gastrointestinal disorders

MDPI

Review

A Survey of Methodologies for Assessing Mast Cell Density and Activation in Patients with Functional Abdominal Pain Disorders

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Abstract: The aim was to assess methods utilized in assessing mast cell involvement in functional abdominal pain disorders (FAPDs), specifically to describe variability in methods utilized to assess both mast cell density and activation and determine if a consensus exists. After a literature search identified 70 manuscripts assessing mast cell density, data were extracted including FAPD diagnosis, site of biopsy, selection of microscopic fields analyzed, selection of mucosal region analyzed, method of mast cell identification, method to assess mast cell density, and if performed, method to assess mast cell activation. There appears to be some consensus favoring inmmunohistochemical stains over histochemical stains for identifying mast cells. Otherwise, considerable variability exists in methodology for assessing mast cell density and activation. Regardless of method, approximately 80% of studies found increased mast cell density and/or activation in comparison to controls with no method being superior. A wide variety of methods have been employed to assess mast cell density and activation with no well-established consensus and inadequate data to recommend specific approaches. The current methodology providing physiologic information needs to be translated to a standard methodology providing clinical information with the development of criteria establishing abnormal density and/or activation, and more importantly, predicting treatment response.

Keywords: mast cells; irritable bowel syndrome; functional dyspepsia

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1. Introduction

Functional abdominal pain disorders (FAPDs), particularly irritable bowel syndrome (IBS) and functional dyspepsia (FD), are highly prevalent conditions resulting in significant morbidity and healthcare costs worldwide. IBS is defined by abdominal pain associated with a change in stool frequency, a change in stool form, or a change in pain intensity with stools [1]. FD is defined by the presence of epigastric pain, epigastric burning, early satiety, or postprandial fullness [2]. FAPDs are considered to result from disordered brain-gut axis function with a wide variety of pathophysiologic contributors including altered neural pathways (both peripheral and central), dysmotility, visceral hypersensitivity, inflammation, dysbiosis, and psychologic dysfunction. Mast cells have been implicated in both IBS and FD, in part due to their location at the interface between the patient and the environment and in part due to their functional connectivity to the multiple systems implicated in the generation of gastrointestinal symptoms [3]. Previous studies have shown increased mast cell density and/or evidence of increased mast cell activation in patients with FAPDs in most, but not all, studies. A meta-analysis of adults with FD demonstrated increased mast cells in the stomach and duodenum [4]. Multiple systematic reviews and/or

meta-analyses have demonstrated increased mast cells in the ileum and colon of adults with IBS [5–8]. In the largest of these reviews, Krammer and colleagues reviewed 36 studies with 30 of these studies demonstrating increased mucosal mast cells in adults with IBS [8]. Mast cells have been specifically linked to the development of visceral hypersensitivity, a pathophysiologic process of central importance in FAPDs [9].

A variety of methods have been employed to assess gastrointestinal mast cells, most commonly methods to determine mast cell density. There are a variety of techniques utilized to identify mast cells including histochemical staining (e.g., utilizing toluidine blue or Alcian blue) or immunohistochemical staining (e.g., utilizing antibodies to tryptase or CD 117, also known as c-kit). However, it is well recognized that mast cells exert their biologic functions primarily through the release of mediators and, thus, density does not give a complete picture of mast cell involvement. A variety of methods are available to assess mast cell activation. These include measuring (1) degranulation (e.g., utilizing transmission electron microscopy) [10–19]; (2) mast cell-derived mediators (e.g., tryptase and histamine) in biologic fluids or intestinal tissue either utilizing techniques such as RNA seq or protein analysis [17,18,20–23]; and, (3) mediators in the supernatant after tissue incubation [16,17,20,24–33].

The aim of the current survey was to assess the methods utilized in assessing mast cell involvement in FAPDs in both adults and children, specifically to describe variability in methods utilized to assess both mast cell density and activation and determine if there appears to be any consensus. Ultimately, the transition of the current state implicating mast cells in the pathophysiology of FAPDs to a clinically useful process of assessing mast cell involvement will require some standardization of methods, definitions of abnormal, and proof of an ability to predict response to treatments directed at mast cells or their mediators. Assessment of current practices represents the first step in this transition.

2. Literature Assessment

We conducted a literature search utilizing PubMed, Google, and Google Scholar employing the keywords “gastrointestinal mast cells”, “irritable bowel syndrome”, and “functional dyspepsia” from 2000 to 2021. Additionally, we cross-referenced bibliographies from all identified manuscripts to identify other relevant manuscripts. While the current manuscript was intended to be a methodologic survey and not a systematic review, we did include all relevant manuscripts identified in previous systematic reviews assessing mast cells in patients with FD and/or IBS [4–8]. Manuscripts were included if they reported on any patients with FAPDs and included an assessment of gastrointestinal mast cell density. Manuscripts were excluded if they did not assess density, even if activation was assessed.

Data extractions were performed independently by 2 reviewers (HF and CF). After comparing results, any discrepancies were resolved. Specific data extracted was first author, country where subjects were evaluated, age group, patient FAPD diagnosis, site of biopsy, selection of microscopic fields analyzed, selection of mucosal region analyzed, method of mast cell identification, method to assess mast cell density, and if performed, method to assess mast cell activation (See Figure 1). In addition, whether density and activation differed from a control group was assessed, primarily to determine how frequently findings related to density were discrepant from findings related to activation.

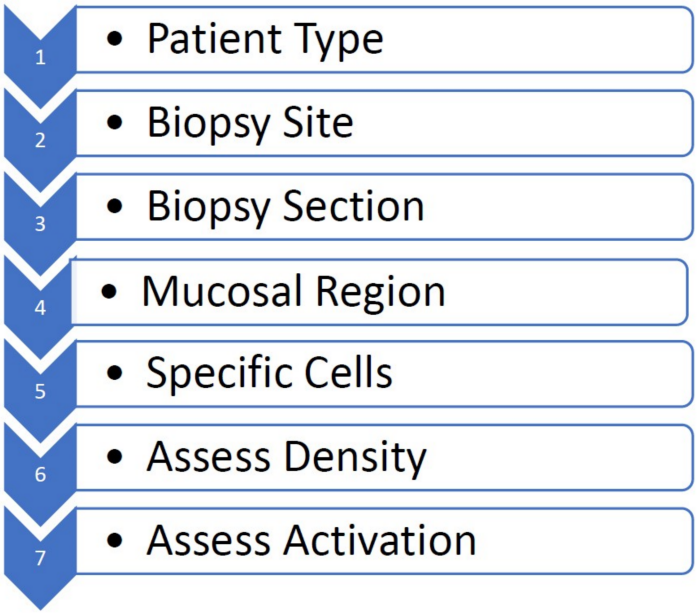

Figure 1. Levels of study design decisions regarding assessment of mast cells in functional abdominal pain disorders.

3. Summary of Methods for Mast Cell Evaluation

A total of 11 studies (1 IBS, 5 FD, 2 FAPD, and 3 of endoscopy patients) in children and adolescents and 59 studies (44 IBS, 14 FD, and 1 with both IBS and FD) in adults were identified [8–78]. The primary findings in pediatric patients are shown in Table 1 and in adults are shown in Tables 2 and 3. In youth, 8/11 (73%) studies identified mast cells with immunohistochemical stains (all utilizing anti-tryptase antibody) and 3/11 studies utilized histochemical stains. In adults, 49/59 (83%) studies identified mast cells with immunohistochemical stains (33 utilizing anti-tryptase, 15 anti-CD117, and one using both) and 10/59 studies utilized histochemical stains, most commonly toluidine blue (7 studies). Mast cell activation was assessed in 2/11 (18%) pediatric studies and 25/59 (43%) of adult studies. The number of microscopic fields assessed varied from 5 to 10 in pediatric studies and 3/20 in adult studies. In pediatric studies, the process for field selection was not stated in 3 studies, random in 2 studies, and through identification of most involved areas in 6 studies. In adult studies, the process for field selection was not stated in 42 studies, random in 11 studies, through identification of most involved areas in 2 studies, and through identification of 'most representative' areas in 4 studies. Other selection criteria in some studies included a requirement that the specimen be well-oriented or that villi be cut transversely. Others also avoided lymphoid follicles or lymphoid aggregates. Additional variability was noted in the mucosal layer evaluated with some assessing only the lamina propria and some assessing both the lamina propria and epithelium. There were also a variety of methods for assessing the mast cell density. While most studies involved manual counting of mast cells, others utilized image analysis to report the percentage of the area occupied by mast cells (either overall or lamina propria only). With manual counting, mast cell density was reported per high power field in some studies, per mm^2 in others, and as a percentage of total immunocytes in another. Densities were also reported by both quantitative and semi-quantitative (density ranges) methods.

Table 1. Summary of pediatric studies assessing mast cell density in functional abdominal pain disorders.

Author	Country	Age Group	Population (N)	Mucosal Sites	Mast Cell ID Method	Number of Microscopic Fields Assessed	Field Selection	Cell Activation Assessed
Yeom et al. [34]	Korea	6–12	FD (56)	Gastric antrum and body; duodenum	Anti-tryptase	5	Most involved area	No
Henderson et al. [35]	USA	5–17	AP-FGID (26)	Upper and lower	Toluidine Blue	10	Random	No
Di Nardo et al. [36]	Italy	4–18	IBS (21)	TI, Ascending and Descending Colon	Anti-tryptase	Not stated	Random	No
Mahjoub et al. [37]	Iran	1–14	Endoscopy patients (86)	Antrum	Giemsa	10	Not stated	No
Schurman et al. [38]	USA	8–17	FD (59)	Antrum and duodenum	Anti-tryptase	5	Most involved area	No
Singh et al. [39]	USA	8–17	FD (114)	Antrum and duodenum	Anti-tryptase	5	Most involved area	No
Schäppi et al. [40]	UK	2–12	FD (16)	Gastric	Anti-tryptase	10	Not stated	Yes
Saad et al. [41]	USA	3.3–17.9	Endoscopy patients: 92% for abdominal pain (41)	Cecum, ascending, transverse, descending and rectosigmoid colon	Anti-tryptase	5	Most involved area	No
Friesen et al. [42]	USA	8–17	FD (30)	Antrum	Anti-tryptase	5–10	Not stated	Yes
Chernetsova et al. [43]	Canada	1–17	Endoscopy patients designated as healthy (38)	Gastric body and antrum, duodenum, TI, cecum, ascending, transverse, descending, and sigmoid colon, and rectum	Hematoxylin-Phloxine-Saffron and Giemsa	Not stated	Most involved area	No
Friesen et al. [44]	USA	8–17	AP-FGID (208)	Antrum and duodenum	Anti-tryptase	5	Most involved area	No

Table 2. Summary of adult studies assessing mast cell density where mast cell activation was not assessed in functional abdominal pain disorders.

Author	Country	Age Group	Population (N)	Mucosal Sites	Mast Cells ID Method	Number of Microscopic Fields Assessed	Field Selection	Density Different from Controls
Goral et al. [45]	Turkey	Mean 35–36 years	IBS (72)	Cecum and rectum	Giemsa	10	Not stated	Yes
De Silva et al. [46]	Sri Lanka	18–59 years	IBS-D (49)	Ileum, cecum, ascending, transverse, descending, and rectum	Giemsa	10	Not stated	Yes
Binesh et al. [47]	Iran	15–76 years	FD (25)	Stomach and duodenum	Giemsa	≥5	Not stated	No
Tunc et al. [48]	Turkey	27–64 years	IBS (11)	Cecum	Toluidine blue	10	Not stated	Yes
Chadwick et al. [49]	New Zealand	19–79 years	IBS (77)	Ascending, transverse, descending, and rectum	Tryptase	15	Not stated	Yes
Wang et al. [50]	China	Mean 42–49 years	IBS-D (20) and IBS-C (18)	Duodenum, jejunum, and TI	Tryptase	6	Not stated	Yes
Yang et al. [51]	China	16–75 years	IBS-D (55)	TI, ascending and sigmoid	Tryptase	Not stated	Not stated	Yes
Chang et al. [52]	USA	18–55 years	IBS-PI (45)	Sigmoid	Tryptase	% of area	Not stated	No
Sohn et al. [53]	Korea	18–72 years	IBS-D (22)	Rectum	Tryptase	Not stated	Most representative	Yes
Ahn et al. [54]	Korea	Median 32 years	IBS-D (83)	Ascending, transverse, descending, sigmoid, and rectum	Tryptase	6	Not stated	Yes
Dunlop et al. [55]	UK	Mean 38–40 years	IBS (75)	Rectum	Tryptase	4	Not stated	Yes
El-Salhy et al. [56]	Norway	18–62 years	IBS (50)	Rectum	Tryptase	10	Random	No
Cremon et al. [57]	Italy	22–75 years	IBS (48)	Descending colon	Tryptase	% of area	Random	Yes
Dunlop et al. [58]	England	Mean 42 years	IBS-PI (28)	Rectum	Tryptase	4	Not stated	No
Bian et al. [59]	China	21–66 years	D-IBS (10)	Descending colon	Tryptase	≥10	Random	Yes
Sundin et al. [60]	Sweden	Mean 32 years	IBS (43)	Sigmoid colon	Tryptase	3	Not stated	No

Table 2. *Cont.*

Author	Country	Age Group	Population (N)	Mucosal Sites	Mast Cells ID Method	Number of Microscopic Fields Assessed	Field Selection	Density Different from Controls
O'Sullivan et al. [61]	Ireland	28–65 years	IBS (14)	Cecum, ascending, descending, and rectum	Tryptase	3	Not stated	Yes
Park et al. [62]	Korea	25–65 years	IBS-D (18)	TI, ascending, and rectum	Tryptase	6	Not stated	Yes
Lee et al. [63]	South Korea	Mean 48 years	IBS (42)	Rectum	Tryptase	5	Not stated	Yes
Kim et al. [64]	Korea	Mean 30–51 years	IBS (18)	Descending, sigmoid, and rectum	Tryptase	5	Not stated	Yes
Giancola et al. [65]	Belgium	18–68 years	FD (13)	Duodenum	Tryptase	4	Random	Yes
Hall et al. [66]	Ireland	18–79 years	FD (62)	Gastric body and antrum	Tryptase	15	Not stated	Yes
Vanheel et al. [67]	Belgium	17–52 years	FD (15)	Duodenum	Tryptase	≥7	Representative	Yes
Tanaka et al. [68]	Japan	Mean 45 years	FD (9)	Duodenum	Tryptase	5	Not stated	No
Vicario et al. [69]	Spain	18–63 years	IBS-D (49)	Jejunum	CD117	8	Not stated	Yes
Braak et al. [70]	Amsterdam	19–65 years	IBS (66)	Ascending and descending colon	CD117	18	Not stated	Yes- decreased
Boyer et al. [71]	France	Mean 54–67 years	IBS (11)	Cecum, transverse, descending, and rectum	CD117	4	Not stated	Not reported
Piche et al. [72]	France	Mean 54 years	IBS (50)	Cecum	CD117	5	Not stated	Yes
Doyle et al. [73]	USA	18–78 years	IBS (100)	Colon	Anti-kit	5	Area of highest density	Yes
Coeffier et al. [74]	France	Mean 44.6 years	IBS (25)	Descending colon	CD117	10	Not stated	Yes
Walker et al. [75]	Sweden	Mean 53 years	FD (51) and IBS (41)	Duodenum	CD117	5	Not stated	Yes
Taki et al. [76]	Japan	Mean 53 years	FD (35)	Duodenum	CD117	≥3	Representative	Yes
Lee et al. [77]	Korea	Mean 36 years	FD (51)	Duodenum	c-KIT	5	Hot spots	No
Wauters et al. [78]	Belgium	18–64 years	FD (45)	Duodenum	c-kit	3	Not stated	Yes

Table 3. Summary of adult studies assessing mast cell density which also assessed mast cell activation in functional abdominal pain disorders.

Author	Country	Age Group	Population (N)	Mucosal Sites	Mast Cells ID Method	Number of Microscopic Fields Assessed	Field Selection	Density Different from Controls	Activation Different from Controls
Park et al. [10]	Korea	Mean 48 years	IBS-D (14)	Cecum and rectum	Toluidine blue	Up to 20	Not stated	Yes	Yes
Liu et al. [11]	China	22–40 years	IBS-D (42)	Rectosigmoid junction	Toluidine blue	5	Random	No	Yes
Xu et al. [12]	China	18–49 years	IBS-D (38)	Rectosigmoid junction	Toluidine blue	5	Random	Yes	No
Yuan et al. [13]	China	Mean 45–47 years	FD (48)	Duodenum	Toluidine blue	Not stated	Not stated	Yes	Yes
Yuan et al. [14]	China	Mean 45–47 years	FD (48)	Duodenum	Toluidine blue	Not stated	Not stated	Yes	Yes
Wang et al. [15]	China	Mean 46 years	FD (141)	Duodenum	Toluidine blue	4-6 random sites, then 5	Random	Yes	Yes
Foley et al. [16]	England	Mean 42 years	IBS-D (20)	Duodenum	Tryptase	Not stated	Not stated	Yes	Yes
Lee et al. [24]	Korea	24–66 years	IBS-D (16)	Rectum	Tryptase	5	Not stated	No	Yes
Barbara et al. [17]	Italy	22–75 years	IBS (44)	Descending colon	Tryptase	Area occupied	Random	Yes	Yes
Balestra et al. [25]	Italy	21–70 years	IBS (37)	Descending colon	Tryptase	% of LP occupied	Random	Yes	Yes
Han et al. [26]	China	18–59 years	PI-IBS (23)	Left colon	Tryptase	≥8	Not stated	Yes-area; No-number	Yes
Cremon et al. [27]	Italy, Spain, France, Croatia, and Bosnia and Herzegovina	Mean 37–40 years	IBS (54)	Proximal descending colon	Tryptase	Not stated	Not stated	Yes	Not reported
Bednarska et al. [28]	Sweden	19–55 years	IBS (32)	30-40 cm from anal verge	Tryptase	Not stated	Not stated	Yes	Yes
Buhner et al. [29]	Italy	27–68 years	IBS (11)	Proximal descending colon	Tryptase	Not stated	Not stated	Yes	Yes

Table 3. *Cont.*

Author	Country	Age Group	Population (N)	Mucosal Sites	Mast Cells ID Method	Number of Microscopic Fields Assessed	Field Selection	Density Different from Controls	Activation Different from Controls
Barbara et al. [30]	Italy	19–70 years	IBS (29)	Proximal descending colon	Tryptase	Not stated	Not stated	Yes	Yes
Li et al. [20]	China	17–65 years	FD (65)	Antrum	Tryptase	10	Not stated	Yes	Yes
Vanheel et al. [79]	Belgium	23–43 years	FD (24)	Duodenum	Tryptase	≥7	Representative	Yes	No
Du et al. [19]	China	Mean 48 years	FD (96)	Duodenum	Tryptase	5	Random	Not reported	No
Cremon et al. [31]	Italy	22–56 years	IBS (25)	Descending colon	Tryptase	Area occupied	Random	Yes	Yes
Klooker et al. [32]	The Netherlands	19–65 years	IBS (29)	Descending and rectum	Tryptase or CD117	18	Not stated	Yes- decreased	Yes- decreased
Martinez et al. [21]	Spain	18–60 years	IBS-D (45)	Jejunum	CD117	Not stated	Not stated	Yes	Yes
Vivinus-Nébot et al. [33]	France	42–58 years	IBS (34)	Cecum	CD117	3	Not stated	Yes	Yes
Lobo et al. [18]	Spain	18–65 years	IBS-D (43)	Jejunum	CD117	10	Not stated	No	Yes
Guilarte et al. [22]	Spain	21–56 years	D-IBS (20)	Jejunum	CD117	8	Not stated	Yes	Yes
Martinez et al. [23]	Spain	18–59 years	IBS-D (25)	Jejunum	CD117	Not stated	Not stated	Yes	Yes

Only 2 pediatric studies compared patients to a control group and both studies demonstrated increased mast cells in the study group [36,39]. Neither assessed activation. Results comparing adult patients to controls are shown in Tables 2 and 3. Overall, density in comparison to controls was reported in 57 studies. Of these, increased density was reported in 45 studies (79%), no difference in 10 studies (18%), and decreased density in 2 studies (4%). One study reported density as both cell count and area occupied by mast cells demonstrating decreased cell counts and increased area occupied in patients with IBS [26]. Increased mast cells in association with an FAPD was found in 26/33 (79%) of studies staining for tryptase, 12/15 (80%) of studies staining for CD117, and 8/10 (80%) of studies utilizing histochemical staining.

Overall activation in comparison to controls was reported in 24 studies (Table 3). Of these, increased activation was reported in 20 studies (83%), decreased activation in one study (4%), and no difference in 3 studies (13%). Twenty-three of these studies reported both density and activation as compared to controls. In 5 studies, there was a discrepancy between density and activation findings. Three studies showed increased activation with no difference in density and two showed increased density with no difference in activation. One study showed increased density by area occupied but not cell density and activation was increased. Seven studies utilized multiple methods to assess activation and findings were concordant between methods in 6 studies [17,18,20,26,28–30]. In the remaining study, supernatant tryptase was increased but degranulation did not differ from controls [28]. Overall activation was assessed by supernatant tryptase in 12 studies, degranulation in 10 studies, tissue tryptase expression or protein analysis in 5 studies, supernatant histamine in 6 studies, assessment of in vitro nerve stimulation in 3 studies, and luminal tryptase in 2 studies.

4. Discussion

A variety of techniques are available for assessing mast cell density in FAPDs and these have been applied with considerable variability. Immunohistochemistry (IHC) staining appears to be the preferred method for identifying mast cells to assess density, with anti-tryptase or anti-CD117 antibodies utilized in 73% of pediatric studies and 83% of adult studies. Histochemical stains for mast cells are known to be less sensitive in identifying mast cells in gastrointestinal mucosa fixed with formalin [80,81]. Not surprisingly, there appears to be a preference for IHC stains. However, these IHC stains are not without limitations and introduce other variability into the literature. For example, 20–30% of tryptase-positive cells in the stomach and colon fail to stain for CD117, creating a challenge in assimilating data obtained utilizing the two different methods [82]. CD117 is present in immature mast cells and a large proportion of mast cells in the stomach and colon do not stain with anti-CD117 [83]. While anti-tryptase appears to identify more mast cells and is the most commonly utilized method, tryptase is expressed late in mast cell maturation and will not identify those mast cells expressing only chymase which are present in the stomach, small bowel, and colon [81–83]. This latter limitation can be overcome by also staining for chymase [81]. Variability is also introduced by the selection of microscopic fields to be assessed. While the process for field selection is often not stated, when reported, it varies from random to "most representative" to most involved. The rationale for most involved is that density is often patchy. Most studies assess mast cells per area, either per high power field (hpf) or per mm^2. The actual area of a hpf varies between microscopes. Others assess the percentage of area occupied by mast cells utilizing digital imaging. While some studies have found a high correlation between manual cell counts and measurement of the percentage of area occupied by mast cells, another study found discordant results between manual counts and area occupied in comparison to healthy controls [26,52].

There are also a variety of methods for assessing mast cell activation. This may be of particular importance as most biologic functions of mast cells are the result of the release of specific mediators generally acting in a concentration-dependent fashion [84]. There appear to be 3 commonly used approaches for assessing activation: (1) assessing

degranulation at a cellular level using light microscopy or at both a cellular and sub-cellular level using electron microscopy, (2) assessing tissue or luminal mast cell mediators, most commonly tryptase and histamine, and (3) functional studies using mucosal supernatants to stimulate enteric nerves in vitro. Regardless of the method, increased activation relative to healthy controls was demonstrated in over 80% of studies. In 22% of studies where activation was assessed by any method, there was a discrepancy between density and activation comparisons to controls. In 2 studies, density alone was increased and in 3 studies, activation alone was increased. Density and activation measurements are not perfectly aligned and both may be needed to get a full picture of mast cell involvement. When multiple methods were utilized to assess activation, these were concordant with each other in 6 of the 7 studies.

While mast cells produce a wide variety of cytokines, chemokines, and other mediators, previous studies have primarily, but not exclusively, focused on tryptase and histamine. This appears justified as both have been implicated in mast cell interactions with sensory nerves. In a series of experiments, Wouters and colleagues established a role for histamine (via H1 receptors) in upregulating TRPV1 which has been highly implicated in visceral hyperalgesia, a central process in FAPDs [9]. Studies have nearly universally found increased tryptase in tissue, tissue supernatants, and luminal fluid. Studies have also demonstrated that supernatants from IBS mucosal biopsies can stimulate submucosal sensory nerves which correlate with serotonin, histamine, and tryptase concentrations and which could be inhibited by H1 receptor antagonists and serine protease inactivation [29,30]. An effect on myenteric nerves is reported to be independent of histamine and serine protease [25]. Mast cell mediators appear to have differential effects on submucosal and myenteric nerves [85]. Methods utilizing sensory nerves are likely a better model for assessing mast cell effects in FAPDs and possibly more representative of effects on pain transmission and visceral hyperalgesia.

There is some evidence to support mast cells as therapeutic targets in IBS and FD utilizing medications to inhibit mediator release (e.g., mast cell stabilizers such as cromolyn or ketotifen or anti-siglec 8) or to inhibit mast cell mediators once released (e.g., histamine or cysteinyl leukotriene inhibitors) [18,32,86–91]. One study utilizing cromolyn and one utilizing ketotifen demonstrated evidence of decreased mast cell activation [18,32]. However, no studies have evaluated whether any measures of mast cell density or activation are predictive of response to any of these medications.

5. Conclusions

A wide variety of methods have been employed to assess mast cell density and activation in patients with FAPDs with no well-established consensus and not enough data to recommend a specific approach. Given that differences in mast cell density and activation between patients with FAPDs and healthy controls are demonstrated in a strong majority of studies, regardless of methodology, this variability may make a more compelling case for mast cells in the pathophysiology of FAPDs in general. Whether this evidence is strong enough to warrant empiric treatment aimed at mast cell stabilization or mediators is up to clinical interpretation. Ideally though, the methodology providing physiologic information would be translated to a standard methodology providing clinical information with the development of criteria establishing abnormal density and/or activation, and more importantly, predicting response to treatment.

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