Actionable biomarkers: the key to resolving disorders of gastrointestinal function

Michael Camilleri , Victor Chedid

INTRODUCTION

A valid biomarker is defined as 'a characteristic that is measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention'.[1] The advent and validation of biomarkers based on identification of organic mechanisms, pathogenesis or pathophysiology have the potential to introduce individualisation in management of disorders of lower gastrointestinal function. An actionable biomarker is a biomarker that is associated with a directed treatment to prevent or reverse symptoms or disease, and this has resulted in a paradigm shift in oncological treatment from a tumour type-focussed approach to a molecularly-directed agnostic one, exploring the role of biological agents targeted to the driver genomic alteration irrespective of the cancer histology.[2] Such actionable biomarkers have been introduced in functional lower gastrointestinal disorders.[3] Importantly, this approach also provides opportunity to provide patients with personalised approach to treatment.

This article highlights the diverse methods to identify the organic pathogenesis (figure 1) and to choose treatments based on approved treatments, off-label treatment with approved medications or, in the future, experimental medications. The a priori criteria for inclusion was based on the evidence of pathobiological relevance, extensive data on normal values, performance characteristics of the biomarker, availability of treatment directed to the biomarker, as well as evidence of efficacy of treatment directed at the specific biomarker. For example, the normal value data in adults for cited measurements are based on the following numbers: gastric emptying (319 (214 females, 105 males));[4] colonic transit (220 (145 females, 75 males); [5] colonic compliance (n = 40);[6] anorectal manometry with normal balloon expulsion (143 (96 females and 47 males));[7] gastric accommodation (354 (230 females, 120 males));[8] 48 hours faecal bile acid excretion (96 (60 females and 36 males))[9] and serum 7αC4 for bile acid diarrhoea (184 (110 female, 74 male)).[10] Some biomarkers were considered, but excluded from extensive discussion, as they did not fulfil all these criteria: intestinal permeability, microbiome, duodenal eosinophilia, colonic mast cell infiltration and cytotoxic lethal binding toxin and vinculin assays in IBS.

Division of Gastroenterology, Mayo Clinic, Rochester, Minnesota, USA

Correspondence to Professor Michael Camilleri, Mayo Clinic, Rochester, MN 55905, USA; camilleri.michael@mayo.edu

ACTIONABLE BIOMARKERS IN LOWER GASTROINTESTINAL DISORDERS

Constipation and rectal evacuation disorders

Mechanisms

Among patients with constipation, it is critically important to evaluate the patient for evacuation disorders.[11] In a review of articles on chronic constipation cited in MEDLINE and PubMed databases from 1999 to 2018 that included a total of 5897 patients with chronic idiopathic constipation in the literature, 22% had slow transit constipation, 39% had normal transit constipation and 39% had rectal evacuation disorders.[12]

Diagnosis and the utility of actionable biomarkers

Predictive factors in identifying rectal evacuation disorders among patients with chronic idiopathic constipation are: the coexistence of urinary symptoms, the presence of poor anal relaxation and increased anal squeeze on digital rectal examination and, on anorectal testing, a rectoanal pressure gradient lower than −40 mm Hg, high anal pressure on straining and a balloon expulsion time of greater than 120 s.[12] There are robust normal data on anorectal function based on high-resolution manometry.[7]

Recent studies have identified additional useful biomarkers. First, measurement of the rectal gas volume or area between the upper border of the symphysis pubis and the lower margins of the sacroiliac joints on CT or on a plain abdominal radiograph indicates likelihood of evacuation disorders.[13] If this area exceeds 900 mm^2, there is a 70% likelihood that the patient has a rectal evacuation disorder, based on comparisons of 65 patients with rectal evacuation disorder and 53 patients with chronic constipation without evacuation disorders.[14] Second, the optimal combination of sensitivity and specificity of the time-based balloon expulsion test is 22 s (respectively, 77.8% and 69.8%); whereas, 60 s expulsion time is associated with high specificity (93%), but relatively low sensitivity (39%).[15] Third, the morphology of air in the distal rectum and anal canal on a sagittal image of the CT scan of the pelvis can provide evidence of a rat-tail sign[16] of 'anal achalasia', first recognised by Sir Arthur Hurst in 1925. Fourth, there are differences in the colonic transit profile, with markers retained in the descending, sigmoid and rectum in patients with rectal evacuation disorders, in contrast to the ascending and transverse colon in patients with slow transit constipation.[17] Colonic transit based on scintigraphy is used to identify slow transit constipation or fast transit diarrhoea based on cut-offs at 24 hours of geometric center <2.1 and >3.8, respectively, in a study of 287 patients with lower functional gastrointestinal disorders.[18] Stool burden on abdominal radiograph has also been recently proposed as a useful surrogate for slow transit constipation.[19]

Management based on biomarkers of bowel dysfunction

Multiple individual studies (for example, references[20–22]) have demonstrated the benefit of biofeedback-based therapy compared with sham feedback and standard therapy for dyssynergic defecation. Although the majority of 17 trials were deemed to be of poor methodological quality and subject to bias, a systematic review and meta-analysis confirmed effectiveness of biofeedback therapy,[23] and more recent analysis demonstrated major symptom improvement in 70% to 80% of patients undergoing biofeedback therapy, with superiority over polyethylene glycol laxatives, diazepam or sham therapy.[24] Long-term studies have shown 55% to 82% of patients maintain symptom improvement.[25] Rao *et al* recently reported equivalence of efficacy between office-based, therapist-guided pelvic floor training for six sessions over 3 months (visits every 2 weeks) and home-based biofeedback, which consisted of 20 min, self-training sessions twice per day with a self-inserted probe and a hand-held monitoring device of anal sphincter pressure and push effort.[26]

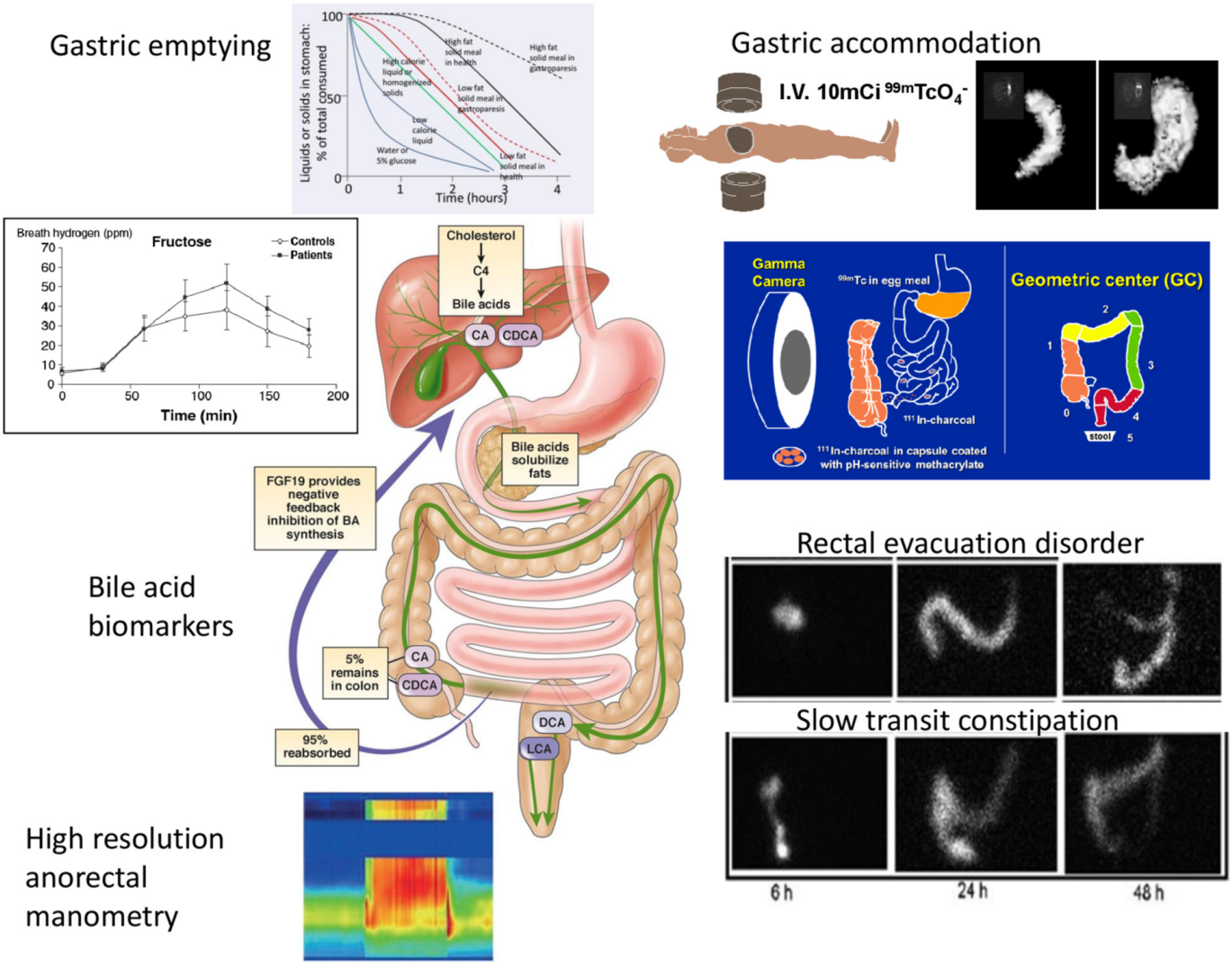

Figure 1 Graphical summary of actionable biomarkers.

Several medications have been shown to normalise colonic transit in functional disorders associated with constipation or diarrhoea, and these results correctly predicted efficacy of the same medications in phase 2B and phase 3 trials (as summarised elsewhere[3]).

Acquired chronic megacolon in adulthood

Diagnosis

Acquired megacolon is a condition involving persistent dilatation and lengthening of the colon in the absence of organic disease. Although relatively rare in adults, particularly in those without a family history or Hirschsprung disease, it is important to identify this condition in clinical practice because its management differs from that of chronic idiopathic constipation. Histological analysis in resected colons showed variable, non-pathognomonic results in different reports,[27] although immunohistochemical studies provide some mechanistic information, suggesting increased excitatory (AChE) and decreased inhibitory (VIP and nitric oxide) innervation.[28 29] Investigations of genetic mechanisms have been inconclusive to date;[30 31] hence, preoperative actionable biomarkers are unavailable, and diagnosis is usually based on exclusion of organic disease and a radiological sigmoid diameter of ~10 cm.[27]

In a series of 24 patients evaluated over 20 years at Mayo Clinic,[6] the mean maximal colonic diameter on abdominal X-ray was 12.7±0.8 cm, and aetiology was idiopathic in 16 and secondary in 8 patients. It is important to note that 10/24 patients had comorbid pelvic floor dyssynergia that required treatment; in addition, conservative treatment was typically ineffective and, therefore, patients were candidates for laparoscopic colectomy with ileorectal anastomosis. The decision to pursue this surgical treatment has to be based on firm diagnostic criteria. The sigmoid colon diameter cut-off of >10 cm on radiological imaging is based on three studies which showed SD ranging from 2 to 3.5 cm. If sigmoid colon diameter is <10 cm, colonic compliance measurements with an infinitely compliant balloon can identify chronic acquired megacolon[32] if the balloon volumes at pressures of 20, 32 and 44 mm Hg distension exceed 273.5, 355.0 and 397.6 mL, respectively.

Management

Patients with megacolon may require colectomy if they do not respond to medical therapy; at the time of the report of 24 adult patients with acquired megacolon, 16 patients required colectomy for symptom relief.[6] This experience is similar to the observed colectomy in five of seven patients with megacolon associated with multiple endocrine neoplasia type 2B.[3333]

Bile acid imbalance in patients presenting with functional diarrhoea or constipation

Bile acid diarrhoea

Mechanisms

The effect of bile acids to increase colonic motility and secretion has been discussed elsewhere.[34] Among patients presenting with functional diarrhoea, a systematic review and meta-analysis estimated that 25% to 33% of patients had bile acid malabsorption,[35] and patients with evidence of bile acid diarrhoea on ^{75}SeHCAT test had great likelihood of responding to a bile acid sequestrant, specifically, cholestyramine.[36] In patients with bile acid malabsorption based on ^{75}SeHCAT retention test, there was >75% likelihood to respond to treatment with a bile acid sequestrant such as cholestyramine.[36]

Diagnosis

Advances in the understanding of the mechanisms leading to bile acid diarrhoea have provided novel approaches for diagnosis, specifically serological tests (reduced fasting serum FGF-19 and increased fasting serum 7αC4).[37 38] The sensitivity and specificity of these tests are lower than the gold standard ^{75}SeHCAT retention test[39] or the total 48 hours faecal bile acid excretion test.[40] Therefore, based on consensus guidelines, measurements of ^{75}SeHCAT retention test or serum 7αC4[41 42] or, based on original data in the USA, measurements of total and individual faecal bile acids collected over 48 hours[34 43] are the most direct ways to identify bile acid diarrhoea among patients with chronic diarrhoea or diarrhoea-predominant irritable bowel syndrome. For the faecal bile acid test, three measurements have similar diagnostic accuracy: total faecal bile acids over 48 hours >2337µmol, faecal primary bile acids (cholic acid (CA)+chenodeoxycholic acid (CDCA)) >10% or combined total faecal bile acids over 48 hours >1000µmol plus >4% faecal primary bile acids (CA+CDCA).[34 43] The application of such tests in clinical evaluation of patients with chronic non-bloody diarrhoea can significantly reduce healthcare utilisation.[44 45]

The alternative approach advocated in patients with suspected bile acid diarrhoea[46] is a therapeutic trial with bile acid sequestrants. However, the precise dose, frequency of administration, duration of treatment for the different sequestrants and the degree of response to confidently diagnose bile acid diarrhoea are unclear. In practice, the unpalatability of the sequestrants is often associated with low compliance and interferes with the 'diagnostic therapeutic trial'. In fact, the Canadian Association of Gastroenterology[42] and the British Society of Gastroenterology[41] guidance documents recommended diagnostic testing over such a 'diagnostic therapeutic trial' approach.

Treatment

Identifying bile acid diarrhoea among patients with chronic functional diarrhoea provides the opportunity to specifically treat the diarrhoea with bile acid sequestrants such as colesevelam or colestipol,[47 48] or with farnesoid X receptor agonists such as obeticholic acid or tropifexor.[49 50]

Bile acids in constipation

Mechanisms

Bile acids are physiological laxatives. A study in IBS-constipation (IBS-C) showed that ~15% of patients with IBS-C had reduced 48 hours faecal total bile acids and deoxycholic acid, which correlated with slower colonic transit.[51]

Diagnosis

Although this has not yet entered routine clinical practice, reduced total and primary faecal bile acids and increased faecal lithocholic acid were significant predictors of decreased faecal weight, frequency and consistency with area under the curve >0.82 (sensitivity >76%, specificity >72%).[51] Among 45 patients with IBS-C, 15% had reduced total bile acids and level of deoxycholic acid in faecal samples collected over 48 hours on a 100 g fat diet, and lower levels of excretion of bile acids into faeces correlated with slower colonic transit.[51]

Treatment

Administration of a colonic release formulation of chenodeoxycholic acid was associated with acceleration of colonic transit, looser stool consistency, increased stool frequency and greater ease of passage in female patients with IBS-C.[52] Increasing colonic bile acids to relieve constipation was achieved with the ileal bile acid transport inhibitor, elobixibat, which accelerated colonic transit[53] and relieved constipation in a 2-week, randomised, placebo-controlled trial and during 52-week, open-label treatment.[54] Treatment with elobixibat in 19 patients with chronic constipation was associated with increased fasting serum C4, increased total and primary faecal bile acids, as well as improvement in the number of spontaneous and complete spontaneous bowel movements, stool consistency scores and other constipation-related symptoms to levels almost comparable to those of a control healthy subject cohort studied simultaneously.[55]

Sensible application of low fermentable oligo-, di-, mono-saccharides and polyols in diet of patients with symptoms of irritable bowel syndrome

Mechanisms

Much has been written about the potential of low fermentableoligo-, di-, monosaccharides and polyols (FODMAPs) in the diet of patients with symptoms of irritable bowel syndrome. Indeed, the National Institute of Clinical Excellence in the UK has strongly recommended diet and, specifically, the low FODMAP diet as the first line of management of patients with irritable bowel syndrome, particularly those with diarrhoea or bloating. Information from rats fed with a high FODMAP diet showed increased rat faecal Gram-negative bacteria and elevated lipopolysaccharides (LPS), and there was induced intestinal pathology as indicated by inflammation, barrier dysfunction and visceral hypersensitivity.[56] These effects were prevented by rifaximin treatment, suggesting that FODMAPs may induce their effects through gut dysbiosis. Table 1 addresses the question: Is the FODMAP hypothesis biologically plausible?

Oligosaccharides of the fructan and galactan groups cannot be metabolised and, therefore, could result in fermentation, production of gas and abdominal symptoms. Among disaccharides, the most prevalent intolerance is hypolactasia and, given the high global population prevalence (approximately 65% of the human population),[57] avoidance of lactose is a logical strategy. Nevertheless, when lactose intake is limited to the equivalent of 240 mL of milk or less a day, symptoms are negligible and the use of lactose-digestive aids unnecessary.[58] Moreover, yoghurt is an auto-digesting source of lactose and results in changes in breath hydrogen after ingestion of lactose or milk in lactase-deficient people.[59]

Sugar alcohols and polyols are most frequently ingested in artificially sweetened drinks, and alternative sweeteners such as aspartame, sucrose and saccharin may provide approaches to relieve diarrhoea and bloating in patients who experience these symptoms with ingestion of drinks containing these artificial sweeteners.

Table 1 Is the FODMAP hypothesis biologically plausible?

Factor	Evidence	Plausible?	Strategy
Oligosaccharides: fructans (polymer of fructose molecules) and galacto-oligosaccharides (galactans)	No enzyme to digest fructose-fructose bonds → fermentation	+	Onions, garlic are common dietary causes of abdominal symptoms
Disaccharides	Lactose intolerance	SPECIFIC Diagnosis	SPECIFIC Rx: Lactase supplements; limit lactose loads
Monosaccharides	Human small bowel has considerable capacity and reserve to absorb for example, glucose and fructose	NONE	Irrelevant
Polyols (sugar alcohols: maltitol, xylitol, erythritol, sorbitol)	Osmotically active → diarrhoea	+	AVOID artificially sweetened drinks; use alternatives such as aspartame, sucralose, saccharin

FODMAP, fermentable oligo-, di-, mono-saccharides and polyols.

Diagnosis

From a clinical and mechanistic perspective, it is also important to address whether the breath tests most commonly used to 'diagnose' saccharide intolerance are sufficiently specific, not in terms of test performance and reproducibility, but in relation to the actual cause of the functional gastrointestinal symptoms. This is illustrated by the major controversy that revolves around the role of fructose malabsorption in the aetiology of the symptoms. The syndrome of hereditary fructose intolerance associated with aldolase B deficiency is a rare autosomal recessive disorder that is seldom the cause of such abdominal symptoms.[60] In contrast, abdominal symptoms are often attributed to fructose intolerance based on fructose breath hydrogen testing. Classical human physiology studies have demonstrated great capacity of the human small intestine to absorb monosaccharides and disaccharides; thus, glucose and lactose absorption is completed in the first 200 cm of the human small bowel,[61] and absorption of glucose is only marginally more efficient than the absorption of fructose in health and in celiac disease,[62 63] since the GLUT-2 transporter uptake of fructose is facilitated by the presence of glucose, galactose and certain amino acids (alanine, proline, glutamine).

Moreover, ingestion of fructose in the diet usually occurs with other foods (eg, fruit or a solid meal) which may retard the emptying of fructose into the small intestine. Indeed, a comparison of fructose and high fructose corn syrup in health and irritable bowel syndrome showed that breath hydrogen curves after fructose were modestly different between controls and patients with irritable bowel syndrome, and breath hydrogen excretion in the presence of high fructose corn syrup was markedly reduced in both groups.[64] In addition, glucose dose-dependently enhanced absorption of fructose, reducing hydrogen in breath.[65] These perspectives led to the recommendation for the more personalised approach to dietary restriction, such as avoidance of individual carbohydrates rather than exclusion of all FODMAPs.[66] In fact, in a placebo-controlled study of patients with IBS, a low FODMAP diet was associated with adequate symptom relief and significantly reduced symptom scores compared with placebo. However, the authors concluded that it was not clear whether changes resulted from collective FODMAP restriction or removal of a single component such as lactose.[67]

Given the potential confounders in the interpretation of breath hydrogen or methane excretion in response to such substrates in the absence of concomitant measurement of oro-cecal transit by scintigraphy,[68 69] a MRI biomarker has been developed that is capable of assessing intestinal and colonic secretory effects and volume changes non-invasively.[70 71] This approach requires further validation since colonic volumes may not correlate with other beneficial effects of a low FODMAP diet.[72]

Treatment

An analysis of the literature confirmed that a low FODMAP diet leads to profound changes in the microbiota and metabolome; however, it was concluded that the duration and clinical relevance of those changes are as yet unknown.[73 74] Due to the heterogeneity of reviewed studies, the influence on patients' gut microbiome composition and/or microbiota metabolites requires additional studies and, despite the microbial changes, there was no measurable effect of a low FODMAP diet on colonic volume in humans.[71]

Other candidate biomarkers

Candidate biomarkers that have been proposed in functional lower gastrointestinal disorders are cytotoxic lethal binding toxin and vinculin assays, mast cell infiltration and intestinal permeability. However, these fall short of the criteria for inclusion. Thus, the pathobiological mechanism(s) that results in symptoms in patients with positive cytotoxic lethal binding toxin and vinculin assays in association with IBS is still unclear, and there is no treatment directed to the toxin or vinculin that reverses IBS. While mast cell infiltration in proximity to nerves may be associated with afferent dysfunction or IBS, there is no evidence of increased infiltration or altered localisation of mast cells in the colonic mucosa of patients with IBS.[75] Positive correlations between mast cells and symptoms were documented in 6 of 30 studies in a systematic review.[76] A systematic review and meta-analysis concluded that changes in mast cell numbers are segmental, sometimes IBS-subtype dependent, and the diagnostic value of the quantification of colonic mucosal cells in IBS requires further investigation.[77] Finally, a pilot study demonstrated efficacy of a mast cell stabiliser in IBS, but there was no placebo or active control treatment arm in the study.[78]

With regard to intestinal permeability, there was evidence of abnormal permeability in IBS (summarised in reference[79]), and a controlled trial of glutamine suggested potential for clinical and mechanistic benefits;[80] however, the methodology for measurement of intestinal and colonic permeability in vivo in humans and the pathobiological significance of in vitro measurements of intestinal permeability are still incompletely resolved.[81]

The potential roles of the microbiome or metabolomics as biomarkers in lower functional gastrointestinal disorders are still unclear, based on systematic reviews and meta-analyses, given the inconsistent data across studies, the inability to identify a microbial signature even in the extremes of phenotype (diarrhoea vs constipation),[82] the lack of clear pathobiological mechanism(s) and the inconsistent efficacy of faecal microbial transplantation.[83]

Functional upper gastrointestinal symptoms: identify actionable biomarkers suggestive of organic mechanisms

In the upper gastrointestinal tract, the predominant actionable biomarkers are gastric emptying, accommodation and sensation. Among almost 1300 patients with upper gastrointestinal symptoms, which included 108 with diabetes mellitus,[84 85] we demonstrated equal proportions with abnormal gastric emptying, impaired gastric accommodation, both, or neither, suggesting increased sensation.

Diagnosis

With regard to *gastric emptying*, it is important to note that an optimal gastric emptying test, typically 4 hour measurement by scintigraphy, is essential. Normal values and performance characteristics of the scintigraphic test have been published.[4]

An optimally measured gastric emptying result was associated with nausea, vomiting and bloating,[86] and there was a significant relationship between the improvement in gastric emptying and the improvement in symptoms on a meta-regression analysis. The slope of the analysis showed that one unit change in symptoms severity (based on standardised mean difference) was associated with a change in gastric emptying $T_{1/2}$ of 20.4 min.[87] Unfortunately, many studies reported in the literature had used measurements over 2 hours, which may not be sufficient to appraise the relationship between gastric emptying and symptoms. Alternative valid approaches to measure gastric emptying are stable isotope-based gastric emptying tests that have the advantage of standardisation and centralised measurements, and are, therefore, applicable particularly in multicentre trials. For emptying of solids, the usual stable isotopes used are ^{13}C-spirulina (a protein derived from a blue-green alga)[88] and ^{13}C-octanoate (a medium chain triglyceride),[89] both of which are easily digested and rapidly absorbed in the upper intestine, metabolised and excreted from the lungs as $^{13}CO_2$, and therefore the rate-limiting step for breath excretion is the rate of gastric emptying. Special attention should be given to the face value of the results (calculated lag time and $T_{1/2}$) and the mathematical formula used to derive these summary results.[90]

In our studies, *gastric accommodation* was measured using SPECT imaging,[91] which unfortunately is not widely available. Other centres use an intragastric barostat or intraluminal high-resolution manometry to measure gastric accommodation.[92 93] However, a nutrient drink test is a surrogate for gastric accommodation and *increased sensation;*[94] thus, a maximum tolerated volume of less than about 750 kilocalories is significantly

Table 2 Actionable biomarkers in lower and upper functional gastrointestinal disorders

Biomarker	Cases (N)	Controls (N)	Cut-offs	Sn	Sp	PPV	NPV	+ LR	-LR	Ref. #
Rectal evacuation disorder										
Digital rectal exam (DRE)	390	72	>2 findings on DRE	83.9	68.1	49	92.8	2.6	0.2	12
Anorectal manometry: rectoanal pressure gradient	74	30	<-40 mm Hg	32.4	100	100	85.5	>10	0.7	108
Balloon expulsion test	74	202	22 s	77.8	69.8	39.2	92.6	2.6	0.3	15
Rectal gas volume	65	53	>20 mL	38.1	89.1	46.6	85.2	3.5	0.7	14
Rectal area on scout film	65	53	>900 mm^2	39.5	73.8	27.4	83.0	1.5	0.8	14
Slow transit constipation										
Stool burden score on abdominal X-ray	145	216	>7	86.9	54.5	27.1	95.5	1.9	0.2	19
Colonic transit with scintigraphy	RED: 390 STC: 61	211	Geometric centre 48 hours <2.1	82	65	31.3	94.9	2.4	0.3	17
Fast transit diarrhoea										
Colonic transit with scintigraphy	139	170	Geometric centre at 24 hours >3.8	31.6	87.2	21.5	91.9	2.5	0.8	18
Bile acid diarrhoea										
^{75}SeHCAT	26	33	3-day retention: 34%	100	94	33	77	1.7	0.9	109
48 hours faecal bile acid	64	30	Primary BA >4% + total BA >1000 µmol/48 hours	46	97	83.6	84.3	15.3	0.5	43
FGF-19	7	23	≤61.7 pg/mL	29	83	29	78	1.3	0.9	40
7aC4	7	23	≥52.5 ng/mL	29	78	33	79	1.7	0.8	40
Carbohydrate maldigestion										
Fructose breath test	108	185	Peak rise in breath H_2 >20 ppm	98	86	26.9	99.9	7.0	0.02	110
Lactose breath test	Total subjects: 41		Peak rise in breath hydrogen >20 µL/L	80	100	100	98.9	∞	0.2	111
Upper GI motor dysfunctions										
Gastric emptying solids at 4 hour with scintigraphy	10	25	>25% retention	100	70	6.4	100	3.3	0	112
Gastric accommodation with SPECT	32	20	PP/fasting ratio >3.0	40.6	95	52.5	92.1	8.1	0.6	113

BA, bile acids; IBS-D, irritable bowel syndrome-diarrhoea; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; RED, rectal evacuation disorder; Sn, sensitivity; Sp, specificity; STC, slow transit constipation.

correlated with impairment of gastric accommodation measured by an intragastric balloon and could serve as a biomarker for accommodation and sensation. Recent attempts to use two-dimensional imaging to estimate gastric accommodation require further validation.[95–97]

Treatment

Treatment of functional upper gastrointestinal symptoms should be based on identification of the actionable biomarkers discussed under 'Diagnosis'. For example, in a randomised, placebo-controlled, cross-over study of 4 weeks duration with a 2-week washout period in between each treatment arm, the 5-HT_4 receptor agonist, prucalopride, was shown to improve symptom control as well as gastric emptying in 28 patients with idiopathic gastroparesis (seven men, age 42.3±2.6 years) and six patients with diabetes (one man).[98] Similarly, another 5-HT_4 receptor agonist, velusetrag, was efficacious in the treatment of diabetic or idiopathic gastroparesis.[99]

The pentapeptide ghrelin agonist, relamorelin, has been shown to accelerate gastric emptying measured by scintigraphy in both type 1 and type 2 diabetes[100] and, in addition, it demonstrated substantial improvement in gastric emptying as well as core diabetic gastroparesis symptoms individually, using a composite total score in a phase 2b trial.[101]

For impaired gastric accommodation, several medications have been associated with enhancement of postprandial gastric relaxation and relief of symptoms in patients with functional dyspepsia: buspirone[102] and acotiamide.[103]

For increased gastric sensation, the dopamine D2 and D3 antagonist, TAK506, significantly increased the volume to fullness compared with baseline with 1 week of treatment.[104] The NK-1 receptor antagonist, aprepitant, improved multiple symptoms of gastroparesis including nausea,[105] and aprepitant has been shown to enhance gastric accommodation rather than affect gastric emptying.[106] Similarly, tradipitant, a novel NK-1 receptor antagonist, improved nausea and other symptoms of gastroparesis in a 4-week, randomised, controlled trial.[107]

SUMMARY OF THE VALIDATION OF PROPOSED BIOMARKERS

Table 2 provides a summary of the sensitivity, specificity, positive predictive value, negative predictive value and positive and negative likelihood ratios for the commonly available actionable biomarkers in lower and upper functional gastrointestinal disorders discussed in this article.[12 14 15 17–19 40 43 108–113] It is important to note the almost uniform high specificity of the biomarkers listed, all of which are actionable in accordance with the criteria used in this article.

CONCLUSION

The impact of approved and experimental medications for functional disorders of the upper and lower gastrointestinal tract is enhanced by the development and validation of actionable biomarkers that facilitate individualisation of treatment based on phenotype. Although there have been advances, it is important to acknowledge the fact that there is high specificity of many of these biomarkers, but sensitivity and positive likelihood ratios are not yet ideal since the average sensitivity of the biomarkers in table 1 is 60% and the average positive likelihood ratio is 5.04. Thus, more work is required in this field to continue to validate biomarkers that are directly related to the pathophysiology, building on the treatise provided in this article.

Acknowledgements The authors thank Mrs Cindy Stanislav for excellent secretarial assistance.

Contributors MC conceived the idea and drafted the manuscript and revised and finalised the manuscript. He is the senior author. VC is the co-author. He was involved in revision and finalising of the manuscript.

Funding MC research work is supported by grants R01-DK115950 and R01- DK122280 from National Institutes of Health. MC is the guarantor of the manuscript, taking responsibility for its accuracy, having access to all of the data and having the decision to publish the paper.

Competing interests MC has received research support for proof of concept single centre or multicentre studies from Allergan, Bird Rock Bio, Novartis and Takeda.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

To cite Camilleri M, Chedid V. *Gut* 2020;**69**:1730–1737.

Received 20 November 2019
Revised 12 February 2020
Accepted 15 February 2020
Published Online First 8 April 2020

Gut 2020;**69**:1730–1737.
doi:10.1136/gutjnl-2019-320325

ORCID iDs
Michael Camilleri http://orcid.org/0000-0001-6472-7514
Victor Chedid http://orcid.org/0000-0001-8548-9228

REFERENCES

1 Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001;69:89–95.
2 Valeri N. Streamlining detection of fusion genes in colorectal cancer: Having "faith" in precision oncology in the (tissue) "agnostic" era. *Cancer Res* 2019;79:1041–3.
3 Camilleri M. Review article: biomarkers and personalised therapy in functional lower gastrointestinal disorders. *Aliment Pharmacol Ther* 2015;42:818–28.
4 Camilleri M, Iturrino J, Bharucha AE, *et al*. Performance characteristics of scintigraphic measurement of gastric emptying of solids in healthy participants. *Neurogastroenterol Motil* 2012;24:1076–e562.
5 Kolar GJ, Camilleri M, Burton D, *et al*. Prevalence of colonic motor or evacuation disorders in patients presenting with chronic nausea and vomiting evaluated by a single gastroenterologist in a tertiary referral practice. *Neurogastroenterol Motil* 2014;26:131–8.
6 O'Dwyer RH, Acosta A, Camilleri M, *et al*. Clinical features and colonic motor disturbances in chronic megacolon in adults. *Dig Dis Sci* 2015;60:2398–407.
7 Oblizajek NR, Gandhi S, Sharma M, *et al*. Anorectal pressures measured with high-resolution manometry in healthy people—Normal values and asymptomatic pelvic floor dysfunction. *Neurogastroenterol Motil* 2019;25:e13597.
8 Breen M, Camilleri M, Burton D, *et al*. Performance characteristics of the measurement of gastric volume using single photon emission computed tomography. *Neurogastroenterol Motil* 2011;23:308–15.
9 Vijayvargiya P, Camilleri M, Burton D, *et al*. Bile and fat excretion are biomarkers of clinically significant diarrhoea and constipation in irritable bowel syndrome. *Aliment Pharmacol Ther* 2019;49:744–58.
10 Donato LJ, Lueke A, Kenyon SM, *et al*. Description of analytical method and clinical utility of measuring serum 7-alpha-hydroxy-4-cholesten-3-one (7aC4) by mass spectrometry. *Clin Biochem* 2018;52:106–11.
11 Lembo A, Camilleri M. Chronic constipation. *N Engl J Med* 2003;349:1360–8.
12 Brandler J, Camilleri M. Pre- and post-test probability to diagnose rectal evacuation disorders based on symptoms, rectal exam, and basic tests. *Clin Gastroenterol Hepatol*. In Press;2019.
13 Park S-Y, Khemani D, Nelson AD, *et al*. Rectal gas volume measured by computerized tomography identifies evacuation disorders in patients with constipation. *Clin Gastroenterol Hepatol* 2017;15:543–52.
14 Park S-Y, Khemani D, Acosta A, *et al*. Rectal gas volume: defining cut-offs for screening for evacuation disorders in patients with constipation. *Neurogastroenterol Motil* 2017;29:e13044.
15 Chedid V, Vijayvargiya P, Halawi H, *et al*. Audit of the diagnosis of rectal evacuation disorders in chronic constipation. *Neurogastroenterol Motil* 2019;31:e13510.
16 Camilleri M. Anal achalasia: the rat tail gas sign. *Clin Gastroenterol Hepatol* 2018;16:e124.
17 Nullens S, Nelsen T, Camilleri M, *et al*. Regional colon transit in patients with dys-synergic defaecation or slow transit in patients with constipation. *Gut* 2012;61:1132–9.
18 Manabe N, Wong BS, Camilleri M, *et al*. Lower functional gastrointestinal disorders: evidence of abnormal colonic transit in a 287 patient cohort. *Neurogastroenterol Motil* 2010;22:293–e82.
19 Cangemi DJ, Flanagan R, Barshop K, *et al*. Colonic stool burden a useful surrogate for slow transit constipation as determined by a radiopaque transit study. *Am J Gastroenterol* 2019;114:519–23.

20 Rao SSC, Seaton K, Miller M, *et al*. Randomized controlled trial of biofeedback, sham feedback, and standard therapy for dyssynergic defecation. *Clin Gastroenterol Hepatol* 2007;5:331–8.
21 Chiarioni G, Whitehead WE, Pezza V, *et al*. Biofeedback is superior to laxatives for normal transit constipation due to pelvic floor dyssynergia. *Gastroenterology* 2006;130:657–64.
22 Heymen S, Scarlett Y, Jones K, *et al*. Randomized, controlled trial shows biofeedback to be superior to alternative treatments for patients with pelvic floor dyssynergia-type constipation. *Dis Colon Rectum* 2007;50:428–41.
23 Woodward S, Norton C, Chiarelli P. Biofeedback for treatment of chronic idiopathic constipation in adults. *Cochrane Database Syst Rev* 2014;3:CD008486.
24 Skardoon GR, Khera AJ, Emmanuel AV, *et al*. Review article: dyssynergic defaecation and biofeedback therapy in the pathophysiology and management of functional constipation. *Aliment Pharmacol Ther* 2017;46:410–23.
25 Lee BH, Kim N, Kang S-B, *et al*. The long-term clinical efficacy of biofeedback therapy for patients with constipation or fecal incontinence. *J Neurogastroenterol Motil* 2010;16:177–85.
26 Rao SSC, Valestin JA, Xiang X, *et al*. Home-Based versus office-based biofeedback therapy for constipation with dyssynergic defecation: a randomised controlled trial. *Lancet Gastroenterol Hepatol* 2018;3:768–77.
27 Cuda T, Gunnarsson R, de Costa A. Symptoms and diagnostic criteria of acquired Megacolon - a systematic literature review. *BMC Gastroenterol* 2018;18:25.
28 Koch TR, Schulte-Bockholt A, Otterson MF, *et al*. Decreased vasoactive intestinal peptide levels and glutathione depletion in acquired megacolon. *Dig Dis Sci* 1996;41:1409–16.
29 Koch TR, Schulte-Bockholt A, Telford GL, *et al*. Acquired megacolon is associated with alteration of vasoactive intestinal peptide levels and acetylcholinesterase activity. *Regul Pept* 1993;48:309–19.
30 Chen B, Knowles CH, Scott M, *et al*. Idiopathic slow transit constipation and megacolon are not associated with neurturin mutations. *Neurogastroenterol Motil* 2002;14:513–7.
31 Camilleri M, Wieben E, Eckert D, *et al*. Familial chronic megacolon presenting in childhood or adulthood: seeking the presumed gene association. *Neurogastroenterol Motil* 2019;31:e13550.
32 Wang XJ, Camilleri M. Chronic megacolon presenting in adolescents or adults: clinical manifestations, diagnosis, and genetic associations. *Dig Dis Sci* 2019;64:2750–6.
33 Gibbons D, Camilleri M, Nelson AD, *et al*. Characteristics of chronic megacolon among patients diagnosed with multiple endocrine neoplasia type 2B. *United European Gastroenterol J* 2016;4:449–54.
34 Vijayvargiya P, Camilleri M. Current practice in the diagnosis of bile acid diarrhea. *Gastroenterology* 2019;156:1233–8.
35 Valentin N, Camilleri M, Altayar O, *et al*. Biomarkers for bile acid diarrhoea in functional bowel disorder with diarrhoea: a systematic review and meta-analysis. *Gut* 2016;65:1951–9.
36 Wedlake L, A'Hern R, Russell D, *et al*. Systematic review: the prevalence of idiopathic bile acid malabsorption as diagnosed by SeHCAT scanning in patients with diarrhoea-predominant irritable bowel syndrome. *Aliment Pharmacol Ther* 2009;30:707–17.
37 Hofmann AF, Mangelsdorf DJ, Kliewer SA. Chronic diarrhea due to excessive bile acid synthesis and not defective ileal transport: a new syndrome of defective fibroblast growth factor 19 release. *Clin Gastroenterol Hepatol* 2009;7:1151–4.
38 Walters JRF, Tasleem AM, Omer OS, *et al*. A new mechanism for bile acid diarrhea: defective feedback inhibition of bile acid biosynthesis. *Clin Gastroenterol Hepatol* 2009;7:1189–94.
39 Pattni SS, Brydon WG, Dew T, *et al*. Fibroblast growth factor 19 in patients with bile acid diarrhoea: a prospective comparison of FGF19 serum assay and SeHCAT retention. *Aliment Pharmacol Ther* 2013;38:967–76.
40 Vijayvargiya P, Camilleri M, Carlson P, *et al*. Performance characteristics of serum C4 and FGF19 measurements to exclude the diagnosis of bile acid diarrhoea in IBS-diarrhoea and functional diarrhoea. *Aliment Pharmacol Ther* 2017;46:581–8.
41 Arasaradnam RP, Brown S, Forbes A, *et al*. Guidelines for the investigation of chronic diarrhoea in adults: British Society of gastroenterology, 3rd edition. *Gut* 2018;67:1380–99.
42 Sadowski DC, Camilleri M, Chey WD, *et al*. Canadian association of gastroenterology clinical practice guideline on the management of bile acid diarrhea. *Clin Gastroenterol Hepatol* 2020;18:24–41.
43 Vijayvargiya P, Camilleri M, Chedid V, *et al*. Analysis of fecal primary bile acids detects increased stool weight and colonic transit in patients with chronic functional diarrhea. *Clin Gastroenterol Hepatol* 2019;17:922–9.
44 Vijayvargiya P, Donato LJ, Calderon G, *et al*. 856 – assessment of the impact of introducing fecal bile acid diagnostic test for bile acid diarrhea in 250 patients in clinical practice and on prediction of response to bile acid Sequestrants. *Gastroenterology* 2019;156:S188–9.
45 Turner JM, Pattni SS, Appleby RN, *et al*. A positive SeHCAT test results in fewer subsequent investigations in patients with chronic diarrhoea. *Frontline Gastroenterol* 2017;8:279–83.
46 Smalley W, Falck-Ytter C, Carrasco-Labra A, *et al*. AGA clinical practice guidelines on the laboratory evaluation of functional diarrhea and diarrhea-predominant irritable bowel syndrome in adults (IBS-D). *Gastroenterology* 2019;157:851–4.
47 Bajor A, Törnblom H, Rudling M, *et al*. Increased colonic bile acid exposure: a relevant factor for symptoms and treatment in IBS. *Gut* 2015;64:84–92.
48 Camilleri M, Acosta A, Busciglio I, *et al*. Effect of colesevelam on faecal bile acids and bowel functions in diarrhoea-predominant irritable bowel syndrome. *Aliment Pharmacol Ther* 2015;41:438–48.
49 Walters JRF, Johnston IM, Nolan JD, *et al*. The response of patients with bile acid diarrhoea to the farnesoid X receptor agonist obeticholic acid. *Aliment Pharmacol Ther* 2015;41:54–64.
50 Camilleri M, Nord SL, Burton D, *et al*. 976 – a double-blind, randomized, placebo-controlled, crossover, multiple-dose study of Tropifexor, a non bile acid FXR agonist, in patients with primary bile acid diarrhea. *Gastroenterology* 2019;156:S204–5.
51 Vijayvargiya P, Busciglio I, Burton D, *et al*. Bile acid deficiency in a subgroup of patients with irritable bowel syndrome with constipation based on biomarkers in serum and fecal samples. *Clin Gastroenterol Hepatol* 2018;16:522–7.
52 Rao AS, Wong BS, Camilleri M, *et al*. Chenodeoxycholate in females with irritable bowel syndrome-constipation: a pharmacodynamic and pharmacogenetic analysis. *Gastroenterology* 2010;139:1549–58.
53 Wong BS, Camilleri M, McKinzie S, *et al*. Effects of A3309, an ileal bile acid transporter inhibitor, on colonic transit and symptoms in females with functional constipation. *Am J Gastroenterol* 2011;106:2154–64.
54 Nakajima A, Seki M, Taniguchi S, *et al*. Safety and efficacy of elobixibat for chronic constipation: results from a randomised, double-blind, placebo-controlled, phase 3 trial and an open-label, single-arm, phase 3 trial. *Lancet Gastroenterol Hepatol* 2018;3:537–47.
55 Nakajima A, Ishizaki S, Kurosu S, *et al*. Elobixibat, ileal bile acid transporter inhibitor, increases fecal bile acids in patients with chronic constipation. *DDW abstract submitted* 2020.
56 Zhou S-Y, Gillilland M, Wu X, *et al*. FODMAP diet modulates visceral nociception by lipopolysaccharide-mediated intestinal inflammation and barrier dysfunction. *J Clin Invest* 2018;128:267–80.
57 Lactose intolerance. Reprinted from Genetics Home Reference. Reviewed: May 2010, Published: October 29, 2019. Lister Hill National Center for Biomedical Communications, U.S. National Library of Medicine, National Institutes of Health, Department of Health & Human Services. Available: https://ghr.nlm.nih.gov/condition/lactose-intolerance [Accessed 1 Nov 2019].
58 Suarez FL, Savaiano DA, Levitt MD. A comparison of symptoms after the consumption of milk or lactose-hydrolyzed milk by people with self-reported severe lactose intolerance. *N Engl J Med* 1995;333:1–4.
59 Kolars JC, Levitt MD, Aouji M, *et al*. Yogurt — an autodigesting source of lactose. *N Engl J Med* 1984;310:1–3.
60 Cox TM, Camilleri M, O'Donnell MW, *et al*. Pseudodominant transmission of fructose intolerance in an adult and three offspring: heterozygote detection by intestinal biopsy. *N Engl J Med* 1982;307:537–40.
61 Borgstrom B, Dahlquist A, Lundh G, *et al*. Studies of intestinal digestion and absorption in the human. *J Clin Invest* 1957;36:1521–36.
62 Holdsworth CD, Dawson AM. The absorption of monosaccharides in man. *Clin Sci* 1964;27:371–9.
63 Holdsworth CD, Dawson AM. Glucose and fructose absorption in idiopathic steatorrhoea. *Gut* 1965;6:387–91.
64 Skoog SM, Bharucha AE, Zinsmeister AR. Comparison of breath testing with fructose and high fructose corn syrups in health and IBS. *Neurogastroenterol Motil* 2008;20:505–11.
65 Rumessen JJ, Gudmand-Høyer E. Absorption capacity of fructose in healthy adults. Comparison with sucrose and its constituent monosaccharides. *Gut* 1986;27:1161–8.
66 Wang XJ, Camilleri M, Vanner S, *et al*. Review article: biological mechanisms for symptom causation by individual FODMAP subgroups - the case for a more personalised approach to dietary restriction. *Aliment Pharmacol Ther* 2019;50:517–29.
67 Staudacher HM, Lomer MCE, Farquharson FM, *et al*. A diet low in FODMAPs reduces symptoms in patients with irritable bowel syndrome and a probiotic restores Bifidobacterium species: a randomized controlled trial. *Gastroenterology* 2017;153:936–47.
68 Yu D, Cheeseman F, Vanner S. Combined oro-caecal scintigraphy and lactulose hydrogen breath testing demonstrate that breath testing detects oro-caecal transit, not small intestinal bacterial overgrowth in patients with IBS. *Gut* 2011;60:334–40.
69 Lin EC, Massey BT. Scintigraphy demonstrates high rate of false-positive results from glucose breath tests for small bowel bacterial overgrowth. *Clin Gastroenterol Hepatol* 2016;14:203–8.
70 Major G, Murray K, Singh G, *et al*. Demonstration of differences in colonic volumes, transit, chyme consistency, and response to psyllium between healthy and constipated subjects using magnetic resonance imaging. *Neurogastroenterol Motil* 2018;30:e13400.
71 Wilkinson-Smith VC, Major G, Ashleigh L, *et al*. Insights into the different effects of food on intestinal secretion using magnetic resonance imaging. *JPEN J Parenter Enteral Nutr* 2018;42:1342–8.
72 Sloan TJ, Jalanka J, Major GAD, *et al*. A low FODMAP diet is associated with changes in the microbiota and reduction in breath hydrogen but not colonic volume in healthy subjects. *PLoS One* 2018;13:e0201410.
73 Staudacher HM, Whelan K. The low FODMAP diet: recent advances in understanding its mechanisms and efficacy in IBS. *Gut* 2017;66:1517–27.

74 Su H, Li Y-T, Heitkemper MM, *et al*. Effects of low-FODMAPS diet on irritable bowel syndrome symptoms and gut microbiome. *Gastroenterol Nurs* 2019;42:150–8.
75 Sundin J, Nordlander S, Eutamene H, *et al*. Colonic mast cell numbers, symptom profile, and mucosal expression of elements of the epithelial barrier in irritable bowel syndrome. *Neurogastroenterol Motil* 2019;31:e13701.
76 Krammer L, Sowa AS, Lorentz A. Mast cells in irritable bowel syndrome: a systematic review. *J Gastrointest Liver Dis* 2019;28:463–72.
77 Bashashati M, Moossavi S, Cremon C, *et al*. Colonic immune cells in irritable bowel syndrome: a systematic review and meta-analysis. *Neurogastroenterol Motil* 2018;30:e13192.
78 Lobo B, Ramos L, Martínez C, *et al*. Downregulation of mucosal mast cell activation and immune response in diarrhoea-irritable bowel syndrome by oral disodium cromoglycate: a pilot study. *United European Gastroenterol J* 2017;5:887–97.
79 Camilleri M, Lyle BJ, Madsen KL, *et al*. Role for diet in normal gut barrier function: developing guidance within the framework of food-labeling regulations. *Am J Physiol Gastrointest Liver Physiol* 2019;317:G17–39.
80 Zhou Q, Verne ML, Fields JZ, *et al*. Randomised placebo-controlled trial of dietary glutamine supplements for postinfectious irritable bowel syndrome. *Gut* 2019;68:996–1002.
81 Camilleri M. Leaky gut: mechanisms, measurement and clinical implications in humans. *Gut* 2019;68:1516–26.
82 Pittayanon R, Lau JT, Yuan Y, *et al*. Gut microbiota in patients with irritable bowel Syndrome—A systematic review. *Gastroenterology* 2019;157:97–108.
83 Moayyedi P. Faecal microbiota transplantation for IBS: still a long way to go. *Lancet Gastroenterol Hepatol* 2019;4:656–7.
84 Park S-Y, Acosta A, Camilleri M, *et al*. Gastric motor dysfunction in patients with functional gastroduodenal symptoms. *Am J Gastroenterol* 2017;112:1689–99.
85 Chedid V, Brandler J, Vijayvargiya P, *et al*. Characterization of upper gastrointestinal symptoms, gastric motor functions, and associations in patients with diabetes at a referral center. *Am J Gastroenterol* 2019;114:143–54.
86 Vijayvargiya P, Jameie-Oskooei S, Camilleri M, *et al*. Association between delayed gastric emptying and upper gastrointestinal symptoms: a systematic review and meta-analysis. *Gut* 2019;68:804–13.
87 Vijayvargiya P, Camilleri M, Chedid V, *et al*. Effects of promotility agents on gastric emptying and symptoms: a systematic review and meta-analysis. *Gastroenterology* 2019;156:1650–60.
88 Szarka LA, Camilleri M, Vella A, *et al*. A stable isotope breath test with a standard meal for abnormal gastric emptying of solids in the clinic and in research. *Clin Gastroenterol Hepatol* 2008;6:635–43.
89 Maes BD, Mys G, Geypens BJ, *et al*. Gastric emptying flow curves separated from carbon-labeled octanoic acid breath test results. *Am J Physiol Gastrointest Liver Physiol* 1998;275:G169–75.
90 Odunsi ST, Camilleri M, Szarka LA, *et al*. Optimizing analysis of stable isotope breath tests to estimate gastric emptying of solids. *Neurogastroenterol Motil* 2009;21:706–38.
91 Bouras EP, Delgado-Aros S, Camilleri M, *et al*. Spect imaging of the stomach: comparison with barostat, and effects of sex, age, body mass index, and fundoplication. single photon emission computed tomography. *Gut* 2002;51:781–6.
92 Tack J, Piessevaux H, Coulie B, *et al*. Role of impaired gastric accommodation to a meal in functional dyspepsia. *Gastroenterology* 1998;115:1346–52.
93 Carbone F, Tack J, Hoffman I. The intragastric pressure measurement: a novel method to assess gastric accommodation in functional dyspepsia children. *J Pediatr Gastroenterol Nutr* 2017;64:918–24.
94 Tack J, Caenepeel P, Piessevaux H. Assessment of meal induced gastric accommodation by a satiety drinking test in health and in severe functional dyspepsia. *Gut* 2003;52:1271–7.
95 Orthey P, Yu D, Van Natta ML, *et al*. Intragastric meal distribution during gastric emptying scintigraphy for assessment of fundic accommodation: correlation with symptoms of gastroparesis. *J Nucl Med* 2018;59:691–7.
96 Orthey P, Dadparvar S, Parkman HP, *et al*. Enhanced gastric emptying scintigraphy to assess fundic accommodation using intragastric meal distribution and antral contractility. *J Nucl Med Technol* 2019;47:138–43.
97 Chedid V, Halawi H, Brandler J, *et al*. Gastric accommodation measurements by single photon emission computed tomography and two-dimensional scintigraphy in diabetic patients with upper gastrointestinal symptoms. *Neurogastroenterol Motil* 2019;31:e13581.
98 Carbone F, Van den Houte K, Clevers E, *et al*. Prucalopride in gastroparesis: a randomized placebo-controlled crossover study. *Am J Gastroenterol* 2019;114:1265–74.
99 Abell T, Kuo B, Esfandyari T, *et al*. 784 – Velusetrag improves Gastoparesis both in symptoms and gastric emptying in patients with diabetic or idiopathic gastroparesis in a 12-week global phase 2B study. *Gastroenterology* 2019;156:S164.
100 Shin A, Camilleri M, Busciglio I, *et al*. The ghrelin agonist RM-131 accelerates gastric emptying of solids and reduces symptoms in patients with type 1 diabetes mellitus. *Clin Gastroenterol Hepatol* 2013;11:1453–9.
101 Camilleri M, McCallum RW, Tack J, *et al*. Efficacy and safety of Relamorelin in diabetics with symptoms of gastroparesis: a randomized, placebo-controlled study. *Gastroenterology* 2017;153:1240–50.
102 Tack J, Janssen P, Masaoka T, *et al*. Efficacy of buspirone, a fundus-relaxing drug, in patients with functional dyspepsia. *Clin Gastroenterol Hepatol* 2012;10:1239–45.
103 Kusunoki H, Haruma K, Manabe N, *et al*. Therapeutic efficacy of acotiamide in patients with functional dyspepsia based on enhanced postprandial gastric accommodation and emptying: randomized controlled study evaluation by real-time ultrasonography. *Neurogastroenterol Motil* 2012;24:540–5.
104 Dukes GE, Scimia C, Kuo B, *et al*. Tolerability and pharmacodynamics of tak-906, a dopamine 2,3 antagonist, in patients with diabetic or idiopathic gastroparesis. *Neurogastroenterol Motil* 2019;31:e13657.
105 Pasricha PJ, Yates KP, Sarosiek I, *et al*. Aprepitant has mixed effects on nausea and reduces other symptoms in patients with gastroparesis and related disorders. *Gastroenterology* 2018;154:65–76.
106 Jacob D, Busciglio I, Burton D, *et al*. Effects of NK1 receptors on gastric motor functions and satiation in healthy humans: results from a controlled trial with the NK1 antagonist aprepitant. *Am J Physiol Gastrointest Liver Physiol* 2017;313:G505–10.
107 Carlin JL, Lieberman VR, Dahal A, *et al*. 951d – Tradipitant, a novel NK-1 receptor antagonist, significantly improved nausea and other symptoms of gastroparesis: results of a multicenter, randomized, double-blind, placebo-controlled phase II trial. *Gastroenterology* 2019;156:S1510–1.
108 Heinrich H, Sauter M, Fox M, *et al*. Assessment of obstructive defecation by high-resolution anorectal manometry compared with magnetic resonance defecography. *Clin Gastroenterol Hepatol* 2015;13:1310–7.
109 Sciarretta G, Furno A, Mazzoni M, *et al*. Post-Cholecystectomy diarrhea: evidence of bile acid malabsorption assessed by SeHCAT test. *Am J Gastroenterol* 1992;87:1852–4.
110 Götze H, Mahdi A. [Fructose malabsorption and dysfunctional gastrointestinal manifestations]. *Monatsschr Kinderheilkd* 1992;140:814–7.
111 Rosado JL, Solomons NW. Sensitivity and specificity of the hydrogen breath-analysis test for detecting malabsorption of physiological doses of lactose. *Clin Chem* 1983;29:545–8.
112 Thomforde GM, Camilleri M, Phillips SF, *et al*. Evaluation of an inexpensive screening scintigraphic test of gastric emptying. *J Nucl Med* 1995;36:93–6.
113 Kim DY, Delgado-Aros S, Camilleri M, *et al*. Noninvasive measurement of gastric accommodation in patients with idiopathic nonulcer dyspepsia. *Am J Gastroenterol* 2001;96:3099–105.