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Duodenal inflammation: an emerging target for functional dyspepsia?

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Abstract

Introduction: Functional dyspepsia (FD) is one of the most common functional gastrointestinal disorders and is classified into postprandial distress and epigastric pain syndrome. Despite the recognition of duodenal inflammation as a potential trigger of symptoms, only limited anti-inflammatory therapies exist.

Areas covered: This narrative review summarizes the recent advances in the pathophysiology and treatment of FD; it identifies potential therapeutic targets and gaps in the field. An electronic literature search was conducted in Pubmed up to 31st of December 2019.

Expert opinion: There is compelling evidence for the role of duodenal inflammation and the eosinophil-mast cell axis in the pathogenesis of dyspeptic symptoms. Traditional prokinetic drugs and neuromodulators target gastric dysmotility and visceral hypersensitivity, but are hampered by limited efficacy and side effects. Independent of acid suppression, the anti-inflammatory action of proton pump inhibitors, which remain the first-line therapy in FD, may also explain their therapeutic effect. Other existing and newly established anti-inflammatory drugs should be investigated while trials including probiotics and selective antibiotics should examine the host microbiome and immune activation. Targeted treatments for potential causes of duodenal pathology such as impaired permeability and dysbiosis, are likely to emerge in the future.

Keywords: Functional dyspepsia, postprandial distress syndrome, epigastric pain syndrome, eosinophil, mast cell, proton pump inhibitor, histamine receptor blocker

Article Highlights

- Inflammation in functional dyspepsia (FD) is characterized by duodenal mucosal eosinophils and mast cells with increased systemic gut homing lymphocytes
- Proton pump inhibitors (PPI) have anti-inflammatory effects in patients with duodenal eosinophilia, which may be the trigger of dyspeptic symptoms
- Inhibition of T-helper(Th)-2 cytokines and eosinophil chemoattractants may improve symptoms related to eosinophil infiltration, similar to eosinophilic oesophagitis
- Treatment with histamine- or leukotriene-receptor antagonists is effective in pediatric patients and randomized-controlled trials are needed in adult patients
- There are no established tight junction regulators for FD but this is a key novel drug target
- Novel probiotics may potentially target impaired barrier function in FD
- Traditional prokinetic drugs and neuromodulators are hampered by limited efficacy and potential side effects

1. Introduction

Functional dyspepsia (FD) is a chronic functional gastrointestinal disorder (FGID) defined by upper abdominal symptoms originating from the gastroduodenal region with no abnormalities on routine investigation[1]. Two subgroups were proposed by the international Rome III consensus and reiterated in the Rome IV revision: postprandial distress syndrome (PDS) with meal-related symptoms of postprandial fullness and early satiation, and epigastric pain syndrome (EPS) with meal-unrelated epigastric pain and burning[2] (Table 1). Up to 20% of the general population experiences dyspeptic symptoms, which overlaps with reflux symptoms and results in more frequent and severe symptoms in up to a third of patients[3]. A recent epidemiological study reported a higher prevalence of FD in the USA (12%) compared to Canada and the UK (both 8%) with a majority of PDS (61%) compared to EPS (18%) or overlapping symptoms of PDS and EPS (21%)[4]. Health impairment and health-care usage were significantly higher in dyspeptic patients[4], which was also reported by a Belgian study with frequent absenteeism and impact on daily life[3]. The loss of work and activity days was confirmed by the US Upper Gastrointestinal Study[5], with significantly increased yearly medical costs ($5138) and increased work absences in a retrospective study[6]. Finally, quality of life in FD is impaired in all main domains (physical, mental and social aspects)[7].

Despite the common occurrence of FD with considerable health care expenses and impact on quality of life, the underlying pathophysiology is unclear. Although changes in gastric function have been studied, the cardinal FD symptoms do not consistently correlate with gastric emptying[8,9]. Moreover, a large tertiary-care study showed a similar prevalence of gastric sensorimotor dysfunction (delayed emptying, impaired accommodation and

hypersensitivity to distention) in the PDS and EPS subgroups (Rome III), the former being traditionally linked to 'dysmotility'-like FD[10]. Recently, reports of subtle duodenal changes with increased mucosal eosinophil infiltration and degranulation in patients with early satiety or PDS[11] have shifted the focus to the duodenum. Indeed, activation of duodeno-gastric reflexes has been implicated in gastric sensorimotor dysfunction[12,13], suggesting a primary role for duodenal pathology. In addition, cellular immune activation with increased 'gut-homing' lymphocytes in the blood of FD patients has been linked to gastric emptying and symptoms[14]. However, the cause of both duodenal and systemic inflammation in FD is still unknown and may include luminal (acid, bile, food and microbiota) or central (stress) triggers. Emerging pre-clinical and clinical data indeed point towards mucosal immune activation by an altered gut microbiota composition or 'dysbiosis' in other FGIDs most notably the irritable bowel syndrome (IBS)[15,16].

Despite recent advances in the understanding of the pathophysiology of FD, current treatment options are limited[17]. The complexity and heterogeneity of the syndrome are a result of the multifactorial pathophysiology, which has not been fully unraveled. With the exception of eradication for *Helicobacter pylori* (Hp) infection with antibiotics and acid suppressant drugs, no cost-effective therapies for FD exist and routine use of proton pump inhibitors (PPI) has been scrutinized due to an increased risk of side effects including enteric infections[18]. Also, traditional prokinetic drugs and neuromodulators are hampered by limited efficacy or side effects, and the lack of effective therapies has recently been recognized by the American College of Gastroenterology (ACG) and Canadian Association of Gastroenterology (CAG)[19]. Despite increasing recognition of duodenal inflammation as a potential trigger of dyspeptic symptoms in several reviews[1,12,13,16,17], none have provided a detailed and mechanistic description of the inflammatory changes and their role as a potential treatment target. However, small trials using anti-inflammatory drugs and randomized controlled trials

of probiotics and selective antibiotics are emerging. In addition to assessing clinical outcomes, these studies should also aim at providing insights in the underlying mechanisms of dyspeptic symptom generation, as discussed below.

In the current narrative review, we report on duodenal inflammation as a pathophysiological mechanism and treatment target for FD patients. An electronic literature search was conducted in Pubmed up to 31st of December 2019. The following keywords were used in various combinations: 'functional dyspepsia', 'duodenum', 'inflammation', 'eosinophil', 'mast cell', 'therapy', 'treatment', 'proton pump inhibitor', 'anti-inflammatory', 'anti-histamine', 'probiotic', 'prokinetic', 'neuromodulator'. In addition, the abstract lists of the conferences of the American Gastroenterology Assocation, United European Gastroenterology and European Society of Neurogastroenterology and Motiliy were searched and manual search was performed of the reference list of the initially selected articles. Only articles in English were reviewed.

2. Inflammation in FD and the eosinophil-mast cell axis

2.1 Mucosal and systemic inflammation

Eosinophils and mast cells are normally present in the GI-tract except for the esophagus, but increased numbers and activation (eg. clustering and degranulation) have been described in FGID, including FD[20]. Duodenal eosinophil infiltration in FD was first described in pediatric patients from the US although controls were not assessed[21], and in the first nested case-control study of adults from Sweden[11]. Interestingly, this early adult study showed increased duodenal eosinophil counts in 'non-ulcer' dyspepsia patients from a population-based cohort of 1,001 subjects undergoing upper GI endoscopy[11]. Replication of these findings confirmed a predominance of duodenal eosinophilia in PDS patients from the UK[22] and Australia[23], but similar prevalence between PDS and EPS in other cohorts[24–

29]. The inflammatory phenotype with predominantly mucosal eosinophils in FD is different from IBS patients with lymphocytes and mast cells in the colon[30] or duodenum[24], although duodenal mast cells can be elevated in FD patients[25–27]. In addition, infiltration of both eosinophils and CCR2-positive macrophages with increased counts surrounding the crypts and focal CD8+ T-cell aggregates were found in post-infectious(pi)- FD patients, indicating a persisting cellular immune response and delayed recovery of the initial infectious episode[31,32]. The higher levels of antibodies to Cytolethal distending toxin B (CdtB, produced by Gram-negative bacteria causing acute gastroenteritis) in an FD sample from the general Australian population also suggest that pi-FD with only subtle histological changes may be more common than previously thought[33].

We have recently summarized the local and systemic inflammatory changes in FD[17,34], which may differ between pediatric and adult patients or after acute onset (Table 2). Regarding systemic immune activation, increased proportions of β7+ T cells were consistently reported in IBS[35] but α4β7 co-expression or increased 'gut-homing' lymphocytes has only been described in FD[14]. This study from Australia showed a higher fraction of CD4+ α4β7+ CCR9+ T-cells in the peripheral blood and increased production of TNF-α, IL-1β and IL-10, which correlated with gastric emptying and symptoms in FD patients[14]. In addition, a Belgian study found increased CD45RA+ (naïve)/ CD45RO+ (activated) lymphocytes in FD patients with a shift towards a T-helper(Th)-2 cytokine profile, including increased IL-5 and IL-13 and decreased IL-10 and IFN-γ expression in stimulated lymphocytes[36]. Importantly, eosinophils are critical effector cells of Th2 allergic inflammation[20]. Although the presence of duodenal eosinophils may help explain the observed link between FD and allergy[22], atopy[37] or autoimmune diseases[38], no mucosal Th2-signal has yet been reported[34]. In contrast, increased TNF-α and IL-1β

production is suggestive of a Th17-response and interactions between Th2- and Th17-signalling may be at play[39].

2.2 Eosinophil and mast cell activation

Eosinophil recruitment in the GI tract is regulated by chemoattractants or chemokines, of which eotaxin-1 (CCL11) is the most important[20]. Both recruited and resident eosinophils are activated by eotaxin, which is constitutively expressed in the intestinal lamina propria[40]. Eosinophils express the eotaxin-receptor or CCR3 as well as integrin α4β7, which is necessary for intestinal recruitment by binding to MadCAM1 (mucosal vascular addressin cell adhesion molecule 1), expressed in gut endothelium [41]. Alongside increased numbers, degranulation of eosinophils has been described in pediatric[21] and adult[11,28,42] FD patients. IL-5 is the strongest driver of the major Th-2 cytokines responsible for eosinophil activation[39,43]. Increased IL-5 and IL-13 production of stimulated lymphocytes in FD patients also point towards a Th2-type profile[36], similar to increased IL-5 expression in CD4+ cells from eosinophilic esophagitis (EoE) patients[44]. Notably, CCR3-expression in peripheral eosinophils correlated with tissue eotaxin-3 expression in EoE patients and as these systemic changes were reversible with remission[44], this may serve as a marker of therapeutic efficacy for other conditions with eosinophil infiltration.

In EoE, the food antigen-induced Th2-response results from an impaired esophageal barrier function with activation of mucosal mast cells and B-cells[45]. Similarly, duodenal eosinophils may be activated by Th2-cells (IL-5) and lead to activation of mast cells through secretion of chemokines such as major basic protein (MBP) from granules[20] (Figure 1). Eosinophil degranulation potentially results from and may also lead to changes in duodenal permeability, as eosinophils have been implicated in both epithelial cell damage and repair[46]. Although activated eosinophils act as antigen-presenting cells to Th2-lymphocytes with immunoglobulin (Ig) class switching of B-cells to proallergic IgE-antibodies via IL-4

and IL-13, evidence is lacking for a role of IgE in symptom generation of FGID, including FD[15]. Nevertheless, food antigens have been proposed as a potential trigger for GI-symptoms[47] and controlled studies are needed to assess the efficacy of elimination diets in FD, similar to EoE patients[48]. Mast cells activated by IL-4 and IL-13 release histamine, which has been implicated in neural activation, smooth muscle contraction and symptom generation in IBS patients[49]. In addition, a link between duodenal eosinophils or mast cells and altered neuronal signaling has been shown in FD patients[26]. The potential roles of mast cell mediators is discussed below and although several anti-inflammatory or –allergy treatments exist, only few existing drugs target the potential causes of duodenal inflammation (Figure 1).

3. Duodenal eosinophils and mast cells as therapeutic targets

3.1 Anti-inflammatory therapies

Acid-suppressive therapy with PPI is the recommended first-line treatment in Hp-negative FD patients or in case of ongoing symptoms after eradication, following the recent ACG and CAG[19] and NICE guidelines[50]. From a recent Cochrane meta-analysis[51], the tendency for higher efficacy of PPI in PDS (RR 0.89; 95% CI 0.77-1.03) vs. EPS (RR 0.99; 95% CI 0.76-1.28) suggests anti-inflammatory effects via suppression of duodenal eosinophils, similar to EoE[52]. PPI may exhibit anti-oxidant properties by binding the vacuolar H+-adenosine triphosphatase (ATPase) and proton receptor GPR65[52]. PPI also inhibit the expression of vascular cell adhesion molecule-1 (VCAM-1), which is recognized by eosinophil ligands[53]. Moreover, the Th2-stimulated (IL-4 and IL-13) secretion of eotaxin-3 by esophageal epithelial cells is regulated by the signal transducer and activator of transcription 6 (STAT6) and blocked by omeprazole[54,55]. As this effect was found for various PPI and in human bronchial and nasal epithelial cell lines through non-gastric H+-K+-ATpase[56], similar mechanisms may occur in the duodenum of FD patients treated with PPI. Indeed, similar

clinical efficacy was reported for different doses or types of PPI[57] and we reported lower eosinophil counts in FD patients on- vs. off-PPI in a cross-sectional study from Australia and a prospective study from Belgium, supporting the anti-inflammatory actions of PPI, although the underlying mechanisms need to be further studied [58,59].

Interestingly, preclinical studies have shown smooth muscle hypercontractility with Th2-cytokines[60] and beneficial effects on gastric motility by blocking eotaxin[61], which has not yet been translated to human subjects. The Th2-derived cytokine IL-5 regulates recruitment and responsiveness of eosinophils to local activating signals, including eotaxin-3[62] and treatment of EoE patients with anti-IL-5 antibodies mepolizumab in adults[63,64] and children[65] or reslizumab in adolescents and children[66] resulted in decreased eosinophil counts. In addition, a monoclonal antibody against the alpha subunit of the IL-4 receptor, which is administered subcutaneously (dupilumab)[67] or intravenously (QAX576)[68], improved eosinophilia by inhibition of the Th2-cytokines IL-4 and IL-13 and the expression of eotaxin-3[68]. However, these biological agents have not been tested in other GI-conditions with increased eosinophils, such as FD. Oral glucocorticoids are the first line therapy for eosinophilic gastroenteritis with resolution of eosinophil infiltration[69]. Although controlled-release budesonide with low systemic bioavailability is used in several inflammatory diseases of the distal GI tract[70], open-capsule budesonide is effective for proximal small intestinal diseases including refractory celiac disease[71]. Trials with topical steroids are awaited in FD patients and should include transcriptomics, as a reversible overexpression of eotaxin-3 and IL-13 was shown with topical steroids in EoE[72]. Antagonists of the chemoattractant receptor-homologous molecule on Th2 cells (CRTH2), which mediates chemotaxis and activation of Th2-cells and eosinophils in response to prostaglandin D2, also showed eosinophil-reducing effects in adult EoE patients with corticosteroid-dependent or –refractory EoE[73]. Finally, the anti- α4β7 integrin antibody

vedolizumab may also be effective in refractory eosinophilic GI diseases[74].

3.2 Anti-allergy therapies

Histamine is the main mast cell-mediator and a potential target of the eosinophil-mast cell-axis in FD. Early clinical reports of histamine(H)1-receptor antagonism in treatment-refractory dyspeptic patients with increased antral mast cells showed a response rate of 79% in an open label study[75]. The H1-receptor antagonist and mast cell-stabilizer ketotifen also improved visceral hypersensitivity, symptoms and quality of life in IBS patients[76] and similar effects were reported with ebastine via reduction of H1-mediated sensitization of the transient receptor potential vanniloid receptor type 1 (TRPV1)[49]. Dual H1- and H2-receptor blockade was effective in a retrospective case series from Australia, especially in FD patients with increased duodenal eosinophil counts[77]. Moreover, combined H1- and H2-receptor blockade with the mast cell stabilizer cromolyn led to an overall response rate of 90% in dyspeptic children with duodenal eosinophilia[78]. Despite the superior anti-secretory effect of PPI compared to H2-receptor antagonists, the resolution of dyspeptic symptoms was comparable between both treatments (RR 0.88, 95% CI 0.74-1.04)[51], suggesting that anti-histamine effects play an additional role. In addition, the H4-receptor was involved in an animal model of post-infectious visceral hypersensitivity[79] and studies in FD are awaited as H4-receptor blockade reduced eosinophils in proof-of-concept trials in asthma and allergy[80]. The H4-receptor is expressed on eosinophils, and has been implicated in mediating chemotaxis, suggesting inhibiton of this receptor may demonstrate some efficacy in FD patients with duodenal eosinophilia[81]. Finally, treatment with the leukotriene(LT)1-receptor antagonist montelukast resulted in significantly higher clinical response rate in pediatric FD patients compared to placebo[82], which was not associated with changes in eosinophil density or activation although changes in duodenal mucosal mast cells or nerve interactions were not studied[83].

4. Potential causes and consequences of duodenal inflammation

4.1 Duodenal sensitivity and barrier defect

The passage of food as chyme from the stomach to the small intestine is regulated by the duodenum, where auto- and paracrine mechanisms are involved in the mucosal defense to acid and luminal digestion of nutrients[84]. The complex acid- and lipid-sensitive duodeno-gastric feedback reflex results in inhibition of gastric motility, thereby protecting the duodenum from excessive acid or lipid exposure[85]. Indeed, intraluminal acid perfusion altered the duodenal mechanosensitivity in healthy subjects[86,87] and resulted in dyspeptic symptoms[88,89]. Treatment with the 5-hydroxytryptamine(5-HT)-3 receptor blocker ondansetron decreased gastric sensitivity to distension during duodenal acid infusion in healthy volunteers, with no effect on gastric volume or dyspeptic symptoms[90]. Although duodenal acid exposure was increased in FD patients, possibly due to delayed duodenal acid clearance, brief duodenal acid infusion did not cause dyspeptic symptoms and the gastric motor response was not affected by ondansetron[91], which is similar to lipid-infusion[92]. While duodenal serotonin content was normal in FD, inducible nitric oxide synthase (iNOS) expression was higher in PDS patients compared to controls and correlated positively with the percentage of degranulating mast cells, suggesting a potential role for iNOS-inihibitors in PDS to improve gastric dysmotility and symptoms[93]. We have shown that duodenal acid perfusion disrupts duodenal integrity in healthy subjects with increased tryptase expression, implicating the involvement of mast cells, although this was not reversed by pretreatment with the mast cell stabilizer disodium cromoglycate (DSCG)[94]. Studies using the local anesthetic benzocaine showed an inhibition of gastric relaxation upon duodenal acid exposure in healthy volunteers, which would disable a protective physiological mechanism in FD patients [95]. A better option may be to target specific receptors of duodenal mucosal primary afferent nerve endings such as TRPV1, considering their role in histamine-induced visceral

hypersensitivity[49]. Duodenal acid sensitivity in FD patients may thus predispose to mast cell-activation and altered permeability and sensitivity[13,96], which could be reversed by more potent anti-allergy treatment.

Besides acid and nutrient sensing in the duodenum, transmucosal passage of luminal content occurs in the proximal small intestine and is regulated by the apical junction complex consisting of tight junctions, adherens junctions and desmosomes[97]. We have demonstrated increased duodenal mucosal permeability in FD patients using Ussing chambers (*ex vivo*) with a decreased expression of tight junctions (zonula occludens 1 (ZO-1) and occludin), adherens junctions (β-catenin and E-cadherin) and desmosomes (desmoglein-2), correlating with the number of mast cells and eosinophils[25]. Mucosal impedance studies (*in vivo*) have also shown correlations between duodenal ZO-1 and IL-1β expression with permeability[98], and IL-1β release by peripheral lymphocytes from FD patients[14] as well as human macrophages and eosinophils [99,100]. However, future studies should include protein analyses as post-translational regulation is important for both barrier-[97] and immune-related genes such as IL-1β[34]. Abnormalities of the apical junction complex have also been reported in the jejunum of diarrhea-predominant IBS patients, which were correlated with mast cell activation and clinical symptoms[101]. Expression of ZO-1 was reduced both at gene- and protein-level, with redistribution to the cytoplasm on confocal microscopy[102]. However, the cause and effect relation between immune activation and permeability is still unknown and requires interventional studies with drugs affecting the duodenal barrier function. Treatment with larazotide acetate reversed the gliadin-induced decrease in the duodenal ZO-1 expression in patients with celiac disease [103] and future trials with drugs in the novel class of tight junction regulators are awaited for FD patients. Finally, oral dietary glutamine supplements reduced symptoms and intestinal hyperpermeability, measured with the urinary lactulose-mannitol ratio in diarrhea-predominant pi-IBS patients, and therefore may have a

role for alleviating symptoms in FD, although the underlying mechanisms are unclear[104].

Regarding potential targets of mast cell-induced intestinal hyperpermeability, it should be noted that no differences in substance P and vasoactive intestinal peptide (VIP) were found in FD patients compared to controls[93]. However, VIP-levels were numerically increased in colonic biopsies and significantly higher in plasma from IBS patients compared to controls with a higher percentage of mast cells expressing the VIP-receptor VPAC1[105]. This study also showed increased bacterial and para-cellular passage in IBS compared to controls with a significant reduction in bacterial passage in both IBS and controls with ketotifen and anti-VPAC. Although VIP may be a key regulatory molecule in the colonic permeability of IBS patients, only ketotifen and not anti-VPAC showed a significant inhibition of the *Salmonella*-induced increase in permeability and decreased occludin-expression[105]. VIP-receptors have been implicated in barrier dysfunction of the human ileum and during stress in rats[106]. Interestingly, the mast cell mediator substance P was found to mediate the effect of psychological stress on the expression of corticotropin releasing hormone (CRH) of mucosal eosinophils in mice, with impaired jejunal permeability after activation of mast cells by eosinophil-derived CRH [107]. We have confirmed the role of CRH and mast-cells in stress-induced small intestinal hyperpermeability in students undergoing psychological stress during an oral exam, as this effect was induced by CRH-administration and blocked by pretreatment with DSCG[108]. Although we did not study immune activation, the eosinophil-mast cell axis is likely to be involved in stress-induced small intestinal hyperpermeability as CRH-receptors are present on both eosinophils and mast cells[109,110]. Therefore, treatment with anti-VPAC seems less promising than mast cell-stabilizers for treating mast-cell related barrier dysfunction and hypersensitivity. Finally, anti-CRH agents should be tested in FD.

4.2 Duodenal bile content and dysbiosis

The release of bile salts in the duodenum has been linked with symptom generation in FD[111], as postprandial onset or worsening of symptoms is common[112]. We have reported decreased fasting primary bile salt concentrations in duodenal aspirates from FD patients compared to controls with very few deconjugated bile salts[113]. Indeed, bacterial deconjugation mainly occurs in the colon, where both primary and secondary bile acids are reabsorbed and excreted in the duodenum after reconjugation in the liver[114]. Although changes in the fecal flora may affect the duodenal bile salt pool, recent studies have focused on the mucosa-associated microbiota (MAM)[115]. As previously mentioned, subtle histological changes in presumed post-infectious FD[31,32] suggest an ongoing immune response to microbial antigens, which could be attributed to dysbiosis in the absence of an infectious episode. Indeed, changes in the duodenal MAM were reported in FD patients from an Australian pilot study with an increase in *Streptococcus* and total bacterial load, which correlated with meal-related symptom severity and quality of life[116]. Interestingly, the effect of Hp-eradication was greater in patients with microscopic duodenitis and with metronidazole[117,118], suggesting an effect on the duodenal microbiome. Besides antibiotics, Hp-eradication includes acid suppressant drugs, which may also affect the microbiome. Overgrowth with oral species such as *Streptococcus* has been reported in the fecal flora of subjects during PPI-therapy[119,120] and an increased risk of enteric infections has been reported with the use of pantoprazole during 3 years[18]. Thus, future studies examining changes in the duodenal microbiome and symptoms during antibiotic and/or acid-suppressive therapy are needed in FD patients.

Probiotics are live micro-organisms that, when ingested in adequate amounts, exert a health benefit on the host and may improve symptoms in FD patients[121]. A recent systematic review and meta-analysis concluded that the evidence supporting probiotics for FD is limited, especially regarding specific strains or species[121]. Treatment with *Lactobacillus gasseri*

OLL2716 (LG21) resulted in significantly higher elimination rates for PDS- but not EPS-like symptoms with a trend for an overall effect on gastric symptoms (p=0.07) in a randomized placebo-controlled trial[122]. Although only changes in the gastric microbiota were studied in a follow-up trial, the proportion of bile acids in gastric fluid samples from FD patients was higher compared to controls and the intestinal-like bacterial profile was restored after LG21[123]. Thus, small intestinal bacterial overgrowth may be present in a subset of FD patients although no standardized diagnosis or treatment is available[124]. Treatment with the non-absorbable and selective antibiotic rifaximin was superior to placebo in Hp-negative FD patients from Hong Kong for the adequate relief of global dyspeptic symptoms at 8 weeks and post-prandial fullness, bloating and belching at 4 weeks[125]. Although the increased solubility of rifaximin with bile salts may contribute to its therapeutic effect in the duodenum of FD patients[126], the exact mechanism of action is unknown and replication in Western populations and from multi-center studies is needed.

5. Gastric dysmotility and gut-brain interactions in FD

5.1 Gastric dysmotility

Targeting gastric dysmotility with prokinetics may be beneficial in FD patients, as delayed gastric emptying was present 23% of FD patients in a large tertiary-care study and prokinetics also affect gastric accommodation and sensitivity, which were abnormal in 37%[10]. Although a recent meta-analysis concluded that prokinetics are effective in reducing ongoing dyspeptic symptoms (RR 0.81; 95% CI 0.74-0.89, NNT=7), significant heterogeneity and publication bias were noted and the effect was less pronounced after removal of cisapride (NNT=12), which was withdrawn from the market due to cardiac adverse events[127]. Prolongation of the QT-interval also limits the use of dopamine-2 (D2) receptor-antagonists including domperidone or metoclopramide and the latter also carries a black box warning due to an increased risk for extrapyramidal side effects[128] (Figure 2). Selective 5-HT-4-receptor

agonists such as prucalopride stimulate both colonic and gastrointestinal motility without adverse cardiac events, as shown in a study from Leuven with improvement in symptoms and overall GI-quality of life compared to placebo in patients with delayed gastric emptying[129]. However, accelerated gastric emptying does not correlate with improved symptoms in all studies, potentially due to overlapping dyspeptic symptoms which do not result from gastric dysmotility[130]. Thus, the effect of prucalopride in FD patients with normal gastric emptying is unclear. Itopride exerts mixed D2- and cholinesterase-inhibiting activity but results from two phase III trials were largely negative, likely related to issues with patient and endpoint selection[131]. However, a study using the Leuven Postprandial Distress Scale (LPDS) suggested efficacy of itopride in overlapping PDS-EPS patients (Rome III) in a placebo-controlled trial[132], which requires replication.

Acotiamide is a mixed presynaptic muscarinic(M)-receptor-antagonist and cholinesterase inhibitor, leading to increased availability of acetylcholine in the synaptic cleft with improved gastric emptying and accommodation[128]. This first in class prokinetic agent was specifically developed for FD and showed beneficial effects in PDS patients from Japan[133] and Europe[134]. Moreover, preclinical studies have shown that acotiamide exerted effects on stress-impaired gastric function via decreased expression of stress-related genes in the brain stem of rats[135]. Treatment with 5-HT-1A receptor agonists (azapirones) such as buspirone and tandospirone also results in anxiolytic effects and improved accommodation, with a significant reduction of both overall and PDS-type symptoms in FD patients[136]. Thus, neuronal receptors and targets may overlap for gastric dysmotility and gut-brain interactions (Figure 2). We have shown that the effect of stress on the GI-tract involved increased permeability and immune activation[108] and that duodenal hyperpermeability in FD patients correlated with gastric emptying to solids, suggesting that gastric dysmotility can be secondary to duodenal pathology[137]. Whether targeting mucosal inflammation would not

only improve symptoms and duodenal pathology but also gastric emptying and accommodation is subject of ongoing research.

5.2 Gut-brain interactions

Neuromodulators or psychotropic drugs are frequently prescribed in FD with proven efficacy for antipsychotics and tricyclic antidepressants (TCA) but only limited high-quality trials for other agents, thus precluding conclusions for efficacy[138]. TCA exhibit properties of both serotonin and noradrenalin reuptake inhibition with additional receptor affinities (eg. 5-HT, H- or M-receptors) contributing to their analgesic and antidepressant effects[139]. Amitriptyline but not the selective serotonin-reuptake inhibitor (SSRI) escitalopram was effective for the treatment of ulcer-like FD or EPS, which was found in patients with normal gastric emptying and suggested an effect on pain and visceral sensitivity rather than gastric motility[140]. Serotonin noradrenalin reuptake inhibitors (SNRI) also block both serotonin and noradrenalin reuptake but despite the absence of additional receptor affinities, side effects such as nausea are still common and led to a high drop-out rate in the largest trial of venlafaxine, showing no benefit over placebo[141]. However, the lower doses used during the study suggest less potent noradrenalin reuptake inhibition and thus pain-modulating effects. Interestingly, absence of anxiety was an independent predictor of patients being symptom-free, supporting a role for anxiety in symptom generation[141]. Also, the increased risk of pi-IBS after an episode of infectious gastroenteritis possibly resulted from a systemic Th2-predominant profile in subjects with anxiety and somatization[142], confirming the role of psychologic comorbidity in FGID and the potential for treatment with neuromodulators. Moreover, the presence of dyspepsia was an independent factor associated with pi-IBS and may also be a result of the predominant systemic Th2-response in anxious individuals[142]. In addition to measuring clinical and psychological outcomes, trials should study effects of neuromodulators on mucosal and systemic immune activation in FD.

Mirtazapine is a tetracyclic antidepressant which blocks the presynaptic α2-noradrenergic receptors, resulting in more noradrenaline and 5-HT release[139]. Additional receptor affinities include 5-HT-3-receptor antagonism with anti-nausea effects (in contrast to venlafaxine) and both H1- and 5-HT-2c-receptor antagonism with increased appetite, weight gain and sedation. These effects were confirmed in a Belgian study with significant improvement of PDS-type symptoms, quality of life and nutrient tolerance in FD patients with weight loss and no anxiety or depression[143]. In addition, GI-specific anxiety was reduced, which may be secondary to improved gastric function or changes in duodenal barrier function, as a correlation was also found between permeability and GI-specific anxiety in another FD cohort[25]. Besides increased appetite, improved gastric accommodation may occur via 5-HT-2c-receptor antagonism and the effect of H1-receptor antagonism on duodenal mast cells and resulting duodeno-gastric reflexes requires further study. Finally, herbal medicine or phytotherapy has been studied in FD patients with the promise of a broader range of pharmacological effects as well as patient acceptance and tolerance. Iberogast (STW5) is composed of 9 different extracts (eg. Iberis, peppermint, chamomile) and was more efficacious than placebo with regard to the severity of the most bothersome GI symptom[144], although its effect on EPS- or PDS-type symptoms and comparative efficacy with existing medical therapies remains to be established.

6. Conclusion

In summary, FD is a common and debilitating condition which imposes a considerable financial and psychological burden on patients. Although progress has been made in the management of this condition, we are far from understanding the underlying causes and finding a potential cure. The lack of effective therapies is illustrated by the absence of targeted treatments, although the underlying pathophysiology with duodenal inflammation

and the eosinophil-mast cell axis in particular is becoming increasingly clear. Anti-inflammatory effects of existing therapies such as PPI are being studied and trials with biologicals and topical steroids are needed. Anti-allergy treatments and drugs aimed at mast cell mediators such as histamine have proven efficacy in pediatric FD and should confirmed in adult populations.

Future drug development in FD should focus on targeting the duodenal pathology rather than the downstream consequences such as gastric dysmotility, also considering the limited efficacy and side effects of prokinetics. Future clinical classifications systems should include different pathophysiological mechanisms in order to allow targeted and not just symptom-based therapy. Studies are needed to confirm and validate the use of duodenal eosinophils and markers of Th2- and Th17-signalling as biomarkers in FD, which may guide diagnosis and therapeutic decisions.

Moreover, treatments for potential causes of duodenal pathology such as impaired permeability and dysbiosis may soon become available.

7. Expert opinion

Through recent advances in the understanding of the pathogenesis of FD, the paradigm of this condition as a functional gastroduodenal disorder with 'no evidence of structural disease' has been challenged. Despite the fact that the Rome IV criteria do not exclude the presence of microscopic changes such as duodenal eosinophilia[2], many studies have not taken duodenal inflammation into account. The absence of associations between duodenal eosinophilia and PDS in certain cohorts may be due to the lack of recognition of postprandial pain as part of the PDS-subgroup[145]. Indeed, Rome IV criteria acknowledge that postprandial epigastric pain or burning may be present in PDS[2]. Based on our previous findings[11], a cut-off of 22 eosinophils per 5 high-power fields (HPF) was proposed for adult FD patients[22], which can

also be expressed per mm^2[23] with >112 duodenal eosinophils per mm^2 associated with a 33-fold increased risk of pediatric FD[146]. Thus, studies should include the latest Rome IV criteria and besides the assessment of duodenal eosinophil and mast cell infiltration at baseline and during therapy, research should focus on signs of active inflammation including measurement of mediators and evaluation of cellular markers of immune activation rather than cell counts. While ultrastructural evaluation of eosinophil and mast cell degranulation provides valuable information[42], more advanced immunohistochemical techniques should be explored[11]. Moreover, future diagnostic assessment should include markers of immune activation in FD patients, both at the mucosal and systemic level, which would improve the classification in novel subgroups and allow the development of targeted treatments.

We have proposed a central role for the duodenal eosinophil-mast cell axis in FD, especially PDS, although the underlying mechanisms are still unclear[17]. Besides being potent acid-suppressant drugs, anti-inflammatory actions of PPI may also explain their therapeutic effect in FD as illustrated in cross-sectional and prospective studies[58,59]. Although reduction of duodenal eosinophilia on-PPI is an interesting finding, further mechanistics studies are needed to unravel the upstream mechanisms in the search for more efficacious therapies. The gap in the knowledge regarding eosinophil recruitment and activation in the duodenal mucosa of FD patients will hopefully be addressed in future studies by using monoclonal antibodies, which target Th2-cell development, cytokine production and eosinophil interactions, similar to EoE patients. Importantly, the identification of systemic markers of Th2-signalling will prove useful as a marker of therapeutic efficacy such as eotaxin-3 expression of peripheral eosinophils[44]. Also, studies including transcriptomics of the duodenal mucosa in FD patients are needed both at baseline and during therapy, as illustrated in EoE with reversible overexpression of IL-13 and eotaxin-3 during treatment with topical steroids[72]. Drugs targeting mast cell-signalling in particular have shown efficacy in FD patients, including

antihistamines[77], cromoglycates[78] and the LT1-receptor antagonist montelukast[82,83]. Trials using dual H1- and H2-blockade are awaited based on positive but preliminary findings from Australia[77]. Finally, the interplay between eosinophils, mast cells and Th2- or Th17-signalling requires further study[39].

Despite the breakthrough of the identification and recognition of duodenal and systemic inflammation in FD, evidence for the trigger to inflammation is less conclusive. Although 5-HT3-receptor blockade with ondansetron had mixed results on duodenal sensitivity, gastric motor function and symptoms in healthy subjects and FD patients, drugs targeting specific receptors on mucosal primary afferent nerve endings such as TRPV1 or VPAC are needed. Based on studies on histamine-induced visceral hypersensitivity in IBS patients, we expect that targeted treatments would be effective in FD patients[95]. The association between duodenal sensitivity and mast cells is further supported by the clinical benefit of anti-allergy treatment in FD patients. Although impaired duodenal barrier function was linked with mast cell activation, the cause and effect relation between permeability and inflammation is unknown and studies with anti-CRH and tight junction regulators are awaited. The limited availability of *ex vivo* or *in vivo* experimental investigations of permeability is a challenge to test this hypothesis but potential mucosal biomarkers include expression of tight junction proteins[25,98]. Thus, studies including the assessment of barrier, immune and neuronal function under different conditions and with specific interventions are awaited in order to disentangle the underlying mechanisms.

Regarding the possible causes, food antigens may be a trigger of eosinophilic infiltration, similar to EoE patients, where acute and/or delayed responses have been observed after mucosal food allergen injection[147]. The same strategy may guide the use of elimination diets, in case similar mechanisms are found in the duodenum of FD patients. Studies using

microbial therapies such as probiotics or selective antibiotics should include their potential effect on mucosal barrier function and immune activation. Indeed, immune activation in FD patients is presumably triggered by an altered gut microbiota composition but how this relates to duodenal (eg. impaired permeability) or gastric (eg. dysmotility) changes is unknown. Thus, the study of gastric dysfunction remains relevant in at least a proportion of patients and the effect of anti-inflammatory therapies on gastric emptying and accommodation should be studied. The limited efficacy of prokinetics is illustrated by the lack of association between symptoms and changes in gastric motor function[10], which can be explained by the fact that gastric dysmotility may be secondary to duodenal pathology[137]. In contrast, some of the effects of prokinetics may be centrally driven due to similar neuronal receptors, and anti-histamine effects of neuromodulators may also affect mucosal mast cells. These overlapping effects are important for future drug development and novel neuromodulators should also take into account the effects on mucosal and systemic immune activation in FD patients.

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Abbreviations: 5-HT: 5-hydroxytryptamine, ACG: American College of Gastroenterology, CGA: Canadian Gastroenterology Association, CeD: celiac's disease: DSCG: disodium cromoglycate, EoE: eosinophilic esophagitis, EPS: epigastric pain syndrome, FD: functional dyspepsia, FGID: functional gastrointestinal disorder, GI: gastrointestinal, H: histamine, Hp: *Helicobacter pylori*, IBS: irritable bowel syndrome, LT: leukotriene, M: muscarinic, MAM: mucosa-associated microbiota, NICE: National Institute for Health and Care Excellence, NNT: number needed to treat, PDS: postprandial distress syndrome, pi: post-infectious, PPI: proton pump inhibitor, TCA: tricyclic antidepressants

Tables and figures

Table 1: Rome IV criteria for functional dyspepsia, including postprandial distress and epigastric pain syndrome.

	Rome IV diagnostic criteria	Duration and onset
FD	1. One or more of the following: a. Bothersome postprandial fullness b. Bothersome early satiation c. Bothersome epigastric pain d. Bothersome epigastric burning	Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis

	AND 2. No evidence of structural disease (including at upper endoscopy) that is likely to explain the symptoms	
PDS	One or both of the following at least 3 days a week: 1. Bothersome postprandial fullness (i.e., severe enough to impact on usual activities) 2. Bothersome early satiation (i.e., severe enough to prevent finishing a regular size meal)	Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis
EPS	One or both of the following at least 1 day a week: 1. Bothersome epigastric pain (i.e., severe enough to impact on usual activities) 2. Bothersome epigastric burning (i.e., severe enough to impact on usual activities)	Criteria fulfilled for the last 3 months, with symptom onset at least 6 months prior to diagnosis.

Abbreviations: EPS: epigastric pain syndrome, FD: functional dyspepsia, PDS: postprandial distress syndrome.

Table 2: Mucosal and systemic immune activation in Functional dyspepsia.

Population	**Finding**	**Reference**
Pediatric FD	Duodenal mucosal eosinophil infiltration and degranulation	Friesen et al. 2002[21] Wauters et al. 2017[146]
	Antral mast cell degranulation	Friesen et al. 2008[148]
Adult FD	Duodenal mucosal eosinophil infiltration and degranulation	Talley et al. 2007[11] Walker et al. 2010[22] Walker et al. 2009[24]

		Vanheel et al. 2014[25] Cirillo et al. 2015[26] Wang et al. 2015[27] Du et al. 2016[28] Tanaka et al. 2016[29] Vanheel et al. 2018[42]
	Duodenal mucosal mast cell infiltration and degranulation	Walker et al. 2009[24] Vanheel et al. 2014[25] Cirillo et al. 2015[26] Wang et al. 2015[27] Yuan et al. 2015[93] Vanheel et al. 2018[42]
	Duodenal mucosal IL-1β expression	Komori et al. 2019[98]
	Circulating α4β7-T cells (gut-homing) with TNF-a, IL-1β and IL-10 production	Liebregts et al. 2011[14]
	CD45RA+/ CD45RO+ lymphocytes with IL-5 and IL-13 and decreased IL-10 and IFN-γ expression	Kindt et al. 2009[32]
Pi- or acute onset FD	Duodenal mucosal eosinophil infiltration	Futagami et al. 2010[31]
	Duodenal mucosal (CCR2+) macrophage infiltration	Futagami et al. 2010[31] Kindt et al. 2009[32]
	Decrease in duodenal mucosal CD4+ cells, no change in CD3+ lymphocytes	Kindt et al. 2009[32]
	CD45RA+/CD45RO+ lymphocytes with increased TNFα-levels	Kindt et al. 2009[32]

	Increased circulating IL-10	Kindt et al. 2009[36]

Abbreviations: FD: functional dyspepsia, pi: post-infectious. PDS: postprandial distress syndrome.

Figure 1: Pathways underlying duodenal pathology in functional dyspepsia patients. An unidentified luminal trigger may initiate and perpetuate immune and neuronal activation, with a central role for the eosinophil-mast cell axis. Duodenal sensitivity to lipids and acids and a mucosal barrier defect are also present. Finally, altered bile content and dysbiosis have been studied and microbital therapies are emerging. Existing and effective treatments (green) as well as potentially effective (yellow) and ineffective (red) treatments are shown on the right.

Abbreviations: 5-HT3: serotonin, CRH: corticotropin releasing hormone, CRTH2: chemoattractant receptor-homologous molecule on Th2 cells, H: histamine, LT: leukotriene, MBP: major basic protein, Th: T-helper, TRPV1: transient receptor potential vanniloid receptor type 1, VIP: vasoactive intestinal peptide, ZO-1: zonula occludens-1

Figure 2: Neuronal receptors underlying gastric dysmotility and gut-brain interactions in FD patients. Existing and effective treatments (green) as well as potentially effective (yellow) and potentially harmful or withdrawn (red) treatments are shown on the right.

Abbreviations: 5-HT: 5-hydroxytryptamine (serotonin), α: α-noradrenalin receptor, D: dopamin receptor, H: histamine receptor, M: muscarinic acetylcholine receptor, NRI: noradrenalin reuptake inhibition, SRI: serotonin reuptake inhibition

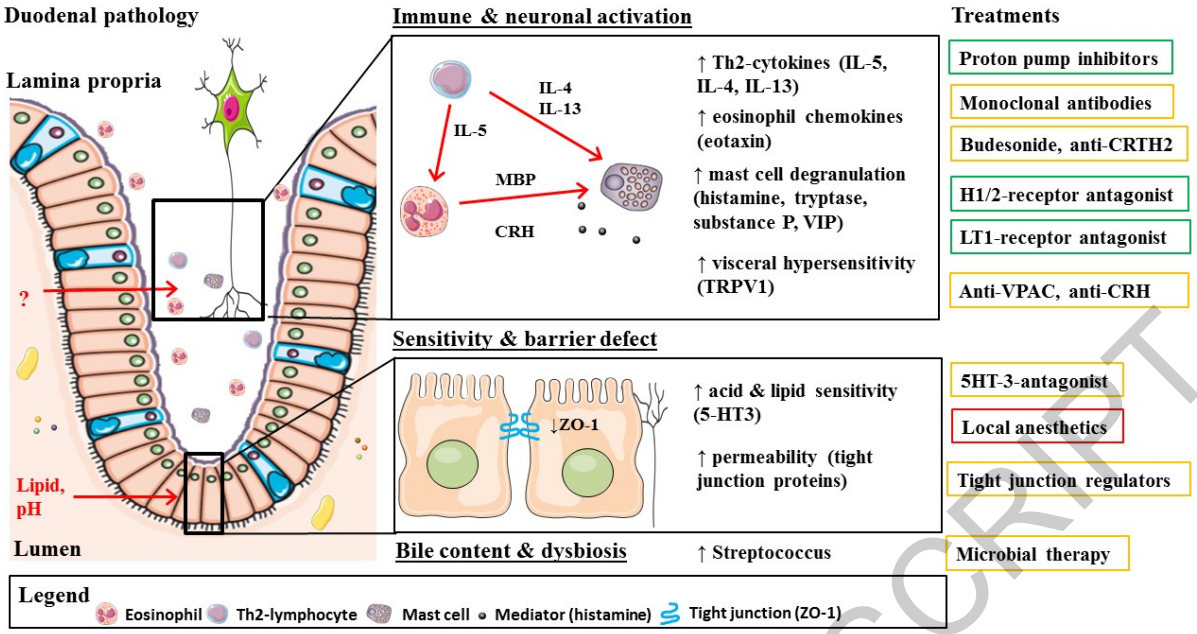

Figure 1

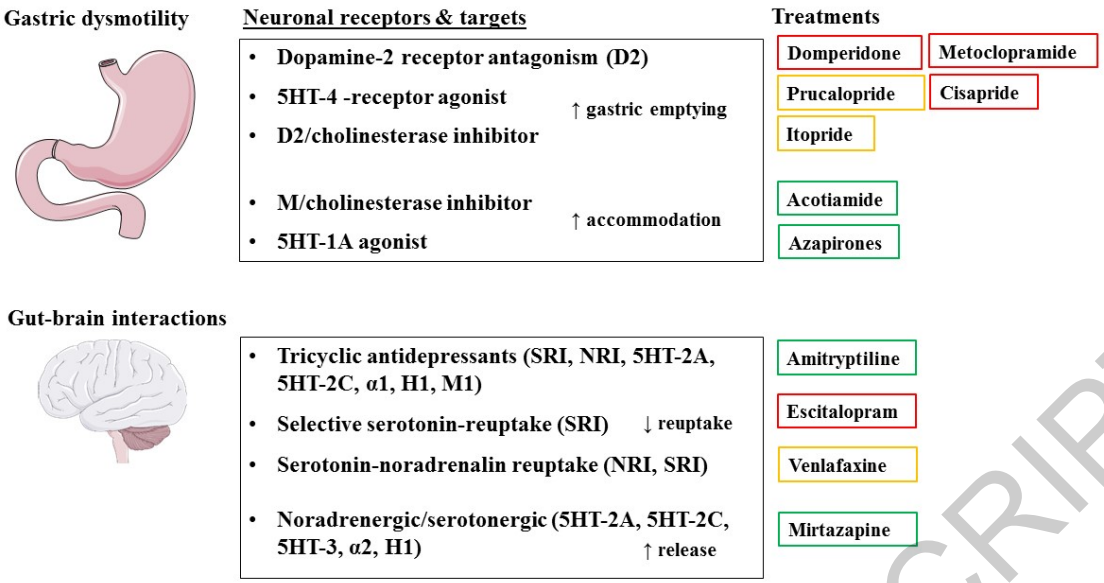

Figure 2