Title: MORPHOLOGICAL AND FUNCTIONAL EVALUATION OF THE GUT IN CYSTIC FIBROSIS

Running title: Gut dysfunction in Cystic Fibrosis

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ABSTRACT

Background: Cystic fibrosis (CF) is a multisystem disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. CFTR is extensively expressed in the intestine and has an important role in the regulation of the viscosity and pH of gut secretions. Several studies have reported a delay in small bowel and colonic transit times in patients with CF which have been attributed to the secretory dysfunction. Our aim was to determine whether intestinal contractility is also affected in these patients.

Methods: Consecutive patients with CF referred to our institution between 2014 and 2017 were prospectively investigated using automated non-invasive techniques for morpho-functional evaluation of the gut developed in our laboratory. On separate days, intraluminal images of the gut were obtained by capsule endoscopy, and external images by abdominal MRI. Analysis of images (endoluminal and external) was performed with original, previously validated programs based on computer vision and machine learning techniques.

Results: Patients with CF (n=16) exhibited important reduction in contractile activity and increased retention of static turbid secretions in the small bowel by endoluminal image analysis. Morpho-volumetric analysis of MRI images found increased ileo-colonic volumes in CF. Significant correlations between abnormalities detected by intraluminal and external imaging techniques were found. The presence and severity of digestive symptoms was not related to abnormal gut function.

Conclusion: Impaired transit and pooling of gut contents in patients with CF is associated to impaired intestinal motility.

KEYWORDS

Cystic fibrosis, intestinal motility, intestinal transit, intestinal secretion, capsule endoscopy, magnetic resonance

HIGHLIGHTS

• In previous studies, delayed gut transit and pooling of contents in CF has been related to impaired regulation of digestive secretory function.

• Our study shows that pooling of content in these patients is associated to impaired intestinal motor function.

• Since the presence and severity of digestive symptoms does not predict abnormal gut function in CF, a positive diagnosis of dysmotility is required for treatment planning.

1. INTRODUCTION

Cystic fibrosis is a multisystem disease caused by a mutation with loss of function in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The role of CFTR is to regulate the water content in epithelial secretions by controlling fluid transport. The loss of CFTR function in cystic fibrosis (CF) mainly manifests as severe respiratory and pancreatic insufficiency. However, CFTR is also extensively expressed in the intestine and has an important role in the regulation of the viscosity and pH of gut secretions.[1] In fact, the first clinical manifestation of CF in newborns may be an impaction of meconium in the small intestine causing acute bowel obstruction. Similarly, children and adults with CF may develop retention of solidified secretions in the small bowel, producing acute or subacute obstruction, a condition named distal intestinal obstruction syndrome (DIOS).

Several studies have reported a delay in small bowel and colonic transit times in patients with FC, which have been attributed to the secretory dysfunction.[2–4] However, it is not known whether intestinal contractility is also affected in these patients. This lack of information may be related to the difficulties in evaluating gut motility, because intestinal manometry, the current gold standard test, is invasive and restricted to only a few referral centers in the world.

In our laboratory we have applied imaging techniques that are conventionally used for the detection of structural abnormalities of the digestive tract to evaluate gut function. Specifically, endoluminal image analysis using

capsule endoscopy and abdominal magnetic resonance imaging (MRI) were applied for the evaluation of small bowel and ileo-colonic region. The advantages of these techniques over intestinal manometry are that they are non-invasive, more sensitive to the detection of motor dysfunctions and that they allow a concomitant evaluation of structure and function.[5,6]

We hypothesized that the combination of internal (endoluminal) and external (MRI) imaging would provide a unique perspective to define the relation between the structural and functional findings associated to cystic fibrosis.

2. METHODS

2.1. Participants

Adult patients with diagnosis of CF referred to the Vall d'Hebron gastroenterological clinic were prospectively recruited between April 2014 and April 2017. The study protocol was approved by the Ethics Committee of the University Hospital Vall d'Hebron, and all participants gave their written informed consent before enrolment. Criteria for inclusion were age> 18 yrs and a confirmed diagnosis of CF. Patients who had received lung transplantation or had current signs of intestinal obstruction were not included in the study.

Endoscopic capsule procedure and abdominal MRI were performed on separate days without previous bowel preparation.

2.2. Clinical evaluation

The previous history of the patients, particularly pancreatic and pulmonary involvement and history of meconium ileus or DIOS syndrome, as well as the specific genetic mutations associated to CF were recorded.

Participants completed a symptom questionnaire which evaluated on a 0 – 4 scale the presence of the following digestive symptoms in the previous 3 months: abdominal pain/discomfort, abdominal distension, early satiation, nausea, vomiting, heartburn, belching, dysphagia, constipation, diarrhea and flatulence; the intensity of each symptom was rated as absent (scored 0), mild (scored 1), moderate (scored 2), severe (scored 3) or invalidating (scored 4). Bowel movement frequency and fecal consistency by the Bristol scale were also evaluated.

2.3. Small bowel evaluation

Studies were performed after discontinuation of medications that could affect gastrointestinal motility for at least 48 h and overnight fast. Endoluminal images were obtained with the Pillcam capsule (Pillcam SB2 video capsule, Given Imaging, Yokneam, Israel). Images were obtained at a fixed 2 per second rate and recorded for a total of 8 hours with the subjects lying comfortably on a hospital bed and the trunk raised 30º above horizontal

Gastric exit of the capsule was determined by visual inspection at 10-minute intervals using a real-time viewer monitor (RAPID Access, Given Imaging, Yokneam, Israel). Forty-five minutes later, participants were instructed to ingest a liquid meal (Ensure HN, Abbott, Zwolle, The Netherlands; 300 ml, 1 Kcal/ml). After the 8 hour procedure, images were transferred to the standard

viewer program (RAPID Reader software v8, Given Imaging, Yokneam, Israel). After visual detection of gastric exit and cecal arrival, small bowel images were selected and examined to detect morphological mucosal abnormalities by an experienced gastroenterologist (CM).

Endoluminal images of the small bowel were analyzed with a computer program specifically developed in our laboratory for the evaluation of intestinal motility. This previously validated program is based on computer vision techniques, which allow the detection and quantification of contractile and non-contractile patterns characterizing small bowel motility. Contractile patterns include phasic intestinal luminal closures, detected as a brief closing of the small bowel lumen within a 9 image sequence (4.5 seconds), and radial mucosal wrinkles, which form around the lumen by the effect of contractions. Non-contractile patterns include the tunnel pattern, characterized by a peripheral band of bright wall and a central open lumen, large and dark; and the wall pattern, characterized by flat wall, smooth and bright, without view of lumen. Small bowel chyme appears as turbid content and is identified by color analysis. Finally, motion of both intestinal walls and content is measured in consecutive images to detect low-motion and high-motion sequences defined by previously established thresholds.[5]

Motility patterns were analyzed in all small bowel images and the proportion of images containing each pattern was calculated. Parameters were also quantified in the pre-meal phase and in 3 equal periods of the postprandial phase (i.e. between the end of the meal and the passage of the capsule into the colon or the end of the recording time).

Among a historical cohort of healthy subjects who had undergone the same endoluminal image analysis procedure, a group of 15 gender- and age-matched healthy subjects was randomly selected as comparator for CF patients.

2.4. Ileo-colonic evaluation

Abdominal MRI examinations were performed by using a 1.5-T MRI system (Avanto, Siemens Healthcare, Erlangen, Germany) and two six-channel phased-array abdominal coils. The subjects were placed in prone position with a 4-element body coil wrapped around the abdomen. The images were obtained using a) a T2-weighted fast sequence in the coronal plane, HASTE technique (1270 ms repetition time, 76 ms echo time, 3.5 mm slice thickness, 256x244 resolution) during two apneas of about 20 seconds each, and b) a T1-weighted VIBE Fat-Sat sequence in coronal plane (3.24 ms repetition time, 1.24 ms echo time, 1.5 mm slice thickness, 320x210 resolution) in one apnea of 12 seconds. No contrast (oral or intravenous) or antiperistaltic drugs were used. All MRI images were reviewed by an expert radiologist (XMe) who reported abdominal radiological findings.

Morpho-volumetric analysis of the terminal ileum and the colon was performed using original software developed for this purpose. The program allows semiautomatic segmentation of the intestine using a region-growing-based algorithm. First, an anisotropic contrast enhancement filter is applied to enhance the boundary of the intestine without loss of inside detail. Then, seed points are placed which expand depending on the gray-level mapping defined by the window-level setting of the images. To facilitate

segmentation, a toolkit was developed that permits enlarging or reducing the segmentation obtained by the region-growing algorithm. Segmentation was correlated in T2 and T1 images. Non-gaseous content was measured in T1 images, and gaseous content was measured by subtracting T1 from T2 volumes. A three-dimensional reconstruction program with 360° rotation over the three dimensions, previously developed for morpho-volumetric analysis of CT images, was adapted for MRI analysis to facilitate measurement of the volume, length, area, perimeter and diameters in selected regions of the intestine. Since the ileal segment detectable by MRI imaging varies, the relative volume of the ileum was normalized as ml/cm, which reflects the average cross-sectional area.

A historical cohort of 13 healthy subjects who had been previously evaluated using the same MRI protocol for intestinal morpho-functional evaluation was used as a comparator for CF patients.

2.5. Statistical Analysis

Statistical analysis was performed with the SPSS 12.0 for Windows statistical package. Mean values (±SE) of the parameters measured were calculated in each group of subjects and compared. Normality of data distribution was evaluated by the Kolmogorov-Smirnov test. Comparisons of parametric, normally-distributed data were made by Student's t-test, paired tests for intragroup comparisons and unpaired tests for intergroup comparisons; otherwise, the Wilcoxon signed rank test was used for paired data within groups, and the Mann-Whitney U test for unpaired data between groups.

Distribution of abnormalities between groups was evaluated by the X^2 test. Continuous variables were correlated using Pearson's R. Differences were considered significant at a P value <0.05.

3. RESULTS

3.1. Clinical data

Sixteen patients (9 women, 7 men; age range 19-59 years) with genetic mutations for CF were included in the study. Pancreatic insufficiency requiring oral enzyme replacement therapy was present in 12 patients. Pulmonary dysfunction (defined as predicted forced expiratory volume (FEV1) <80%) was present in 13 patients. One patient had a history of meconium ileus and 2 of DIOS syndrome which had occurred more than 12 months before entering the study. Body mass index (BMI) was below 18.5 kg/m^2 in 4 patients, all with pancreatic insufficiency and pulmonary dysfunction. Genotype and clinical features are summarized in Table 1.

Patient number	Genotype	Sweat chloride (mmol/L)	FEV1%	Pancreatic insufficiency	BMI (kg/m2)	Meconium ileus	DIOS	Diabetes	Hepatic involvement
1	G542X/dF508	80	73	Yes	25,0	No	No	No	Yes
2	dF508/dF508	100	64	Yes	24,1	No	No	No	No
3	dF508/E1308X	120	31	Yes	16,9	No	No	No	No
4	G542X/1811+11.2KbA>C	90	30	Yes	17,6	No	No	No	No
5	dF508/R75Q	104	109	Yes	24,5	No	No	No	No
6	dF508/dF508	120	64	Yes	17,6	No	No	No	No
7	dF508/2729+5A	120	85	No	23,5	No	No	No	No
8	V754M/D1152H	75	100	No	21,1	No	No	No	No
9	2183AA>G/2869insG	100	75	Yes	21,0	No	No	No	No
10	dF508/dF508	112	54	Yes	20,4	Yes	No	No	Est
11	dF508/dF508	112	75	No	23,0	No	No	No	No
12	dF508/R334W	106	45	No	19,0	No	No	No	No
13	711+1G>T/W1282X	111	75	Yes	19,5	No	No	No	No
14	dF508/dF508	119	45	Yes	17,7	No	Yes	No	No
15	dF508/Q890X	80	66	Yes	21,3	No	No	Yes	Yes
16	dF508/712-1G>T	102	72	Yes	21,6	No	Yes	No	Yes

Table 1. Genotype and clinical features of CF patients

Digestive symptoms in the previous 3 months were reported by all but two patients. The most prevalent symptoms were abdominal distension (n=9) and abdominal pain (n=8) (Figure 1). Severity of symptoms was moderate in most patients (2.2 ± 0.3 mean score on the 0-4 scale) and none rated symptoms as invalidating.

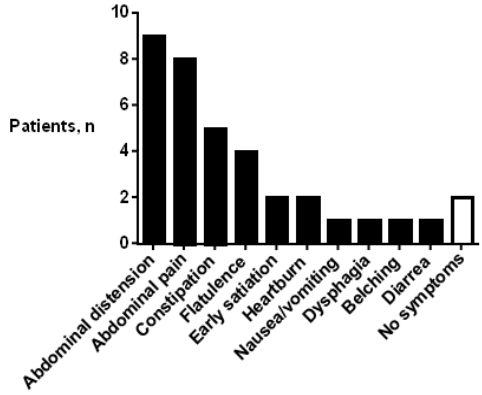

Figure 1: Digestive symptoms in CF patients. Each column shows the number of patients with each symptom as recorded on a questionnaire which evaluated the presence of digestive symptoms in the previous 3 months.

3.2. Capsule endoscopy

3.2.1. Capsule transit and mucosal morphology in the small bowel

In patients, total small bowel transit time was markedly increased: only in 7 patients the capsule reached the colon during the 8 h recording period and in them total small bowel transit time was 292 ± 33 min (vs 188 ± 29 min in healthy subjects; p=0.041).

Visual inspection of the endoscopic capsule images detected erosive duodenitis in 3 patients and mild patchy erythema in 3 patients. No other significant findings were detected in the rest of CF patients.

3.2.2. Functional evaluation

In the cohort of healthy subjects endoluminal imaging provided a clear view of the inner intestinal walls, with only one quarter of images containing turbid intestinal content which were predominantly located in the distal small bowel. By contrast, in patients with CF more than half of small bowel images contained turbid secretions (58 ± 6% vs 28 ± 4% in healthy subjects; $p < 0.001$), Interestingly, excessive pooling of contents also occurred in the proximal segments of the small bowel observed during the first part of the postprandial period (52 ± 7% of turbid secretions in vs 19 ± 3% in healthy subjects; $p < 0.001$). In patients, turbid secretions were frequently observed during prolonged periods of time (26 ± 4% of recorded images corresponding to >5 min long turbid sequences vs 16 ± 3% in healthy subjects; $p = 0.026$).

As compared to healthy subjects, patients with CF exhibited an important reduction in small bowel contractile activity, reflected by a reduction in luminal closures (2.3 ± 0.3 vs 5.2 ± 0.3% of small bowel images; $p < 0.001$) and a decreased proportion of closures with radial wrinkles (12 ± 1 vs 27 ± 3 %; $p = 0.001$) Reduced contractile activity in CF was also detected when specifically analyzing bowel segments without turbid secretions (5.9 ± 0.5 vs 7.4 ± 0.5% of clear small bowel images; $p = 0.038$). In healthy subjects meal ingestion induced a contractile response, and this response was virtually absent in CF patients, who exhibited reduced number of contractile events after the meal and thorough the whole postprandial period (49 ± 20% increase in contractile actively in health vs -9 ± 14% in patients; $p < 0.001$) (Figure 2).

Static sequences, characterized by null motion of intestinal walls and content, were observed in healthy subjects, but were more frequent in patients with CF (16 ± 7% of all intestinal images vs 7 ± 2% in health; $p = 0.029$). Static

turbid secretions were rare in healthy subjects and only detected in the last segments of the small bowel (corresponding to the last third of the postprandial period) whereas in CF, images with static turbid secretions were increased also in proximal segments of the bowel (13 ± 2% of all intestinal images vs 3 ± 1 % in healthy subjects; p = 0.004) (Figure 2).

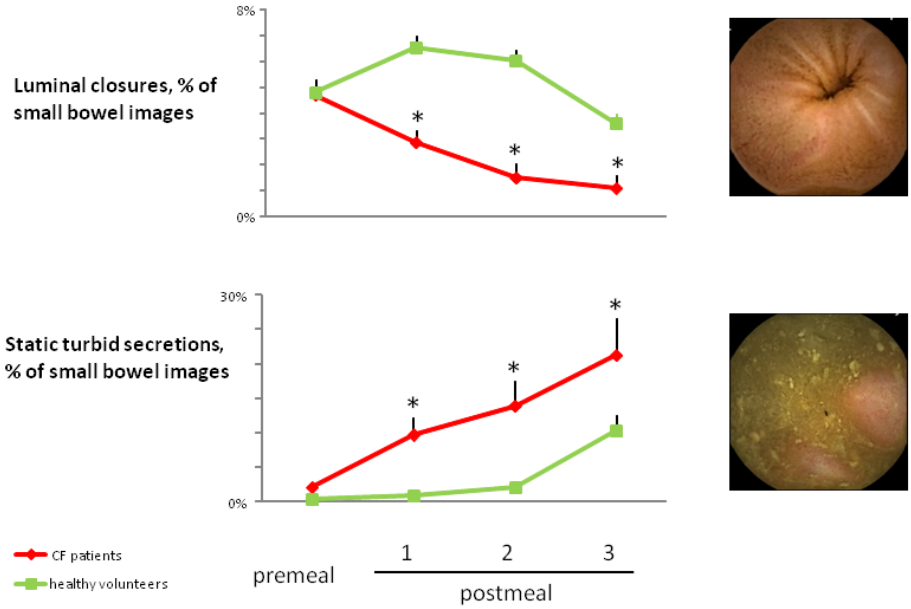

Figure 2: Evaluation of intestinal motility by computerized analysis of endoluminal images obtained by capsule endoscopy. On the upper left, number of luminal closures throughout the small bowel recording. Note the reduced number of contractile events in patients after the meal and throughout the whole postprandial period compared to healthy subjects. On the upper right, an image of an intestinal contraction by capsule endoscopy. On the lower left, amount of immobile turbid secretions throughout the small bowel recording. Note that static turbid secretions are very rare in healthy subjects, and only observed in the last segments of the small bowel (corresponding to the last third of the postmeal period) whereas in cystic fibrosis, static turbid secretions are present in the whole small bowel except in the first segments of the small bowel (corresponding to the premeal period). On the lower right, an image showing intestinal content by capsule endoscopy.

3.3. Abdominal MRI

3.3.1. General structure

Abdominal MRI detected typical CF radiological abnormalities in 14 of 16 patients. Pancreatic atrophy was present in 12 patients and was associated with replacement of the pancreas by fatty tissue in 10 patients. Two patients also had multiple cystic lesions of the pancreas. Hepatic abnormalities were rare; no patient had signs of cirrhosis and only one had hepatic steatosis. In one patient multiple bile duct strictures were observed, similar to those of sclerosing cholangitis.

The small intestinal wall was found to be thickened in 10 patients, and in half of them this was also associated to thickening of the colonic walls. Seven patients were found to have an appendicular mucocele. No intestinal strictures or signs of fibrosing colonopathy were observed. None of the patients had signs of distal intestinal obstructive syndrome.

3.3.2. Morpho-volumetric analysis of the ileo-colonic region

Significant differences in colonic and ileal volume were found between patients and healthy subjects (Figure 3). Total colonic volume was 47% larger in patients with CF ($p=0.019$), and was particularly increased in the right and transverse colon (69% and 46% larger respectively; $p<0.028$ for both) (Figure 4). To note no differences in gas content were observed. The normalized size of the terminal ileum (measured as ml / cm of length) was 81% larger in patients than in healthy subjects ($p=0.001$). Differences in ileal volume were by-and-

large related to differences in solid/liquid content (37±4 vs 22±2 ml in healthy subjects; p=0.024); the volume of gas, although small, was also significantly larger in patients than in healthy subjects (4.9±1.5 ml vs 1.0±0.5 ml, respectively; p=0.024).

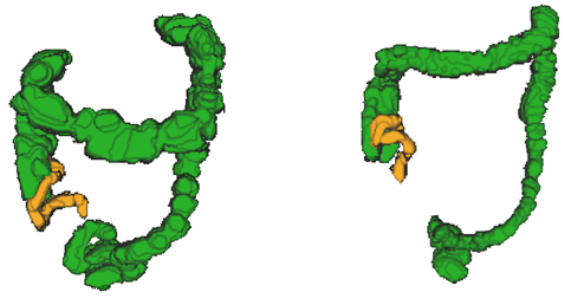

Figure 3. Examples of morpho-volumetric analysis of the colon (green) and terminal ileum (yellow) in a cystic fibrosis patient (left) and in a healthy subject (right). Note larger colonic volumes in the cystic fibrosis patient.

3.4. Correlations between small bowel and ileo-colonic imaging features

The amount of turbid secretions in the small bowel detected by endoluminal image analysis (proportion of turbid images) correlated with the luminal volumes measured by MRI in the ileum (R=0.59; p=0.046) and in the colon (R=0.68; p=0.016) (Figure 4). Furthermore, motor activity in the small bowel before the meal correlated with the volume of content measured in proximal colon by MRI: patients with lower number of luminal closures exhibited larger pooling of solid content in the right (R=-0.68; p=0.015) and transverse colon (R=-0.75; p=0.005) (Figure 5).

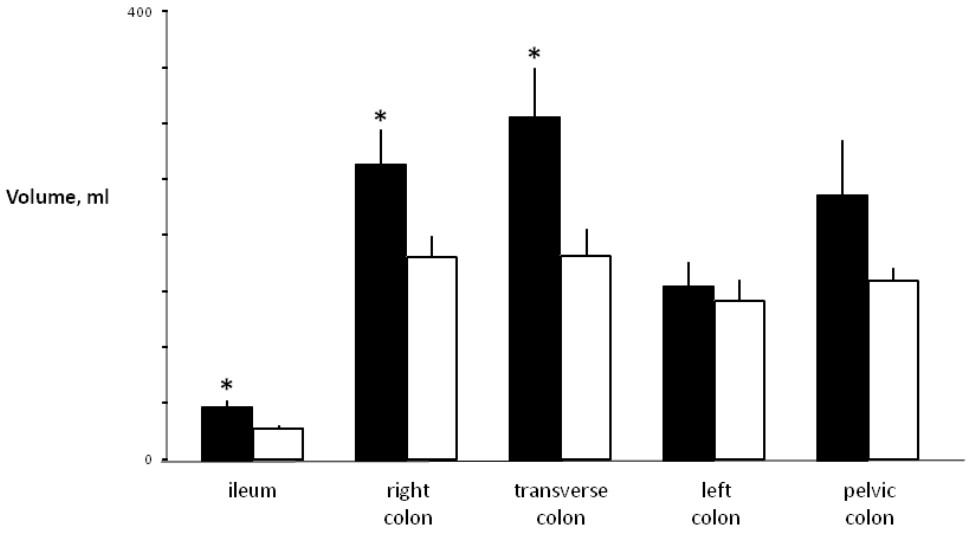

Figure 4: Morpho-volumetric analysis of abdominal MRI images in cystic fibrosis and healthy subjects. Patients with cystic fibrosis have increased ileal and colonic volumes.

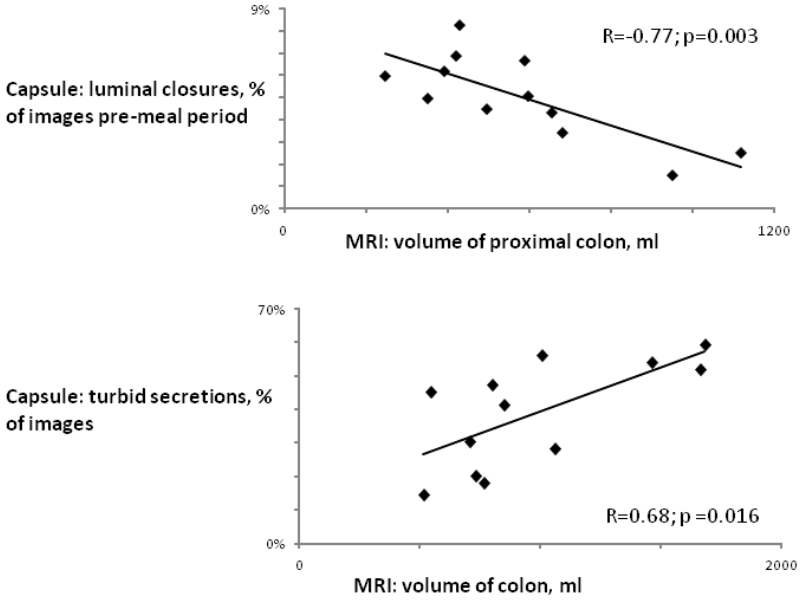

Figure 5: Correlation between functional parameters measured in the small bowel and colon by computerized analysis of capsule endoscopy and abdominal MRI images in CF patients. Upper panel: The number of luminal closures in the pre-meal period (corresponding to the proximal small bowel) correlated inversely with the volume of the proximal colon (ascending and transverse colon). Lower panel: The amount of turbid secretions in the small bowel correlated with the total volume of the colon.

3.5. Relation between clinical and functional parameters

Patients with pancreatic insufficiency had larger ileal volumes by MRI than those with normal pancreatic function (4.7±0.5 ml/cm vs 2.6±0.2 ml/cm; p=0.033). Likewise, small bowel function was more severely affected in patients with pancreatic insufficiency than in those without, as shown by a reduced intestinal luminal occlusions (1.9±0.2% of luminal closures vs 3.7±1.2%, respectively; p=0.023) with less radial mucosal wrinkles (2.3±0.4% of images with wrinkles vs 5.3±0.2%, respectively; p=0.027), and more presence of turbid content (64±5% of images with turbid content vs 29±13%, respectively; p=0.028).

3.6. Relation between digestive symptoms and gut dysfunction

Patients complaining of flatulence had larger colonic volumes by MRI than those who did not have this symptom (1465±145 ml vs 738±56 ml in; p=0.002) and this was due to a significant increase in both solid (1177±157 ml vs 645±52 ml in; p=0.021) and gas content (288±34 ml vs 92±34 ml in; p=0.001). No other associations between intestinal volumes and symptoms were detected. Furthermore, the type or intensity of digestive symptoms did not predict abnormal intestinal motility by endoluminal motility analysis.

4. DISCUSSION

Our study provides direct evidence of impaired intestinal motility in patients with CF as well as a quantitative assessment of intraluminal retention of intestinal contents. Previous studies showed delayed transit in the small bowel and the colon in patients with CF using scintigraphy or transit markers.[2–4] Our data show that delayed transit is associated to retention of intraluminal contents, and that slow motion and pooling are not only related to the secretory dysfunction and thickening of intraluminal contents, but also to a motor dysfunction of the gut.

Analysis of the endoluminal images obtained by capsule endoscopy showed an overall hypodynamic motility pattern in the small bowel in CF, manifested by reduced intestinal contractility and more presence of chyme, visualized as turbid intraluminal material. Patients not only presented more images with turbid content, but turbid content was also present in more proximal segments of the small bowel and exhibited reduced motion. Complementary confirmation was obtained by morpho-volumetric analysis of intestinal MRI images which detected increased ileal and colonic volumes related to pooling of contents.

Using the same endoluminal image analysis procedure, similar types of dysfunctions have been previously observed in patients with severe intestinal dysmotility diagnosed by intestinal manometry.[7] Likewise, studies using abdominal imaging showed that patients with severe dysmotility also exhibit pooling of intestinal contents.[8] The motor dysfunction in CF patients affected both the small bowel and the colon and the abnormalities independently

detected by external and internal imaging in different segments of the gut showed correlations, indicating that dysmotility in CF is a pan-enteric disorder. Parameters of intestinal dysmotility were related to pancreatic dysfunction, but not to the presence and severity of digestive symptoms. The lack of correlations between symptoms and objective measurement of dysmotility has been observed in other conditions, e.g., in patients with functional bowel disorders, symptoms were not predictive of abnormal motility detected by endoluminal image analysis, and no correlations between specific motor patterns and clinical parameters were found.[9] The fact that the type or severity of symptoms does not predict abnormal motility indicates that the diagnosis of intestinal dysmotility should be performed using appropriate motility tests.

Intestinal dysmotility may be related to inflammation of the gut wall. Inflammatory biomarkers have been shown to be present in fecal samples and intestinal secretions in CF. Fecal calprotectin levels are elevated in patients with CF and are associated to pancreatic insufficiency which in our study was also associated to worse intestinal motility.[10,11] Furthermore, histological analysis of the intestinal wall has shown that enteric inflammation in CF reaches the muscular layers and neuronal plexuses.[12] These histological abnormalities are in fact very similar to those detected in intestinal samples of patients with severe intestinal motility disorders.[13,14]. The mechanism by which the mutation of the CTFR gen in CF leads to inflammation of the gut wall is not clear. Intestinal dysbiosis has been suggested as a potential causing factor;[15] however, current evidence also indicates a direct role of CFTR in the abnormal inflammatory response of CF patients, specifically due to dysfunctional macrophage activity.[16]

We acknowledge that in this exploratory pilot study we compared the prospective data in CF to cohorts of historical controls of healthy subjects. However, the abnormalities observed were concordant with independent techniques and the magnitude of the differences with healthy subjects were substantial and consistent. The current gold standard test, intestinal manometry, is an invasive technique that involves oro-jejunal intubation. The use of non-invasive diagnostic techniques, such as capsule endoscopy and abdominal MRI, is particularly suited for the evaluation of adult CF patients in whom the disease is generally at advanced stages with restricted indications for invasive tests.

The recent advances in the pharmacological management of digestive neuromuscular disorders with new prokinetics and intestinal secretagogues may be applied to the CF patients with confirmed dysmotility.[17] This is especially important in patients with CF in whom indications for medications should be well substantiated. Moreover, there is also a need for objective biomarkers of therapeutic response to the new CFTR modulators and gastrointestinal outcome measures are considered a promising option.[18]

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DECLARATION OF INTEREST

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