Gastro-intestinal Manifestations of Cystic Fibrosis

Gastro-intestinal manifestations of cystic fibrosis
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Gastro-intestinal Manifestations of Cystic Fibrosis

Gastro-intestinale Manifestaties van Cystische Fibrose
(met een samenvatting in het Nederlands)

Proefschrift

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voor Linde en Imre

Table of contents

Chapter 1

General introduction and outline of the thesis

Cystic fibrosis (CF) is a common autosomal recessive genetic disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. CFTR acts as a chloride channel and absent or reduced CFTR activity results in thickened and viscous secretions in lung, pancreas, intestine and liver.[1] In most CF patients this results in severe lung disease and pancreatic insufficiency. In addition, meconium ileus, distal intestinal obstruction syndrome, constipation and liver cirrhosis are frequent complications of CF.[1,2]

Intestinal obstruction syndromes in cystic fibrosis

The survival of CF patients has dramatically improved over the last decades, because of centralized management of CF patients in specialized CF centers, more aggressive use of antibiotics and intensive nutritional support.[3] With the improved life expectancy other manifestations of CF are becoming clinically more relevant. Meconium ileus at birth, distal intestinal obstruction syndrome (DIOS) and constipation are an interrelated group of manifestations, ranging from severe to mild intestinal obstruction and possibly sharing a common pathophysiology.[4-6] However, definitions of DIOS and constipation were not consistent. They included post-neonatal distal small bowel obstruction (also called meconium ileus equivalent)[7], but also encompassed a variety of other intestinal symptoms, including palpable caecal masses, abdominal pain, intussusception and volvulus[8,9] or even included constipation, which is common in CF[4], but received little attention in the literature.

Meconium ileus at birth occurs in 10-20% of all CF patients and is clearly influenced by genetic factors, since a large twin study demonstrated that monozygous twins show a greater concordance for meconium ileus than dizygous twins.[5] Partially this variation could be explained by the CFTR genotype mutations, such as homozygosity for the delta F508 deletion, the most common CFTR mutation in CF patients, which is strongly associated with the presence of meconium ileus.[5] Also non-CFTR genes (i.e. modifier genes) influence the risk for developing meconium ileus at birth[5,10,11], although a solid association between a causal modifier gene and meconium ileus in genome wide association studies has yet to be discovered. [5,10,11] A possible candidate gene for influencing intestinal obstruction in CF is CLCA1 and its orthologue in mice Clca3. These genes encode a calcium activated chloride channel. Recent studies showed an important role for CLCA1/ Clca3 in intestinal obstruction in CF; the expression of Clca3 in the intestine of CF mice, which all die of intestinal obstruction, is decreased[12,13] and up-regulation of Clca3 in CF mice results in ameliorated intestinal disease and improved survival.[12]

Medical interventions in cystic fibrosis: longitudinal effect on growth, bacterial colonization and pulmonary function

CF patients receive a variety of medications (i.e. antibiotics, corticosteroids, pancreatic enzyme replacement, gastric acid (GA) inhibition (proton pump inhibitors or histamine-2 receptor antagonists), ursodeoxycholic acid (UDCA) and laxatives). All have the potential to interact with each other and the vulnerable host, who is at risk to develop severe lung disease and poor nutritional status. However, little attention is paid to the interactions of commonly used medications in CF.

In CF patients GA inhibition might be given to optimize fat malabsorption[14] or for gastroesophageal reflux disease (GERD). However GA inhibition might potentially affect pulmonary function in CF since this was found to be associated with an increased risk for pulmonary infections in non-CF patients.[15-17] In CF patients the effect of GA inhibition on the pulmonary function was unclear so far; in cross-sectional studies of CF patients with GERD pulmonary function (forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC)) is reduced[18,19], while pulmonary function (FEV_1, FVC and maximum expiratory flow at 50% of FVC (MEF_{50})) was not different during 1 year of GA inhibition treatment in a study of 14 CF patients with persistent fat malabsorption.[20]

A second medication which is commonly used for CF related liver disease, ursodeoxycholic acid (UDCA), might also have side effects. CF related liver disease originates by inspissated bile, which leads to bile duct obstruction and progressive periportal fibrosis, eventually causing liver cirrhosis in 10% of all CF patients.[21] UDCA therapy supposedly improves bile viscosity through a changed bile acid composition.[22] However, UDCA has an impaired capacity to form mixed micelles, which may negatively influence fat absorption and growth in CF patients.[23] Nevertheless a small observational study reported an improvement of weight and body mass index (BMI).[24] UDCA may also influence pulmonary function and bacterial colonization, since UDCA has anti-inflammatory and immuno-modulatory properties.[25] In addition, a small retrospective study reported lower Pseudomonas aeruginosa (PA) colonization in meconium ileus patients who receive early UDCA compared to patients who receive UDCA at the onset of CF related liver disease.[26]

Finally optimal nutrition is essential for CF patients, as poor nutritional status is associated with severe morbidity and impaired survival in CF patients.[27] Furthermore, nutritional status is closely related to pulmonary function.[28,29] Malnutrition in CF is the consequence of a high caloric expenditure in combination with fat malabsorption due to exocrine pancreatic insufficiency. Therefore the current recommendation is to provide a high caloric diet with 120-150% of the recommended daily allowance (RDA) for energy

intake[30] and optimize fat absorption with pancreatic enzyme replacement, adding GA inhibition if necessary.[14] However, the relationship between energy intake, nutritional status and pulmonary function is unclear, since several nutritional interventions, although showing a positive effect on weight gain, seem to have no effect on pulmonary function.[31-34]

Aims of this thesis

This thesis consists of two parts. The aim of the first part is to give a clear definition of constipation and DIOS in CF and to determine the incidence, prevalence and risk factors (both genetic and environmental) of the intestinal obstruction syndromes (constipation, DIOS and meconium ileus). The aim of the second part is to investigate the effect of medical interventions (GA inhibition, UDCA and energy intake) on growth, bacterial colonization and pulmonary function.

Outline of this thesis

Part 1: Intestinal obstruction syndromes in cystic fibrosis

In **chapter 2** the European Society for Pediatric Gastroenterology, Hepatology and Nutrition CF working Group definitions for DIOS and constipation in CF are described. Also, characteristics of 39 pediatric European (Belgian, Dutch, French, Italian, Israeli and Polish) DIOS patients were given, and the incidence during the years 2001 to 2005 is described. Subsequently, in **chapter 3** the incidence, prevalence and risk factors of constipation in the pediatric CF population of the UMC Utrecht were determined. **Chapter 4** describes the association between the p.S357N variant in the CLCA 1 gene and meconium ileus in a cohort of European (Dutch, German and Italian) pediatric CF patients.

Part 2: Medical interventions in cystic fibrosis: longitudinal effect on growth, bacterial colonization and pulmonary function

In **chapter 5** and **chapter 6** the longitudinal effect on growth, bacterial colonization and pulmonary function of respectively GA inhibition (for GERD or fat malabsorption) and UDCA in pediatric CF patients is determined. At last, the complex relationship between nutritional status, pulmonary function and energy intake is investigated in **chapter 7**.

This thesis concludes with a summary and general discussion (**chapter 8**) in which also directions for future research are given.

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Part I

Intestinal obstruction syndromes in Cystic Fibrosis

Chapter 2

Defining DIOS and Constipation in Cystic Fibrosis With a Multicentre Study on the Incidence, Characteristics, and Treatment of DIOS

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Abstract

Objectives: Various definitions for distal intestinal obstruction syndrome (DIOS), meconium ileus equivalent, and constipation in patients with cystic fibrosis (CF) are used. However, an unequivocal definition for DIOS, meconium ileus equivalent, and constipation is preferred. The aims of this study were, therefore, to seek consensus on the definitions for DIOS and constipation in patients with CF and to determine the incidence, characteristics, and treatment of DIOS in a cohort of pediatric patients with CF.

Methods: During the 2005 European Society for Pediatric Gastroenterology, Hepatology, and Nutrition meeting in Porto a group of pediatric gastroenterologists discussed the definition of DIOS and constipation in CF. Subsequently, all patients younger than or equal to 18 years with complete DIOS according to the definition agreed upon and diagnosed during the years 2001 to 2005 in 8 CF centres were studied.

Results: Distal intestinal obstruction syndrome was defined as an acute complete or incomplete faecal obstruction in the ileocaecum, whereas constipation was defined as gradual faecal impaction of the total colon. Fifty-one episodes of DIOS in 39 patients were recorded, giving an overall incidence of 6.2 (95% confidence interval, 4.4–7.9) episodes per 1000 patient-years. Of the 39 patients with DIOS, 20% experienced a relapse, 92% were pancreatic insufficient, 44% had a history of meconium ileus at birth, and 82% had a severe genotype. Conservative treatment was effective in 49 of 51 DIOS episodes (96%).

Conclusions: The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition CF Working Group definitions of DIOS and constipation in CF are specific and make a clear distinction between these 2 entities. The incidence of DIOS in the present study was considerably higher than reported previously.

Introduction

In patients with cystic fibrosis (CF), a syndrome of postneonatal distal small bowel obstruction caused by meconium-like stool plugs was first described in 1945. At that time this condition was named meconium ileus equivalent (MIE).[1] Later the term "distal intestinal obstruction syndrome" (DIOS) was introduced, referring to a range of clinical conditions due to partial or complete bowel obstruction.[2] Subsequently, DIOS and MIE were sometimes used as interchangeable terms[3], but more commonly DIOS also encompassed a variety of other intestinal symptoms, including palpable caecal masses, abdominal pain, intussusception, and volvulus.[4,5] The definition of DIOS sometimes also included constipation, which is a common condition in CF that also causes abdominal pain and distension, and responds to conservative medical treatment.[6] Because of these varied definitions, comparing studies on incidence and other characteristics of these conditions is difficult.

Consequently, consensus on the definition for DIOS, MIE, and constipation in CF is essential. The aims of this report were, therefore, to seek consensus on the definitions for these conditions in patients with CF. Subsequently, the incidence, characteristics, and treatment results of DIOS in a pediatric CF cohort from 8 centres was investigated.

Methods

European Society for Pediatric Gastroenterology, Hepatology and Nutrition CF Working Group

A group of pediatric gastroenterologists with an interest in gastrointestinal manifestations of CF, convened at the 38th annual meeting of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) in Porto in June 2005 to seek consensus on the definition of DIOS and constipation in patients with CF.

The ESPGHAN CF Working Group suggested that the term MIE is redundant because both MIE and DIOS, as defined in Porto, refer to the same condition. The ESPGHAN CF Working Group also made a distinction between complete and incomplete DIOS. Complete DIOS was defined as the combination of (1) complete intestinal obstruction, as evidenced by vomiting of bilious material and/or fluid levels in small intestine on an abdominal radiography with (2) a faecal mass in ileo-caecum and (3) abdominal pain or distension or both. Incomplete or impending DIOS was defined as (1) a short history (days) of abdominal pain or distension or both and (2) a faecal mass in ileocaecum, but without signs of complete obstruction. Constipation was defined as (1) abdominal pain or distension or both or (2a) a decline in the frequency of bowel movements in the last few weeks to months or (2b) increased consistency of stools in the last few weeks or months or both, whereas (3) the symptoms are relieved by the use

Table 1: ESPGHAN CF Working Group definition for DIOS in Cystic Fibrosis

ESPGHAN CF Working Group definition for DIOS in Cystic Fibrosis	
#1	complete intestinal obstruction as evidenced by vomiting of bilious material and/or fluid levels in small intestine on an abdominal radiography
#2	faecal mass in ileo-caecum
#3	abdominal pain and/or distension
Complete DIOS: #1, #2 and #3	
Incomplete/ Impending DIOS: #2 and #3, without #1	

Table 2: ESPGHAN CF Working Group definition for Constipation in Cystic Fibrosis

ESPGHAN Working Group definition for Constipation in Cystic Fibrosis	
#1	abdominal pain and/or distension
#2a	reduced frequency of bowel movements in the last few weeks or months
#2b	increased consistency of stools in the last few weeks or months
#3	symptoms 1 and 2 are relieved by the use of laxatives
Constipation: #1 or #2a or #2b and #3	

of laxatives. If a plain abdominal radiography is performed, the faecal content of the total colon should be increased. These 2 sets of definitions also make a distinction between the fairly acute onset of symptoms as seen in complete and impending DIOS, and the more gradual onset of symptoms as usually seen in constipation (summarized in Tables 1 and 2).

Patients

At the 38th annual meeting of the ESPGHAN, questionnaires to determine frequency, risk factors, and treatment of complete DIOS were given to members of the ESPGHAN CF Working Group. Information was obtained from 8 medical centres: Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands; Hopital Necker-Enfants-Malades, Paris, France; Hopital Robert Debre, Paris, France; Universitair Ziekenhuis Brussel, Brussels, Belgium; University Hospital of Ghent, Ghent, Belgium; Poznan University of Medical Sciences, Poznan, Poland; Fondazione IRCCS Policlinico, Mangiagalli, Regina Elena, University of Milan, Milan, Italy, and the Hadassah University Hospitals, Hebrew University Jerusalem, Israel/Sheba Medical Center, Tel Aviv-University, Tel Hashomer, Israel.

The following parameters were recorded: number and characteristics (pancreatic status, meconium ileus at birth) of patients with CF with a DIOS episode that was diagnosed at an age younger than or equal to 18 years between 2001 and 2005, the treatment instituted, as well as the mean number of patients with CF younger than or equal to 18 years enrolled in each of the participating medical centres between 2001 and 2005.

Genetic Classification

Cystic fibrosis transmembrane conductance regulator (CFTR) mutations were classified into 5 classes (class I–class V) on the basis of primary mechanism of defective CFTR function. *Cystic fibrosis transmembrane conductance regulator* mutations class I, II, and III were considered severe mutations and CFTR mutations class IV and V were considered mild mutations.[7-10] With this classification, the patients were classified into 3 groups according to the probable effect of their mutations on CFTR function, regardless of clinical severity. The first group consisted of patients with a severe genotype, defined as 2 severe CFTR mutations. Within this group, DF508 homozygous patients were also analysed separately. The second group consisted of patients with a mild genotype, defined as at least 1 mild mutation. The third group consisted of patients with an undetermined genotype, defined as 1 undetermined mutation and 1 severe mutation.

Clinical Manifestations

Pancreatic insufficiency was defined as abnormal faecal fat excretion in stool samples

collected during a 72-hour period.[11] When these results were not available patients with faecal elastase-1 concentrations lower than 100mg/g of stool were considered to have pancreatic insufficiency.[12,13] Also, a history of meconium ileus at birth was recorded.

Diagnosis of CF

The diagnosis of CF had been established on the basis of characteristic clinical findings (typical pulmonary or gastrointestinal disease) in combination with an elevated sweat chloride concentration of more than 60 mmol/L (quantative pilocarpine iontophoresis) or 2 disease-causing mutations within the CFTR gene.[14,15]

Statistical Analysis

The incidence of DIOS was given in episodes per 1000 patient-years with 95% confidence limits.

Results

During a 5-year period (2001–2005), 51 episodes of complete DIOS were diagnosed in 39 patients. The overall incidence of DIOS in the 8 participating centres was 6.2 episodes per 1000 patient-years (95% confidence interval, 4.5–7.9) (Table 3). The mean age of the patients during an episode was 9.0 years (range 0.1–17.9 years). A subdivision into 6 age categories was made (Table 4) and the frequency of DIOS episodes among these categories ranged from 10% to 22%.

The CFTR genotypes in patients are shown in Table 5. The vast majority, 32 patients (82%), had a severe genotype, of which 21 patients were homozygous for DF508. One patient had a mild genotype (3%), whereas the genotype was unknown in 6 patients (15%). Among the 39 patients with DIOS, 8 (20%) experienced more than 1 episode during

Table 3: Incidence of DIOS in Cystic Fibrosis in the medical centers

Center	**Patient years**	**DIOS (episodes/ patients)**	**Incidence (episodes/1000 patient years)**
UMCU	1165	9/8	7.7
Necker-Enfants-Malades	1750	13/9	7.4
Robert Debre	900	5/5	5.6
Universitair Ziekenhuis Brussel	396	5/5	12.6
University Hospital of Ghent	750	1/1	1.3
Poznan University of Medical Sciences	450	5/3	11.1
Hadassah Medical Organization/ Sheba Medical Center	1250	5/3	4.0
University of Milan	1600	8/5	5.0
Total	**8261**	**51/39**	**6.2 (95%CI 4.5-7.9)**

Table 4: Age categories of the 51 DIOS episodes in the 8 medical centers

Age categories DIOS episodes	**Frequency of DIOS episodes**
0-3 years	10 (20%)
3-6 years	8 (16%)
6-9 years	9 (18%)
9-12 years	5 (10%)
12-15 years	11 (22%)
15-18 years	8 (16%)

the 5-year period (2001–2005); 5 patients experienced 2 episodes, 2 patients experienced 3 episodes, and 1 patient experienced 4 episodes. Thirty-six patients (92%) were pancreatic insufficient and 17 (44%) had a history of meconium ileus (Table 5).

Most patients were treated with meglumine diatrizoate (Gastrografin; Schering AG, Berlin, Germany) enema (33%), polyethylene glycol (PEG) lavage (20%), an enema with or without an oral laxative (22%), or oral laxatives (16%). Although the participating centres differed widely in the treatment of DIOS all were effective; only 2 patients (4%) needed surgery in the treatment of a DIOS episode (Table 6). One patient with pancreas insufficiency and a history of meconium ileus was treated surgically at age 2.9 years and experienced 2 more episodes of DIOS at age 3.3 and 3.6 years, whereas the other patient with pancreas insufficiency and without a history of meconium ileus was treated surgically at age 0.24 years and did not experience a relapse.

Table 5: Clinical Features associated with DIOS in Cystic Fibrosis in the 8 medical centers

	UMCU	**Enfants-Malades**	**Robert Debre**	**Brussels**	**Ghent**	**Poznan**	**Hadassah/ Sheba**	**Milan**	**Total**
DIOS Patients	8	9	5	5	1	3	3	5	39
Patients with > 1 episode of DIOS	1	4	0	0	0	1	1	1	8 (20%)
Genotype									
Severe	8	8	3	5	1	1	3	3	32 (82%)
[DF508/DF508	6	6	2	4	0	0	0	3	21 (54%)]
Mild	0	0	1	0	0	0	0	0	1 (3%)
Undetermined	0	1	1	0	0	2	0	2	6 (15%)
Clinical Manifestations									
Exocrine PI	8	8	5	5	0	2	3	5	36 (92%)
Meconium Ileus	7	1	4	2	0	1	0	2	17 (44%)

Table 6: Treatment of DIOS episodes in the 8 medical centers

Treatment	UMCU	Enfants-Malades	Robert Debre	Brussels	Ghent	Poznan	Hadassah/Sheba	Milan	Total
Conservative	2	0	0	0	0	0	0	0	2 (4%)
Oral laxatives	0	0	0	0	0	0	1	7	8 (16%)
Poly ethylene glycol lavage + oral laxatives	2	2	0	0	1	0	4	1	10 (20%)
Enema + oral laxatives	3	0	0	1	0	4	0	0	8 (16%)
Enema – oral laxatives	1	0	0	2	0	0	0	0	3 (6%)
Gastrografin enema ± oral laxatives	0	10	5	2	0	0	0	0	17 (33%)
Barium enema + oral laxatives + poly ethylene glycol lavage	1	0	0	0	0	0	0	0	1 (2%)
Surgery	0	1	0	0	0	1	0	0	2 (4%)

Discussion

The ESPGHAN CF Working Group definitions for DIOS and constipation in CF are specific, which should simplify future comparison of different aspects of these conditions. Previous definitions of DIOS sometimes included constipation, which overestimated the incidence of genuine obstruction, whereas the real incidence of constipation in CF was difficult to estimate. Therefore, a distinction between constipation and DIOS was made in the current ESPGHAN CF Working Group definitions.

In this study, the overall incidence of DIOS in pediatric patients with CF was 6.2 episodes per 1000 patient-years (95% confidence interval, 4.5–7.9). Andersen et al.[4] reported a lower incidence of DIOS (2.15 per 1000 patient-years) in patients with CF ages 0 to 20 years using a definition identical to ours. The patients in the present study, encompassing 2001 to 2005, were probably treated with a higher dose of pancreas enzyme replacement therapy than in the 1976 to 1986 period.[4] Littlewood et al.[16] indeed suggests that the current more aggressive treatment with pancreas supplements may result in less undigested food in the intestine and thus promote faecal impaction, which seems to be in agreement with our results. Nevertheless the role of pancreas enzyme replacement therapy and steatorrhea in the pathogenesis of DIOS remains controversial. Pancreatic insufficiency or poorly controlled steatorrhea is also thought to induce more sticky intestinal mucus and DIOS.[17-19] In addition, Rosenstein and Langbaum[20] and Andersen et al.[4] reported that the incidence of DIOS did not change after the introduction of the more efficient acid resistant, enteric-coated, encapsulated microspheres of pancreatic enzymes.

The present study found a higher frequency (44%) of meconium ileus at birth in patients with DIOS than the 15% to 28% found in other studies.[1,17,21] This could be because of the stricter definition of DIOS in the present multicentre study compared with the reviewed studies.[1,17,21] The relation between MI at birth and the subsequent development of DIOS is also corroborated by Blackman[22] who reported a significant correlation between these 2 entities. In 2 smaller studies a similar trend was observed, which did not reach statistical significance.[23,24] Additionally, similar pathological defects, such as slow intestinal transit[25] and impaired intestinal secretion, may contribute both to the development of intestinal obstruction in DIOS and to meconium ileus.

Treatment with Gastrografin enema, PEG lavage, oral laxatives, or an enema with or without laxatives was effective in almost all of our patients with DIOS. Interestingly, although treatment schedules differed widely between centres, the preferred treatment in each centre was effective. This seems to indicate that removal of the sticky intestinal contents from the ileocaecum can be obtained effectively through different medical methods. In general, we prefer a step-up approach starting with oral laxatives with or

without an enema: Treat the patient with PEG lavage, when this is not effective. Consider surgery if these conservative treatments are not successful.

The low frequency of mild genotype in patients with DIOS (3%) in our study is in concordance with the reported association between severe genotype and DIOS.[17,22] This may indicate that a severely impaired intestinal chloride secretion, as a result of major CFTR dysfunction, plays an important role in this condition. However, the relation between severe genotype and DIOS is not absolute, because patients with a mild genotype may still develop DIOS. Genes other than the CFTR gene, modifier genes, may also influence the severity of the gastrointestinal phenotype of CF and thus DIOS[26,27], although the CF Twin and Sibling Study in the United States[22] reported no significant differences in concordance rates between monozygotic twins and siblings, indicating that genetic factors other than CFTR genotype do not play a major role in DIOS. Nevertheless, meconium ileus was clearly influenced by modifier genes, so DIOS, which is associated, may still have a small genetic component. Clearly, further studies are necessary to investigate the role of modifier genes in the gastrointestinal phenotype in patients with CF.

In conclusion, the current definitions of DIOS and constipation in CF are specific and make a strict distinction between DIOS and constipation, which should make comparison for different aspects of these conditions between centres straightforward. Using the newly established definition for DIOS, we could already establish that the current incidence is higher than previously estimated, which could be caused by the more aggressive treatment with pancreatic enzymes used. We also found that conservative treatment was effective in almost all patients with DIOS.

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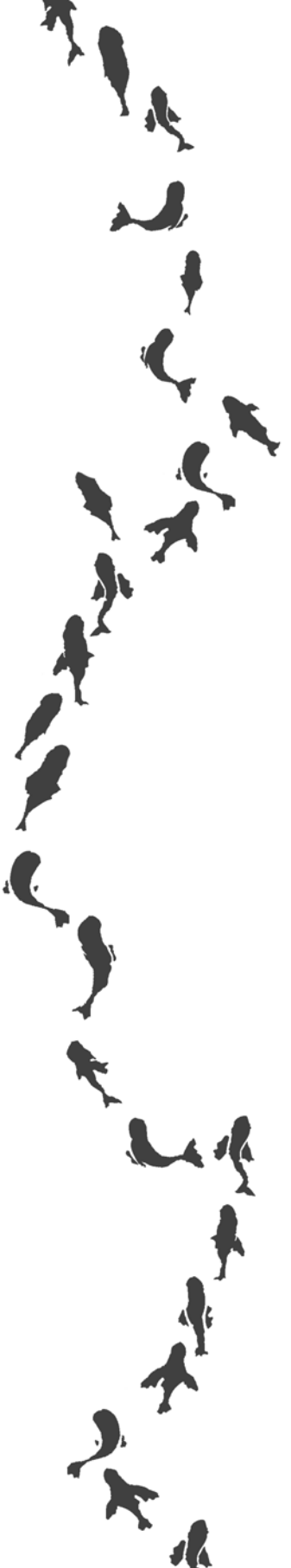

Chapter 3

Constipation in pediatric Cystic Fibrosis patients: An underestimated medical condition

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Abstract

Objectives: The aims of this study were to determine prevalence, risk factors and treatment of constipation in patients with Cystic Fibrosis (CF), as well as the diagnostic value of abdominal radiography.

Methods: A cohort of 214 pediatric CF patients was investigated. Furthermore, 106 abdominal radiographs of CF patients with or without constipation were independently assessed by three observers on two separate occasions using the Barr and Leech scores.

Results: The prevalence of constipation was 47%. Low total fat absorption and meconium ileus were independent risk factors for constipation in CF, while fiber and fluid intake were not associated. In CF patients the inter and intraobserver variabilities of the Barr and Leech scores were poor to moderate.

Conclusion: Constipation is a significant medical issue in CF and was associated with low total fat absorption and a history of meconium ileus. Finally, abdominal radiography seems of little value in the regular follow-up of CF patients.

Introduction

Constipation is one of the gastrointestinal manifestations of Cystic Fibrosis (CF). It is characterized by a reduced stool frequency and increased consistency, usually in combination with abdominal pain and distension and generally responds well to conservative medical treatment.[1,2] The frequency of this condition in CF is unclear. The only study addressing this problem so far reported a prevalence of 26% in patients aged 0–20 years.[2]

The main etiological factor for constipation in CF patients seems to be an altered intestinal fluid composition, caused by a defective expression of the Cystic Fibrosis transmembrane regulator (CFTR) protein in the gut.[3,4] In addition it is generally thought that a more aggressive treatment with pancreas supplements would result in more compact feces leading to fecal impaction[5,6], although no correlation between pancreas supplement dose and constipation was found.[7]

Key elements in the diagnosis of constipation are a careful medical history and physical examination. In addition abdominal radiography is frequently performed when constipation is suspected. In this respect several scoring systems, like the Barr[8] and the Leech[9] scores, are available to assess the severity of fecal impaction. However the diagnostic value of these scores has only been investigated in patients with functional constipation, but not in CF.

Recently the ESPGHAN CF Working Group made a strict distinction between the (sub)acute complete ileocecal obstruction, as seen in the distal intestinal obstruction syndrome (DIOS) and the gradual fecal impaction of the total colon in constipation.[1] DIOS patients are treated generally successfully with intensive laxative treatment (meglumine diatrizoate enema, polyethylene glycol lavage, oral laxatives or an enema).[1] It seems logical that in constipated CF patients generally a milder laxative regime will be used, but no such data are available at present.

The aims of this study were therefore to determine the prevalence of constipation, its risk factors and diagnostic value of abdominal radiography in patients with CF. In addition we listed laxative treatment used.

Methods

Prevalence and risk factors

A retrospective cohort study of all pediatric CF patients (age ≤18 years) under treatment at the University Medical Center Utrecht, The Netherlands on January 1st, 2006 was performed according to the guidelines of the medical ethics board of the University Medical Center Utrecht, The Netherlands.

For constipation and DIOS the recently published definitions of the ESPGHAN CF Working Group were used.[1] Constipation was defined as (1) abdominal pain and/or distension or (2a) a reduced frequency of bowel movements in the last few weeks and/or (2b) increased consistency of stools in the last few weeks, while (3) the symptoms are relieved by the use of laxatives. DIOS was defined as the combination of (1) complete intestinal obstruction, as evidenced by vomiting of bilious material and/or fluid levels in small intestine on an abdominal radiograph with (2) a fecal mass in ileocecum and (3) abdominal pain and/or distension. Incomplete or impending DIOS was defined as (1) a short history (days) of abdominal pain or distension or both and (2) a fecal mass in ileocecum, but without signs of complete obstruction.

Three-day dietary records (3 consecutive days including 1 weekend day) were completed by patient and/or family every year and analyzed by a registered dietitian (J.W.). The dietary data used for analysis were obtained 0 to 6 months before patients presented for the first time with constipation and were compared with the last available dietary data obtained in the patients without constipation or DIOS (complete or incomplete). Nutrient intake was expressed as percentage of the gender- and age-specific Reference Daily Intake (RDI).[10] Total fat absorption was calculated from the mean daily fat intake of three-day dietary records and the daily fecal fat output and was expressed as percentage of the mean daily fat intake.

For the determination of CFTR genotypes only alleles with known mutations were analyzed. Then the CFTR genotypes of the CF patients were subdivided into two groups: the first group consisted of patients with a severe genotype, defined as 2 severe CFTR mutations (class I–III) and the second group consisted of patients with a mild genotype, defined as at least 1 mild mutation (class IV–V).[11] The distribution of DF508 homozygous patients was also examined.

Abdominal radiography

Between April and December 2006 all pediatric CF patients (age ≤18 years) who visited the outpatient clinic for the annual check-up in the University Medical Center Utrecht underwent abdominal radiography regardless of the presence or absence of abdominal

symptoms or constipation. A retrospective analysis of this group was subsequently performed according to the guidelines of the medical ethics board of the University Medical Center Utrecht, The Netherlands. Three observers; a medical student (S.F.), an experienced pediatric radiologist (F.B.) and an experienced pediatric gastroenterologist (F.K.) independently assessed the abdominal radiographs taken. The three observers were blinded to the study objective and each abdominal radiograph was evaluated on two separate occasions, 3 weeks apart. Each abdominal radiograph was scored according to two different scoring systems and before the first scoring, the different systems were not discussed by the observers. The first method, described by Barr et al.[8], quantifies the amount of feces in four different bowel segments (ascending colon, transverse colon, descending colon and rectum) and is scored respectively from 0 to 2, 0 to 5, 0 to 5 and 0 to 5. Also the consistency of the feces, i.e. granular or rocky stools is scored respectively from 0 to 3 and 0 to 5. Constipation is defined as a score ≥10. The second method was described more recently by Leech et al.[9] In this system the colon is divided into three colonic segments (the right colon, the left colon and the rectosigmoid segment) and the amount of feces in each segment is scored from 0 to 5. Constipation is defined as a score ≥9.

The presence of radiological constipation in the first evaluation of all three observers according to the Barr and Leech scores were compared with the presence of constipation according to the ESPGHAN criteria[1] as gold standard and the sensitivity, specificity, positive and negative predictive values (PPV and NPV) were calculated. Furthermore, the interobserver variability of the Barr and Leech scores was calculated using the first evaluation of the three observers and the intraobserver variability was calculated using both evaluations of the observers.

Statistical analysis

Data were described as mean and standard deviation for ordinal values, and absolute and relative frequencies for nominal values. Logistic regression was used to test the effect of potential risk factors and variables contributing significantly ($p<0.05$) were included in the multivariate analysis. Unweighted kappa coefficients were calculated as indicators of inter and intraobserver variabilities for nominal variables (presence or absence of radiological constipation) and weighted kappa coefficients were calculated for ordinal variables (amount of points scored by the observers). Kappa coefficients <0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80, and 0.81–1.00 were considered to indicate poor, fair, moderate, good and very good agreement, respectively.[12] Values were considered significant if $p<0.05$. The weighted kappa coefficient was calculated using R software (Free Software Foundation Inc., Boston,

MA, USA), while all other statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Prevalence and risk factors

The study group consisted of 230 pediatric CF patients (age≤ 18 years) under treatment at the University Medical Center Utrecht, The Netherlands on January 1st, 2006. Within this study group, 107 patients (47%) had a history of constipation, while 46 patients (20%) were constipated at January 1st, 2006.

Sixteen patients with a history of DIOS (complete or incomplete) were excluded in the analysis of risk factors. The characteristics of the 107 constipation patients were compared with 107 CF patients without a history of constipation or DIOS (complete or incomplete) and are reported in Table 1.

Meconium ileus was significantly more frequent in patients with a history of constipation than in patients without constipation (13% vs. 5%, p=0.038). In the subgroup of the 19 meconium ileus patients surgical treatment for meconium ileus was more common in constipation patients than in patients without constipation (71% vs. 60%), although no statistical significance was reached (p=1.00). Also pancreatic insufficiency

Table 1: Clinical characteristics associated with constipation in Cystic Fibrosis

Characteristics	All patients	Constipation	Controls	P-value	OR (95%CI)
Patient number	214	107	107		
Gender (Male)	119 (56%)	61 (57%)	58 (54%)	0.68	1.10 (0.65-1.92)
Age diagnosis CF [2]	1.31 (2.08)	1.12 (1.64)	1.49 (2.43)	0.20	0.92 (0.80-1.05)
Current age [2]	9.96 (4.64)	10.31 (4.37)	9.60 (4.90)	0.26	1.03 (0.98-1.10)
CFTR genotype					
Severe	174 (81%)	93 (87%)	81 (76%)	0.34 [1]	1.64 (0.60-4.51) [1]
DF508/DF508	130 (61%)	73 (68%)	57 (53%)	0.25 [1]	1.83 (0.66-5.11) [1]
Mild	17 (8%)	7 (7%)	10 (9%)		
Clinical manifestations					
Pancreas insufficiency	207 (97%)	106 (99%)	101 (94%)	0.091	0.16 (0.019-1.34)
Meconium Ileus	19 (9%)	14 (13%)	5 (5%)	0.038	3.07 (1.07-8.86)
Dietary intake					
Fiber (% RDI) [2]	0.58 (0.19)	0.58 (0.23)	0.58 (0.17)	0.93	0.92 (0.14-6.27)
Fluid (% RDI) [2]	0.87 (0.21)	0.89 (0.21)	0.86 (0.21)	0.46	1.88 (0.35-10.00)
Total fat absorption [2]	0.89 (0.08)	0.86 (0.09)	0.90 (0.07)	0.012	0.003 (0.00-0.28)

[1] severe genotype or DF508 homozygous vs. mild genotype

[2] mean (SD)

was more common in the constipated group than in control CF patients (99% vs. 94%), although this difference was not significant (p=0.091). However total fat absorption was significantly lower in patients with constipation than in patients without constipation (0.86±0.09 vs. 0.90±0.07, p=0.012). All other variables (current age, age at diagnosis of CF, gender, CFTR genotype, mean fiber intake and mean fluid intake) were not significantly different between patients with or without constipation.

Logistic regression analysis showed that meconium ileus at birth (p=0.024; OR 4.69, 95%CI 1.22–18.0) and low total fat absorption (p=0.010; OR 0.002, 95%CI 0.000–0.24) were indeed both independently associated with constipation.

Abdominal radiography

Abdominal radiography was performed in the 106 CF patients who visited the outpatient clinic for the annual check-up in the University Medical Center Utrecht, The Netherlands between April and December 2006. Of the 106 patients 36 (34%) were constipated and 70 (66%) were not constipated according to the ESPGHAN criteria[1]. With this criterion as a gold standard sensitivity, specificity, PPV and NPV of radiological constipation according to the different scoring systems generally was low (Table 2). For the Barr score the observers reported sensitivities ranging from 0.14 to 0.61, specificities ranging from 0.43 to 0.96, PPV ranging from 0.35 to 0.63 and NPV ranging from 0.68 to 0.71. For the Leech score the observers report sensitivities ranging from 0.11 to 0.72, specificities ranging from 0.34 to

Table 2: Sensitivity, specificity and positive and negative predictive value of the presence of radiological constipation according to the Barr and Leech score in children with Cystic Fibrosis

	Radiologist	**Gastroenterologist**	**Medical Student**
Barr score			
Sensitivity	0.61	0.14	0.53
Specificity	0.43	0.96	0.59
Positive predictive value	0.35	0.63	0.40
Negative predictive value	0.68	0.68	0.71
Leech score			
Sensitivity	0.72	0.11	0.50
Specificity	0.34	0.93	0.63
Positive predictive value	0.36	0.44	0.41
Negative predictive value	0.71	0.67	0.71

0.93, PPV ranging from 0.36 to 0.44 and NPV ranging from 0.67 to 0.71.

Furthermore, the inter and intraobserver variabilities of the three observers according to the two different scoring systems generally were low too. The inter and intraobserver variabilities of the Barr and Leech scores for the presence of radiological constipation (Barr score <10 vs. ≥10 and Leech score <9 vs.≥9) ranged from an unweighted kappa coefficient of 0.08 (poor) to 0.44 (moderate) and the inter and intraobserver variabilities of the Barr and Leech scores for the amount of points scored ranged from a weighted kappa coefficient of 0.09 (poor) to 0.55 (moderate).

Treatment of constipation patients

In our patient group 58% had had at least 1 oral laxative (OL), 26% 2 OL, 8% 3 OL, 6% 4 OL and 2% 5 OL. Generally patients started with lactulose or polyethylene glycol. If the effect was insufficient one or two additional OLs were added. In 53 patients (50%) at least once an enema was necessary and in 14 patients (13%) intestinal lavage. Eight patients received a stimulant laxative for a short period.

Discussion

In this study we determined the prevalence, risk factors and treatment of constipation in a cohort of CF patients, as well as the diagnostic value of abdominal radiography in this condition.

One hundred and seven out of 230 patients (47%) had a history of constipation, while 46 out of 230 patients (20%) were constipated at January 1st, 2006. Prevalence numbers of constipation in CF are scarce; only one study published in 1986 has reported prevalence numbers of constipation in CF.[2] This study observed that constipation had been present in 26% of all CF patients aged 0–20 years[2], which is significantly lower than prevalence in the present study ($p < 0.001$). While it is possible that the prevalence of constipation has increased over time, it is as likely that laxatives are prescribed more easily nowadays in CF patients suspected of constipation, especially as current laxatives are almost devoid of side effects. As both in our definition for constipation and in the definition of Rubinstein et al.[2] the use of laxatives is a key component, such a change in practice might result in the increasing prevalence numbers we here describe.

In the current study we found that meconium ileus was independently associated with constipation. An association between meconium ileus and DIOS has been reported previously.[13] It seems indeed logical that both meconium ileus, DIOS and constipation in CF are an interrelated group of manifestations, ranging from severe to mild intestinal obstruction and sharing a common pathophysiology.

The relationship between pancreatic insufficiency or poorly controlled steatorrhea and constipation is unclear and conflicting results have been published. In general, it is thought that constipation is correlated with highly dosed pancreatic supplements.[5,6] However this is not supported by Baker et al.[7], who report no correlation between constipation and the dosage of pancreatic supplements. We now found that constipation patients have a lower total fat absorption than control patients, although both patient groups (with and without constipation) had an adequate control of steatorrhea with a mean total fat absorption of 86% and 90% respectively.[14] Slow intestinal transit and malabsorption may allow undigested food to enter the colon over a prolonged period.[15,16] This could lead, in combination with impaired intestinal secretion[3,4], to sticky intestinal mucus and constipation.

Finally, in concordance with a report describing Belgian CF patients[17], fiber intake was not correlated with constipation in CF. Furthermore, no differences in the fluid intake between patients with or without constipation were found, despite the general opinion that inadequate fluid intake is an etiological factor of constipation in CF.[2]

In children with CF the Barr and Leech scores have poor sensitivity, specificity,

PPV and NPV for diagnosing constipation, with a considerable overlap in Barr and Leech scores between constipated and nonconstipated patients. In addition, poor inter and intraobserver variabilities were found when scoring abdominal radiographs in CF patients. Similar results have been published in patients with functional constipation; a systematic review showed a low diagnostic value of abdominal radiography (sensitivity 60–80% and specificity 35–90%)[18], while the inter and intraobserver variabilities of the different scoring systems are poor too.[19,20] Consequently, abdominal radiography is not recommended as a standard diagnostic tool in the regular gastrointestinal follow-up of CF patients. However, abdominal radiography is useful to differentiate between constipation and the distal intestinal obstruction syndrome in CF patients with acute abdominal pain.[1]

Currently, polyethylene glycol seems to be the preferred initial treatment for both constipation and DIOS (complete and incomplete), because it is as effective and does not have the side effects that are inherent to lactulose (flatulence and abdominal cramps).[21]

In conclusion, constipation is a significant medical issue in CF patients, with a prevalence of 47%. Furthermore, we found that low total fat absorption and meconium ileus were independent risk factors for constipation in CF, while fiber and fluid intake were not associated with constipation in CF. In addition, the diagnostic value of abdominal radiography in CF is limited. Abdominal radiography is therefore not recommended in the regular follow-up of CF patients.

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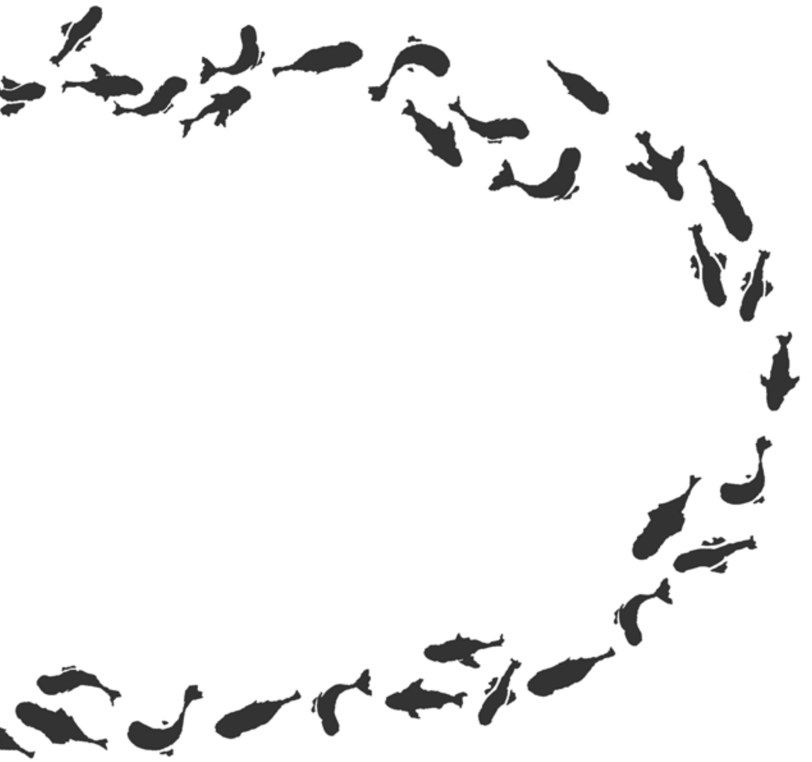

Chapter 4

Association of the CLCA1 p.S357N variant with meconium ileus in European patients with Cystic Fibrosis

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Abstract

In *Cftr*-/- mice that mostly die because of intestinal obstruction, intestinal expression of Clca3 is decreased, whereas upregulation of Clca3 results in amelioration of intestinal disease. The aim of the study was to investigate whether the p.S357N variant in *CLCA1*, the human orthologue of *Clca3*, acts as a modifier gene in a cohort of 682 European patients with cystic fibrosis (CF)–99 patients with meconium ileus. The 357SS genotype was significantly overrepresented in both patients with meconium ileus and also with a severe *CFTR* genotype (P=0.009) and in p.F508del homozygotes (P=0.002). This suggests that *CLCA1* has similar important functions in CF-related intestinal obstruction in humans as in *Cftr*-/- mice.

Introduction

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the *CF transmembrane conductance regulator gene* (*CFTR*). Of all of the patients with CF, a minority of 10% to 20% will develop meconium ileus, an intestinal obstruction in the neonatal period caused by increased viscosity of luminal secretions.[1,2] This gastrointestinal phenotype can be explained partially by variation in the *CFTR* genotype, such as homozygosity for the delta F508 deletion, the most common *CFTR* variant in patients with CF that is strongly associated with the presence of meconium ileus. Also, a modifier locus for meconium ileus (*Cfm1*) on chromosome 7 has been described in a murine CF model[3], and subsequently, several markers on human chromosome 19, the region syntenic to the mouse locus, showed significant linkage with the presence of meconium ileus in 185 CF sibling pairs.[4] However, in a genome wide analysis, including more than 1000 patients, this reported linkage between *CFM1* and meconium ileus could not be replicated.[1] Thus, a role of *CFM1* in the development of meconium ileus is likely not possible. Consequently, other genes have to be involved, because meconium ileus is clearly the result of both genetic and environmental factors.[1]

A possible candidate gene for influencing intestinal obstruction in CF is *CLCA1*, the human orthologue of murine *Clca3*. In human rectal CF epithelium, *CLCA1* is strongly expressed. Family-based analysis suggests that *CLCA1* interacts with *CFTR* because the CLCA cluster modifies the electrophysiological properties of rectal epithelium in patients with CF.[5] Furthermore, in severely affected *CFTR* knockout mice that mostly die of intestinal obstruction[6,7], the expression of Clca3 in the intestine is decreased[8,9], whereas upregulation of Clca3 in these mice results in ameliorated intestinal disease and improved survival.[9] Therefore, we investigated whether *CLCA 1* acts as a modifier gene in patients with CF and also with meconium ileus.

Methods

Patients

The European cohort consisted of pediatric and adult patients with CF treated at the University Medical Center Utrecht, the Charite University Hospital, and the University of Milan. This study was done with the approval of the medical ethical review committees of the 3 universities.

The diagnosis of CF had been established on the basis of characteristic clinical findings (typical pulmonary or gastrointestinal disease) in combination with persistently elevated sweat chloride concentrations (>60 mmol/L quantitative pilocarpine iontophoresis) and/or 2 pathologic *CFTR* alterations.[10,11]

Meconium ileus was defined as lack of passage of stool within 24 hours after birth, evidence of obstruction on abdominal radiography, and subsequent treatment for this obstruction.[1]

CFTR Genotype

The *CFTR* genotype was subdivided into 5 classes and was classified as severe (class I-III) and mild (class IV-V)[12]. Furthermore, patients homozygous for F508del were analyzed as a separate subgroup.

In the association analysis between meconium ileus and *CFTR* genotype, the frequency of meconium ileus was compared between patients with a severe genotype (class I-III) and a mild genotype (class IV-V), and between F508del homozygotes and patients with a mild genotype. Patients with an unknown *CFTR* genotype or unknown *CFTR* genotype class were excluded.

The p.S357N variant in CLCA1

The *c.1070G>A* (p.S357N) (rs2734705) variant was selected because it leads to an alteration in *CLCA 1* and is a common variant (www.ensembl.org). The p.S357N variant is not in linkage disequilibrium with other common variants.

From all of the study participants, genomic DNA was extracted from peripheral blood leukocytes. The laboratory staff was blinded for the phenotype (meconium ileus) of each DNA sample. The p.S357N variant in *CLCA 1* was determined in the European patients with CF by melting curve analysis using fluorescence resonance energy transfer probes and the LightCycler (Roche Diagnostics, Mannheim, Germany) (primers 5-TGCTCTA CATTAAGGCAgCCACT-3 and 5-CACATCTCACAGTAAATGCCG-3, and the hybridization probes 5-CCCATGTACAAAATGAACTCATAC-FL and LC610-GATAAACAGTGGCAGTGACAGGGACAC-ph) or TaqMan SNP genotyping assays (assay

identification number C__16070505_10; Applied Biosystems, Nieuwerkerk a/d Ijssel, the Netherlands) on an Applied Biosystems 7500 real-time polymerase chain reaction system.

Statistical analysis

The wild-type genotype (357SS) was used as reference genotype. Frequencies of the p.S357N variant were compared between cases and controls, and odds ratios were calculated (SS vs SN and SS vs NN). Mantel-Haenszel statistics (PEPI program version 4.0, available at http://sagebrushpress.com/PEPI.html) were used to determine P values for the pooled European analysis and to test heterogeneity between the Dutch, German, and Italian populations. Values were considered significant if $P<0.05$. Verification of Hardy-Weinberg equilibrium of genotypes was performed using the Pearson χ^2 test.

Results

Patients

The population consisted of 682 European patients with CF, of which 294, 212, and 176 were, respectively, Dutch, German, and Italian nationals. Of all European patients with CF, 99 (15%) suffered from meconium ileus, of which 42, 21, and 36 were respectively Dutch, German, and Italian nationals.

Mantel-Haenszel statistics showed no heterogeneity between the Dutch, German, and Italian populations for all of the variables, including the *CFTR* genotype and the *CLCA 1* p.S357N variant, and therefore data were pooled.

Characteristics of the European patients with CF with and without meconium ileus are described in Table 1. In the European population, age was significantly lower in patients with than inpatients without meconium ileus (P<0.001). No differences were found between meconium ileus and sex.

After excluding patients with an unknown *CFTR* genotype or genotype class, the European population consisted of 551 patients of which 79 (14%) experienced meconium ileus at birth. In the European cohort, we found an association between meconium ileus and severe *CFTR* genotype (P=0.003) and between meconium ileus and F508del homozygosity (P=0.002) (Table 1).

The p.S357N CLCA 1 variant and meconium ileus

The genetic distribution for the p.S357N variants was in Hardy-Weinberg equilibrium. Frequencies of p.S357N in patients with CF were comparable with the CEU-HapMap

Table 1: Characteristics of European cystic fibrosis patients

Characteristics	**All patients**	**Meconium ileus**	**Non meconium ileus**	**P-value**
Total patients	682	99	583	
Age (years) mean (SD)	21.2 (12.1)	16.5 (8.9)	22.0 (12.4)	<0.001
Male (%)	367 (53%)	49 (50%)	318 (54%)	0.432
CFTR Genotype				
Total patients	551	79	472	
Mild genotype	59 (11%)	1 (1%)	58 (12%)	
Severe genotype	492 (89%)	78 (99%)	414 (88%)	0.003
F508del homozygous	371 (67%)	60 (76%)	311 (66%)	0.002

SD=standard deviation

samples (www.ensembl.org). In all of the patients with CF, without stratifying for *CFTR* genotype, a trend towards significance was found between meconium ileus and the p.S357N variant (SS vs SN, P=0.076) (Table 2). Because meconium ileus was associated with *CFTR* genotype, further analysis was done after stratification for genotype (severe genotype and F508del homozygotes). Stratification for mild *CFTR* genotype was not possible because of low numbers.

In the European population, the 357SS genotype was found to be significantly overrepresented in patients with meconium ileus, both when analyzing patients with severe genotype (SS vs SN, P=0.009) and when analyzing patients with F508del homozygotes (SS vs SN, P=0.002) (Table 2).

Table 2: Genotype distributions of S357N in patients with/without meconium ileus

CFTR Genotype subgroup	**Total Patients (meconium ileus)**	**S357N**	**Meconium ileus (%)**	**Non-meconium ileus (%)**	**P-value**	**OR (95%CI)**
All CFTR genotypes	682 (99)	SS	75 (75.8)	408 (70.0)	-	Reference
		SN	18 (18.2)	160 (27.4)	0.076	0.61 (0.36-1.06)
		NN	6 (6.1)	15 (2.6)	0.108	2.25 (0.83-6.14)
Severe genotype	492 (78)	SS	62 (79.5)	288 (69.6)	-	Reference
		SN	10 (12.8)	117 (28.3)	0.009	0.40 (0.20-0.81)
		NN	6 (7.7)	9 (2.2)	0.029	3.31 (1.08-10.12)
F508del homozygous	371 (60)	SS	51 (85.0)	217 (69.8)	-	Reference
		SN	5 (8.3)	89 (28.6)	0.002	0.25 (0.10-0.64)
		NN	4 (6.7)	5 (1.6)	0.053	3.61 (0.91-14.38)

Discussion

We found an association between meconium ileus in European patients with CF and the *c.1070G>A* (p.S357N) *CLCA 1* variant. The wild-type genotype, 357SS, was found to be significantly overrepresented in patients with meconium ileus and also with a severe genotype or F508del homozygosity. So far, this study represents the first report of an association between a *CLCA 1* variant and meconium ileus in CF.

In European patients with CF, meconium ileus was strongly associated with a severe *CFTR* genotype and F508del homozygosity that is in concordance with a large twin and sibling study.[1] Because of the strong effect of the *CFTR* genotype on the development of meconium ileus, we stratified the patients according to the *CFTR* genotype. The association between the *CLCA 1* p.S357N variant and meconium ileus was significant in the subgroup with a severe genotype and in the p.F508del homozygote patients, whereas in the total group of patients with CF only a trend towards significance was observed, indicating that meconium ileus is a consequence of interaction between the *CFTR* genotype and other genes such as *CLCA 1*. This is consistent with the evidence that *CFTR* and modifier genes interact in influencing the risk of developing meconium ileus.[1]

Recently, the CF twin and sibling study in the United States identified regions of suggestive linkage for modifier genes that cause meconium ileus at chromosome 4, 8, and 11 or protect from meconium ileus at chromosomes 20 and 21 by genome wide analysis.[1] However, *CLCA 1* that is located at chromosome 1 is not positioned in one of these regions, suggesting the involvement of additional loci in the development of meconium ileus.

It was initially suggested that CLCA1 and Clca3 were Ca^{2+}- activated Cl^- channels.[13] Subsequent studies indicated that CLCA1 and Clca3 are, rather, soluble secreted globular proteins[14], likely involved in protein–protein interactions and extracellular signalling.[14,15] Although the exact function of Clca3 is unknown, it is obvious that Clca3 plays a major role in intestinal obstruction in *Cftr* knockout mice. In congenic C57BL/6 *Cftr*-/- mice that usually die of severe intestinal obstruction[6,7], the intestinal expression of Clca3 is decreased[8,9], whereas correction for this Clca3 deficiency results in amelioration of intestinal pathology and survival.[9]

The results of the *Cftr*-/- mice studies show that Clca3 has an important function in the development of intestinal obstruction in mice.[8,9] Furthermore, through genetic and expression studies it was concluded that CLCA1, the human orthologue of murine Clca3, could be involved in the human rectal CFTR independent residual chloride secretion.[5] Our study showed that the p.S357N variant in *CLCA 1* is associated with meconium ileus

in humans. This change in amino acid residue at position 357 may affect physicochemical properties of the CLCA1 protein and consequently its function and/ or expression, suggesting that CLCA1 may contribute in a similar manner to intestinal obstruction in patients with CF with meconium ileus as in *Cftr* knockout mice.

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Part 2

Medical interventions in Cystic Fibrosis: longitudinal effect on growth, bacterial colonization and pulmonary function

Chapter 5

Gastric acid inhibition for fat malabsorption or gastroesophageal reflux disease in cystic fibrosis: longitudinal effect on bacterial colonization and pulmonary function

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Abstract

Objectives: To investigate bacterial colonization and pulmonary function longitudinally in patients with cystic fibrosis (CF) receiving drugs for gastric acid (GA) inhibition for fat malabsorption or for gastroesophageal reflux disease (GERD).

Methods: A retrospective cohort study of 218 pediatric patients with CF was performed. Multilevel modeling was used to perform longitudinal analysis of forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), maximum expiratory flow at 50% of FVC (MEF_{50}), and maximal mid-expiratory flow between 25% and 75% of FVC ($MMEF_{25-75}$). Cox regression was used to calculate *Pseudomonas aeruginosa*- and *Staphylococcus aureus*-free survival.

Results: Patients with CF and GA inhibition had a significantly smaller yearly decline of MEF_{50} and $MMEF_{25-75}$ compared with control subjects. Other pulmonary function parameters and *P aeruginosa* or *S aureus* acquisition or colonization were not different from that of control subjects. GERD was associated with a significantly reduced pulmonary function (FEV_1 and FVC) and an earlier acquisition of *P aeruginosa* and *S aureus*.

Conclusions: GA inhibition did not affect pulmonary function or bacterial acquisition and therefore is not contraindicated in patients with CF. GA inhibition might improve pulmonary function with time, because the decline of MEF_{50} and $MMEF_{25-75}$ was less pronounced. GERD was associated with a reduced pulmonary function and an earlier acquisition of *P aeruginosa* and *S aureus*. Therefore the diagnosis and treatment of GERD should be aggressively pursued in patients with CF.

Introduction

Most patients with cystic fibrosis (CF) have exocrine pancreatic insufficiency, which is treated with pancreas enzyme replacement therapy. Gastric acid (GA) inhibition via proton pump inhibitors or histamine-2 receptor antagonists is added when fat absorption remains insufficient despite an adequate dosage of pancreas enzyme replacement.[1,2] Gastroesophageal reflux disease (GERD) is another reason to start drugs for GA inhibition in patients with CF.

In various populations without CF, GA inhibition has been shown to be associated with an increased risk for pulmonary infections (eg, intensive care patients treated with histamine-2 antagonists for the prevention of stress ulcers have an increased risk of pneumonia[3] and more recently, an increased risk for community-acquired pneumonia has been reported in both pediatric patients with GERD and adult patients receiving GA inhibition for various reasons).[4,5]

The effect of GA inhibition on pulmonary function in patients with CF is unclear; cross-sectional analysis showed that GA inhibition for GERD in patients with CF is associated with a reduced forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) [6,7], and FEV_1, FVC, and maximum expiratory flow at 50% of FVC (MEF_{50}) did not change during 1 year of GA inhibition treatment in a small study of patients with CF with fat malabsorption.[8]

Therefore the aim of this study was to longitudinally investigate pulmonary function and bacterial colonization in a large cohort of pediatric patients with CF who were receiving drugs for GA inhibition for either fat malabsorption or GERD.

Methods

A retrospective cohort study of all pediatric patients with CF (age ≤18 years) treated in the CF Center of the University Medical Center Utrecht on January 1, 2007, was performed according to the guidelines of the medical ethics board of the University Medical Center Utrecht.

Patients were classified in 3 groups on the basis of the history of GA inhibition (proton pump inhibitors or histamine- 2 receptor antagonists). The first group consisted of patients receiving GA inhibition because of insufficient effect of pancreas enzyme replacement therapy (fat absorption coefficient <85%, with impaired growth or weight gain). The second group used GA inhibition for GERD, defined as abnormal results of 24-hour esophageal pH monitoring or esophagitis on esophageal histology.[9] The performance of esophageal pH monitoring or esophageal endoscopy was indicated on the basis of clinical signs of gastroesophageal reflux. The third group had no history of GA inhibition (control subjects). Patients for whom the indication for GA inhibition could not be retrieved were excluded. In the subsequent analysis, these clinical characteristics were analyzed: sex, current age, age at diagnosis of CF, *cystic fibrosis transmembrane regulator* (*CFTR*) genotype severity, lower airway culture results (especially *Pseudomonas aeruginosa* [PA] or *Staphylococcus aureus* [SA]), presence of meconium ileus at birth, distal intestinal obstruction syndrome, and liver cirrhosis.

All pulmonary function tests and body mass index (BMI) z-scores from the first visit at the CF center were retrieved. Patients with at least 2 pulmonary function measurements were included in the longitudinal analysis. Pulmonary function assessments and BMI z-scores of patients with GERD or patients with fat malabsorption during GA inhibition treatment were compared with the pulmonary function tests of patients without a history of GA inhibition.

Pulmonary function (FEV_1, FVC, MEF_{50}, $MMEF_{25-75}$) was measured with spirometry and converted to percentage of predicted values.[10] Patients with at least 1 PA- and SA- positive culture test (ever) were identified, and the date of the first positive culture test was determined. Patients were considered to be colonized with PA or SA when the monthly culture test results (sputum or cough swab) in a 3-year period were ≥50% positive.[11] Patients with <2 culture tests were excluded. Patients with 2 severe *CFTR* mutations (class I-III) were considered to have a severe *CFTR* genotype.[12] Distal intestinal obstruction syndrome was defined according the ESPGHAN CF Working Group criteria.[13] Patients with a nodular liver margin on ultrasound scanning were classified as having cirrhosis.

Statistical analysis

Categorical variables were analyzed with the Pearson χ^2 or Fisher exact test, and continuous variables were analyzed with the Mann-Whitney U test. Logistic regression was used to determine risk factors for GERD or fat malabsorption requiring GA inhibition. Cox regression analysis was used to analyze the effect of the treatment groups on PA- and SA-free survival.

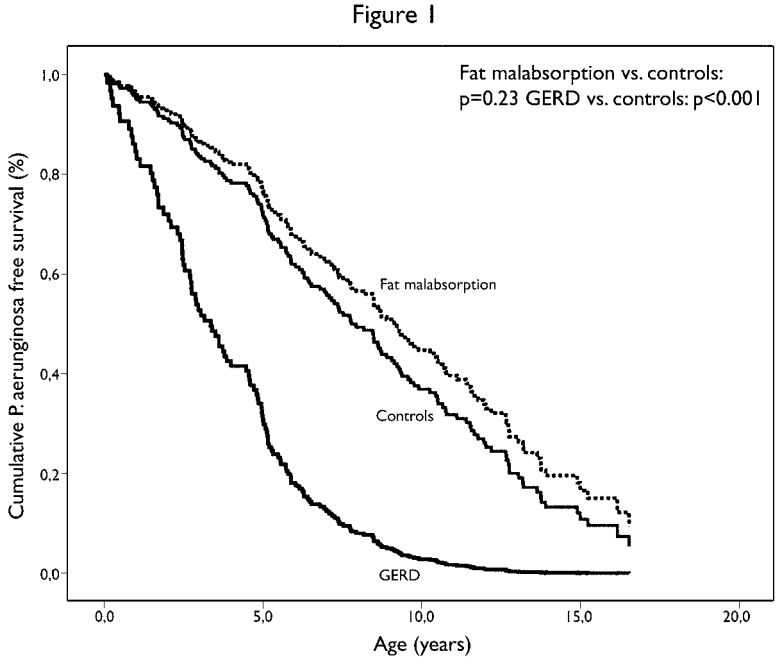
Multilevel linear regression modeling was used to perform longitudinal analysis of pulmonary function and nutritional status (BMI z-score), because it allows inclusion of variable numbers of measurements per child and adjusts for irregularly timed and missing observations.[14-16] In this model, yearly decline of pulmonary function and nutritional status and intercept at 10 years of age were compared in the 3 treatment groups, and adjustments for confounders (sex, age, severe *CFTR* genotype, PA and SA colonization, BMI z-score for pulmonary function, FEV_1 for nutritional status) were made. Also, when necessary, adjustments were made for the remaining variables (age at diagnosis of CF, meconium ileus, distal intestinal obstruction syndrome, cirrhosis).[15,16] For the longitudinal analysis of pulmonary function intercept, age and BMI z-score were specified as random effects, and for the longitudinal analysis of nutritional status intercept, age and FEV_1 were specified as random effects to allow for individual differences.

Results

The study group consisted of 228 pediatric patients with CF. Seventy-nine patients (35%) received drugs for GA inhibition for fat malabsorption, 12 patients (5%) received drugs for GERD, and 127 patients had no history of GA inhibition (56%). Ten patients (4%), for whom the indication for GA inhibition could not be retrieved, were excluded from the cohort analysis.

Patients receiving drugs for GA inhibition for fat malabsorption were younger at the time of diagnosis of CF (P = .001) and had a higher frequency of a severe *CFTR* genotype (P = .011) than control subjects. Furthermore, there was a trend for a higher prevalence of liver cirrhosis in patients who had GA inhibition for fat malabsorption (P = .06). Other variables (sex, current age, meconium ileus, distal intestinal obstruction syndrome) were not significantly different (Table 1). In the logistic regression analysis, only age at diagnosis of CF was found to be significantly different (P = .036; odds ratio [OR], 1.3; 95% CI, 1.0-1.5).

Figure 1. Cumulative free survival of the first infection with PA between patients receiving drugs for GA inhibition for fat malabsorption or GERD and patients without a history of GA inhibition (control subjects)

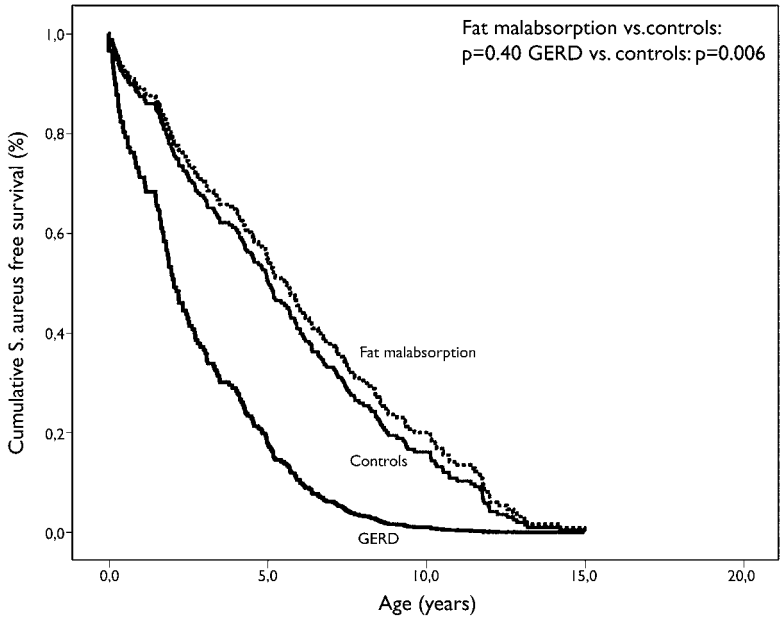


Patients with GERD receiving GA inhibition were younger (P = .036) and the frequency of meconium ileus was higher (P = .009) compared with control subjects. Also, a trend was observed for age at diagnosis of CF (P = .07). Other variables (sex, *CFTR* genotype, distal intestinal obstruction syndrome, cirrhosis) were not significantly different between patients with GERD and control subjects (Table 1). With logistic regression, meconium ileus was shown to be the only variable independently over-represented in patients with GA inhibition for GERD (P = .012; OR, 5.4; 95% CI, 1.4-20.1).

For 210 of the 218 patients in the cohort analysis, ≥2 lower airway culture test results were available. The percentage of patients with positive culture results for PA and SA did not differ from control subjects in patients receiving GA inhibition for fat malabsorption or for GERD. This was found for both at least 1 PA and SA infection and for colonization with PA and SA (Table 1). With Cox regression analysis, the age of first PA and SA infection and age of PA and SA colonization was shown not to differ from control subjects in patients with GA inhibition for fat malabsorption (first infection PA and SA;

Figure 2. Cumulative free survival of the first infection with SA between patients receiving drugs for GA inhibition for fat malabsorption or GERD and patients without a history of GA inhibition (control subjects)

Figure 2



Figures 1 and 2; colonization PA, P = .87 and SA, P = .25). However, patients with GERD had an earlier onset of first acquisition with PA and SA than control subjects (PA: P < .001; hazard ratio, 3.6; 95% CI, 1.8-7.0; and SA: P = .006; hazard ratio, 2.5; 95% CI, 1.3-4.9; Figures 1 and 2). There were no differences in the age of PA and SA colonization (PA: P = .44; SA: P = .11).

Of the 218 patients, 181 had at least 2 measurements of FEV_1, FVC, and BMI z-scores, and for 179 of the 218 patients at least 2 measurements of MEF_{50} and $MMEF_{25-75}$ were available (Table 2). Pulmonary function parameters (FEV_1, FVC, MEF_{50}, $MMEF_{25-75}$) and BMI z-score at 10 years of age did not differ between patients with fat malabsorption requiring GA inhibition and control subjects. However, yearly decline of MEF_{50} (P = .032) and $MMEF_{25-75}$ (P = .027) was significantly improved in patients with fat malabsorption compared with control subjects, and yearly decline of FEV_1, FVC, and BMI z-score showed no significant difference. However, at 10 years of age, patients with GERD receiving GA inhibition had a significant reduced FEV_1 (P = .025) and FVC (P = .007) compared with control subjects, but MEF_{50} and $MMEF_{25-75}$, and BMI z-scores at the age of 10 years did not differ significantly, nor did the yearly decline of pulmonary function parameters (FEV_1, FVC, MEF_{50}, $MMEF_{25-75}$) and BMI z-score. All longitudinal analyses of pulmonary function were adjusted for sex, age, BMI z-score, severe *CFTR* genotype, PA and SA colonization, and all longitudinal analyses of nutritional status were adjusted for sex, age, severe *CFTR* genotype, PA and SA colonization, and FEV_1.

Table 1: Baseline characteristics of patients receiving gastric acid inhibition for fat malabsorption, gastroesophageal reflux disease (GERD) and patients without a history of gastric acid inhibition (controls)

Characteristics	**Fat malabsorption**	**GERD**	**Controls**	**P-value[1]**	**OR (95%CI)**	**P-value[2]**	**OR (95%CI)**
Number	79	12	127				
Gender (male)	46 (58%)	6 (50%)	67 (53%)	0.44	1.2 (0.7; 2.2)	0.86	0.90 (0.27; 2.9)
Current age (years)[3]	10.0 (4.4)	7.1 (4.9)	10.2 (4.6)	0.64	0.99 (0.93; 1.1)	***0.036***	0.87 (0.76; 0.99)
Age at diagnosis of CF (years)[3]	0.7 (1.4)	0.7 (1.4)	1.5 (2.2)	***0.001***	0.76 (0.62; 0.93)	0.07	0.72 (0.42; 1.2)
Severe *CFTR* genotype	71 (90%)	11 (92%)	96 (76%)	***0.011***	2.8 (1.2; 6.6)	0.30	3.6 (0.44; 28.6)
Meconium ileus	11 (14%)	5 (42%)	13 (10%)	0.42	1.4 (0.60; 3.3)	***0.009***	6.3 (1.7; 22.6)
Distal intestinal obstruction syndrome	8 (10%)	1 (8%)	6 (5%)	0.13	2.3 (0.76; 6.8)	0.48	1.8 (0.20; 16.6)
Cirrhosis	12 (15%)	1 (8%)	9 (7%)	0.06	2.3 (0.94; 5.9)	1.00	1.2 (0.14; 10.3)
Ever *P. aeruginosa* positive	50 (65%)	10 (83%)	91 (75%)	0.12	0.61 (0.33; 1.1)	0.73	1.6 (0.34; 7.9)
P. aeruginosa colonization	18 (23%)	3 (25%)	32 (26%)	0.63	0.85 (0.44; 1.6)	1.00	0.93 (0.24; 3.6)
Ever *S. aureus* positive	72 (94%)	10 (83%)	115 (95%)	0.75	0.75 (0.22; 2.6)	0.15	0.26 (0.05; 1.5)
S. aureus colonization	37 (48%)	5 (42%)	65 (54%)	0.44	0.80 (0.45; 1.4)	0.43	0.62 (0.19; 2.0)

[1]Fat malabsorption vs. controls; [2]GERD vs. controls; [3]mean (SD)

Table 2: Longitudinal analysis of pulmonary function and nutritional status in patients receiving gastric acid inhibition for fat malabsorption, gastro-esophageal reflux disease (GERD) and patients without a history of gastric acid inhibition (controls) and adjusted for confounders.

Variables	**Groups**	**Number of patients (number of tests)**	**Variables at age 10 (95% CI)**	**P-value[1]**	**Yearly decline (95% CI)**	**P-value[1]**
FEV_1	Fat malabsorption	61 (570)	92.9 (86.0; 99.8)	0.65	-1.3 (-2.2; -0.5)	0.10
	GERD	8 (86)	76.2 (61.7; 90.7)	***0.025***	-1.0 (-3.4; 1.5)	0.34
	Controls	112 (1996)	91.7 (86.1; 97.3)	-	-2.2 (-2.7; -1.6)	-
FVC	Fat malabsorption	61 (570)	96.7 (90.8; 102.5)	0.43	-0.3 (-1.1; 0.5)	0.87
	GERD	8 (86)	82.5 (70.3; 94.8)	***0.007***	0.4 (-1.8; 2.7)	0.48
	Controls	112 (1989)	98.4 (93.6; 103.1)	-	-0.4 (-0.9; 0.1)	-
MEF_{50}	Fat malabsorption	61 (556)	75.5 (64.0; 87.2)	0.11	-1.1 (-2.6; 0.4)	***0.032***
	GERD	7 (83)	62.8 (38.7; 86.8)	0.61	-1.8 (-6.1; 2.5)	0.58
	Controls	111 (1869)	68.5 (59.0; 78.1)	-	-3.0 (-4.0; -2.0)	-
$MMEF_{25-75}$	Fat malabsorption	61 (531)	67.1 (54.1; 80.0)	0.11	-2.1 (-3.8; 0.4)	***0.027***
	GERD	7 (75)	54.2 (27.7; 80.7)	0.68	-1.2 (-6.4; 3.9)	0.24
	Controls	111 (1588)	59.4 (48.7; 70.0)	-	-4.4 (-5.5; -3.2)	-
BMI z-score	Fat malabsorption	61 (570)	-0.92 (-1.30; -0.53)	0.84	0.00 (-0.04; 0.04)	0.74
	GERD	8 (86)	-0.99 (-1.70; -0.28)	0.77	0.06 (-0.06; 0.17)	0.27
	Controls	112 (1996)	-0.89 (-1.22; -0.56)	-	-0.01 (-0.04; 0.01)	-

[1]Fat malabsorption or GERD vs. controls

Discussion

We report the longitudinal assessment of bacterial colonization and pulmonary function (FEV_1, FVC, MEF_{50}, $MMEF_{25-75}$) in patients with CF receiving drugs for GA inhibition for fat malabsorption or GERD.

In our pediatric population with CF, GA inhibition for fat malabsorption did not affect pulmonary function at 10 years of age nor did culture results: both pulmonary function at 10 years of age and PA and SA acquisition or colonization did not differ between patients and control subjects. This confirms and extends earlier findings showing that FEV_1, FVC, and MEF_{50} were not different after 1 year of GA inhibition in 14 patients with CF with fat malabsorption.[10] Although in our study the decline of pulmonary function for FEV_1 and FVC was identical between patients and control subjects, the MEF_{50} and $MMEF_{25-75}$ showed significantly less decline with time than in control subjects, indicating a possible favorable effect on peripheral airway obstruction. Consequently, GA inhibition is not contraindicated in patients with CF with fat malabsorption and might have an advantageous effect on some aspects of the decline in pulmonary function.

We have shown that patients with CF receiving GA inhibition for GERD have reduced pulmonary function at the age of 10 years, which supports an earlier cross-sectional cohort study.[6] The differences in FEV_1 and FVC, reflecting airway obstruction and hyperinflation, probably are the effect of GERD itself and not the GA inhibition, because this medication did not affect pulmonary function in patients with fat malabsorption. Before initiation of treatment in patients with CF and GERD, GA aspiration might increase bacterial adherence of PA in airway epithelium, as shown in mice studies.[17,18] In our CF population, patients with GERD had earlier first acquisition of PA and SA. However, onset of colonization was not different between patients with GERD and control subjects, which probably is the consequence of early aggressive antibiotic intervention in patients with a first PA or SA infection at our medical center. Only a few patients became colonized with these microorganisms during the study period. Changes in pulmonary function with time did not differ between patients with CF and GERD and control subjects, indicating that GA inhibition is sufficient to prevent additional pulmonary function decline. However, the pronounced reduced pulmonary function at 10 years of age in patients with GERD suggests that an earlier diagnosis and treatment of GERD might be necessary to prevent the initial pulmonary damage.

No difference between patients with severe fat malabsorption requiring GA inhibition and control subjects was shown with longitudinal analysis of nutritional status, suggesting that the combination of pancreas enzyme replacement and GA inhibition is adequate in the treatment of severe fat malabsorption, at least in our CF population.

In this study, GERD was independently associated with meconium ileus. This is supported by a study reporting a similar trend toward an association between meconium ileus and reflux episodes.[19] Furthermore, gastrointestinal motility disorders are frequently reported in CF: CF patients with symptoms suggesting GERD have esophageal motility defects[20] and in the general CF population, prolonged intestinal transit time is described.[21,22] Although intestinal transit time in meconium ileus patients is unknown, the association between GERD and meconium ileus suggests common defects in gastrointestinal motility.[21,22]

In conclusion, GA inhibition for fat malabsorption is not associated with a compromised pulmonary function at 10 years of age, as compared with control subjects, nor is it associated with PA and SA colonization. Consequently it is not contraindicated in patients with CF with fat malabsorption. In addition, GA inhibition for fat malabsorption might even be favorable because the yearly decline of MEF_{50} and $MMEF_{25-75}$ was significantly less in our patients compared with that in control subjects. GA inhibition for GERD in patients with CF is associated with a reduced pulmonary function at 10 years of age and an earlier acquisition of both PA and SA. Most likely this is caused by GERD, not by GA inhibition. Therefore diagnosis and treatment of GERD should be aggressively pursued in patients with CF.

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Chapter 6

Ursodeoxycholic acid in cystic fibrosis: longitudinal effects on fat absorption, growth, bacterial colonization and pulmonary function

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Abstract

Objectives: To investigate longitudinally the effects of ursodeoxycholic acid (UDCA) on fat absorption, growth, bacterial colonization and pulmonary function in a large cohort of pediatric cystic fibrosis (CF) patients.

Methods: Retrospectively a cohort of 207 pediatric CF patients was studied. Multilevel modeling was used to analyze the longitudinal effect of UDCA on the coefficient of fat absorption (CFA), weight, height and body mass index (BMI) z-scores, forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC). Differences in *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) free survival in patients with or without UDCA were analyzed.

Results: CF patients receiving UDCA had a significantly reduced CFA, weight, height and BMI z-score at 10 years of age compared to patients without UDCA. Furthermore, UDCA patients had a worse yearly change of weight z-score, height z-score, FEV_1 and FVC, although they had a later acquisition of PA and SA.

Conclusion: Our data suggest serious side effects of UDCA in pediatric CF patients, since UDCA was negatively associated with fat absorption, growth and pulmonary function. Considering the questionable positive effects of UDCA, this generates an urgent need for well performed randomized clinical trails with UDCA in CF patients.

Introduction

Cystic Fibrosis (CF) is a multi-systemic and common autosomal recessive genetic disease caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene. A large majority of patients has exocrine pancreatic insufficiency, which is treated with pancreatic enzyme replacement therapy (PERT), if necessary in combination with gastric acid (GA) inhibition.[1] Lung disease is common and responsible for most morbidity and mortality in these patients. However with the improved life expectancy nowadays other manifestations of CF are becoming clinically more relevant.[2] In particular liver disease is the single most important non-pulmonary cause of death, as it accounts for about 2.5% of overall mortality in CF patients.[3] CF related liver disease originates by inspissated bile, which leads to bile duct obstruction and progressive periportal fibrosis, eventually causing liver cirrhosis in 10% of all CF patients.[4] Ursodeoxycholic acid (UDCA) therapy is commonly used for CF related liver disease as it supposedly improves bile viscosity by changing bile acid composition.[5]

However, UDCA has additional pharmacological properties such as an impaired capability to form mixed micelles which may negatively influence fat absorption and growth in CF patients.[6] Nevertheless, a small observational study reported an improvement of weight and body mass index (BMI) in CF patients receiving UDCA.[7] In addition UDCA may influence pulmonary function and bacterial colonization, given its anti-inflammatory and immuno-modulatory properties.[8] A small retrospective study indeed reported that meconium ileus patients who receive early UDCA showed a significant lower of *Pseudomonas aeruginosa* (PA) colonization rate than patients who receive UDCA at the onset of CF related liver disease.[9]

Therefore the aim of this study was to longitudinally investigate the effects of UDCA on fat absorption, growth, bacterial colonization and pulmonary function in a large cohort of pediatric CF patients.

Methods

Study population

A longitudinal, retrospective study was performed of all pediatric CF patients under treatment at the University Medical Center, Utrecht, the Netherlands on January 1st, 2007 according to the guidelines of the medical ethics board of the University Medical Center Utrecht.

Using clinical files for all CF patients a history of chronic UDCA use was determined. The indication to start UDCA was CF related liver disease (ranging from liver enzyme abnormalities to liver cirrhosis). Additionally, the following clinical characteristics were retrieved: gender, age, age at diagnosis of CF, *CFTR* genotype (severe vs. mild/unknown)[10], first positive culture or colonization with PA or *Staphylococcus aureus* (SA)[11], GA inhibition use (proton pump inhibitors and/or histamine-2-receptor antagonists) for gastroesophageal reflux disease (GERD) or fat malabsorption[10], presence of meconium ileus (MI) at birth, distal intestinal obstruction syndrome (DIOS)[12] and liver cirrhosis[10]. Baseline characteristics of patients receiving UDCA and patients without a history of UDCA use were compared. Patients receiving GA inhibition because of fat malabsorption or GERD were compared with patients without a history of GA inhibition.[10] Patients, in whom the indication for GA inhibition could not be retrieved were excluded. Also patients with < 2 lower airway cultures were excluded.

Cumulative free survival of first infection and colonization of Pseudomonas aeruginosa and Staphylococcus aureus

Secondly, cumulative PA and SA free survival (first infection or colonization) was compared between patients with or without UDCA use. Patients with at least 1 PA and SA positive culture (ever) were identified and the date of the first positive culture was determined. Patients were considered to be colonized with PA or SA when the monthly cultures (sputum or cough swab) in a three year period were ≥ 50% positive.[11]

Longitudinal analysis of fat absorption, growth and pulmonary function

Additionally, for each visit PERT dose (lipase units/kilogram/day), coefficient of fat absorption (CFA), growth variables (z- score weight, height and body mass index (BMI)) and pulmonary function tests (forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC)) were retrieved. To determine the longitudinal effect of UDCA on fat absorption, patients with at least two reliable measurements of PERT dose and CFA were included. To determine the longitudinal effect of UDCA on growth and pulmonary function, patients with at least two reliable measurements of both BMI z-score and pulmonary

function were included.

The CFA, expressed as percentage of fat intake, was calculated from fat intake, determined in three-day dietary intake records by a registered dietician (JW), and faecal fat excretion, determined in three-day stool collections. Pulmonary function was measured by spirometry and converted to percentage of predicted values.[13] Height was measured with a stadiometer to the nearest 0.5 centimeter (Holtain, Crymisch, UK) and weight was measured using a digital weight scale to the nearest 0.1 kilogram. Weight, height and BMI z-scores were calculated using the growth analyzer 3.0 software of the Dutch Growth Foundation (available at http://www.growthanalyser.org).

Statistical analysis

Categorical variables were analyzed using Pearson's chi-square or Fisher's exact test and continuous variables were analyzed with the Mann-Whitney U test. Cox regression analysis was used to analyze the effect of UDCA on PA and SA free survival and if necessary adjustments were made for the other variables.

Multilevel linear regression modeling (Linear Mixed Models, SPSS Inc., Chicago, IL, USA, version 15.0 for Windows) was used for the longitudinal analysis, which is appropriate for analyzing longitudinal data, since it allows for variable numbers of measurements per patient and adjusts for irregularly timed and missing data.[14] In this model yearly change of CFA, growth or pulmonary function and intercept at 10 years of age were compared between patients with or without UDCA, while adjustments for confounders (age, *CFTR* genotype, PERT dose, GA inhibition for fat malabsorption or GERD, liver cirrhosis, MI and DIOS for CFA[1,15-19]; gender, age, *CFTR* genotype, PA and SA colonization, FEV_1 for growth variables[10]; gender, age, *CFTR* genotype, PA and SA colonization, GA inhibition for fat malabsorption or GERD, BMI z-score for pulmonary function[10]) were made. The remaining variables were investigated and adjustments were made if a statistically significant contribution was present. The following variables were specified as random effects to allow individual differences: intercept, age and PERT dose for CFA, intercept, age and FEV_1 for growth variables and intercept, age and BMI z-score for pulmonary function.[10] Values were considered statistically significant if the p-value was <0.05.

Results

Study population

The study group consisted of 228 pediatric CF patients of whom 207 (91%) met the inclusion criteria for the cohort analysis. The baseline data of patients with or without UDCA use are shown in table 1. A history of UDCA use was significantly associated with male gender, a higher age and GA inhibition to improve fat malabsorption. Fourteen of the 27 patients (52%) started GA inhibition to improve fat malabsorption after UDCA treatment was initiated. As expected all liver cirrhosis patients received UDCA. Other variables were not significantly different between patients with or without UDCA.

Cumulative free survival of first infection and colonization of Pseudomonas aeruginosa and Staphylococcus aureus

Cox regression analysis showed that patients with UDCA had a later onset of first acquisition with PA and SA than patients without UDCA (PA: p<0.001; hazard ratio 0.34, 95% CI 0.20-0.59 and SA: p=0.014; hazard ratio 0.55, 95%CI 0.34-0.89; figure 1 and 2). Furthermore, UDCA patients had a later SA colonization age than patients without UDCA (p=0.003; hazard ratio 0.49, 95%CI 0.31-0.78; supplementary file). However, PA colonization age did not differ between the two groups (p=0.17, supplementary file).

Table 1: Baseline characteristics of patients receiving ursodeoxycholic acid (UDCA) and patients without a history of ursodeoxycholic acid (controls)

Characteristics	**UDCA**	**Controls**	**P-value**
Number	54 (26%)	153 (74%)	
Gender (male)	37 (69%)	75 (49%)	***0.013***
Current age (years)[1]	12.2 (4.0)	9.4 (4.5)	***<0.001***
Age at diagnosis of CF (years)[1]	1.2 (2.2)	1.2 (1.9)	0.89
Severe *CFTR* genotype	48 (89%)	120 (78%)	0.091
GA inhibition for fat malabsorption	27 (52%)	48 (31%)	***0.013*** [2]
GA inhibition for GERD	3 (6%)	9 (6%)	0.71 [2]
Meconium ileus	10 (19%)	14 (9%)	0.065
Distal intestinal obstruction syndrome	5 (9%)	5 (3%)	0.13
Liver Cirrhosis	21 (39%)	0 (0%)	***<0.001***
Ever PA positive	39 (72%)	109 (71%)	0.89
PA colonization	14 (26%)	38 (25%)	0.87
Ever SA positive	52 (96%)	143 (94%)	0.74
SA colonization	32 (59%)	74 (48%)	0.17

[1]mean (SD),[2] GA inhibition for GERD or fat malabsorption vs. controls (no history of GA inhibition)

Figure 1: Cumulative free survival of the first infection with P. aeruginosa between patients with and without ursodeoxycholic acid use (adjusted for age at diagnosis of cystic fibrosis, gastric acid inhibition for gastroesophageal reflux disease and liver cirrhosis)

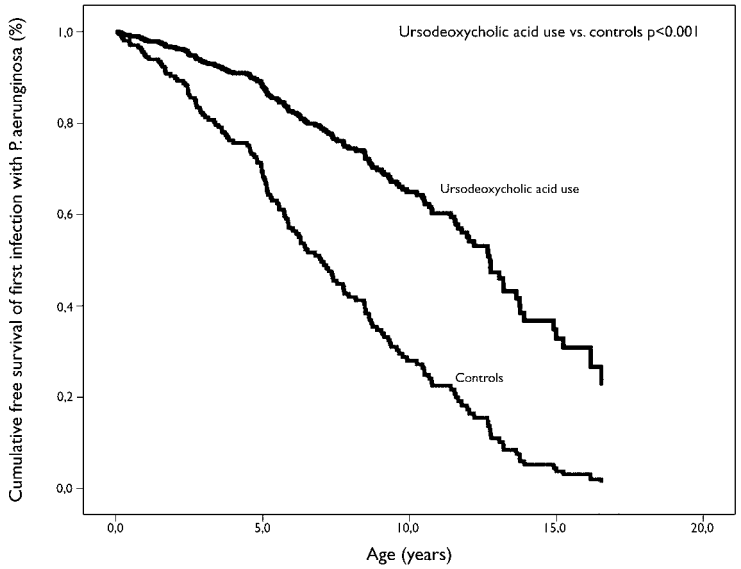
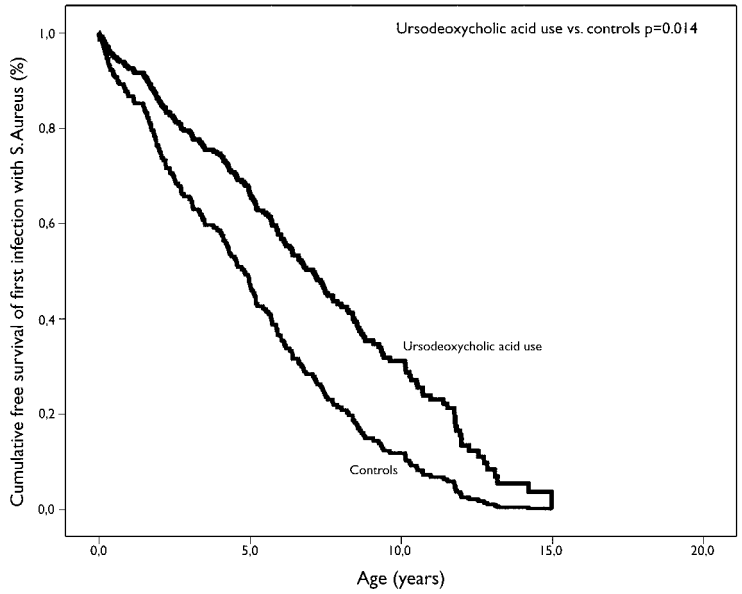
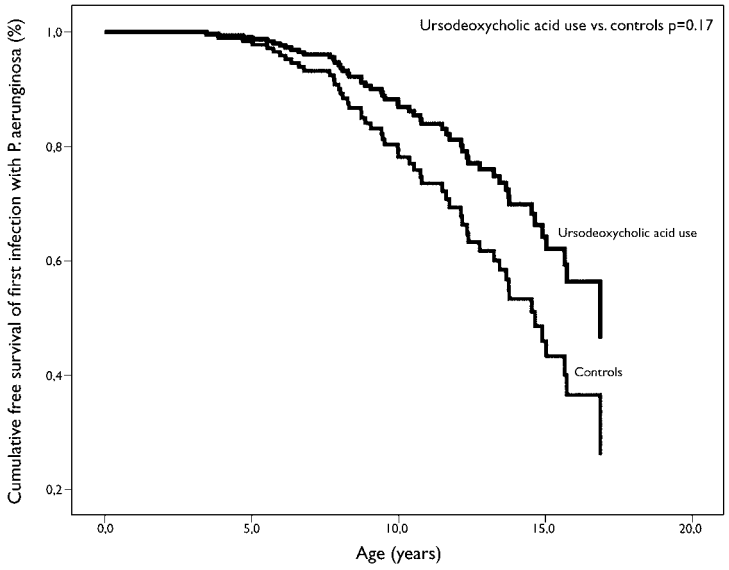


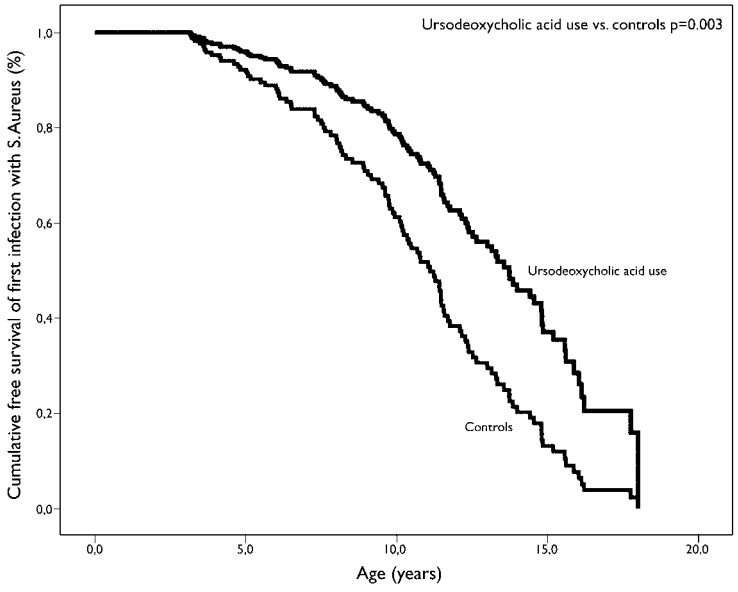
Figure 2: Cumulative free survival of the first infection with S. aureus between patients with and without ursodeoxycholic acid use (adjusted for age at diagnosis of cystic fibrosis and gastric acid inhibition for gastroesophageal reflux disease)



Supplementary figure 1: Cumulative free survival of colonization with P. aeruginosa between patients with and without ursodeoxycholic acid use (adjusted for age at diagnosis of cystic fibrosis and liver cirrhosis)



Supplementary figure 2: Cumulative free survival of colonization with S. aureus between patients with and without ursodeoxycholic acid use (no adjustments were necessary)



Longitudinal analysis of fat absorption, growth and pulmonary function

Of the 207 patients 176 met the inclusion criteria for the longitudinal analysis of the CFA and 181 patients had at least two measurements of length, weight, FEV_1 and FVC (table 2).

UDCA patients had a significant reduced CFA, weight, height and BMI z-score at 10 years of age compared to patients without UDCA, while pulmonary function at 10 years of age (FEV_1 and FVC) did not differ. Furthermore, in UDCA patients the yearly change of weight z-score, height z-score, FEV_1 and FVC was worse. However the yearly change of CFA and BMI z-score was not significantly different between the two groups (table 2).

Table 2: Longitudinal analysis of coefficient of fat absorption (CFA), growth and pulmonary function in patients with or without ursodeoxycholic acid (UDCA)

Outcome	**Groups**	**Number of patients (number of tests)**	**Outcome at age 10 (95% CI)**	**P-value**	**Yearly change (95% CI)**	**P-value**
CFA	UDCA	34 (142)	83.3 (78.0; 88.6)[1]	***<0.001***[1]	0.01 (-0.97; 0.99)[1]	0.75[1]
	Controls	142 (782)	87.6 (84.5; 90.7)[1]	-	0.10 (-0.35; 0.54)[1]	-
Weight z-score	UDCA	43 (495)	-1.46 (-1.90; -1.02)[2]	***<0.001***[2]	-0.052 (-0.12; 0.024)[2]	***0.016***[2]
	Controls	138 (2073)	-1.24 (-1.58; -0.90)[2]	-	-0.017 (-0.065; 0.031)[2]	-
Height z-score	UDCA	43 (495)	-1.00 (-1.52; -0.47)[3]	***<0.001***[3]	-0.03 (-0.10; 0.04)[3]	***0.018***[3]
	Controls	138 (2073)	-0.82 (-1.27; -0.38)[3]	-	-0.0026 (-0.052; 0.04)[3]	-
BMI z-score	UDCA	43 (495)	-1.20 (-1.65; -0.74)[4]	***0.004***[4]	-0.068 (-0.15; 0.013)[4]	0.060[4]
	Controls	138 (2073)	-1.03 (-1.37; -0.69)[4]	-	-0.037 (-0.085; 0.012)[4]	-
FEV_1	UDCA	43 (495)	93.4 (84.0; 102.8)[5]	0.77[5]	-2.7 (-4.6; -0.79)[5]	***0.006***[5]
	Controls	138 (2073)	93.9 (87.8; 100.0)[5]	-	-1.5 (-2.5; -0.44)[5]	-
FVC	UDCA	43 (490)	98.1 (90.3; 106.0)[5]	0.48[5]	-0.65 (-2.40; 1.08)[5]	***0.011***[5]
	Controls	138 (2071)	99.2 (94.3; 104.1)[5]	-	0.38 (-0.57; 1.32)[5]	-

[1]adjusted for age, age at diagnosis of CF, *CFTR* genotype, PA colonization, PERT dose, GA inhibition for fat malabsorption or GERD, liver cirrhosis, MI and DIOS

[2]adjusted for gender, age, *CFTR* genotype, PA and SA colonization, FEV_1, GA inhibition for fat malabsorption or GERD and liver cirrhosis

[3]adjusted for gender, age, age at diagnosis of CF, *CFTR* genotype, PA and SA colonization, FEV_1, GA inhibition for fat malabsorption or GERD and liver cirrhosis

[4]adjusted for gender, age, *CFTR* genotype, PA and SA colonization, FEV_1, GA inhibition for fat malabsorption or GERD

[5]adjusted for gender, age, *CFTR* genotype, PA and SA colonization, BMI z-score, GA inhibition for fat malabsorption or GERD and liver cirrhosis

Discussion

In our cohort of pediatric CF patients a strong negative association was found between the use of UDCA and both fat absorption and growth using longitudinal analysis. The negative association between UDCA treatment and fat absorption and growth has not been reported previously, but is an important significant finding since UDCA is commonly used in CF patients. Until now three smaller studies found no association between the CFA and treatment with UDCA[7,20,21], while one observational study of 8 CF patients found an improvement in weight and BMI after 6 months of UDCA treatment. However, in the latter study no comparison was made with a control group.[7] The decreased fat absorption we found is not that surprising, as UDCA is less hydrophobic compared to the more dominant bile acids, cholic acid and chenodeoxycholic acid, and therefore has an impaired capability to form mixed micelles[6], which is an essential step in fat absorption. That a reduced CFA results in a decreased growth, both for height, weight and BMI, is also logical, as in most CF patients energy intake necessary for an uncompromised growth is already below recommendations. Reducing CFA through the use of UDCA will only aggravate this situation.

In our cohort UDCA patients had a later onset of first infection with PA and SA and delayed colonization with SA, suggesting an anti-inflammatory and immuno-modulatory role of UDCA. Our data confirm the findings of a small retrospective study reporting a lower PA acquisition in CF patients who started early with UDCA compared to patients who received UDCA at the onset of liver disease.[9] In non-CF populations more reports of the anti-inflammatory and immuno-modulatory capacities of UDCA have appeared, especially describing a reduction of rejection of solid organs in transplanted patients.[22-24]

However, the decreased or delayed bacterial acquisition does not result in better lung function. On the contrary, the yearly change of FEV_1 and FVC was worse in UDCA patients than in patients without UDCA. In our cohort apparently the negative association between UDCA and fat absorption and growth outweighs the protective effect on PA and SA acquisition. In agreement with our findings, a large longitudinal German study showed that malnutrition was more negatively associated with pulmonary function than PA colonization.[25]

The use of UDCA for the treatment of CF related liver disease is a contentious subject. Several studies showed a positive effect of UDCA on the liver biochemistry[20,26,27], liver histology[26], hepatic excretory function and bilairy drainage[28]. Because of the favorable effect on elevated liver enzymes and no reports of significant disadvantages, UDCA is currently widely prescribed at CF Centers and potentially has a role in the prevention of

liver cirrhosis in CF. However, the effect of UDCA on end stage liver disease and survival in CF patients has yet to be established.[29] Additionally, a recent report showed that patients with primary sclerosing cholangitis receiving high dosed UDCA had an higher frequency of varices, death or eligibility for liver transplantation than controls. [30] Together with our report of serious side effects of UDCA use in CF patients, this generates an urgent need for well-performed randomized clinical trails with UDCA in CF patients.

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Chapter 7

Energy intake is positively correlated with pulmonary function in children with cystic fibrosis, but only in those at risk for malnutrition and a FEV_1<100%

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Submitted

Abstract

Objectives: Our aim was to investigate the relationship between energy intake, nutritional status and pulmonary function in an analysis of cystic fibrosis (CF) patients with a body mass index (BMI) z-score between 0SD and -1SD.

Methods: To this end a retrospective study of 82 paediatric CF-patients was performed. The inclusion criteria were: availability of at least two visits within a period of two years, a BMI z-score between 0SD and -1SD and an age >4 years at first visit. Exclusion criteria were: prednisone and/or intravenous antibiotics between or during the visits.

Results: In an extensive analysis of these patients a significant correlation was found between change in energy intake and BMI z-score change, while change in energy intake and change in forced expiratory volume in 1 second (FEV_1) did not correlate. Interestingly, in the subgroup of patients with a compromised pulmonary function (initial FEV_1<100 %) change in energy intake was significantly correlated with FEV_1 change (n= 41, p=0.02), but not so in patients with a FEV_1>100%.

Conclusion: Our data suggests that only a subgroup of CF-patients will have improvement of pulmonary function when increasing energy intake. This suggests that a more individualized approach to nutritional management in CF can be considered.

Introduction

Nutritional status and lung disease are the two main determinants for prognosis in cystic fibrosis (CF) patients and both are closely related: e.g. in (severely) malnourished patients FEV_1 is compromised, as is survival.[1,2] Therefore the current recommendation for CF patients is to provide a high energy diet, with 120-200% of the recommended daily allowance (RDA) and to optimize fat absorption with adequate pancreatic enzyme replacement and additional gastric acid inhibition if necessary.[3,4]

However the majority of CF patients have difficulties meeting these energy intake recommendations[5], sometimes resulting in behavioural problems regarding food intake and early introduction of tube feeding. As the relationship between energy intake, nutritional status and pulmonary function is unclear in well-nourished CF patients and in those with a BMI slightly below the mean[6,7], it is questionable if these recommendations should be applied to all CF patients. We hypothesized that a tailored fit nutritional management might be more appropriate for subgroups of patients with CF. Therefore the aim of this study was to investigate the relationship between energy intake, nutritional status and pulmonary function in an extensive analysis of patients at risk for malnutrition, i.e. those with a body mass index (BMI) z-score between 0.0SD and -1.0SD.

Methods

A retrospective study was performed of all paediatric CF patients under treatment at the University Medical Center, Utrecht, the Netherlands on January 1st, 2007 according to the guidelines of the medical ethics board of the University Medical Center Utrecht.

The effect of energy intake on BMI z-score and forced expiratory volume in 1 second (FEV_1) in patients at risk for malnutrition (BMI z-score between 0.0SD and -1.0SD) was studied. CF patients with two reliable and simultaneous measurements of energy intake (% RDA), BMI z-score and FEV_1 within a period of two years and an initial BMI z-score between 0.0SD and -1.0SD were included, but only if patients were >4 years and could perform a reliable pulmonary function test. Patients who received prednisone (oral or intravenous) and/or intravenous antibiotics for a pulmonary exacerbation and/or active allergic bronchopulmonary aspergillosis between or during the two visits were excluded. Also patients eligible for liver or lung transplantation and patients receiving surgery between the two visits were excluded. Coefficient of fat absorption (CFA) and pancreatic enzyme replacement dose (PERT) (lipase units/kilogram/day) had to be known at each visit. Using the clinical charts the following characteristics were recorded: gender, current age, *cystic fibrosis transmembrane regulator (CFTR)* genotype (severe vs. mild/unknown)[8], liver cirrhosis[8], meconium ileus, colonization with *Pseudomonas aeruginosa* (PA) or *Staphylococcus aureus* (SA)[9].

Mean energy intake was determined by a registered dietician (J.W.) using three-day dietary intake records and was expressed as percentage of the RDA. The CFA, expressed as percentage of fat intake, was calculated from mean fat intake, determined in three-day dietary intake records, and mean faecal fat excretion, determined in three-day stool collections. Pulmonary function was measured by spirometry and converted to percentage of predicted values[10]. Height was measured with a stadiometer to the nearest 0.5 centimetre (Holtain, Crymisch, UK) and weight was measured using a digital weight scale to the nearest 0.1 kilogram. Weight, height and BMI z-scores were calculated using the growth analyzer 3.0 software of the Dutch Growth Foundation (available at http://www.growthanalyser.org).

Statistical analysis

To test for differences between the two visits categorical variables were analyzed using Pearson's chi-square or Fisher's exact test and continuous variables were analyzed with the Wilcoxon rank test. To analyze correlations between change in energy intake and change in BMI z-score or FEV_1 Pearson's correlation or Spearman rank correlation was used. Also was investigated if CFA and PERT dose change was correlated with energy intake change

(and thereby act as confounders). Statistical analysis were made using SPSS software package version 15.0 (SPSS Inc. Chicago IL, USA). Values were considered statistically significant if the p-value was <0.05.

Results

The study group consisted of 228 paediatric CF patients of which 82 (36%) met the inclusion criteria for the extensive analysis in patients at risk for malnutrition (BMI z-score between 0.0SD and -1.0SD). The clinical characteristics between the first and second visit were not different (table 1). In all CF patients the change in energy intake and the change in BMI z-score between the two visits correlated (figure 1a), while energy intake change did not correlate with FEV_1 change (figure 1b). CFA and PERT dose did not act as confounders, since energy intake change did not correlate with CFA change (r=0.001, p=0.99) and PERT dose change (r=0.13, p=0.26).

As we hypothesized that in patients with a suboptimal pulmonary function (FEV_1<100%) changes in energy intake would have a larger effect, we subsequently analyzed subgroups with a FEV_1<100% and >100% (both 41 patients). For both subgroups a trend was observed for a correlation between energy intake change and BMI z-score change (figure 1c). More importantly, when analyzing the relationship between changes in pulmonary function and energy intake a clear correlation was observed in patients

Table 1: Characteristics of cystic fibrosis patients at risk for malnutrition (BMI z-score between 0.0SD and -1.0SD)

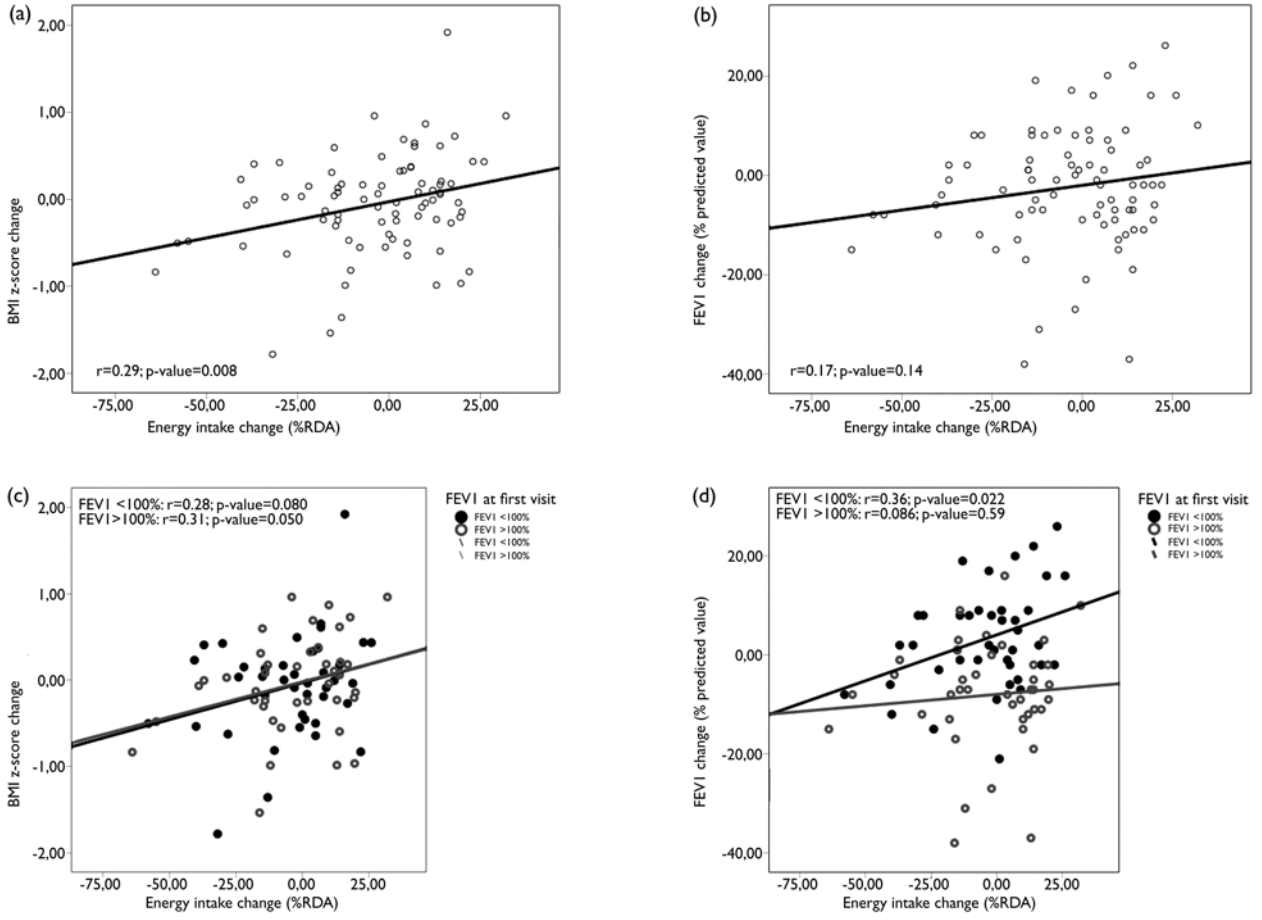
	1st visit	**2nd visit**	**p-value**
Number	82	-	-
Gender (male)	47 (57%)	-	-
Age (years)*	8.9 (3.3)	10.0 (3.3)	<0.001
Severe *CFTR* genotype	70 (85%)	-	-
Meconium ileus	11 (13%)	-	-
Liver Cirrhosis	4 (5%)	4 (5%)	1.0
PA colonization	7 (9%)	10 (12%)	0.44
SA colonization	23 (28%)	27 (33%)	0.50
Energy intake (% RDA)*	111.0 (21.5)	107.2 (19.5)	0.34
FEV_1 (% of predicted value)*	100.4 (16.1)	98.0 (14.8)	0.084
BMI z-score*	-0.38 (0.31)	-0.43 (0.67)	0.48
Coefficient of fat absorption*	88.1 (6.6)	88.3 (6.8)	0.82
PERT dose (1000 U/kg/day)*	5.0 (2.4)	5.1 (2.3)	0.87

*mean (SD)

with initially a FEV_1<100%, while in patients with initially a FEV_1>100% no correlation was observed (figure 1d). Interestingly when stratifying the FEV_1<100% group for gender (23 males and 18 females) we only found a significant correlation between energy intake change and FEV_1 change in female patients ($r=0.50$, $p=0.035$), but not in male patients ($r=0.18$, $p=0.40$).

Figure 1: Correlation between energy intake change (%RDA) and BMI z-score change or FEV_1 (% of predicted values) change in cystic fibrosis patients at risk for malnutrition (BMI z-score between 0.0SD and -1.0SD). (a) energy intake change vs. BMI z-score change in all patients, (b) energy intake change vs. FEV_1 change in all patients, (c) energy intake change vs. BMI z-score change in patients with a FEV_1 below or above 100% at first visit, (d) energy intake change vs. FEV_1 change in patients with a FEV_1 below or above 100% at first visit.

Figure 1


(a)
BMI z-score change
2,00
1,00
0,00
-1,00
-2,00
r=0.29; p-value=0.008
-75,00
-50,00
-25,00
0,00
25,00
Energy intake change (%RDA)
(b)
FEV1 change (% predicted value)
20,00
0,00
-20,00
-40,00
r=0.17; p-value=0.14
Energy intake change (%RDA)
(c)
FEV1 <100%: r=0.28; p-value=0.080
FEV1>100%: r=0.31; p-value=0.050
FEV1 at first visit
FEV1 <100%
FEV1 >100%
FEV1 <100%
FEV1 >100%
BMI z-score change
Energy intake change (%RDA)
(d)
FEV1 <100%: r=0.36; p-value=0.022
FEV1 >100%: r=0.086; p-value=0.59
FEV1 at first visit
FEV1 <100%
FEV1 >100%
FEV1 <100%
FEV1 >100%
FEV1 change (% predicted value)
Energy intake change (%RDA)

Discussion

In the extensive analysis of all patients at risk for malnutrition (BMI z-score between 0.0SD and -1.0SD) a correlation was found between change in energy intake and BMI z-score change, while no correlation was found between change in energy intake and FEV_1 change. Interestingly, in the subgroup of patients with a suboptimal pulmonary function (initial FEV_1<100%) change in energy intake was significantly correlated with FEV_1 change, especially in female patients.

Traditionally, female CF patients have a disadvantage regarding morbidity and mortality.[11] The explanation of this gender gap is unclear, but psychosocial and/or hormonal differences are suggested.[12] Nevertheless some suggest that with improvement of care closing of this gender gap is possible.[13] Our observation supports this, as particularly in female patients with a suboptimal pulmonary function increasing energy intake had a positive effect on FEV_1.

The relationship between energy intake, nutritional status and pulmonary function seems to be more complex than initially thought. Many reports have shown that very malnourished CF patients (approximately with BMI z-score <-2SD) benefit from high caloric intake using enteral, if necessary through nasogastric or gastrostomy tube feeding, or parenteral nutritional interventions.[14-18] All these studies reported improvement of the nutritional status, but some also reported improvement of pulmonary function.[14,16,18] Subsequently, intensive nutritional support was also initialized in less malnourished patients (approximately with BMI z-score between 0SD and -2SD). In this group the increase in caloric intake, which was supported by behavioural treatment and/ or nutritional education, showed an improvement in nutritional status, although no improvement of pulmonary function was found.[7] Nevertheless, the threshold for intensive nutritional support with high caloric intake and/or tube feeding has become lower with time, as studies showed that increasing caloric intake leads to BMI improvement in all patients with a BMI z-score <0SD. The effect of nutritional intervention on pulmonary function in these patients is lacking, since only severe malnourished patients had pulmonary function improvement after more calories. Despite these ambiguous study results, the North American CF guidelines for caloric intake cover all CF patients[4], although the European guideline suggests that a normal energy intake may be sufficient for children with well-controlled absorption and mild chest infections.[3] Taken into account that the majority of patients have difficulties meeting the energy intake recommendations[5], we believe that a more individualized approach to nutritional management in CF regarding energy intake is appropriate. Our study supports this approach showing that in patients with a BMI between 0.0SD and -1.0SD only those with

a FEV_1<100% benefit from increasing caloric intake, as judged from an improvement in pulmonary function.

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Chapter 8
Summarizing Discussion

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Partially based on an invited review in Current Gastroenterology Reports, 2011;13:265-70

In the first part of the thesis we reported incidence and prevalence numbers, risk factors and treatment of the distal intestinal obstruction syndrome (DIOS) and constipation in cystic fibrosis (CF) patients (**chapter 2 and 3**). Additionally, in **chapter 4** we reported that a polymorphism in the *CLCA1* gene is associated with meconium ileus in CF patients, suggesting that this gene is in fact a modifier for meconium ileus. In the second part of the thesis (**chapter 5-7**) we have reported the longitudinal effect on growth, bacterial colonization and pulmonary function of several medical interventions (gastric acid inhibition, ursodeoxycholic acid and dietary energy intake).

Intestinal obstruction syndromes in cystic fibrosis

Intestinal obstruction syndromes are significant issues for CF patients. Historically, meconium ileus at birth was associated with a worse survival and compromised growth and pulmonary function.[1] However, these long term outcomes have improved dramatically and nowadays no differences between meconium ileus and non meconium ileus patients are noticed.[2,3] Later in life DIOS and more frequently constipation become issues in CF patients (**chapter 2 and 3**).

Incidence and prevalence

Studies investigating the incidence and prevalence numbers of DIOS and constipation are difficult to compare because of the different definitions used.[4-7] We therefore participated in developing consensus guidelines for diagnosing DIOS and constipation, which should simplify comparison of different aspects of these conditions (**chapter 2**). When using these definitions, both incidence (2.2-6.2 episodes per 1000 patient years) and prevalence (7-8%) of DIOS in childhood are low (**chapter 2 and 3**) and comparable to figures reported earlier by authors using the same definitions.[6,8] Constipation, however turned out to be quite frequent in our population (47%; **chapter 3**), and also more frequent than the 26% prevalence rate reported earlier.[8]

Risk factors

The main risk factor for developing meconium ileus is the *CF transmembrane regulator* (*CFTR*) genotype, although *non-CFTR* genes seem to be involved too, such as the *CLCA1* gene, as reported in **chapter 4** (see also Blackman et al.[9]). Pancreatic insufficiency could also play a role, since almost all (95%) meconium ileus patients are born pancreatic insufficient.[9] However the contribution of this risk factor is difficult to study, as it co-segregates with a severe *CFTR* genotype.[9] In addition, a disturbed intestinal motility might contribute to the development of meconium ileus; indeed these patients have a higher chance to develop other gastro-intestinal motility disorders, like reflux disease (GERD),

later in life (**chapter 5** and Heine et al.[10]) However, these risk factors might be linked as some of the non-*CFTR* genes contributing to meconium ileus might in fact be involved in gastro-intestinal motility.

After birth meconium ileus patients are prone to develop DIOS and constipation (**chapter 2 and 3** and Blackman et al.[9]). Risk factors for these conditions include abdominal surgery, as meconium ileus patients treated surgically develop more frequently DIOS than meconium ileus treated medically (34% vs. 20%).[9] This could be due to adhesions developing after surgery. However, patients requiring surgery might also have a more severe clinical phenotype. Patients without a history of meconium ileus also develop DIOS and constipation; in fact this is the largest group. In these patients fat malabsorption and impaired motility seem to be important risk factors (**chapter 3**). The fat malabsorption in combination with slow intestinal transit, as frequently seen in the CF population[11,12], could promote accumulation of fecal material. In contrast to meconium ileus, genetic factors (*CFTR* and *non-CFTR*) seem to play a limited role in DIOS and constipation in CF, as we could not find an association between a severe *CFTR* genotype and constipation (**chapter 3**) and a large twin study shows a low concordance rate for DIOS in monozygous twins and an equally low concordance rate in dizygous twins.[9]

Treatment

Most DIOS episodes can be treated conservatively with intensive laxative treatment (oral laxatives and/or enema or polyethylene glycol lavage); surgical intervention is only rarely necessary (**chapter 2**, Rubinstein et al.[8] and Dray et al.[13]).

For constipation polyethylene glycol is to be preferred, as it is at least as effective as lactulose, but does not have the side effects associated with this laxative (flatulence and abdominal cramps).[14] For DIOS patients treatment is still largely empiric as there are no trials. It is generally recommended to start with an oral laxative (polyethylene glycol) with or without an enema, and to restore adequate hydration, both in patients with impending DIOS and in those with complete DIOS, at least in patients who are not vomiting.[15] When this is not effective, or in more severe DIOS episodes, intestinal lavage with polyethylene glycol or a balanced electrolyte osmotic solution is started orally or via nasogastric tube. Surgery should be used only if conservative treatment is not successful, but with early aggressive medical management this is seldom required.

As most DIOS patients have more than one episode (**chapter 2**), continuation of the laxative treatment (polyethylene glycol) after the first DIOS episode can be considered and seems indeed logical, although no evidence for this approach is available. Furthermore, dehydration and fat malabsorption (coefficient of fat absorption below 85%)

should be avoided to prevent recurrence. Finally in transplantation patients, who are at risk for developing DIOS, pre-transplant bowel preparation and early post-operative start of enteral feeding, seems appropriate as well as adequate use of pancreatic enzymes and prophylactic polyethylene glycol.[16]

Future directions

Reports of DIOS and constipation are scarce and studies were difficult to compare because of different definitions used. The recently published ESPGHAN definition of DIOS and constipation (**chapter 2**) will facilitate further research and can be used to elucidate more reliably the incidence, prevalence and risk factors for DIOS and constipation. A multinational study is currently underway to study these aspects of DIOS prospectively. Furthermore, it is interesting to determine intestinal transit times in patients with meconium ileus, DIOS and constipation to investigate the role of intestinal motility in these groups of CF patients.

A relative new research topic in CF is intestinal inflammation. In the majority of CF patients intestinal inflammation is present as evidenced directly by capsule endoscopy or indirectly by elevated fecal calprotectin levels.[17,18] More specifically, ileal biopsies from both meconium ileus and DIOS patients also show signs of intestinal inflammation, especially in the myenteric ganglion cells and myocytes.[19] This intestinal inflammation may play a role in the development of intestinal obstruction in CF, either directly, or through delaying intestinal transit time. Therefore it might be worthwhile to investigate interventions to reduce the intestinal inflammation. In fact it was already shown that with probiotics fecal calprotectin levels in CF patients can be reduced.[17]

Also the role of CLCA1 in intestinal obstruction in CF patients should be further clarified. In mice the role of clca3, the rodent homologue of CLCA1, in intestinal obstruction is clear; severely affected *CFTR* knockout mice, which mostly die because of intestinal obstruction, have a decreased expression of clca3 while up-regulation of clca3 leads to amelioration of intestinal disease and improved survival.[20] In humans we showed that a genetic variant in the coding region of *CLCA1* is associated with meconium ileus (**chapter 4**). However it is unclear whether this variant leads to changes in function and expression of CLCA1, especially in CF patients with meconium ileus, DIOS or constipation.

Finally, as noted before, the use of prophylactic laxative treatment after the first DIOS episode has not yet been investigated. Although it seems logical to continue laxative treatment and avoid dehydration and fat malabsorption, a clinical trial is necessary to prove these assumptions. However, it might be difficult to recruit sufficient patients for a decent clinical trial, as DIOS incidence is low (**chapter 2**).

Medical interventions in cystic fibrosis

Gastric acid inhibition in CF patients, through proton pump inhibitors or histamine-2 receptor antagonists, is added when fat absorption remains insufficient despite an adequate dosage of pancreas enzyme replacement.[21,22] GERD is another reason to start drugs for gastric acid inhibition in patients with CF. However, it was suggested that gastric acid inhibition might negatively affects pulmonary condition, since it is associated with increased pulmonary infections in non-CF patients.[23-25] We now showed that gastric acid inhibition for fat malabsorption is in fact safe to use in CF patients, since it does not influence bacterial colonization with *P. aeruginosa* and *S. aureus,* and might even have an advantageous effect on the decline in pulmonary function (**chapter 5**). In the same study we found that in CF patients with symptomatic GERD pulmonary function at 10 years of age was significantly reduced, despite adequate gastric acid inhibition (**chapter 5**). In this group an earlier diagnosis and treatment of GERD might prevent or reduce the pulmonary damage, which intervention might even be extended to CF patients with asymptomatic GERD. However, and in contrast with symptomatic GERD, frequency of asymptomatic GERD is unknown in CF patients, although some suggest a higher frequency of silent GERD.[26] Furthermore it is unknown whether GERD is the cause of the reduced pulmonary function or that GERD is secondary to a worse (pulmonary) phenotype. In fact *CFTR* genotype could also play a role since most GERD patients in our Medical Center had a severe *CFTR* genotype (92%), compared to 76% in patients without GERD (**chapter 5**). The assumption that increased chest physiotherapy or frequent coughing provoke reflux by increasing intra-abdominal pressure[27], was recently shown to be incorrect.[26]

Ursodeoxycholic acid (UDCA) therapy is commonly used for CF related liver disease as it supposedly improves bile viscosity and reduces bile toxicity by changing bile acid composition.[28] However, UDCA also has an impaired capability to form mixed micelles in the small intestine, which may negatively influence fat absorption and growth in CF patients.[29] We now showed that UDCA treatment is indeed associated with compromised fat absorption, growth and pulmonary function, independently of liver cirrhosis (**chapter 6**). This is an important finding since the mitigating effect of UDCA on development and progression of liver disease is only an assumption that has never been proven (as discussed in **chapter 6**). Nevertheless UDCA is widely used among CF patients. As patients with CF related liver disease are already at risk for malnutrition[30], this generates an urgent need for well-performed randomized clinical trials with UDCA in CF patients.

Nutrition is an important issue in CF since it affects pulmonary function and survival.[31,32] In severely malnourished CF patients most studies show improvement of both nutritional status and pulmonary function after nutritional interventions.[33-37] However

few well-performed studies have been published in patients with a body mass index (BMI) z-score above -2SD.[38,39] For these patients guidelines for energy intake are mainly based on opinions of experts in the field, which seem to be largely based on results obtained in severely malnourished CF patients.[40,41] Our study now showed that patients with a BMI z-score between 0 and -1 SD and a FEV_1<100% do have pulmonary improvement after increasing energy intake, but not the patients with a FEV_1>100% (**chapter 7**). In this group a less aggressive recommendation for nutritional intervention might be appropriate. We also expect that in the near future more studies in well nourished CF patients and in those with a BMI slightly below the mean will become available, so that it will be possible to provide a more tailored fit nutritional approach.

Future directions

CF patients with symptomatic GERD have earlier bacterial acquisition and compromised pulmonary function as compared to CF patients without GERD (**chapter 5** and Stringer et al.[42]). Earlier diagnosis and treatment of GERD in this group might prevent pulmonary damage. In contrast to symptomatic GERD, the prevalence of asymptomatic GERD in CF patients is unknown as is the effect of gastric acid inhibition on pulmonary function.

A clinical trial investigating the effect of UDCA on the development and progression of CF related liver disease is urgently needed, as so far it has only been proven that UDCA can improve raised transaminases and GGT.[43-45] This is especially pressing as we have showed severe side effects of UDCA treatment (**chapter 6)**. In addition, identifying factors that predict the development of CF related liver disease and/or a severe course of this condition is necessary, as this allows treatment to be initiated early to prevent (further progression of) liver disease. In a recent study 80% of the children with CF related liver disease showed progression and within this group 25% died or underwent a liver transplantation.[30] In this study no factors predicting the clinical course were found, although irregularity of the liver margin was suggested to predict more severe disease.[30] Interestingly in 20% of the patients the clinical course was benign and surprisingly these patients even had normalization of liver and spleen ultrasonography in adulthood.[30] Promising tools to predict the clinical course more accurately are transient elastography (FibroScan), a non-invasive technique that measures liver stiffness, and serum markers for fibrosis (like the FibroTest), although there is too little evidence yet to incorporate these techniques into regular clinical practice.[46-48] At present, only with a careful clinical follow-up it will become clear in whom liver disease will progress and in whom it will have a benign course.

As discussed above we believe that our study (**chapter 7**) contributes to a more

tailored advice for nutritional support in the well-nourished CF patient and in those with a BMI slightly below the mean. Unnecessary emphasis on a very high caloric intake might be avoided in these subgroups. It is to be expected that in the near future more subgroups will be identified in which this is justified. For the severely malnourished CF patients appetite stimulants have shown beneficial effects on both nutritional status and pulmonary function.[49] These stimulants may also increase caloric intake and positively influence growth and pulmonary function in less severely malnourished CF patients, thereby avoiding or reducing the need for tube feeding.

Conclusion

In this thesis several aspects of the intestinal obstructions syndromes in CF (meconium ileus, DIOS and constipation) have been described. Furthermore we investigated the effect of medical interventions (gastic acid inhibition, UDCA and dietary energy intake) on growth, bacterial colonization and pulmonary function in CF patients. The main conclusion from each chapter is listed below.

Highlights

- The consensus guidelines issued by the ESPGHAN CF Working Group for diagnosing DIOS and constipation are strict and should simplify comparison between centers of different aspects of these conditions (**chapter 2**)
- Constipation in CF is common and associated with meconium ileus and fat malabsorption (**chapter 3**)
- The S357N variant in the *CLCA1* gene is associated with meconium ileus in European CF patients (**chapter 4**)
- Gastric acid inhibition for fat malabsorption does not affect bacterial colonization and might even have an advantageous effect on the decline in pulmonary function (**chapter 5**)
- Gastroesophageal reflux disease is associated with earlier bacterial acquisition and a worse pulmonary function (**chapter 5**)
- Ursodeoxycholic acid treatment is longitudinally associated with fat malabsorption and a compromised growth and pulmonary function (**chapter 6**)
- CF patients with a BMI z-score between 0 and -1 SD and a FEV_1<100% have pulmonary improvement after increasing caloric intake, but not CF patients with a FEV_1>100% (**chapter 7**)

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Chapter 9

Summary in Dutch - Nederlandse samenvatting

Cystische fibrose (CF), of taaislijmziekte, is een relatief veelvoorkomende erfelijke ziekte, welke voorkomt bij 1 op de 4750 pasgeborenen. Een mutatie (een "foutje in het DNA") in het CFTR gen is verantwoordelijk voor deze ziekte. Dit gen codeert voor een eiwit (CFTR) welke zorgt voor het chloor transport in de lichaamscellen. Kenmerkend voor deze ziekte is dat alle sappen die organen uitscheiden (secreties) minder vocht bevatten en dus taaier worden. Het is een systeemziekte, wat betekent dat dit taaie slijm op meerdere plaatsen in het lichaam voorkomt. De meest belangrijke aangedane organen zijn de longen en de alvleesklier. Daarnaast zijn er ook vaak ziekteverschijnselen van lever en de darmen.

Bijna alle patiënten hebben last van taaislijm in hun longen. Dit taaie slijm is moeilijker op te hoesten en de longen zijn daardoor gevoeliger voor infecties. CF patiënten worden dus frequent behandeld met antibiotica voor ernstige longinfecties. Bij sommige infecties lukt het echter niet om de bacterie met antibiotica te verwijderen, men spreekt dan van kolonisatie. Bij elke infectie ontstaat longschade, resulterend in een steeds slechtere longfunctie.

Daarnaast hebben de meeste CF patiënten problemen met de opname van vetten uit de voeding (vet malabsorptie). Doordat de verteringssappen die de alvleesklier uitscheidt ook taaier zijn, worden deze moeilijker uitgescheiden en is de alvleesklier vaak al voor de geboorte door zijn eigen verteringssappen verwoest. Bij deze patiënten komen er dan geen verteringssappen meer in de darmen. Deze verteringssappen bevatten enzymen om vetten af te breken (lipasen); omdat deze niet meer bij CF patiënten met een kapotte alvleesklier aanwezig zijn, kunnen deze patiënten vetten minder goed verteren en opnemen. Om ervoor te zorgen dat ze vet toch redelijk goed kunnen verteren wordt deze groep behandeld met alvleesklier-enzymen (m.n. lipasen), die voorzien zijn van een laagje zodat ze pas in de darm vrijkomen. Ondanks deze behandeling zijn CF patiënten toch vaak in een slechte voedingstoestand.

Het merendeel van de CF patiënten overlijdt uiteindelijk ten gevolge van een slechte longfunctie in combinatie met een slechte voedingstoestand. Om de prognose van deze groep te verbeteren is de CF zorg geconcentreerd in grotere centra, waar meer ervaring aanwezig is. Verder worden er eerder en langer antibiotica gebruikt en is er veel aandacht voor voedingstherapie. Hierdoor is de overleving in de afgelopen 10 jaar enorm verbeterd.

Bij CF patiënten komen ten gevolge van het taaie slijm in de darmen een aantal ziektebeelden voor die gepaard gaan met onvoldoende doorstroming van de ontlasting in de darmen, de zogenaamde intestinale obstructie syndromen: meconium ileus, distaal intestinaal obstructie syndroom (DIOS) en obstipatie. Bij de geboorte heeft een gedeelte van de CF patiënten al last van een meconium ileus, dit is een ernstige obstructie in de

darmen veroorzaakt door taai meconium (eerste ontlasting). Later in het leven komt obstipatie (verstopping) frequent voor. DIOS is een zeer ernstige verstopping met een ophoping van ontlasting ter hoogte van de overgang van dunne naar dikke darm (ileo-coecale overgang). Hoewel een DIOS minder frequent voorkomt dan obstipatie, zijn de verschijnselen wel veel ernstiger.

In dit proefschrift beschrijven we in het **eerste gedeelte** het voorkomen en de risicofactoren voor intestinale obstructiesyndromen (meconium ileus, DIOS en obstipatie) bij CF patiënten (**hoofdstuk 2, 3 en 4**). In het **tweede gedeelte** van het proefschrift laten we het effect op lange termijn zien van verschillende medische interventies (zuurremming, ursodeoxycholzuur en energie inname) op de groei, bacteriële kolonisatie en longfunctie bij CF patiënten (**hoofdstuk 5, 6 en 7**).

Intestinale obstructiesyndromen bij CF

Intestinale obstructiesyndromen kunnen problemen geven bij CF patiënten. Vroeger hadden patiënten met meconium ileus een slechtere overleving met een verminderde groei en longfunctie. Tegenwoordig is er echter geen verschil meer in groei, longfunctie en overleving tussen CF patiënten die geboren zijn met of zonder meconium ileus. Na de geboorte is DIOS en in het bijzonder obstipatie een veel voorkomend probleem bij CF patiënten (**hoofdstuk 2 en 3**).

Voorkomen van DIOS en obstipatie

De studies die het voorkomen van DIOS en obstipatie bij CF patiënten rapporteren zijn moeilijk met elkaar te vergelijken, omdat er verschillende definities gebruikt worden. Daarom hebben we in **hoofdstuk 2** met een Europese werkgroep nieuwe internationaal geaccepteerde definities van DIOS en obstipatie bij CF vastgesteld. Als we deze nieuwe definities gebruiken zien we dat de incidentie (het aantal nieuwe gevallen per jaar) en prevalentie (het aantal bestaande gevallen per periode) laag zijn. De incidentie is 2.2-6.2 nieuwe gevallen per 1.000 patiëntjaren en de prevalentie is 7-8% (**hoofdstuk 2 en 3**). Obstipatie bleek veelvoorkomend te zijn in onze populatie; 47% van alle kinderen met CF had tenminste één periode met obstipatie doorgemaakt (**hoofdstuk 3**).

Risicofactoren

De belangrijkste risicofactor voor het ontwikkelen van meconium ileus is de ernst van

de CFTR mutatie. CFTR mutaties kunnen onderverdeeld worden in ernstige en milde mutaties. Wanneer je twee ernstige mutaties hebt, spreekt men van een ernstig CFTR genotype. In **hoofdstuk 4** laten we zien dat een ernstig CFTR genotype geassocieerd is met het ontwikkelen van meconium ileus. Andere hebben al eerder laten zien dat ook andere genen (dus niet het CFTR gen) een rol spelen bij de ontwikkeling van meconium ileus. In **hoofdstuk 4** tonen we aan dat ook het CLCA1 gen een rol speelt bij de ontwikkeling van meconium ileus.

Patiënten die een meconium ileus na de geboorte doorgemaakt hebben, lopen op latere leeftijd een groter risico om obstipatie of een DIOS te ontwikkelen (**hoofdstuk 2 en 3**). Echter de grootste groep patiënten met een DIOS of obstipatie heeft geen voorgeschiedenis van meconium ileus. In deze groep lijken vet malabsorptie en een verminderde darmmotiliteit (beweeglijkheid van de darmen) belangrijke risicofactoren te zijn (**hoofdstuk 3**). Vet malabsorptie gecombineerd met een trage darmmotiliteit, zoals vaak gezien wordt bij CF patiënten, zorgen voor een ophoping van ontlasting in de darmen.

Behandeling

De meeste DIOS episoden kunnen conservatief behandeld worden met intensieve laxerende therapie (macrogol), waardoor chirurgie zelden noodzakelijk is (**hoofdstuk 2**).

De voorkeursbehandeling voor obstipatie zijn macrogolen, omdat het minder bijwerkingen heeft als lactulose en tenminste even effectief is. De behandeling van een DIOS is gebaseerd op gezamenlijke klinische ervaring van specialisten, omdat er nog geen vergelijkend onderzoek naar de verschillende behandelingen is gedaan. Het advies is om te starten met macrogolen met of zonder een klysma bij een milde DIOS. Wanneer dit niet effectief is, of bij een ernstige DIOS wordt een hoge dosering macrogol via een neusmaagsonde gegeven. Bij onvoldoende effect is chirurgie vervolgens een optie. Dit is gelukkig maar zelden noodzakelijk.

Omdat DIOS patiënten vaak meer dan één episode doormaken (**hoofdstuk 2**), wordt na een eerste episode de laxerende therapie (macrogol) vaak enige tijd gecontinueerd. Om herhaling te voorkomen, wordt verder geadviseerd om de vet malabsorptie zo goed mogelijk te behandelen. Daarnaast wordt bij patiënten die een longtransplantatie ondergaan pre- en postoperatief gestart met laxerende therapie (macrogol) en wordt er tijdig gestart met optimale voeding en de toediening van alvleesklier-enzymen (lipase).

Medische interventie bij CF

Bij CF patiënten wordt zuurremming (proton pomp remmer of histamine-2-receptor antagonist) toegevoegd wanneer de vetvertering en opname onvoldoende is ondanks optimale toediening van alvleesklier-enzymen (lipase). Gastro-oesofagale reflux (GOR) (terugvloeien van zure maaginhoud in de slokdarm) is een andere reden om zuurremming te starten. Zuurremming zou echter de longfunctie van CF patiënten negatief kunnen beïnvloeden, aangezien zuurremming geassocieerd is met het krijgen van longontsteking bij patiënten zonder CF. In **hoofdstuk 5** laten we zien dat deze angst onterecht is. Zuurremming is veilig om te geven bij kinderen met CF, aangezien het de longfunctie en de kolonisatie van de bacteriën P. aeruginosa en S. aureus niet beïnvloedt.

CF gerelateerde leverziekte ontstaat doordat ook de gal taaier wordt en dus minder vocht bevat. Door de taaie gal verstoppen de kleine galgangen in de lever en krijg je daar ophoping van gal. Omdat dit toxisch (giftig) is leidt dit to vaak tot leverfibrose (er wordt meer bindweefsel in lever gevormd) en in 10% van alle CF patiënten uiteindelijk tot levercirrose, een ernstige verlittekening van de lever. Ursodeoxycholzuur wordt veel voorgeschreven voor deze problemen, omdat dit medicament de gal minder toxisch maakt en dus minder leverbeschadiging zou geven. Gal is echter ook verantwoordelijk voor vetemulsie, wat een essentiële stap voor de vertering van vetten is. Toediening van ursodeoxycholzuur zorgt er mogelijk voor dat de vetemulsie minder goed verloopt en zou dus de vet absorptie en groei negatief kunnen beïnvloeden. In **hoofdstuk 6** laten we zien dat behandeling met ursodeoxycholzuur inderdaad geassocieerd is met een slechtere vet absorptie, groei en longfunctie. Dit is een belangrijke bevinding, omdat nog onduidelijk is of ursodeoxycholzuur wel effectief is voor de behandeling van CF gerelateerde leverziekte. Op korte termijn geeft dit medicament namelijk wel verbetering van de leverfunctie, maar lange termijn studies ontbreken. Desondanks wordt het in elk CF Centrum voorgeschreven. Doordat wij nu laten zien dat het gebruik van ursodeoxycholzuur ook nadelige effecten heeft, is de noodzaak voor een goed opgezette studie naar de effecten van ursodeoxycholzuur bij CF patiënten nog verder toegenomen.

Tenslotte is voeding belangrijk bij de behandeling van CF, omdat in het algemeen een goede voedingtoestand de longfunctie en overleving verbetert. Dit geldt vooral voor ernstig ondervoede CF patiënten; bij hen laten de meeste studies een verbetering in longfunctie zien na voedingsinterventies. Echter over minder ondervoede patiënten (BMI z-score> -2SD) zijn er weinig goede studies gepubliceerd. Voor deze groep kinderen zijn adviezen ten aanzien van voeding gebaseerd op de meningen van specialisten. In **hoofdstuk 7** laten we nu zien dat patiënten met een BMI z-score tussen 0 en -1SD en een slechte longfunctie (FEV_1<100%), een verbetering laten zien van de longfunctie bij

het verhogen van de energie-inname. Dit effect zien we niet bij patiënten met een goede longfunctie (FEV_1<100%). Voor deze groep patiënten is wellicht een minder voortvarend beleid ten aanzien van energie-inname mogelijk. Wij verwachten dat er op korte termijn meer studies gepubliceerd zullen worden over voedingsbeleid bij CF patiënten met een BMI dat normaal of net onder het gemiddelde is, zodat het mogelijk wordt om beleid te maken dat meer afgestemd is op de individuele patiënt.

To conclude

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Curriculum Vitae

Hubert van der Doef was born the 17th of November in 1981 in Nijmegen. After completing high school at the Canisius College in 1999, he studied medicine at the University of Utrecht. During his study years he became interested in pediatrics and pediatric gastroenterology in particular. He has performed several research projects on liver disease, distal intestinal obstruction syndrome and constipation in pediatric cystic fibrosis patients throughout this period. After graduating medical school in September 2006 these research projects were continued in the PhD project "Gastro-intestinal Manifestations of Cystic Fibrosis" under supervision of dr. R.H.J. Houwen and prof. dr. E.E.S. Nieuwenhuis at the department of pediatric gastroenterology of the Wilhelmina's Children's Hospital in Utrecht, which resulted in this thesis. In the spring of 2007, throughout his PhD project, he has performed research at the genetic laboratory of prof. H. Witt at the Charité Hospital in Berlin, Germany for three months. Subsequently, in January 2009 he started the specialist registrar training in pediatrics at the Wilhelmina's Children's Hospital in Utrecht (dr. J. Frenkel and prof dr. E.E.S. Nieuwenhuis).

Hubert van der Doef is married to Linde and has one son: Imre (2008).

Hubert van der Doef werd op 17 november 1981 geboren te Nijmegen. Na het behalen van zijn middelbare schooldiploma aan het Canisius College te Nijmegen in 1999, startte hij met de studie geneeskunde aan de Universiteit Utrecht. Tijdens zijn studie trok de kindergeneeskunde en met name de kindergastro-enterologie zijn aandacht. Hij heeft in deze tijd wetenschappelijk onderzoek verricht naar leverziekten, het distaal intestinaal obstructie syndroom en obstipatie bij kinderen met cystische fibrose. Dit wetenschappelijk onderzoek zette hij na het afronden van zijn studie geneeskunde in september 2006 voort in het promotieonderzoek "Gastro-intestinal Manifestations of Cystic Fibrosis", onder supervisie van dr. R.H.J. Houwen en prof. dr. E.E.S. Nieuwenhuis bij afdeling kinder-MDL van het Wilhelmina Kinderziekenhuis (UMC Utrecht). Hetgeen geresulteerd heeft in dit proefschrift. Tijdens zijn promotieonderzoek heeft hij in het voorjaar van 2007 drie maanden onderzoek gedaan op het genetisch laboratorium van prof. H. Witt in het Charité Ziekenhuis te Berlijn, Duitsland. Vanaf januari 2009 is hij begonnen aan de opleiding tot kinderarts vanuit het Wilhelmina Kinderziekenhuis (UMC Utrecht, opleider dr. J. Frenkel en prof. dr. E.E.S. Nieuwenhuis), waarbij hij zijn perifere stage gelopen heeft in het Gelderse Vallei Ziekenhuis te Ede (opleider dr. J.G. van Enk).

Hubert van der Doef is getrouwd met Linde en heeft een zoon: Imre (2008).

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List of abbreviations

BMI	body mass index
CI	confidence interval
CF	cystic fibrosis
CFA	coefficient of fat absorption
CFTR	cystic fibrosis transmembrane conductance regulator
CFM1	cystic fibrosis modifier 1
DIOS	distal intestinal obstruction syndrome
ESPGHAN	European Society for Pediatric Gastroenterology, Hepatology, and Nutrition
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
GA	gastric acid
GERD	gastroesophageal reflux disease
MEF_{50}	maximum expiratory flow at 50% of forced vital capacity
MI	meconium ileus
MIE	meconium ileus equivalent
$MMEF_{25-75}$	maximal mid-expiratory flow between 25 and 75% of forced vital capacity
NPV	negative predictive value
OL	oral laxative
OR	odds ratio
PA	Pseudomonas aeruginosa
PEG	polyethylene glycol
PERT	pancreatic enzyme replacement therapy
PI	pancreatic insufficiency
PPV	positive predictive value
RDA	recommend daily allowance
RDI	reference daily intake
SD	standard deviation
SA	Staphylococcus aureus
UDCA	ursodeoxycholic acid