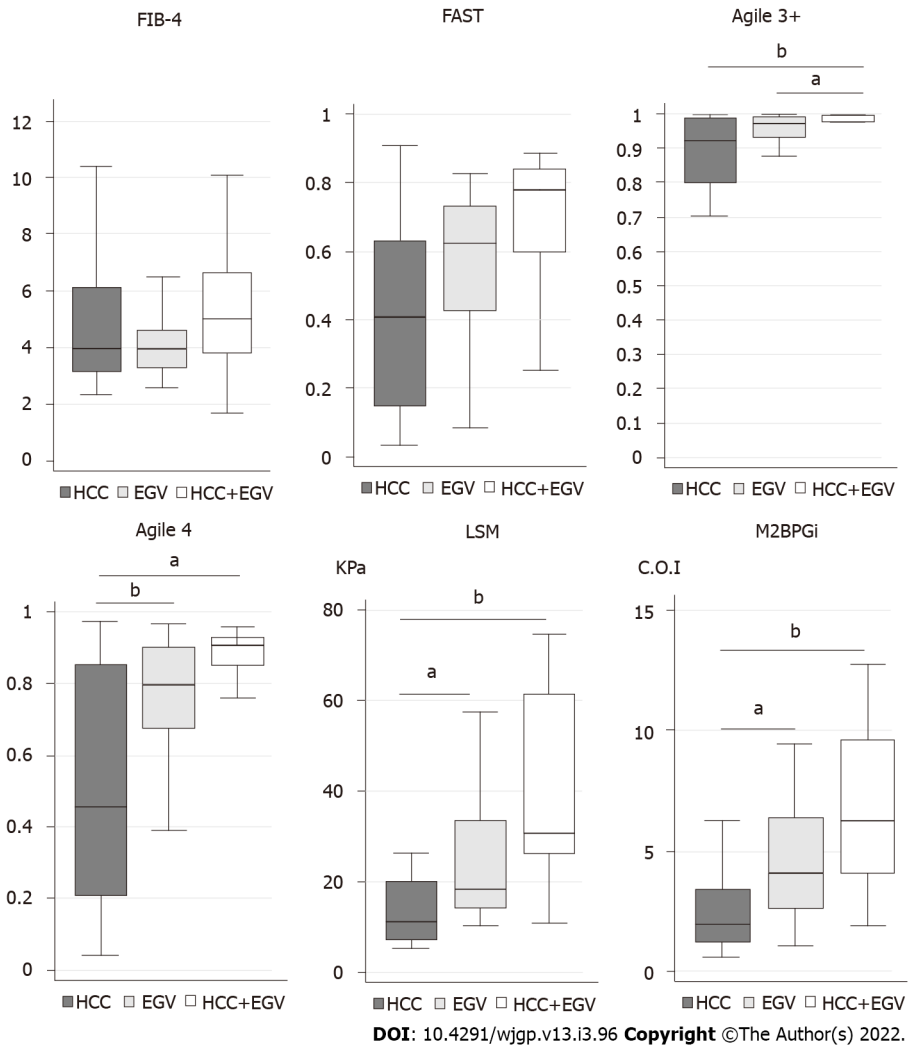
EGV: Esophagogastric varices; HCC: Hepatocellular carcinoma; PPV: Positive predictive value; NPV: Negative predictive value; M2BPGi: Mac2-binding protein glycosylation isomer; FAST: FibroScan-AST; FIB-4: Fibrosis-4; LSM: Liver stiffness measurement.

patients. At the first triage using the FIB-4 index at 1.3 (high to intermediate-risk group), we could narrow the risk group to 120 patients, in whom all patients with HCC and/or EGV were included. In the first step, the prevalence of HCC and EGV was 22% (26/120) and 21% (25/120), respectively. Then, we narrowed the patients using Agile 3+ at the second step, in which all patients with HCC and/or

Table 4 The prevalence of esophagogastric varices in Baveno VI criteria and its derivatives

	Baveno VI		Exp. Baveno VI		New NASH C.C	
	LSM	platelet	LSM	platelet	LSM	platelet
	< 20	150 <	< 25	110 <	< 30	110 <
EGV/rule in (*n*)	12/26		8/13		6/9	
EGV/rule out (*n*)	13/165		17/178		19/182	

Exp. Baveno VI: Expanded Baveno VI; New NASH C.C: New NASH cirrhosis criteria; LSM: Liver stiffness measurement (KPa); Platelet count ($\times 10^9$/L); EVG: Esophagogastric varix.

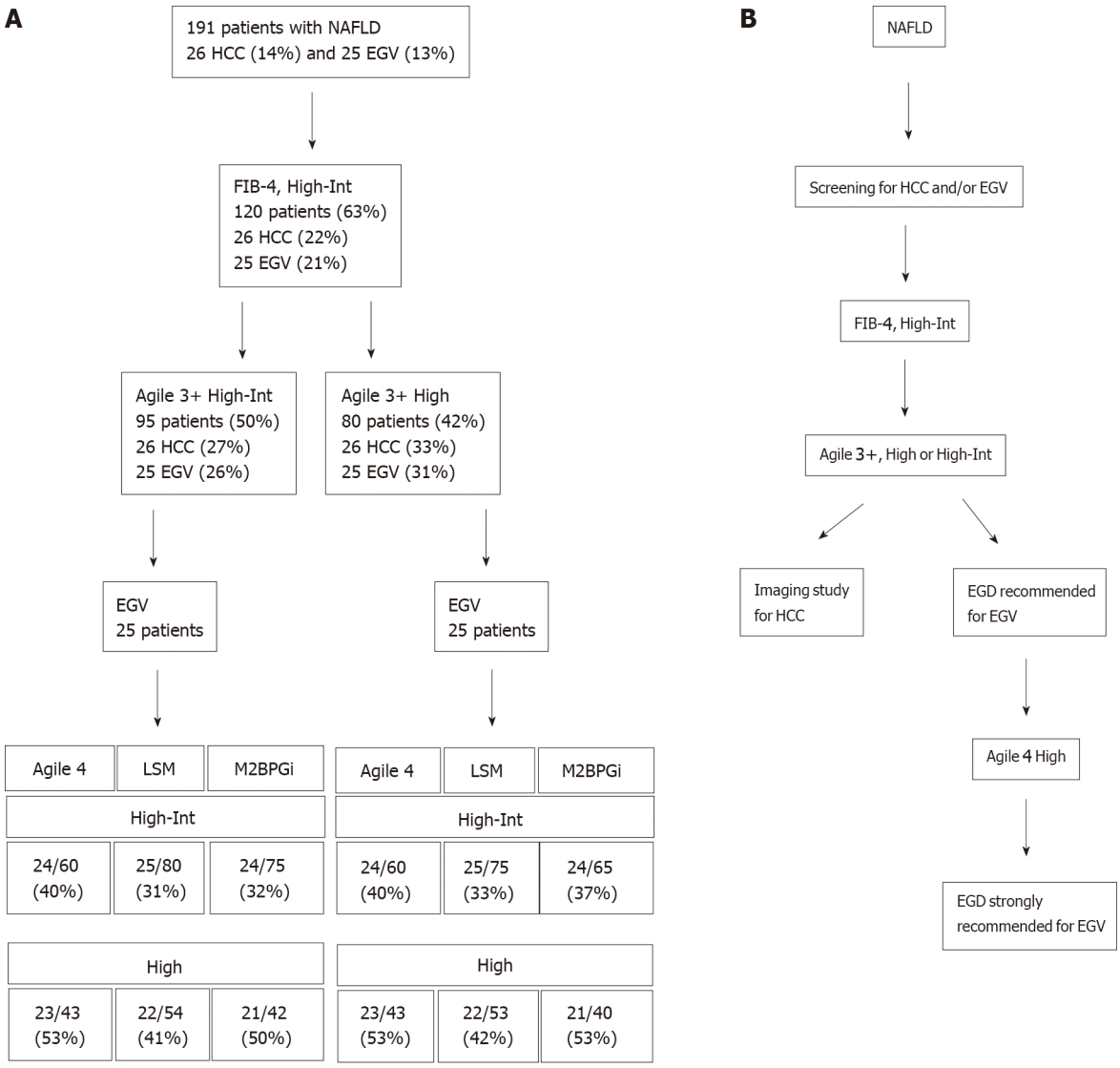


DOI: 10.4291/wjgp.v13.i3.96

Figure 1 Scores (Fibrosis-4, FibroScan-AST, Agile 3+, Agile 4) and values (Liver stiffness measurement, Mac2-binding protein glycosylation isomer) of patients with hepatocellular carcinoma (*n* = 17), esophagogastric varices (*n* = 16), and both hepatocellular carcinoma and esophagogastric varices (*n* = 9). $^aP < 0.05$, $^bP < 0.01$. HCC: Hepatocellular carcinoma; EGV: Esophagogastric varices; LSM: Liver stiffness measurement; FAST: FibroScan-AST; M2BPGi: Mac2-binding protein glycosylation isomer.

EGV were included. When the high to intermediate-risk group of Agile 3+ was used, the prevalence of HCC was 27% (26/95) and 26% (25/95), respectively. When the high-risk group of Agile 3+ was used, the prevalence of HCC was 33% (26/80) and 31% (25/80), respectively. Because the low-risk group of Agile 4, LSM, and M2BPGi included patents with HCC, further narrowing was difficult without missing patients with HCC.

Then, we attempted to narrow the patients with EGV. The high to intermediate and high-risk of Agile 3+ groups subsequently narrowed the patients with EGV. Although the high to intermediate-risk group of LSM successfully narrowed the risk group without missing patients with EGV, the prevalence was a



DOI: 10.4291/wjgp.v13.i3.96

Figure 2 Flow chart. A: A flowchart in sorting nonalcoholic fatty liver disease (NAFLD) patients using the fibrosis-4 index, Agiles, and other fibrosis markers; B: A proposal algorithm to narrow the high-risk group of NAFLD patients with hepatocellular carcinoma and/or esophagogastric varices. HCC: Hepatocellular carcinoma; EGV: Esophagogastric varices; LSM: Liver stiffness measurement; M2BPGi: Mac2-binding protein glycosylation isomer; NAFLD: Nonalcoholic fatty liver disease.

small increase, up to 33% (25/75). In contrast, high-risk group of Agile 4 could concentrated the patients with EGV. Although the high-risk group of Agile 4 missed two patients (8%), the prevalence of patients with EGV increased to 53% (23/43). Thus, Agile 4 is a good tool to further narrow the risk group of patients with EGV.

Based on our results, sorting patients using the FIB-4 index, Agile 3+, and Agile 4 is a potential screening method to narrow the high-risk group of NAFLD patients with comorbidities (Figure 2B).

DISCUSSION

The requirement for NITs to narrow the risk group of patients with comorbidities is expanding because a quarter of people in the world have NAFLD, a risk factor for HCC and/or EGV. The FIB-4 index, which is simple and inexpensive, was used in the first triage to narrow the high-risk group of NAFLD patients with comorbidities. However, there remain many patients even after triage. In the present study, we demonstrated that Agile 3+ and Agile 4, VCTE-based scoring systems, were good tools for further narrowing the high-risk group of patients with HCC and/or EGV at the second and third steps, respectively.

Agile 3+, developed by Yonoussi's group, was suitable to narrow the risk group of patients with HCC and/or EGV in the present study. Agile 3+ has been designed to optimize PPV and reduce cases of intermediate stage (Gray zone) among patients with advanced liver fibrosis[11]. Our data demonstrated

that Agile 3+ had high sensitivity and high NPV for HCC and EGV. Although the number of patients in the high-risk group of Agile 3+ was larger than that of other scoring systems and fibrosis markers, Agile 3+ did not miss the patients with HCC and/or EGV, which is contrast to other tools, including FAST, Agile 4, LSM, and M2BPGi. Indeed, all patients with HCC and/or EGV were included in the high-risk group of Agile 3+, suggesting that Agile 3+ is useful for screening patients with HCC and/or EGV. Because the background liver of NAFLD patients with HCC is often characterized by less fibrosis[18], fibrosis markers sometimes fail to identify patients with HCC. Some patients with HCC were included in the low-risk group of Agile 4, LSM, and M2BPGi. The Agile 3+ scoring system includes age, AST, ALT, platelet count, LSM, sex, and diabetes. Because old age and diabetic individuals are prone to HCC [19], it is reasonable to include these variables in the scoring system to find HCC.

Agile 4, also developed by Yonoussi's group, was suitable to narrow the high-risk group of patients with EGV. Agile 4 was designed to identify patients with NASH cirrhosis. Agile 4 showed high specificity and high PPV for EGV. There were 23 (92%) and 24 patients (96%) with EGV in the high- and high to intermediate-risk groups, respectively. We also applied our patient group to the Baveno VI criteria, expanded Baveno VI criteria, and New NAFLD-cirrhosis criteria, which are combinations of LSM and platelet count. However, more than half of the patients were included in the rule-out group. In the Asian cohort, the Baveno VI criteria performed better than the expanded Baveno VI criteria[20], suggesting that Asian people may have EGV at lower LSM and higher platelet counts than people in the USA and Europe. Although it remains unknown why the Baveno VI criteria and its derivatives did not work in the present study, further studies are required. As a result, Agile 4 can be used at the third step to identify patients with EGV.

FAST failed to narrow the high-risk group of patients with HCC and/or EGV. FAST showed low sensitivity to identify such patients. In addition, there were 10 (38%) with HCC and 6 patients (23%) with EGV in the low-risk (rule out) group, respectively. FAST, designed for identifying patients with NAFLD activity score ≥ 4 and fibrosis stage (F ≥ 2), is calculated using LSM, CAP, and AST. However, the FAST score did not include risk factors for HCC, including age, sex, and diabetes. The association between the grade of CAP, fat content in the liver, and HCC remains unknown. Izumi *et al*[21] reported that CAP was significantly lower in the HCC group than in the non-HCC group in patients with NAFLD. Indeed, our data revealed that CAP tended to be low in patients with HCC (data not shown). Thus, FAST is unlikely suitable for the screening of patients with HCC and/or EGV. However, patients with high FAST scores should be followed up because these patients have a risk of progressive NASH in the future.

There are a couple of limitations in the present study. Our study is a single-center study, and the number of patients examined was small. Thus, the bias of NAFLD population is noted. In a previous study, the proportions of patients in the low- and high-risk FIB-4 index groups were 58.3% and 10.2%, respectively, among patients with biopsy-proven NAFLD[22]. The proportions in the present study showed small size of the low-risk group (37.2%) but large size of the high-risk group (36.1%). In addition, a total of 42 patients (22.0%) had HCC and/or EGV among patients with NAFLD. Because our hospital is a referral center, patients with comorbidities were aggregated into our hospital. In addition, the present study counted patients with histories of HCC and/or EGV, suggesting that scores of FIB-4 and Agile 3+ may be higher than those when comorbidities first developed. Thus, prospective study will clarify the significance of Agiles for finding patients with HCC and/or EGV. At least, the stream from FIB-4 index to Agiles worked in narrowing the high-risk patients with HCC and/or EGV in the present study.

CONCLUSION

In conclusion, Agile 3+ and Agile 4 can narrow the high-risk group of patients who may have HCC and/or EGV after triaging by the FIB-4 index. Because Agile 3+ and Agile 4 share common parameters, including LSM and clinical data, they have a potential use in screening for such patients.

ARTICLE HIGHLIGHTS

Research background

It is necessary to narrow the high-risk group of nonalcoholic fatty liver disease (NAFLD) patients with comorbidities, including hepatocellular carcinoma (HCC) and esophagogastric varices (EGV).

Research motivation

Although the fibrosis-4 index is an excellent formula to narrow the high-risk group, there remain many patients to be ruled out.

Research objectives
This study aimed to assess the utility of VCTE-based scoring systems to narrow the risk group of nonalcoholic fatty liver disease with comorbidities.

Research methods
We performed a cross-sectional study to investigate the usefulness of VCTE-based scoring systems and other fibrosis markers to narrow the high-risk group of patients with NAFLD.

Research results
The high-risk group of Agile 3+ could narrow the patients with HCC and/or EGV without missing one patient. The high-risk group of Agile 4 showed a high PPV for patients with EGV.

Research conclusions
The brand new VCTE-based scoring systems, Agile 3+ and Agile 4, are useful to narrow the NAFLD patient group, in which patients may have HCC and/or EGV.

Research perspectives
Agile 3+ and Agile 4 will be used for screening of NAFLD patients with HCC and/or EGV.

ACKNOWLEDGEMENTS

We thank Eri Noguchi (Jichi Medical University) for her excellent assistance.

FOOTNOTES

Author contributions: Miura K designed the study and performed acquisition, analysis, interpretation of data, and drafted the initial manuscript; Maeda H participated in acquisition and analysis of data; Morimoto N participated in acquisition of data; Watanabe S participated in acquisition of data; Tsukui M participated in acquisition of data; Takaoka Y participated in acquisition of data; Nomoto H participated in acquisition of data; Goka R participated in acquisition of data; Kotani K, a specialist of biostatistics, reviewed the statistical analysis and revised the draft carefully; Yamamoto H revised the draft carefully.

Institutional review board statement: The present study was reviewed and approved by the Institutional Review Board of Jichi Medical University (20-175).

Informed consent statement: A written informed consent was waived because of the retrospective nature of this study. Instead, opt-out consent documents were shown on the website of Jichi Medical University for patients who did not wish to participate in the study.

Conflict-of-interest statement: There are no conflict of interest to report.

Data sharing statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy policies.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

Country/Territory of origin: Japan

ORCID number: Kouichi Miura 0000-0001-8036-6544; Hiroshi Maeda 0000-0003-4558-3935; Naoki Morimoto 0000-0001-9600-7407; Shunji Watanabe 0000-0002-5449-6553; Mamiko Tsukui 0000-0002-2407-0842; Yoshinari Takaoka 0000-0003-3753-6063; Hiroaki Nomoto 0000-0002-3776-2418; Rie Goka 0000-0003-4359-9220; Kazuhiko Kotani 0000-0001-8119-633X; Hironori Yamamoto 0000-0002-3601-1153.

Corresponding Author's Membership in Professional Societies: The Japanese Society of Gastroenterology, No. 030879.

S-Editor: Liu JH

L-Editor: A
P-Editor: Liu JH

REFERENCES

1 **Ioannou GN**. Epidemiology and risk-stratification of NAFLD-associated HCC. *J Hepatol* 2021; **75**: 1476-1484 [PMID: 34453963 DOI: 10.1016/j.jhep.2021.08.012]
2 **Magaz M**, Baiges A, Hernández-Gea V. Precision medicine in variceal bleeding: Are we there yet? *J Hepatol* 2020; **72**: 774-784 [PMID: 31981725 DOI: 10.1016/j.jhep.2020.01.008]
3 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]
4 **Eguchi Y**, Wong G, Akhtar O, Sumida Y. Non-invasive diagnosis of non-alcoholic steatohepatitis and advanced fibrosis in Japan: A targeted literature review. *Hepatol Res* 2020; **50**: 645-655 [PMID: 32307859 DOI: 10.1111/hepr.13502]
5 **Sterling RK**, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M; APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317-1325 [PMID: 16729309 DOI: 10.1002/hep.21178]
6 **Sumida Y**, Yoneda M, Tokushige K, Kawanaka M, Fujii H, Imajo K, Takahashi H, Eguchi Y, Ono M, Nozaki Y, Hyogo H, Koseki M, Yoshida Y, Kawaguchi T, Kamada Y, Okanoue T, Nakajima A; Japan Study Group Of Nafld Jsg-Nafld. FIB-4 First in the Diagnostic Algorithm of Metabolic-Dysfunction-Associated Fatty Liver Disease in the Era of the Global Metabodemic. *Life (Basel)* 2021; **11** [PMID: 33672864 DOI: 10.3390/life11020143]
7 **Sato Y**, Yoshihisa A, Kanno Y, Watanabe S, Yokokawa T, Abe S, Misaka T, Sato T, Suzuki S, Oikawa M, Kobayashi A, Yamaki T, Kunii H, Nakazato K, Saitoh SI, Takeishi Y. Liver stiffness assessed by Fibrosis-4 index predicts mortality in patients with heart failure. *Open Heart* 2017; **4**: e000598 [PMID: 28674631 DOI: 10.1136/openhrt-2017-000598]
8 **Paternostro R**, Reiberger T, Bucsics T. Elastography-based screening for esophageal varices in patients with advanced chronic liver disease. *World J Gastroenterol* 2019; **25**: 308-329 [PMID: 30686900 DOI: 10.3748/wjg.v25.i3.308]
9 **Hsu C**, Caussy C, Imajo K, Chen J, Singh S, Kaulback K, Le MD, Hooker J, Tu X, Bettencourt R, Yin M, Sirlin CB, Ehman RL, Nakajima A, Loomba R. Magnetic Resonance vs Transient Elastography Analysis of Patients With Nonalcoholic Fatty Liver Disease: A Systematic Review and Pooled Analysis of Individual Participants. *Clin Gastroenterol Hepatol* 2019; **17**: 630-637.e8 [PMID: 29908362 DOI: 10.1016/j.cgh.2018.05.059]
10 **Newsome PN**, Sasso M, Deeks JJ, Paredes A, Boursier J, Chan WK, Yilmaz Y, Czernichow S, Zheng MH, Wong VW, Allison M, Tsochatzis E, Anstee QM, Sheridan DA, Eddowes PJ, Guha IN, Cobbold JF, Paradis V, Bedossa P, Miette V, Fournier-Poizat C, Sandrin L, Harrison SA. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. *Lancet Gastroenterol Hepatol* 2020; **5**: 362-373 [PMID: 32027858 DOI: 10.1016/S2468-1253(19)30383-8]
11 **Younoussi ZM**, Harrison SA, Newsome PN, Chan WK, Yilmaz Y, de Ledinghen V, Costentin C, Zheng MH, Wong V, Elkhashab M, Huss R, Myers RP, Foucquier J, Labourdette A, Destro M, Fourier CD, Miette V, Sandrin L, Boursier J, Sanyal A. Development and validation of Agile 3+: novel FibroScan based score for the diagnosis of advanced fibrosis in patients with non-alcoholic fatty liver disease. *J Hepatol* 2021; **75** Supple 2: S 257
12 **Younoussi ZM**, Harrison SA, Newsome PN, Chan WK, Yilmaz Y, Zheng MH, Wong V, Elkhashab M, Kersey K, Myers RP, Foucquier J, Labourdette A, Destro M, Fourier CD, Miette V, Sandrin L, Sanyal A, and NASH Clinical Research Network, NIDDK, NIH. Improving diagnosis of cirrhosis in patient with NAFLD by combining liver stiffness measurement by vibration-controlled transient elastography and routine biomarkers: A global derivation and validation study. AASLD 2020 Late-breaking Abstracts LB12
13 **Kamath PS**, Mookerjee RP. Individualized care for portal hypertension: Not quite yet. *J Hepatol* 2015; **63**: 543-545 [PMID: 26150255 DOI: 10.1016/j.jhep.2015.07.001]
14 **Augustin S**, Pons M, Maurice JB, Bureau C, Stefanescu H, Ney M, Blasco H, Procopet B, Tsochatzis E, Westbrook RH, Bosch J, Berzigotti A, Abraldes JG, Genescà J. Expanding the Baveno VI criteria for the screening of varices in patients with compensated advanced chronic liver disease. *Hepatology* 2017; **66**: 1980-1988 [PMID: 28696510 DOI: 10.1002/hep.29363]
15 **Petta S**, Sebastiani G, Bugianesi E, Viganò M, Wong VW, Berzigotti A, Fracanzani AL, Anstee QM, Marra F, Barbara M, Calvaruso V, Cammà C, Di Marco V, Craxì A, de Ledinghen V. Non-invasive prediction of esophageal varices by stiffness and platelet in non-alcoholic fatty liver disease cirrhosis. *J Hepatol* 2018; **69**: 878-885 [PMID: 29802949 DOI: 10.1016/j.jhep.2018.05.019]
16 **Beppu K**, Inokuchi K, Koyanagi N, Nakayama S, Sakata H, Kitano S, Kobayashi M. Prediction of variceal hemorrhage by esophageal endoscopy. *Gastrointest Endosc* 1981; **27**: 213-218 [PMID: 6975734 DOI: 10.1016/s0016-5107(81)73224-3]
17 **Hashizume M**, Kitano S, Yamaga H, Koyanagi N, Sugimachi K. Endoscopic classification of gastric varices. *Gastrointest Endosc* 1990; **36**: 276-280 [PMID: 2365213 DOI: 10.1016/s0016-5107(90)71023-1]
18 **Kawada N**, Imanaka K, Kawaguchi T, Tamai C, Ishihara R, Matsunaga T, Gotoh K, Yamada T, Tomita Y. Hepatocellular carcinoma arising from non-cirrhotic nonalcoholic steatohepatitis. *J Gastroenterol* 2009; **44**: 1190-1194 [PMID: 19672551 DOI: 10.1007/s00535-009-0112-0]
19 **Huang DQ**, El-Serag HB, Loomba R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 223-238 [PMID: 33349658 DOI: 10.1038/s41575-020-00381-6]
20 **Sharma S**, Agarwal S, Gunjan D, Kaushal K, Anand A, Saraya A. Deciding Among Noninvasive Tools for Predicting Varices Needing Treatment in Chronic Liver Disease: An Analysis of Asian Cohort. *Am J Gastroenterol* 2020; **115**: 1650-

1656 [PMID: 32516202 DOI: 10.14309/ajg.0000000000000722]

21 **Izumi T**, Sho T, Morikawa K, Shigesawa T, Suzuki K, Nakamura A, Ohara M, Kawagishi N, Umemura M, Shimazaki T, Kimura M, Nakai M, Suda G, Natsuizaka M, Ogawa K, Kudo Y, Nishida M, Ono K, Baba M, Furuya K, Sakamoto N. Assessing the risk of hepatocellular carcinoma by combining liver stiffness and the controlled attenuation parameter. *Hepatol Res* 2019; **49**: 1207-1217 [PMID: 31219667 DOI: 10.1111/hepr.13391]

22 **Sumida Y**, Yoneda M, Hyogo H, Itoh Y, Ono M, Fujii H, Eguchi Y, Suzuki Y, Aoki N, Kanemasa K, Fujita K, Chayama K, Saibara T, Kawada N, Fujimoto K, Kohgo Y, Yoshikawa T, Okanoue T; Japan Study Group of Nonalcoholic Fatty Liver Disease (JSG-NAFLD). Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol* 2012; **12**: 2 [PMID: 22221544 DOI: 10.1186/1471-230X-12-2]

World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Pathophysiol 2022 May 22; 13(3): 107-113

DOI: 10.4291/wjgp.v13.i3.107

ISSN 2150-5330 (online)

CASE REPORT

Gastric cancer with concurrent pancreatic schwannoma: A case report

Mateus Barradas Ribeiro, Emerson Shigueaki Abe, André Kondo, Adriana Vaz Safatle-Ribeiro, Marina Alessandra Pereira, Bruno Zilberstein, Ulysses Ribeiro Jr

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: De Raffele E, Italy; Wang YJ, China

Received: November 25, 2021
Peer-review started: November 25, 2021
First decision: January 12, 2022
Revised: January 23, 2022
Accepted: March 26, 2022
Article in press: March 26, 2022
Published online: May 22, 2022

Mateus Barradas Ribeiro, Emerson Shigueaki Abe, André Kondo, Adriana Vaz Safatle-Ribeiro, Marina Alessandra Pereira, Bruno Zilberstein, Ulysses Ribeiro Jr, Department of Gastroenterology, Instituto do Cancer, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Sao Paulo 01249000, Brazil

Corresponding author: Ulysses Ribeiro Jr, PhD, Chief Doctor, Surgeon, Department of Gastroenterology, Instituto do Cancer, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Av Dr Arnaldo 251, Sao Paulo 01249000, Brazil.
ulysses.ribeiro@hc.fm.usp.br

Abstract

BACKGROUND

The differential diagnosis of abdominal masses is somewhat troublesome, especially when there is a malignancy to be evaluated. We report herein a unique case of gastric adenocarcinoma concurrent with a pancreatic schwannoma. Correct assessment of intraoperative findings is essential for adequate tumor staging and to decide the proper management of a concurrent pancreatic lesion.

CASE SUMMARY

Computed tomography scan performed for gastric cancer staging revealed a solid and cystic pancreatic mass that had no signs of local invasiveness. Surgical resection of the pancreas was decided preoperatively since a radical approach of the gastric tumor could be performed. There were no signs of distant metastases, and the large pancreatic mass was in contact with the posterior gastric wall. Histopathological study revealed a pancreatic schwannoma, which is an uncommon neoplasm that arises from Schwann cells around peripheral nerves.

CONCLUSION

Therefore, pancreatic masses deserve special attention regarding the differential diagnosis in patients with gastric cancer. The presence of a large pancreatic mass should not preclude the potentially curative intent of the gastric cancer treatment.

Key Words: Stomach neoplasms; Gastric adenocarcinoma; Schwannoma; Pancreas; Case report

Core Tip: We display here the first case of synchronous gastric cancer and pancreatic schwannoma, highlighting the relevance of the differential diagnosis in approaching pancreatic masses in the context of staging gastric neoplasm. Correct intraoperative staging was essential in treatment decision-making.

Citation: Ribeiro MB, Abe ES, Kondo A, Safatle-Ribeiro AV, Pereira MA, Zilberstein B, Ribeiro Jr U. Gastric cancer with concurrent pancreatic schwannoma: A case report. *World J Gastrointest Pathophysiol* 2022; 13(3): 107-113
URL: https://www.wjgnet.com/2150-5330/full/v13/i3/107.htm
DOI: https://dx.doi.org/10.4291/wjgp.v13.i3.107

INTRODUCTION

Accurate staging is essential in gastric cancer treatment decision-making, and any lymph nodes or masses observed in staging assessment should be investigated[1]. Schwannomas, also referred to as neurilemmomas, are rare neoplasms that arise from Schwann cells around peripheral nerves, usually epineurium of either autonomic sympathetic or parasympathetic fibers[2,3]. Pancreatic locations are unusual, with about 70 cases reported in the last 40 years, and most of them are benign. However, malignancy can be found in up to 15% of cases, especially in lesions greater than 6 cm[3-5]. Schwannomas are usually well-encapsulated firm masses, and two-thirds may undergo degenerative changes, which can be cystic formation, calcification, and hemorrhage, among others[2,6]. Due to these alterations, they can mimic cystic pancreatic lesions or metastasis of a different primary site tumor in radiologic investigation, including gastric cancer.

CASE PRESENTATION

Chief complaints

A 73-year-old woman presented with epigastric pain and weight loss.

History of present illness

She had a history of non-insulin-dependent diabetes mellitus, arterial hypertension, and elevated cholesterol level.

History of past illness

She did not report a history of other previous illnesses.

Personal and family history

She was unaware of a family history of cancer.

Physical examination

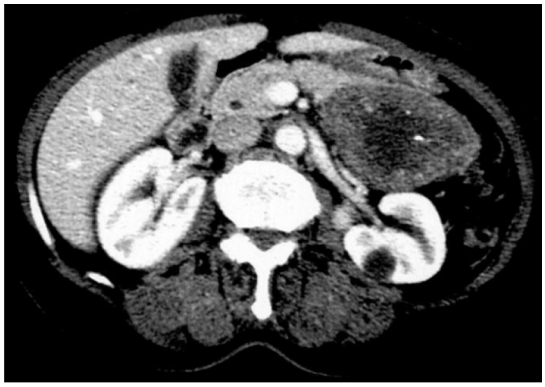
Abdominal examination did not detect any marked change.

Laboratory examinations

All laboratory data were normal, including hemoglobin of 12.2 g/dL. Serum amylase was 50 U/mL, serum CEA was 1.3 ng/mL, and CA19-9 was 12.7 U/mL.

Imaging examinations

Upper gastrointestinal endoscopy revealed an ulcerated and infiltrative (Borrmann III) lesion measuring 4 cm in the lesser curvature extending to the posterior wall of the antrum and body region. Biopsy revealed a moderately differentiated adenocarcinoma. Preoperative evaluation using computed tomography (CT) scan showed a well-defined 8 cm × 5 cm solid and cystic tumor in the body and tail of the pancreas in close contact to the posterior wall of the gastric body. No sign of infiltration in the surrounding tissue was detected. No liver mass, peripancreatic lymph node swelling, or free peritoneal fluid was detected (Figure 1).



DOI: 10.4291/wjgp.v13.i3.107

Figure 1 Computed tomography scan showing solid and cystic tumor in the body and tail of the pancreas (pancreatic schwannoma).

MULTIDISCIPLINARY EXPERT CONSULTATION

Laparotomy disclosed a localized gastric tumor in the body and a distinct solid, well-encapsulated tumor at the body of the pancreas without signs of inflammation or neoplastic infiltration. However, the lesion was in close contact to the posterior gastric wall (Figures 2 and 3). Due to the locoregional infiltration of the gastric tumor, absence of distant metastases, and proximity to a large pancreatic lesion, a total gastrectomy with D2 lymph node dissection plus distal pancreatectomy and splenectomy was performed. The final gastric cancer stage was pT2N0, with 0/73 lymph nodes examined (Figure 4). The cut surface of the excised 8 cm pancreatic tumor was pale yellow with hemorrhage foci. On microscopic examination, the lesion showed spindle cells with Antoni A and B patterns and was strongly positive for S100 protein (Figure 5).

FINAL DIAGNOSIS

Gastric adenocarcinoma and concurrent pancreatic schwannoma.

TREATMENT

Total gastrectomy with D2 lymph node dissection, plus distal pancreatectomy and splenectomy.

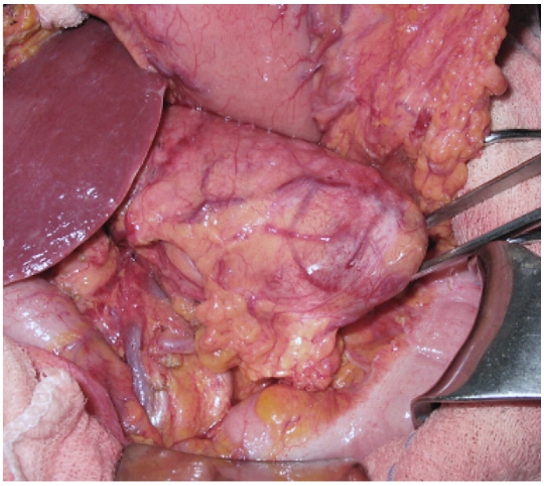
OUTCOME AND FOLLOW-UP

The patient recovered without any complication, and she was discharged after 12 d. After 44 mo of follow-up, the patient has no evidence of recurrence.

DISCUSSION

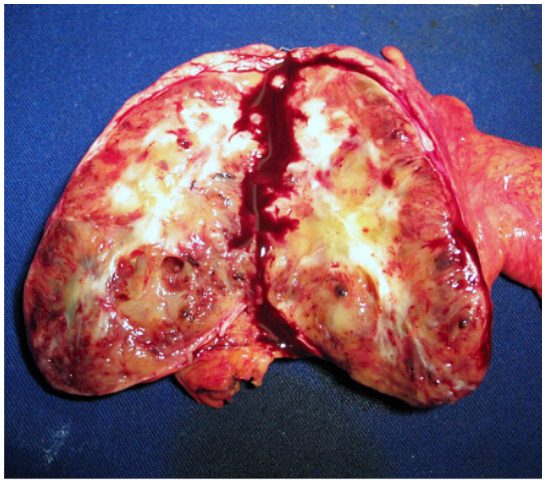
In this case report, the patient presented unspecific symptoms including epigastric pain and weight loss. Therefore, it was not possible to define if these symptoms were related to the gastric cancer or if it was a symptomatic case of pancreatic schwannoma. Pancreatic schwannoma appear to be indolent, corroborating its benign nature, and around one-third of the pancreatic schwannomas are asymptomatic. Abdominal pain is the most displayed symptom, ranging from 30% to 57% of patients. Other symptoms are reported less frequently, such as back pain, jaundice, anorexia, vomiting, weight loss, anemia, abdominal mass, and gastrointestinal bleeding[7,8].

CT scan performed for gastric cancer staging showed a solid and cystic pancreatic mass, and it was necessary to make differential diagnosis with a primary pancreatic neoplasm or metastases from the gastric tumor. CT scan may be beneficial in pancreatic schwannoma initial evaluation, and most of them revealed low density or cystic masses, as presented in this case[9,10]. Moreover, magnetic resonance imaging appears to be more helpful in characterizing schwannomas by their typical encapsulation, hypointensity on T1-weighted images, and hyperintensity on T2-weighted images[11,12]. These characteristics are typical radiological features of Antoni A areas, suggesting that these should be classified as



DOI: 10.4291/wjgp.v13.i3.107 **Copyright** ©The Author(s) 2022.

Figure 2 Laparotomy view of pancreatic body mass.



DOI: 10.4291/wjgp.v13.i3.107 **Copyright** ©The Author(s) 2022.

Figure 3 Macroscopic examination showed a well-encapsulated, pale yellow solid pancreatic tumor with areas of hemorrhage.

solid hypervascularized tumors of the pancreas. Meanwhile, type Antoni B tumor areas are characterized by a significant cystic component, in which differential diagnosis must be made from a large amount of pancreatic cystic neoplasms[9,12]. Fluorodeoxyglucose-positron emission tomography-CT usually demonstrates a hypermetabolic appearance[8,9]. Complementary magnetic resonance imaging and fluorodeoxyglucose-positron emission tomography-CT were not performed in this patient but would be helpful in better characterizing morphological tumoral features.

Endoscopic ultrasound-guided fine needle aspiration may be useful, but this method remains controversial due to high false-negative rate. In two reviews, only 44% and 50% of patients were correctly diagnosed with pancreatic schwannoma[4,8].

Intraoperative analysis is also a helpful tool in diagnosis, especially to ensure negative margins and correct resection of pancreatic neoplasms, as demonstrated in this case. One review showed that 47% of pancreatic schwannomas were correctly diagnosed, and 33% were reported as benign[8], showing that the intraoperative assessment of these tumors may aid the decision making in these cases.

Surgical treatment includes Whipple procedure (pancreaticoduodenectomy) or distal pancreatectomy with or without splenectomy, either because a definite diagnosis was not made pre- or intraoperatively or due to large tumor size[13,14] (Table 1). Enucleation should be considered a surgical option when preoperative histopathology confirms the diagnosis. However, a tumor size larger than 6.0 cm, vascular encasement, or visceral invasion should elicit suspicion of malignant transformation, and a more radical approach should be chosen[4].

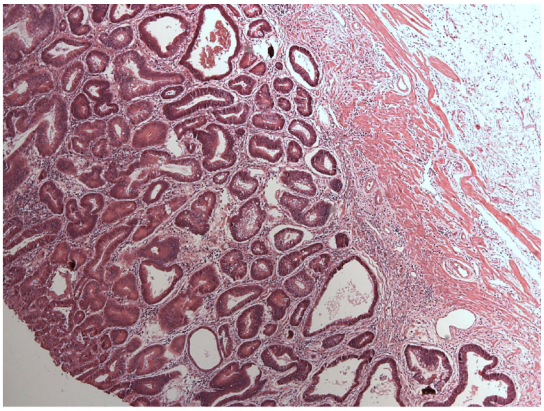
Gastrectomy with D2 lymph node dissection is a gold standard treatment considering the gastric neoplasm; however due to the pancreatic tumor size and the proximity to the posterior gastric wall harboring the tumor, it was decided to perform a partial pancreatectomy with splenectomy in addition

Table 1 Summary of literature review on pancreatic schwannoma surgical management

Ref.	Type of study	Number of patients	Case presented in the article		Literature review presented in the article				
			Moment of diagnosis	Surgery performed	Size (cm)	Mean size/range (cm)	Type of surgery performed			Malignancy, %
							Types of pancreatectomy or pancreatoduodenectomy, %	Enucleation	Surgical resection otherwise non-specified, %	
Paranjape *et al* [3], 2004	Case report and review	40	Postoperative	Enucleation	3.5	8.79	27 (67.5)	4 (10.0)	5 (12.5)	5 (12.5)
Ma *et al*[4], 2017	Case report and review	68	Postoperative	Whipple pancreaticoduodenectomy	6 × 5	6.1 ± 5.7 (1-33)	40 (59.0)	8 (12.0)	14 (21.0)	8 (12.0)
Su *et al*[5], 2016	Case report and review	65	Intraoperative frozen pathology	Central pancreatectomy	1.6 × 1.1 × 1.1	5.83 ± 4.59 (1-20)	40 (61.5)	9 (13.8)	13 (20.0)	5 (7.7)
Gupta *et al*[6], 2009	Case report and review	37	Postoperative	Whipple pancreaticoduodenectomy	7.9 × 8.3	-	19 (51.3)	6 (16.2)	9 (24.3)	-
Moriya *et al* [7], 2012	Case report and review	47	Intraoperative frozen pathology	Enucleation	4 × 4 × 3	6.2 ± 5.1 (1-20)	25 (53.0)	7 (15.0)	12 (26.0)	5 (11.0)
Zhang *et al*[8], 2019	Case report and review	75	Postoperative	Central pancreatectomy	2.8 and 4.0	5.5 ± 5.0 (1.0-30.0)	45 (60.0)	11 (15.0)	14 (19.0)	4 (5.0)
Watanabe *et al* [9], 2018	Case report	1	Postoperative	Subtotal stomach-preserving pancreaticoduodenectomy	5.4 × 5.4	-	-	-	-	-
Wang *et al* [11], 2019	Case report	1	Postoperative	Distal pancreatectomy with splenectomy	2.0 × 2.0 × 1.8	-	-	-	-	-
Shi *et al*[14], 2021	Case series and systematic review	6	Postoperative	Pancreaticoduodenectomy 5 (83%) and distal pancreatectomy 1 (17%)	3.7 (range 2.0-6.4)	4.3 ± 2.2 (1.4-10)	-	-	-	-
Kimura *et al* [15], 2021	Case report	1	Postoperative	Distal pancreatectomy with splenectomy	1.1 × 0.8	-	-	-	-	-

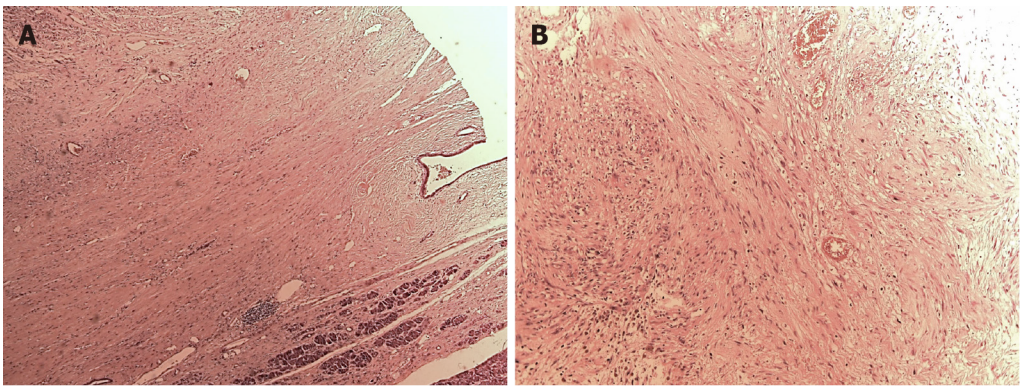
to the gastric resection.

Microscopically, schwannomas are divided in two main subareas: Antoni A areas, displaying an organized hypercellular component, characterized by closely packed spindle cells with occasional nuclear palisading; and Antoni B areas, featuring a hypocellular component with loose myxoid stroma, often with degenerative changes[4,7]. Immunohistochemistry is crucial to the differential diagnosis since immunostaining is strongly positive for S-100 protein, vimentin, and CD56 and negative for cytokeratin AE1/AE3, desmin, smooth muscle myosin, CD34, and CD117[4,7,15]. In this case, diagnosis was confirmed by the presence of these typical findings in pathology: Antoni A and B areas as well as immunohistochemistry with strong S-100 (+) staining.



DOI: 10.4291/wjgp.v13.i3.107 **Copyright** ©The Author(s) 2022.

Figure 4 Representative area of moderately differentiated gastric adenocarcinoma. Hematoxylin and eosin; Magnification × 50.



DOI: 10.4291/wjgp.v13.i3.107 **Copyright** ©The Author(s) 2022.

Figure 5 Microscopic examination. A and B: Representative areas of pancreatic schwannoma; Hematoxylin and eosin; Magnification × 20).

Pancreatic schwannomas usually have good prognosis, showing no rates of recurrence over a mean follow-up of 19 mo[4,8].

CONCLUSION

Therefore, we present the first case of synchronous gastric cancer and pancreatic schwannoma reported in the literature. Intraoperative staging examination was decisive in the adequate management of this patient. The presence of a large pancreatic mass should not preclude the potentially curative intent of the gastric cancer treatment.

FOOTNOTES

Author contributions: Ribeiro MB contributed to the study design and drafting of the manuscript; Abe ES, Kondo A, and Safatle-Ribeiro AV contributed to data retrieval and manuscript review; Pereira MA and Zilberstein B contributed to manuscript review; Ribeiro Jr U conceived the study and contributed to critical analysis and manuscript review.

Informed consent statement: Informed consent was waived by the local Ethics Committee because of the retrospective nature of the study.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest that might be relevant to the contents of this manuscript.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

Country/Territory of origin: Brazil

ORCID number: Mateus Barradas Ribeiro 0000-0001-8702-0079; Emerson Shigueaki Abe 0000-0001-6054-5705; André Kondo 0000-0003-4842-3363; Adriana Vaz Safatle-Ribeiro 0000-0001-7686-8859; Marina Alessandra Pereira 0000-0002-6865-0988; Bruno Zilberstein 0000-0002-1809-8558; Ulysses Ribeiro Jr 0000-0003-1711-7347.

S-Editor: Liu JH
L-Editor: Filipodia
P-Editor: Liu JH

REFERENCES

1 **Ramos MFKP**, Pereira MA, Yagi OK, Dias AR, Charruf AZ, Oliveira RJ, Zaidan EP, Zilberstein B, Ribeiro-Júnior U, Cecconello I. Surgical treatment of gastric cancer: a 10-year experience in a high-volume university hospital. *Clinics (Sao Paulo)* 2018; **73**: e543s [PMID: 30540120 DOI: 10.6061/clinics/2018/e543s]
2 **Skovronsky DM**, Oberholtzer JC. Pathologic classification of peripheral nerve tumors. *Neurosurg Clin N Am* 2004; **15**: 157-166 [PMID: 15177315 DOI: 10.1016/j.nec.2004.02.005]
3 **Paranjape C**, Johnson SR, Khwaja K, Goldman H, Kruskal JB, Hanto DW. Clinical characteristics, treatment, and outcome of pancreatic Schwannomas. *J Gastrointest Surg* 2004; **8**: 706-712 [PMID: 15358332 DOI: 10.1016/j.gassur.2004.05.010]
4 **Ma Y**, Shen B, Jia Y, Luo Y, Tian Y, Dong Z, Chen W, Li ZP, Feng ST. Pancreatic schwannoma: a case report and an updated 40-year review of the literature yielding 68 cases. *BMC Cancer* 2017; **17**: 853 [PMID: 29241452 DOI: 10.1186/s12885-017-3856-6]
5 **Xu SY**, Sun K, Owusu-Ansah KG, Xie HY, Zhou L, Zheng SS, Wang WL. Central pancreatectomy for pancreatic schwannoma: A case report and literature review. *World J Gastroenterol* 2016; **22**: 8439-8446 [PMID: 27729750 DOI: 10.3748/wjg.v22.i37.8439]
6 **Gupta A**, Subhas G, Mittal VK, Jacobs MJ. Pancreatic schwannoma: literature review. *J Surg Educ* 2009; **66**: 168-173 [PMID: 19712917 DOI: 10.1016/j.jsurg.2008.12.001]
7 **Moriya T**, Kimura W, Hirai I, Takeshita A, Tezuka K, Watanabe T, Mizutani M, Fuse A. Pancreatic schwannoma: Case report and an updated 30-year review of the literature yielding 47 cases. *World J Gastroenterol* 2012; **18**: 1538-1544 [PMID: 22509087 DOI: 10.3748/wjg.v18.i13.1538]
8 **Zhang X**, Siegelman ES, Lee MK 4th, Tondon R. Pancreatic schwannoma, an extremely rare and challenging entity: Report of two cases and review of literature. *Pancreatology* 2019; **19**: 729-737 [PMID: 31153779 DOI: 10.1016/j.pan.2019.05.460]
9 **Watanabe T**, Araki K, Ishii N, Igarashi T, Watanabe A, Kubo N, Kuwano H, Shirabe K. A Surgically Resected Pancreatic Schwannoma with Obstructive Jaundice with Special Reference to Differential Diagnosis from Other Cystic Lesions in the Pancreas. *Case Rep Gastroenterol* 2018; **12**: 85-91 [PMID: 29606941 DOI: 10.1159/000485559]
10 **Yu RS**, Sun JZ. Pancreatic schwannoma: CT findings. *Abdom Imaging* 2006; **31**: 103-105 [PMID: 16132429 DOI: 10.1007/s00261-005-0345-1]
11 **Wang S**, Xing C, Wu H, Dai M, Zhao Y. Pancreatic schwannoma mimicking pancreatic cystadenoma: A case report and literature review of the imaging features. *Medicine (Baltimore)* 2019; **98**: e16095 [PMID: 31192973 DOI: 10.1097/MD.0000000000016095]
12 **Novellas S**, Chevallier P, Saint Paul MC, Gugenheim J, Bruneton JN. MRI features of a pancreatic schwannoma. *Clin Imaging* 2005; **29**: 434-436 [PMID: 16274899 DOI: 10.1016/j.clinimag.2005.04.017]
13 **Shi Z**, Cao D, Zhuang Q, You R, Li X, Li Z, Li Y, Huang X. MR imaging features of pancreatic schwannoma: a Chinese case series and a systematic review of 25 cases. *Cancer Imaging* 2021; **21**: 23 [PMID: 33588954 DOI: 10.1186/s40644-021-00390-x]
14 **Kimura K**, Adachi E, Toyohara A, Omori S, Ezaki K, Ihara R, Higashi T, Ohgaki K, Ito S, Maehara SI, Nakamura T, Fushimi F, Maehara Y. Schwannoma mimicking pancreatic carcinoma: A case report. *World J Clin Cases* 2021; **9**: 4453-4459 [PMID: 34141813 DOI: 10.12998/wjcc.v9.i17.4453]
15 **Weiss SW**, Langloss JM, Enzinger FM. Value of S-100 protein in the diagnosis of soft tissue tumors with particular reference to benign and malignant Schwann cell tumors. *Lab Invest* 1983; **49**: 299-308 [PMID: 6310227]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com