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REVIEW

Cystic fibrosis: An update for clinicians. Part 1: Nutrition and gastrointestinal complications

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Abstract

During three decades, the demographics of cystic fibrosis (CF) has undergone a significant change. Advances in nutritional and pulmonary management allow the vast majority of patients reaching adulthood today. With increasing survival, new and previously less common aspects of CF are encountered by the clinician expanding the concept of CF as a multisystem disease. The first part of this two-part review will focus on the nutritional and gastrointestinal aspects of the CF phenotype and outline core principles of diagnosis and care.

Introduction

Cystic fibrosis (CF) is a hereditary condition that is transmitted in an autosomal recessive manner and affects close to 1 in 3000 live births in Australia,[1] with significant variation of incidence within Europe and North America.[2] The diagnosis is based on the recommendations of the US CF Foundation that include characteristic clinical features of the disorder, a positive family history plus an elevated sweat chloride greater or equal to 60 mmol/L and/or two CF-disease causing mutations. These were modified with the introduction of neonatal screening.[3] Before 1970, many of those affected with CF died in infancy with a median age of survival of merely 8 years. Over the past four decades, this has improved substantially with estimates that more than 90% of the CF population born from 1990 onwards will reach the age of 40 years.[4,5] Much of this improved survival in CF is due to centralized and multidisciplinary care both of children and adults with earlier diagnosis through neonatal screening programs[6] and the effective management of lung disease and nutrition.

The aim of this two-part series is to provide the reader with a summary and update of the nutritional issues and luminal gastrointestinal manifestations in the first part followed by pancreatic and hepatobiliary complications in the second.

Epidemiology and pathophysiological aspects

CF is the commonest autosomal recessive inherited disorder in the Western world. The main abnormality is in epithelial chloride and other electrolyte transport that was described in the 1980s[7] and led to the discovery of the "CF gene" in 1989.[8] This referred to mutations in the CF transmembrane conductance regulator (CFTR) gene that was localized on the long arm of chromosome 7. The CFTR protein was later shown to be the cyclic adenosine monophosphate-stimulated chloride channel in epithelial tissue.[9] The key function of CFTR chloride conductivity is its role in hydration of exocrine gland and epithelial surface secretions. The maintenance of mucous fluidity is complex, and CFTR is part of a macromolecular complex ("interactome"[10]), participating in the regulation of other ion channels, receptors, and transporters such as the epithelial Na-channel as well as HCO_3 secretion. Significant progress has been made recently by elucidating the key role of CFTR-driven HCO_3 transport in promoting expansion, hydration, and reducing viscosity of secreted epithelial mucins.[11] Non-epithelial sites of CFTR expression have expanded our understanding of CFTR function beyond pure hydration of epithelial surface layers: CFTR is also expressed in leukocytes, osteoblasts, islet cells, and proximal renal tubules, and thereby involved in pulmonary and intestinal immunoregulation,[12] bone mass accrual,[13] and tubular protein reabsorption.[14] CFTR dysfunction thus leads to a pleiotropic, multisystem clinical syndrome.

Advances in molecular analysis has led to the identification of over 1960 mutations,[15] with only a small percentage of this number proven to cause CF and the majority having unknown functional consequences. Mutations affecting different aspects of CFTR synthesis, intracellular trafficking, degradation, and function have been classified into severe (class 1–3) and mild (class 4–6) mutations (Table 1,[16]). The commonest mutation is referred to as

Table 1 Classification system of mutations and functional effect of mutations on cystic fibrosis transmembrane conductance regulator (CFTR protein)

Risk stratification	Class of mutation	Functional effect of mutation
High risk	I	Defective CFTR production due to premature termination of RNA synthesis.
CFTR genotype	II	Defective CFTR trafficking with protein degradation. Absence of functional CFTR protein on apical cell surface.
	III	Defective CFTR channel regulation with absent function even though CFTR is able to reach the apical cell surface.
Low risk	IV	Decreased CFTR channel conductance even though CFTR is able to reach the apikal cell surface.
CFTR genotype	V	Reduced CFTR synthesis, thus reduced density of functional CFTR in surface membrane.
	VI	Decreased CFTR membrane stability, thus reduced density of functional CFTR in surface membrane.

Adapted from Boyle *et al.*[16]

p.F508del, a class 2 mutation. Of those recorded in international registries with genetic information approximately 90% carry at least one copy of p.F508del, 50% are homozygous for p.F508del, and another 40% are compound heterozygotes with p.F508del and a non-p.F508del mutation.[5,17]

Significant effort has been spent on translating CFTR mutations into clinical symptoms. While there is strong correlation between allelic CFTR variation and degree of pancreatic exocrine dysfunction, this is less pronounced for other gastrointestinal manifestations and pulmonary disease. The variability in disease phenotype of patients with similar genotype suggests a role of genetic modifiers.[18] Understanding and classifying the impact of CFTR mutation on channel function is equally crucial for drug design and therapy, for example a recent breakthrough using VX-770, a "potentiator" of ion function in G551D-CFTR mutations, has been shown to improve lung function and normalize sweat chloride secretion.[19] Developments such as this[20] will further impact on the outcome of this disorder in the future.

The gastrointestinal tract is one of the earliest systems affected in the course of CF and stagnant, unexpanded, viscid mucus, as a consequence of deficient surface fluid and bicarbonate flux is also underlying most CF-related gastrointestinal pathology.[21] In our discussion, we would like to distinguish pancreatic, hepatobiliary, intestinal-luminal complications as well as nutritional aspects of the CF phenotype (Fig. 1).

Nutrition

General nutritional guidance. Nutritional compromise and growth failure are key and early clinical features in untreated CF. There is now general agreement about the importance of nutritional status as an independent determinant of lung function and survival.[22] Aggressive nutritional management is an important aspect of the multidisciplinary approach to CF and has contributed to an impressive increase in median survival age during the last three decades. Timely intervention within the first year of life is crucial as early gain of a nutritional advantage is maintained later in life.[23] Nutritional management recommendations have thus entered all international guidelines.[24–26]

Epidemiology. However, inadequate nutrition remains an ongoing clinical challenge and particularly affects the cohort of pancreatic-insufficient patients and those during infancy and adolescence.[27,28] Results from the Australian CF data registry from 2012 demonstrate that young children aged 2–5 years were normally nourished, but with increasing age, z scores and median centiles for both weight, height, and body mass index (BMI) decrease steadily.[1,17] For adults, there is a gender disadvantage for females with up to 25–30% being underweight compared with 15–17% of males.[5,17] This might partly explain the overall survival disadvantage of female patients with CF.[29]

Pathophysiology. The origin of the nutritional deficit is a result of a chronic mismatch between energy needs and dietary intake with a complex interplay of interdependent factors (Fig. 2).[30–32]

First, anorexia is a frequent issue and can be consequent to a variety of factors such as pulmonary exacerbations and chronic inflammation, micronutrient deficiency (zinc, selenium, iron), sodium depletion, or abdominal discomfort due to gastroesophageal reflux (GOR) or constipation. Disturbed eating attitudes and behaviors, for example as a consequence or alongside depressive episodes and issues of body image during adolescence, can be equally important determinants of reduced dietary intake.[33] Second, inadequate pancreatic enzyme replacement therapy (PERT) mainly because of adherence issues delayed intestinal dissolution of microspheres due to duodenal hyperacidity,[34] lack of adequate intraduodenal bile acid concentrations due to biliary disease, or mucosal dysfunction due to small bowel bacterial overgrowth (SBBO) all contribute to excessive malabsorption of protein and fat and thus to increased fecal energy losses.[35] Third, energy expenditure is frequently increased[36] and is typically negatively correlated with nutritional status and pulmonary function.[30] Expenditure rates are a function of increased respiratory effort and pulmonary inflammation going along with systemic hypercytokinemia. Lastly, nutritional status and energy balance display genetic control dependent[37] and separate[38] of allelic CFTR variation. The CFTR mutation class, for example, independently contributes to an elevated resting energy expenditure that is increased in female and pancreatic insufficient (PI) patients.[37] The overall dysbalance can only partially be compensated by a reduction in physical activity as well as a delay of growth and pubertal development.[31] The vicious circle between protein catabolism and respiratory deterioration will invariably lead to death.

Management. International recommendations[24–26,39] have aimed to standardize assessment and surveillance, diagnostic approaches, and nutritional intervention in CF patients. However, there is no overall consensus with regard to the use of anthropometric indices and their cut-offs, estimation of energy needs, or supplementation regimes reflecting an overall lack of evidence-based data. The aim

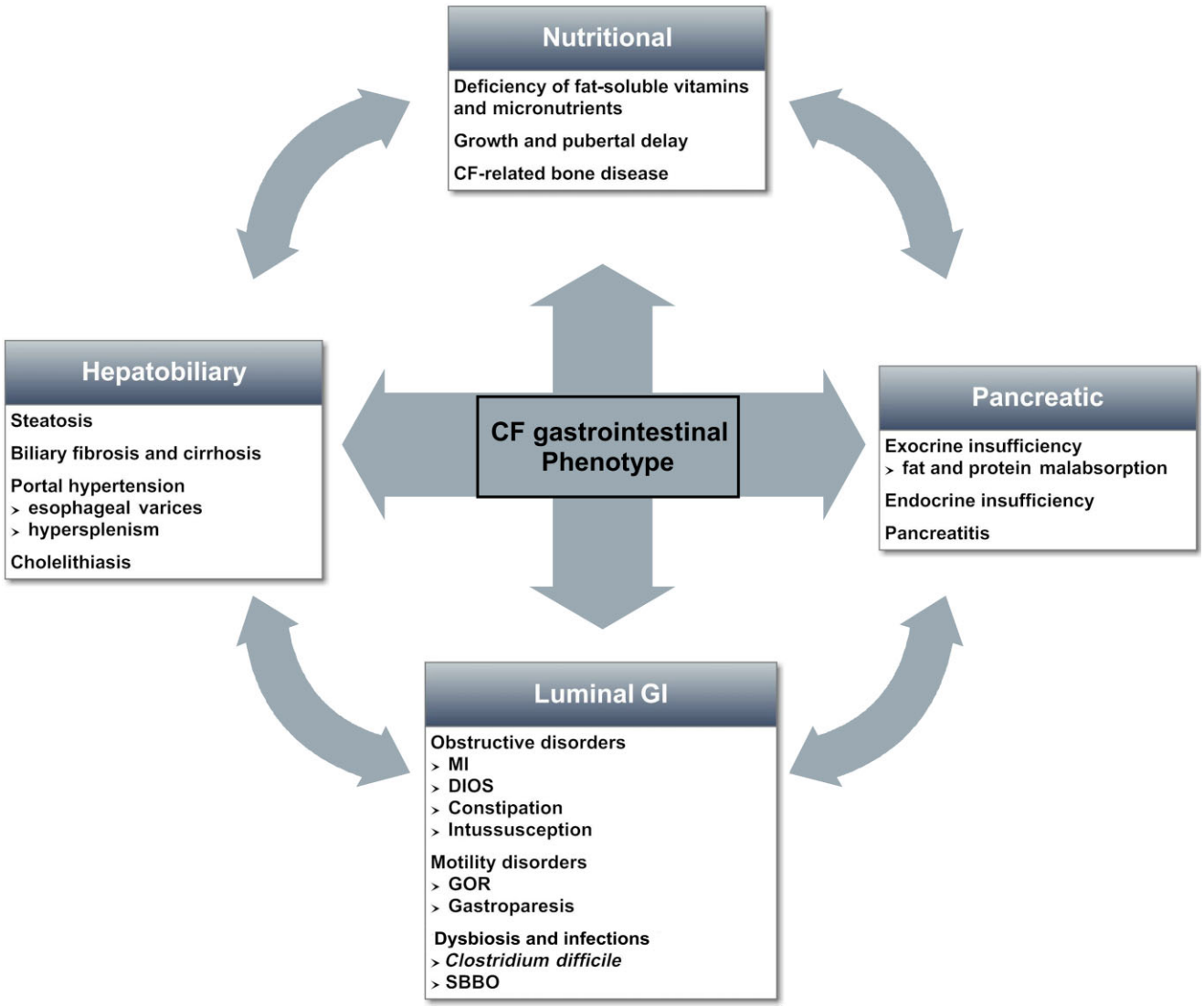

Figure 1 Cystic fibrosis GI phenotype: multisystem disease manifestations within the gastrointestinal tract. CF, cystic fibrosis; DIOS, distal intestinal obstruction syndrome; GI, gastrointestinal; GOR, gastroesophageal reflux; MI, meconium ileus; SBBO, small bowel bacterial overgrowth.

of nutritional management is to secure age-appropriate growth and weight gain with accrual of adequate fat-free mass in order to allow for normal pubertal development and better pulmonary outcome.[40]

Surveillance nutritional assessment aims at early identification of patients who are at risk of nutritional failure. Traditionally, the percent ideal bodyweight has been recommended for assessment of relative weight for height proportion.[41] However, its use is methodologically flawed because of its misclassification of underweight in short and tall children.[42] BMI percentile better reflects and predicts changes in forced expiratory volume.[43] When evaluating height status in children with CF, height centiles should be adjusted for genetic potential.[44] Thus, the following anthropometric indices and targets have been suggested for surveillance:[25]

1 to assess for linear growth in childhood—determination of height for age percentile (aim: ≥ 25, centile);
2 to assess for weight for stature proportion—below 2 years of age weight for length percentile method (aim: ≥ 50. centile by 2 years of age); between 2 and 18 years BMI percentile method (aim: ≥ 50, centile); in adulthood absolute BMI method (aim males ≥ 23 kg/m^2, females ≥ 22 kg/m^2).

Macronutrient requirements. Over 85% of CF patients are PI with associated fat malabsorption of over 7% of the total oral intake.[32] Overall energy requirements can vary considerably between patients and are closely linked to clinical condition including intake, expenditure, and losses. The caloric target is between 110% and 200% of the recommended daily allowance,[25] but individual estimation of energy requirements is mandatory and should take the particular clinical situation and previous growth pattern into account. This can usually be met by establishing an unrestricted high-calorie, high-protein diet. PERT is adjusted to fat

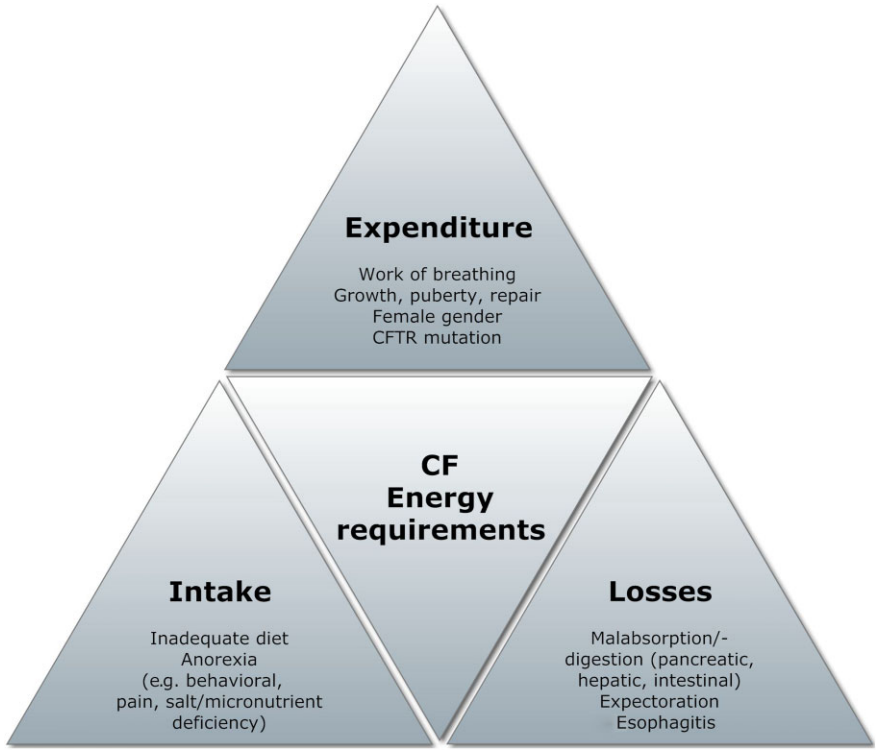

Figure 2 Factors determining energy requirements in cystic fibrosis CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator.

content and not vice-versa. Breast-feeding is associated with better respiratory function[45] and is therefore encouraged in all infants. Solids are introduced at the usual time around 4–6 months.

Fat-soluble vitamins. Both PI and CF-related liver disease are risk factors for fat-soluble vitamin deficiency. There is international agreement that supplementation should be implemented from the time of diagnosis[24,25] as deficiency states are related to deterioration of bone health (Vitamin D), immune function (Vitamin A, D), and inflammation (Vitamin E). The definition of reference ranges, however, is controversial internationally. Target levels for vitamin D are, for example, > 75 nmol/L (30 ng/L) in the US[46] and the UK, and > 50 nmol/L in Australia.[47] There are no robust randomized trials with regard to supplementation regimes. While intermittent stoss oral vitamin D appears to be effective in establishing adequate levels,[48] there is a lack of data confirming safety of such an approach.[49] Current recommendations for vitamin supplementation for Australian CF patients with PI are summarized in Table 2.

Clinical assessment. Regular surveillance of nutritional progress from time of diagnosis is of paramount importance. Monitoring intervals are age-dependent: 1- and 2-weekly measurements until thriving, then 4 weekly visits during the first year of life dependent on metabolic stability followed by 3-monthly anthropometric assessments beyond infancy. The status quo assessment at every clinical visit will correlate anthropometric measurements with a thorough history and symptom assessment.[50] The aim is to determine degree and duration of a possible mismatch between energy needs and intake in order to calculate nutritional catch-up and maintenance. Review of intake, adherence with supplement prescriptions, and adequacy of PERT (for further information, see part 2) are cornerstones of CF surveillance. Times of increased energy requirements during infancy, adolescence, and pregnancy warrant adjustments accordingly. A down-crossing of centiles is a warning sign and should trigger a thorough screen for adherence with PERT first and foremost followed by assessment of CF complications such as pulmonary infection, liver disease, or diabetes mellitus that may be contributing to poor weight gain or loss.

Table 2 Fat-soluble vitamin supplementation in the pancreatic insufficient cystic fibrosis patient[26]

Age	Routine dosing fat-soluble vitamins			
	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (IU)	Vitamin K (μg)
0–12 months	1500–2000	400–1000	40–80	150–500
1–3 years	1500–2500	400–1000	50–150	150–500
4–7 years	2500–5000	400–1000	150–300	300–500
8–18 years	2500–5000	400–1000	150–500	300–500
Adults	2500–5000	400–1000	150–500	300–500

Biochemical assessment. Deficiencies of fat-soluble vitamins are well documented in PI patients of all ages and particularly in those who are non-adherent with PERT therapy. Routine assessment of fat-soluble vitamin levels, also in the pancreatic sufficient (PS) CF patient, is recommended annually; a 3-monthly check should occur after a change in dose. Declining levels in the PS patient can be an early indicator of a change in phenotype with evolving exocrine PI. It is important, however, to recognize the limitations of the available micronutrient monitoring strategies as the

Table 3 Nutritional intervention criteria in CF patients (particular caution should be applied in all cases of short stature, adjusted height < 5. centile)

Category	Criteria indicating nutritional intervention			
	< 2 years	2–18 years	> 18 years	Interventions
At target = Routine nutritional care	Weight/length centile > 50.C (aim to achieve until 2 years of age) OR Weight and length centile-parallel and within 2 centile bands of each other AND No weight loss	BMI ≥ 50.C AND No weight loss	BMI ≥ 23 kg/m^2 (male) BMI ≥ 22 kg/m^2 (female) AND No recent weight loss	Routine nutritional care and surveillance, preventative counseling (adherence, warning signs)
At risk = non-invasive nutritional intervention	Weight/length centile 10.–25.C OR Weight or length centile plateau or any weight loss	BMI 10.–50.C OR Weight loss over 1–3 months OR Weight gain plateau over 2–4 months	BMI 20 to < 23 kg/m^2 (male) BMI 20 to < 22 kg/m^2 (female) OR 5% weight loss 2/12	Evaluation of adherence medical evaluation re CF complications Non-invasive interventions (goal-setting re dietary intake, increase energy density, oral supplements) Discussion next line options and time lines
At failure = invasive nutritional intervention	Weight/length centile < 10.C OR Weight > 2 bands below lengths centile OR Failure of non-invasive measures to improve nutritional status	BMI < 10.C OR Weight loss ≥ 2 centile bands OR No weight gain over 6 months OR Failure of non-invasive measures to improve nutritional status	BMI < 19.0 kg/m^2 (USA) BMI < 18.5 kg/m^2 (Europe) OR 5% weight loss over 2 months despite non-invasive measure to improve nutritional status	Further nutritional and medical and psychological evaluation re factors affecting nutritional state Consider introduction of enteral feeding (nasogastric, gastrostomy)

Adapted from[24–26,42,53]

BMI, body mass index; CF, cystic fibrosis.

biomarkers are influenced by inflammatory response as in the case of vitamin A,[51] zinc, and selenium, or have low sensitivity in the case of prothrombin time as a surrogate for vitamin K depletion. The role and clinical significance of more sensitive markers of deficiency such as PIVKA-II or undercarboxylated osteocalcin are under evaluation.[52]

Importantly, in hot climates or following exercise, rates of sweating are high and sodium losses substantial and require to be replaced. This requirement increases with the surface area of the patient and hence age. Adequacy of replacement can be measured using a spot-urine sodium determination (target: > 10 mmol/L). Other micronutrients that are affected adversely by CF include iron, zinc, and vitamin B_{12}, and levels should be included as part of an annual review.[32]

Nutritional intervention. Longitudinal monitoring of anthropometry and lung function, clinical symptoms, and biochemical data aid in categorizing the individual nutrition risk and in establishing a staged nutritional intervention plan (Table 3). European[24] and American[25] consensus guidelines use the same indices; however, different individual cut-offs are applied to define nutritional failure. Non-invasive nutritional intervention includes education and behavioral therapy, increasing the energy density of food and oral supplements for patients at risk of nutritional failure. Invasive interventions refer to, for example, enteral overnight nutrition through nasogastric tube or gastrostomy, and are usually reserved for patients with nutritional failure. Large-scale, controlled, prospective data regarding enteral feeding strategies are missing.[54,55] Registry data and systematic reviews seem to support the early use of nasogastric and gastrostomy feeding.[56] Timing of supplementation should be adjusted to the regular meals and should not be used to replace them; overnight feeds are the preferred method and should cover 30–50% of the estimated requirements.[57] Institution of supplemental feeding should not be delayed as the results are usually disappointing in those with advanced nutritional compromise.[31] Weight targets and malnutrition cut-offs in patients with CF are summarized in (Table 3).

CF-related bone disease. CF-related bone disease has emerged as an important feature of the CF phenotype and was first described in 1979.[58] Inadequate accrual of bone mass originates in childhood. Cross-sectional studies suggest the prevalence of low bone mineral density (BMD) to be close to 50% in children; osteopenia is reported in up to 85% of adults with advanced disease.[59]

The evolution of low BMD in CF is multifactorial. CF leads to an imbalance between bone formation and absorption by disrupting the complex interplay of caloric intake, vitamin and micronutrient availability, physical activity, and pubertal development.

Systemic hypercytokinemia because of chronic suppurative pulmonary disease may play an additional role during this process.[58] Pro-inflammatory cytokines stimulate osteoclastogenesis, upregulate the parathyroid calcium-sensing receptor with subsequent hyperparathyroidism, and increase renal and intestinal calcium and phosphate loss.[60] More recently, it has been demonstrated that bone formation is under genetic control of CFTR; CFTR inactivation in osteoblasts leads to a decrease in osteoblast differentiation and osteoprotegerin synthesis.[61]

Early identification of reduced BMD (g/cm^2) and bone mineral content (BMC [g]) is important in order to facilitate timely intervention. Dual-energy X-ray absorptiometry is the diagnostic tool of choice with lumbar spine and total body (children), and lumbar spine and proximal hip (adults) being the most accurate and reproducible skeletal measurement sites.[58,62] Results are reported as standard deviation (Z) scores (relating the patient's BMD to an age, gender-matched healthy population) for children or as T scores (relating Z score with peak adult BMD) for adults.

International consensus guidelines have recently been reviewed aiming for consensus regarding timing of first screening and subsequent screening intervals.[58,62] As 90% of bone mass accrual occurs until the end of puberty, a baseline assessment during the prepubertal phase at around 8–10 years of age appears warranted to allow timely intervention before adolescent growth spurt. Those with normal BMD (Z score > -1) warrant a repeat study every 5 years; those with Z scores of -2 to -1 every 2 years; those with Z scores < -2 or a history of low-impact fractures every year.

Evidence-based treatment data are scarce. In view of its complex pathophysiology, a multifaceted, preventative approach is necessary with an aim to attain and maintain normal BMD. This includes aggressive nutritional management, monitoring of vitamin D and calcium intake and supplementation as well as encouragement of physical exercise. Pharmacological intervention with growth hormone as an anabolic agent has shown promising results, particularly in the prepubertal group of patients with low BMD, as it improves intermediate outcome figures such as pulmonary function, height, weight, and BMC. Long-term data with regard to glucose tolerance and diabetes as well as timing and duration are still missing.[59] The experience with bisphosphonates is limited to adult patients with CF and has shown promising results.[63] Longitudinal studies with larger populations are necessary to determine its effect on fracture rate and survival.

Luminal disease

Disorders of motility

Gastrooesophageal reflux. GOR disease is a common phenomenon in patients with CF and more frequent compared with healthy controls. The prevalence has been reported to be 25% in infants, and between 25% and 85% in children and adults, respectively.[64,65] In addition to an increased number of transient lower esophageal sphincter relaxations, secondary pathophysiological mechanisms have been suggested, such as increased gastroesophageal pressure gradient because of lower inspiratory intrathoracic pressure,[66] chronic cough, and chest physiotherapy. Synchronous monitoring of esophageal pH, impedance, and manometry however confirmed that reflux indeed occurs mostly as a primary phenomenon and not secondarily to excessive cough.[65]

The clinical relevance of GOR in CF is controversial. A significant proportion of up to 40% occurs silently, not causing any immediate symptoms.[65] GOR can lead to esophagitis, stricturing disease, and hypoproteinemia, and may contribute to nutritional failure in CF (see Fig. 2). The impact of GOR on progression of CF airway disease is still controversial.[65] There is increasing evidence, however, that airway exposure to bile acids, pepsin, and acid due to microaspiration may play an important role as a non-alloimmunogenic factor in the evolution of chronic graft dysfunction and bronchiolitis obliterans syndrome in lung transplantation.[67]

With a view to treatment, there is currently no evidence to support a different approach to GOR compared with non-CF patients. Conservative treatment with a proton-pump inhibitor is the usual first choice of treatment. However, the clinician needs to be aware that there is an increased risk of gastrointestinal bacterial overgrowth[68] and possible pulmonary infections as seen in the non-CF population.[69] Long-term use may adversely affect bone health in susceptible populations.[70] Fundoplication has been reported to be beneficial in decreasing reflux-cough sequence and respiratory exacerbations.[71] Yet, morbidity of the procedure was high in a group of pediatric patients with CF.[72] Fundoplication may therefore be reserved for high-risk patients such as the group of lung transplant candidates where it appears to decrease the postoperative decline in lung function parameters.[73] Further studies to reliably diagnose microaspirations following GOR, for example through biomarkers, to investigate the mechanism and pathophysiology of lung injury and to sufficiently prove efficiency of available treatment are urgently needed.[74]

Gastroparesis. The effect of CF on gastrointestinal motility is complex and only incompletely understood. Medication such as antibiotics, mucosal inflammation,[75] as well as SBBO[76] all impact on gastrointestinal transit. Normal, rapid as well as delayed gastric emptying consistent with gastroparesis has been reported in children and adults with CF. These inconsistent results can be explained by the multifactorial pathogenesis and methodological issues of published studies, such as the small size of study population and the implemented diagnostic approach. Scintigraphic studies are still considered as the diagnostic gold standard.[77] Gastroparesis has been previously described as a common phenomenon in the group of lung-transplanted patients and may be associated with the increased incidence of GOR and its pulmonary complications.[64] Hence, early investigation and intervention with standard dietetic and prokinetic therapy, review of PERT compliance, and good glycemic control in diabetic patients will be warranted.

Disorders related to intestinal dysbiosis, infection, and inflammation. The gastrointestinal tract is a tightly regulated ecosystem maintained by normal-pattern motility, an intact epithelial barrier and secretion of well-hydrated, expandable mucins. This provides the matrix for the symbiotic interaction and homoeostasis of commensal bacteria and host immune system, and controls bacterial density, composition, and location in the proximal and distal intestine.[21] In CF, disruption

of this unique milieu, for example by delayed intestinal transit, luminal hyperacidity, and frequent antibiotic use, can result in abnormal microbial colonization and infection of small bowel or colon. Animal studies suggest a vicious circle involving abnormal mucus, exaggerated bowel dysmotility, and dysbiosis.[76]

Small bowel bacterial overgrowth. SBBO is defined as gram-negative, colonic-type bacterial colonization of the small bowel. The prevalence is considered to be high in CF patients.[78] However, no adequately validated diagnostic test for SBBO is currently available.[79] Culture of duodenal and jejunal fluid is often considered to be the diagnostic gold standard but is methodologically flawed due to lack of standardization and difficulties avoiding contamination with upper gastrointestinal flora during small bowel endoscopic access. Breath testing is commonly used as a non-invasive diagnostic method and uses the capability of bacteria to ferment carbohydrates, subsequently releasing H_2 and methane.[80] Specific CF-related issues such as delayed gastric emptying and intestinal transit as well as chronic antibiotic use may compromise the validity of the test. Overall, sensitivity and specificity are poor.[80] Therefore, in clinical practice, a treat-outcome strategy is frequently applied using a clinical response to treatment as a surrogate marker for the diagnosis of pre-existing SBBO.

The clinical phenotype of SBBO in CF overlaps with symptoms resulting from PI. Malabsorption with weight loss, diarrhea, and abdominal distension may result from bacterial deconjugation of bile acids, enterotoxic mucosal injury, and inflammation.[21] SBBO should therefore be considered in the situation of ongoing abdominal symptoms and persistent fat malabsorption refractory to increments in PERT.[35]

Currently, no evidence-based treatment guidelines are available for SBBO in CF and management remains primarily empiric. Antibiotics covering gram-negative, anaerobic bacteria should be preferentially used. The available data includes absorbable (e.g. amoxicillin-clavulanate, metronidazole, fluorochinolones) as well as non-absorbable antibiotics (e.g. aminoglycosides such as neomycin, gentamycin, rifamyxin). In view of high recurrence rates after successful treatment, rotating antibiotic treatment regimes with different oral agents have been proposed.[81] These regimes, however, carry a high risk of bacterial resistance, and *Clostridium difficile* (CD) infection and efficacy remains to be validated. There is currently still insufficient evidence suggesting the use of probiotics.

Colonic dysbiosis. Similar to small-intestinal dysbiosis, the perturbation of the large-intestine microbiome in CF has attracted more attention in recent years as the introduction of culture-independent analytical methodologies, such as species-specific polymerase chain reaction, 16S rRNA gene pyrosequencing, and phylogenetic microarrays, allows detailed profiling of microbial communities.[82] Feces is frequently used as an easily accessible surrogate sample to investigate large-bowel microbial colonization. CF has a strong impact on colonic microbiota diversity leading to qualitative and quantitative compositional changes shaped by disease severity.[83] Important discriminants of dysbiosis in CF are a significant reduction in species richness and evenness, a decrease in relative abundance of beneficial species involved (*Lactobacillus* sp., Bifidobacteria, *Faecalibacteria* sp.) and the increase in non-protective or even harmful strains (*Escherichia coli*, *Bacteroides fragilis*).[84–86]

The important role of colonic microbiota in health and disease is increasingly appreciated as it is involved in immediate intestinal physiology, the evolution and shaping of mucosa-associated and systemic immune system, and the maintenance of metabolic homoeostasis. Interestingly, abnormal colonization of the CF intestine during infancy precedes the development and changes of airway microbiota.[87] The microbial communities of large and small intestine, and respiratory tract may therefore need to be seen as cross-talking members of an integrated whole-body microbial ecosystem.[88] Manipulation of abnormal intestinal microbiota in CF by normalizing chloride channel function and fluid flux,[89] dietary intervention,[87] or probiotic supplementation[90] may thus become relevant for treatment pathways of CF respiratory disease in future.

Clostridium difficile infection. Since 1978, CD has been recognized as cause of antibiotic-associated diarrhea contributing to over 90% of pseudomembranous colitis cases.[91] During the past two decades, incidence as well as severity of disease phenotype has dramatically increased.[92] Highly virulent strains with antibiotic resistence and increased toxin production have emerged. Gastrointestinal dysbiosis due to broad-spectrum antibacterial therapy with disruption of colonization resistence, use of proton pump inhibitors, hospitalization, and immunodeficiency—primary or secondary to immunosuppression or severe underlying illnesses—are important risk factors for this infection.[92]

Colonization with toxicogenic CD occurs in up to 50% of CF patients compared with 3% in the healthy adult population.[93] Progression to symptomatic infection is however less frequently seen, possibly as a consequence of inactivation of CFTR-related Cl-secretion, and can atypically present with symptoms of constipation or distal intestinal obstruction syndrome (DIOS).[94] Instead, after lung transplantation, the incidence of symptomatic infection increases dramatically with 30% affected compared with 1–2% of all hospitalized patients.[95] The disease phenotype is frequently complicated and maybe blunted by steroid and immunosuppressive treatment.[96]

International treatment guidelines have been recently revised.[97,98] Treatment is staged and adapted to disease severity and number of recurrences that can occur in up to one third of patients. Preceding antibiotic treatment should be reviewed and narrowed, or discontinued, if possible. Oral metronidazole is still the first choice for first-episode mild diarrheal disease without systemic symptoms. Severe and recurrent disease warrants oral, nasogastric, or rectal vancomycin. If there is significant abdominal distension, there may be an advantage of combining this with intravenous metronidazole that reaches the intestinal lumen through biliary clearance and intestinal exsudation. Fidaxomicin, a first-in-class macrocyclic antibiotic, may be a promising alternative for non-life-threatening, recurrent infections as it seems to carry less risk of commensal colonic microbiota disruption and transmission of multiresistent enterococci (vancomycin-resistant enterococci).[98] Fecal transplantation has recently been shown to be a therapeutic option for recurrent clostridium difficile infection.[99] Surgery is reserved for complicated disease.

Intestinal inflammation and associated gastrointestinal disorders. There is increasing evidence to support the presence

of chronic inflammation to be an important feature of the CF intestine. Capsule and conventional endoscopy allow to document macroscopic and histological inflammatory mucosal changes;[100,101] raised fecal markers of inflammation provide indirect evidence of intestinal inflammation.[102] The picture of the underlying complex pathophysiology is however still incomplete. The evolution of mucosa-associated and systemic immune homoeostasis with appropriate innate and adaptive immune responses necessitates gut colonization with a diverse and balanced microbiota after birth.[103] The altered luminal environment in CF interferes with this process from early on, leading to dysbiosis and thus disrupting the tolerogenic host-immunity/gut microbiota partnership. In addition, CFTR inactivation itself triggers innate and adaptive immune dysfunction and pro-inflammatory responses in CFTR-deficient cells.[12] An impaired intestinal barrier function, as demonstrated in CFTR-deficient mice,[104] may further increase the antigenic load and fuel the pro-inflammatory momentum.

Importantly, other inflammatory enteropathies, such as celiac[105,106] and Crohn's disease,[21,107] are more prevalent in CF patients (3× and 17×, respectively, *vs* prevalence of celiac and Crohn's disease in general population). All three conditions show similarities in pathophysiology with view to intestinal inflammation, for example the disruption of microbiome and intestinal epithelial barrier function.[108] As neither particular abdominal symptoms nor laboratory tests[109] help to discriminate these from CF, it is important to perform diagnostic endoscopy early in those with CF who do not respond to optimized treatment.

Disorders of intestinal obstruction. Meconium ileus (MI), DIOS, and constipation represent a group of clinical syndromes with a variable degree of intestinal obstruction on the background of increased mucous viscosity and slow intestinal transit.[110] Beyond mucus dehydration, there might also be a contributory role of transmural inflammation, fibrosis, and intestinal dysmotility.[111]

Meconium ileus. MI is defined as early-onset neonatal intestinal obstruction at the level of the terminal ileum caused by inspissated intraluminal meconium. There is a spectrum of underlying pathologies, with CF still representing the majority of cases. Usually between 10% and 20% of all children with diagnosis of CF will present with MI during the neonatal period. There is a clear correlation with severe CFTR mutations[112] and PI, but linkage and association studies also suggest contribution of genetic modifiers beyond allelic CFTR variation.[18] Clinically, two forms of MI are distinguished: simple obstruction with failure to pass meconium during the first 24–48 h after birth and complex disease in 40% of patients, where there is additional clinical evidence of perforation, meconium peritonitis, or volvulus.[113] Available treatment experience mostly relies on small-size, single-centre-based cohorts. Enema reductions are commonly used in simple MI. Diluted gastrografin seems to be most efficacious with success rates of up to 40%.[113] Failure to relieve obstruction or complex MI necessitates surgical intervention such as primary anastomosis after resection or fashioning of a double-barreled stoma. Long-term nutritional and pulmonary outcome and survival of CF patients with and without a history of MI is comparable.[114]

Distal intestinal obstruction syndrome. DIOS is defined as a situation of acute fecal obstruction of the ileocecum beyond the neonatal period. It is characterized by a short history of colicky abdominal pain and a palpable mass in the right lower quadrant of the abdomen and can present as a partial or complete obstruction. The majority of these patients are PI, and it is seen in patients with genotypes associated with severe phenotypes.[115] The disease is age-related with an increase of incidence and life-time prevalence during adulthood.[110] Further risk factors are a previous history of MI and major surgery such as lung transplantation.[115] Treatment is usually medical for both complete and incomplete obstruction using polyethylene glycol solutions, oral laxative, or gastrografin enemas.[69] Surgical management remains the last resort.

Constipation. Constipation is very common in the CF population affecting nearly half of pediatric[116] and the majority of adult patients. Similarly to DIOS, it is associated with PI. Patients present with hypogastric abdominal pain or distension.

Clinical complaints usually include a long-standing history of increased stool consistency and/or decreased frequency. Fecal material will be predominantly palpable in the left lower quadrant. Abdominal radiography may assist in distinguishing constipation from DIOS with stool distributed through the entire colon rather than just the ileocecal region.[117] Treatment is with oral laxative agents.

Intussusception. Intussusception is relatively common and occurs with a prevalence of 1% in the CF population.[118] It is an important differential diagnosis in pediatric and adult CF patients with acute or recurrent abdominal pain. Mean age of patients is older than in idiopathic cases. The clinical phenotype in adults is frequently more insidious with recurrent abdominal pain than in children who typically present acutely with an acute abdomen. It is usually ileocolonic, but cases of appendiceal or small bowel intussusception have been reported. Inspissated ileocecal feces, localized areas of dysmotility and inflammation, as well as appendiceal mucocele or inversion play an important part in pathogenesis and can act as lead points. Abdominal ultrasound and potentially computed tomography will be important to distinguish other important clinical entities such as DIOS, volvulus, and appendicitis (Table 4). In the absence of complications, enema reduction is the treatment modality of choice. Recurrence rates are high.[117]

Table 4 Differential diagnosis of abdominal pain in cystic fibrosis

Abdominal pain in cystic fibrosis	
Cause	Condition
Malabsorptive	Insufficient PERT
Obstructive	Constipation
	DIOS
	Intussusception
	Cholelithiasis
Inflammatory	Appendicitis
Infectious	*Clostridium difficile*
	Small bowel bacterial overgrowth
Malign	Gastrointestinal cancer

DIOS, distal intestinal obstruction syndrome; PERT, pancreas enzyme replacement therapy.

Appendicitis. While the overall incidence of appendicitis is lower within the CF population, its clinical picture can mimick one of the mentioned other obstructive conditions (Table 4). Diagnosis is frequently delayed, possibly masked as a consequence of antibiotic use, and complications are more common.[119]

Gastrointestinal cancer. CF is now recognized to carry a significantly increased risk of gastrointestinal tumors including esophageal, gastric, hepatobilary, small intestinal, and colonic, particularly after transplantation.[120,121] The exact pathogenesis of malignancy is unclear but maybe related to long-standing chronic intestinal inflammation with an abnormal intestinal epithelial proliferation rate.[122] Decreased expression of hydroxyprostaglandin dehydrogenase in the CF intestine, a tumor suppressor gene involved in prostaglandin degradation[21] or promotion of epithelial-to-mesenchymal transition[123] are potential pathophysiological candidates. Particular screening or monitoring recommendations have not entered any CF treatment guidelines as yet. Nevertheless, should gastrointestinal malignancy be included in the differential diagnosis of CF patients with otherwise unexplainable abdominal symptoms.

Conclusion and summary

The first part of this series highlights the importance of nutrition in the outcome of children and adults with CF and summarizes the importance of luminal gastrointestinal complications as a major contributor to the morbidity of CF. Last, but not least, is the now-recognized issue of malignancy in these patients that is likely to be of clinical importance as survival improves. There is an imperative for multicentre collaboration to establish evidence-based guidelines for the clinical management of CF-related gastrointestinal disease and nutrition. In the second part of this series, pancreatic and hepatobiliary issues related to CF will be discussed.

References

1 Bell SC, Bye PTP, Cooper PJ *et al*. Cystic fibrosis in Australia, 2009: results from a data registry. *Med. J. Aust.* 2011; **195**: 396–400.
2 Salvatore D, Buzzetti R, Baldo E *et al*. An overview of international literature from cystic fibrosis registries. Part 3. Disease incidence, genotype/phenotype correlation, microbiology, pregnancy, clinical complications, lung transplantation, and miscellanea. *J. Cyst. Fibros.* 2011; **10**: 71–85.
3 Farrell PM, Rosenstein BJ, White TB *et al*. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. *J. Pediatr.* 2008; **153**: S4–14.
4 Cystic Fibrosis Foundation. *Cystic Fibrosis Foundation Patient Registry: 2012 annual data report*. 2013. Cited 28 Feb 2014. Available from URL: http://www.cff.org/UploadedFiles/aboutCFFoundation/AnnualReport/2012-Annual-Report.pdf
5 Cystic Fibrosis Canada. *2012 Annual Report*. 2013. Cited 28 Feb 2014. Available from URL: http://www.cysticfibrosis.ca/wpcontent/uploads/2013/10/CFC_AnnualReport2012_EN_FINAL_singles.pdf
6 Lim M, Wallis C, Price JF *et al*. Diagnosis of cystic fibrosis in London and South East England before and after the introduction of newborn screening. *Arch. Dis. Child.* 2013; **99**: 197–202.
7 Gaskin KJ, Durie PR, Corey M, Wei P, Forstner GG. Evidence for a primary defect of pancreatic HCO3-secretion in cystic fibrosis. *Pediatr. Res.* 1982; **16**: 554–7.
8 Kerem BR, Rommens JM, Buchanan JA *et al*. Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989; **245**: 1073–80.
9 Bear CE, Li CH, Kartner N *et al*. Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). *Cell* 1992; **68**: 809–18.
10 Wu Y, Wang S, Li C. In vitro analysis of PDZ-dependent CFTR macromolecular signaling complexes. *J. Vis. Exp.* 2012; (66): e4091. doi: 10.3791/4091.
11 Yang N, Garcia MA, Quinton PM. Normal mucus formation requires cAMP-dependent HCO3- secretion and Ca2+-mediated mucin exocytosis. *J. Physiol.* 2013; **591**: 4581–93.
12 Gifford AM, Chalmers JD. The role of neutrophils in cystic fibrosis. *Curr. Opin. Hematol.* 2014; **21**: 16–22.
13 Le Henaff C, Gimenez A, Hay E, Marty C, Marie P, Jacquot J. The F508del mutation in cystic fibrosis transmembrane conductance regulator gene impacts bone formation. *Am. J. Pathol.* 2012; **180**: 2068–75.
14 Jouret F, Courtoy PJ, Devuyst O. Segmental and subcellular distribution of CFTR in the kidney. *Methods Mol. Biol.* 2011; **741**: 285–99.
15 *Cystic Fibrosis Mutation Database*. Cited 28 Feb 2014. Available from URL: http://www.genet.sickkids.on.ca/cftr/Home.html
16 Boyle MP, De Boeck K. A new era in the treatment of cystic fibrosis: correction of the underlying CFTR defect. *Lancet Respir. Med.* 2013; **1**: 158–63.
17 Cystic Fibrosis Australia. *Cystic Fibrosis in Australia 2012: 15th Annual Report from the Australian Cystic Fibrosis Data Registry*. 2013. Cited 28 Feb 2014. Available from URL: http://www.cysticfibrosis.org.au/media/wysiwyg/CF-Australia/medicaldocuments/ACFDR_2012/ACFDR_Annual_Report_2012r.pdf
18 Knowles MR, Drumm M. The influence of genetics on cystic fibrosis phenotypes. *Cold Spring Harb. Perspect. Med.* 2012; **2**: a009548.
19 Accurso FJ, Rowe SM, Clancy JP *et al*. Effect of VX-770 in persons with cystic fibrosis and the G551D-CFTR mutation. *N. Engl. J. Med.* 2010; **363**: 1991–2003.
20 Ong T, Ramsey BW. Modifying disease in cystic fibrosis: current and future therapies on the horizon. *Curr. Opin. Pulm. Med.* 2013; **19**: 645–51.
21 De Lisle RC, Borowitz D. The cystic fibrosis intestine. *Cold Spring Harb. Perspect. Med.* 2013; **3**: a009753.
22 Yen EH, Quinton H, Borowitz D. Better nutritional status in early childhood is associated with improved clinical outcomes and survival in patients with cystic fibrosis. *J. Pediatr.* 2013; **162**: 530–5 e1.
23 Farrell PM, Kosorok MR, Rock MJ *et al*. Early diagnosis of cystic fibrosis through neonatal screening prevents severe malnutrition and improves long-term growth. Wisconsin Cystic Fibrosis Neonatal Screening Study Group. *Pediatrics* 2001; **107**: 1–13.
24 Sinaasappel M, Stern M, Littlewood J *et al*. Nutrition in patients with cystic fibrosis: a European Consensus. *J. Cyst. Fibros.* 2002; **1**: 51–75.
25 Stallings VA, Stark LJ, Robinson KA, Feranchak AP, Quinton H. Evidence-based practice recommendations for nutrition-related management of children and adults with cystic fibrosis and pancreatic insufficiency: results of a systematic review. *J. Am. Diet. Assoc.* 2008; **108**: 832–9.
26 Dieticians Associations of Australia—Cystic Fibrosis Interest Group. *Australasian Clinical Practice Guideline for Nutrition in Cystic Fibrosis*. 2006. Cited 28 Feb 2014. Available from URL: http://daa.asn.au/wp-content/uploads/2012/09/Guidelines_CF-Final.pdf
27 Lucidi V, Alghisi F, Raia V *et al*. Growth assessment of paediatric patients with CF comparing different auxologic indicators: a

multicentre Italian study. *J. Pediatr. Gastroenterol. Nutr.* 2009; **49**: 335–42.
28 Powers SW, Patton SR, Byars KC *et al*. Caloric intake and eating behavior in infants and toddlers with cystic fibrosis. *Pediatrics* 2002; **109**: E75-5.
29 Mehta G, Macek M Jr, Mehta A. Cystic fibrosis across Europe: EuroCareCF analysis of demographic data from 35 countries. *J. Cyst. Fibros.* 2010; **9** (Suppl. 2): S5–21.
30 Pencharz PB, Durie PR. Pathogenesis of malnutrition in cystic fibrosis, and its treatment. *Clin. Nutr.* 2000; **19**: 387–94.
31 Dodge JA, Turck D. Cystic fibrosis: nutritional consequences and management. *Best Pract. Res. Clin. Gastroenterol.* 2006; **20**: 531–46.
32 Gaskin KJ. Nutritional care in children with cystic fibrosis: are our patients becoming better? *Eur. J. Clin. Nutr.* 2013; **67**: 558–64.
33 Morton AM. Symposium 6: young people, artificial nutrition and transitional care. The nutritional challenges of the young adult with cystic fibrosis: transition. *Proc. Nutr. Soc.* 2009; **68**: 430–40.
34 Gelfond D, Ma C, Semler J, Borowitz D. Intestinal pH and gastrointestinal transit profiles in cystic fibrosis patients measured by wireless motility capsule. *Dig. Dis. Sci.* 2012; **11**: 333–42.
35 Wouthuyzen-Bakker M, Bodewes FA, Verkade HJ. Persistent fat malabsorption in cystic fibrosis; lessons from patients and mice. *J. Cyst. Fibros.* 2011; **10**: 150–8.
36 Kalnins D, Pencharz PB, Grasemann H, Solomon M. Energy expenditure and nutritional status in pediatric patients before and after lung transplantation. *J. Pediatr.* 2013; **163**: 1500–2.
37 Magoffin A, Allen JR, McCauley J *et al*. Longitudinal analysis of resting energy expenditure in patients with cystic fibrosis. *J. Pediatr.* 2008; **152**: 703–8.
38 Bradley GM, Blackman SM, Watson CP, Doshi VK, Cutting GR. Genetic modifiers of nutritional status in cystic fibrosis. *Am. J. Clin. Nutr.* 2012; **96**: 1299–308.
39 Sermet-Gaudelus I, Mayell SJ, Southern KW. Guidelines on the early management of infants diagnosed with cystic fibrosis following newborn screening. *J. Cyst. Fibros.* 2010; **9**: 323–9.
40 Stephenson AL, Mannik LA, Walsh S *et al*. Longitudinal trends in nutritional status and the relation between lung function and BMI in cystic fibrosis: a population-based cohort study. *Am. J. Clin. Nutr.* 2013; **97**: 872–7.
41 Borowitz D, Baker RD, Stallings V. Consensus report on nutrition for pediatric patients with cystic fibrosis. *J. Pediatr. Gastroenterol. Nutr.* 2002; **35**: 246–59.
42 Lai HJ. Classification of nutritional status in cystic fibrosis. *Curr. Opin. Pulm. Med.* 2006; **12**: 422–7.
43 Zhang Z, Lai HJ. Comparison of the use of body mass index percentiles and percentage of ideal body weight to screen for malnutrition in children with cystic fibrosis. *Am. J. Clin. Nutr.* 2004; **80**: 982–91.
44 Zhang Z, Shoff SM, Lai HJ. Incorporating genetic potential when evaluating stature in children with cystic fibrosis. *J. Cyst. Fibros.* 2010; **9**: 135–42.
45 Colombo C, Costantini D, Zazzeron L *et al*. Benefits of breastfeeding in cystic fibrosis: a single-centre follow-up survey. *Acta Paediatr.* 2007; **96**: 1228–32.
46 Tangpricha V, Kelly A, Stephenson A *et al*. An update on the screening, diagnosis, management, and treatment of vitamin D deficiency in individuals with cystic fibrosis: evidence-based recommendations from the Cystic Fibrosis Foundation. *J. Clin. Endocrinol. Metab.* 2012; **97**: 1082–93.
47 Paxton GA, Teale GR, Nowson CA *et al*. Vitamin D and health in pregnancy, infants, children and adolescents in Australia and New Zealand: a position statement. *Med. J. Aust.* 2013; **198**: 142–3.
48 Shepherd D, Belessis Y, Katz T, Morton J, Field P, Jaffe A. Single high-dose oral vitamin D(3) (stoss) therapy—a solution to vitamin D deficiency in children with cystic fibrosis? *J. Cyst. Fibros.* 2013; **12**: 177–82.
49 Sanders KM, Nicholson GC, Ebeling PR. Is high dose vitamin D harmful? *Calcif. Tissue Int.* 2013; **92**: 191–206.
50 Munck A. Nutritional considerations in patients with cystic fibrosis. *Expert Rev. Respir. Med.* 2010; **4**: 47–56.
51 Greer RM, Buntain HM, Lewindon PJ *et al*. Vitamin A levels in patients with CF are influenced by the inflammatory response. *J. Cyst. Fibros.* 2004; **3**: 143–9.
52 Dougherty KA, Schall JI, Stallings VA. Suboptimal vitamin K status despite supplementation in children and young adults with cystic fibrosis. *Am. J. Clin. Nutr.* 2010; **92**: 660–7.
53 Lai HJ, Shoff SM. Classification of malnutrition in cystic fibrosis: implications for evaluating and benchmarking clinical practice performance. *Am. J. Clin. Nutr.* 2008; **88**: 161–6.
54 Conway S, Morton A, Wolfe S. Enteral tube feeding for cystic fibrosis. *Cochrane Database Syst. Rev.* 2012; (12): CD001198.
55 Smyth RL, Walters S. Oral calorie supplements for cystic fibrosis. *Cochrane Database Syst. Rev.* 2012; (10): CD000406.
56 Woestenenk JW, Castelijns SJ, van der Ent CK, Houwen RH. Nutritional intervention in patients with cystic fibrosis: a systematic review. *J. Cyst. Fibros.* 2013; **12**: 102–15.
57 Kalnins D, Wilschanski M. Maintenance of nutritional status in patients with cystic fibrosis: new and emerging therapies. *Drug Des. Devel. Ther.* 2012; **6**: 151–61.
58 Aris RM, Merkel PA, Bachrach LK *et al*. Guide to bone health and disease in cystic fibrosis. *J. Clin. Endocrinol. Metab.* 2005; **90**: 1888–96.
59 Sermet-Gaudelus I, Castanet M, Retsch-Bogart G, Aris RM. Update on cystic fibrosis-related bone disease: a special focus on children. *Paediatr. Respir. Rev.* 2009; **10**: 134–42.
60 Klein GL. Gut–bone interactions and implications for the child with chronic gastrointestinal disease. *J. Pediatr. Gastroenterol. Nutr.* 2011; **53**: 250–4.
61 Stalvey MS, Clines KL, Havasi V *et al*. Osteoblast CFTR inactivation reduces differentiation and osteoprotegerin expression in a mouse model of cystic fibrosis-related bone disease. *PLoS ONE* 2013; **8**: e80098.
62 Sermet-Gaudelus I, Bianchi ML, Garabedian M *et al*. European cystic fibrosis bone mineralisation guidelines. *J. Cyst. Fibros.* 2011; **10** (Suppl. 2): S16–23.
63 Conwell LS, Chang AB. Bisphosphonates for osteoporosis in people with cystic fibrosis. *Cochrane Database Syst. Rev.* 2012; (4): CD002010.
64 Pauwels A, Blondeau K, Mertens V *et al*. Gastric emptying and different types of reflux in adult patients with cystic fibrosis. *Aliment. Pharmacol. Ther.* 2011; **34**: 799–807.
65 Blondeau K, Pauwels A, Dupont L *et al*. Characteristics of gastroesophageal reflux and potential risk of gastric content aspiration in children with cystic fibrosis. *J. Pediatr. Gastroenterol. Nutr.* 2010; **50**: 161–6.
66 Pauwels A, Blondeau K, Dupont LJ, Sifrim D. Mechanisms of increased gastroesophageal reflux in patients with cystic fibrosis. *Am. J. Gastroenterol.* 2012; **107**: 1346–53.
67 Fisichella PM, Davis CS, Lowery E, Ramirez L, Gamelli RL, Kovacs EJ. Aspiration, localized pulmonary inflammation, and predictors of early-onset bronchiolitis obliterans syndrome after lung transplantation. *J. Am. Coll. Surg.* 2013; **217**: 90–101.
68 Sheen E, Triadafilopoulos G. Adverse effects of long-term proton pump inhibitor therapy. *Dig. Dis. Sci.* 2011; **56**: 931–50.

69 Gelfond D, Borowitz D. Gastrointestinal complications of cystic fibrosis. *Clin. Gastroenterol. Hepatol.* 2013; **11**: 333–42.
70 Abraham NS. Proton pump inhibitors: potential adverse effects. *Curr. Opin. Gastroenterol.* 2012; **28**: 615–20.
71 Fathi H, Moon T, Donaldson J, Jackson W, Sedman P, Morice AH. Cough in adult cystic fibrosis: diagnosis and response to fundoplication. *Cough* 2009; **5**: 1–9.
72 Boesch RP, Acton JD. Outcomes of fundoplication in children with cystic fibrosis. *J. Pediatr. Surg.* 2007; **42**: 1341–4.
73 Abbassi-Ghadi N, Kumar S, Cheung B *et al*. Anti-reflux surgery for lung transplant recipients in the presence of impedance-detected duodenogastroesophageal reflux and bronchiolitis obliterans syndrome: a study of efficacy and safety. *J. Heart Lung Transplant.* 2013; **32**: 588–95.
74 Garrity ER Jr. Gastroesophageal reflux disease and bronchiolitis obliterans syndrome: where are we today? *J. Heart Lung Transplant.* 2013; **32**: 579–80.
75 De Lisle RC, Meldi L, Roach E, Flynn M, Sewell R. Mast cells and gastrointestinal dysmotility in the cystic fibrosis mouse. *PLoS ONE* 2009; **4**: e4283.
76 de Lisle RC, Sewell R, Meldi L. Enteric circular muscle dysfunction in the cystic fibrosis mouse small intestine. *Neurogastroenterol. Motil.* 2010; **22**: 341-e87.
77 Waseem S. Gastroparesis: current diagnostic challenges and management considerations. *World J. Gastroenterol.* 2009; **15**: 25–37.
78 Borowitz D, Durie PR, Clarke LL *et al*. Gastrointestinal outcomes and confounders in cystic fibrosis. *J. Pediatr. Gastroenterol. Nutr.* 2005; **41**: 273–85.
79 Grace E, Shaw C, Whelan K, Andreyev HJ. Review article: small intestinal bacterial overgrowth—prevalence, clinical features, current and developing diagnostic tests, and treatment. *Aliment. Pharmacol. Ther.* 2013; **38**: 674–88.
80 Braden B. Methods and functions: breath tests. *Best Pract. Res. Clin. Gastroenterol.* 2009; **23**: 337–52.
81 Malik BA, Xie YY, Wine E, Huynh HQ. Diagnosis and pharmacological management of small intestinal bacterial overgrowth in children with intestinal failure. *Can. J. Gastroenterol.* 2011; **25**: 41–5.
82 Gerritsen J, Smidt H, Rijkers GT, de Vos WM. Intestinal microbiota in human health and disease: the impact of probiotics. *Genes Nutr.* 2011; **6**: 209–40.
83 Schippa S, Iebba V, Santangelo F *et al*. Cystic fibrosis transmembrane conductance regulator (CFTR) allelic variants relate to shifts in faecal microbiota of cystic fibrosis patients. *PLoS ONE* 2013; **8**: e61176.
84 Duytschaever G, Huys G, Bekaert M, Boulanger L, De Boeck K, Vandamme P. Cross-sectional and longitudinal comparisons of the predominant fecal microbiota compositions of a group of pediatric patients with cystic fibrosis and their healthy siblings. *Appl. Environ. Microbiol.* 2011; **77**: 8015–24.
85 Scanlan PD, Buckling A, Kong W, Wild Y, Lynch SV, Harrison F. Gut dysbiosis in cystic fibrosis. *J. Cyst. Fibros.* 2012; **11**: 454–5.
86 Duytschaever G, Huys G, Bekaert M, Boulanger L, De Boeck K, Vandamme P. Dysbiosis of bifidobacteria and *Clostridium* cluster XIVa in the cystic fibrosis fecal microbiota. *J. Cyst. Fibros.* 2013; **12**: 206–15.
87 Madan JC, Koestler DC, Stanton BA *et al*. Serial analysis of the gut and respiratory microbiome in cystic fibrosis in infancy: interaction between intestinal and respiratory tracts and impact of nutritional exposures. *mBio* 2012; **3**: e00251–12.
88 Rogers GB, Carroll MP, Hoffman LR, Walker AW, Fine DA, Bruce KD. Comparing the microbiota of the cystic fibrosis lung and human gut. *Gut Microbes* 2010; **1**: 85–93.
89 Musch MW, Wang Y, Claud EC, Chang EB. Lubiprostone decreases mouse colonic inner mucus layer thickness and alters intestinal microbiota. *Dig. Dis. Sci.* 2013; **58**: 668–77.
90 Mortaz E, Adcock IM, Folkerts G, Barnes PJ, Paul Vos A, Garssen J. Probiotics in the management of lung diseases. *Mediators Inflamm.* 2013; **2013**: 751068.
91 Aslam S, Musher DM. An update on diagnosis, treatment, and prevention of *Clostridium difficile*-associated disease. *Gastroenterol. Clin. North Am.* 2006; **35**: 315–35.
92 Kachrimanidou M, Malisiovas N. *Clostridium difficile* infection: a comprehensive review. *Crit. Rev. Microbiol.* 2011; **37**: 178–87.
93 Peach SL, Borriello SP, Gaya H, Barclay FE, Welch AR. Asymptomatic carriage of *Clostridium difficile* in patients with cystic fibrosis. *J. Clin. Pathol.* 1986; **39**: 1013–18.
94 Binkovitz LA, Allen E, Bloom D *et al*. Atypical presentation of *Clostridium difficile* colitis in patients with cystic fibrosis. *AJR Am. J. Roentgenol.* 1999; **172**: 517–21.
95 Riddle DJ, Dubberke ER. *Clostridium difficile* infection in solid organ transplant recipients. *Curr. Opin. Organ Transplant.* 2008; **13**: 592–600.
96 Theunissen C, Knoop C, Nonhoff C *et al*. *Clostridium difficile* colitis in cystic fibrosis patients with and without lung transplantation. *Transpl. Infect. Dis.* 2008; **10**: 240–4.
97 Cohen SH, Gerding DN, Johnson S *et al*. Clinical practice guidelines for *Clostridium difficile* infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). *Infect. Control Hosp. Epidemiol.* 2010; **31**: 431–55.
98 Debast SB, Bauer MP, Kuijper EJ; Committee. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): update of the treatment guidance document for *Clostridium difficile* infection (CDI). *Clin. Microbiol. Infect.* 2014; **20** (Suppl. 2): 1–26.
99 van Nood E, Vrieze A, Nieuwdorp M *et al*. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N. Engl. J. Med.* 2013; **368**: 407–15.
100 Werlin SL, Benuri-Silbiger I, Kerem E *et al*. Evidence of intestinal inflammation in patients with cystic fibrosis. *J. Pediatr. Gastroenterol. Nutr.* 2010; **51**: 304–8.
101 Shah N, Tan HL, Sebire N, Suri R, Leuven K. The role of endoscopy and biopsy in the management of severe gastrointestinal disease in cystic fibrosis patients. *Pediatr. Pulmonol.* 2013; **48**: 1181–9.
102 Lee JM, Leach ST, Katz T, Day AS, Jaffe A, Ooi CY. Update of faecal markers of inflammation in children with cystic fibrosis. *Mediators Inflamm.* 2012; **2012**: 948367.
103 Walker WA. Initial intestinal colonization in the human infant and immune homeostasis. *Ann. Nutr. Metab.* 2013; **63** (Suppl. 2): 8–15.
104 De Lisle RC. Disrupted tight junctions in the small intestine of cystic fibrosis mice. *Cell Tissue Res.* 2014; **355**: 131–42.
105 Fluge G, Olesen HV, Gilljam M *et al*. Co-morbidity of cystic fibrosis and celiac disease in Scandinavian cystic fibrosis patients. *J. Cyst. Fibros.* 2009; **8**: 198–202.
106 Bresso F, Askling J, Astegiano M *et al*. Potential role for the common cystic fibrosis DeltaF508 mutation in Crohn's disease. *Inflamm. Bowel Dis.* 2007; **13**: 531–6.
107 Lloyd-Still JD. Crohn's disease and cystic fibrosis. *Dig. Dis. Sci.* 1994; **39**: 880–5.
108 Camilleri M, Madsen K, Spiller R, Greenwood-Van Meerveld B, Verne GN. Intestinal barrier function in health and gastrointestinal disease. *Neurogastroenterol. Motil.* 2012; **24**: 503–12.
109 Hasosah M, Davidson G, Jacobson K. Persistent elevated tissue-transglutaminase in cystic fibrosis. *J. Paediatr. Child Health* 2009; **45**: 172–3.

110 van der Doef HP, Kokke FT, van der Ent CK, Houwen RH. Intestinal obstruction syndromes in cystic fibrosis: meconium ileus, distal intestinal obstruction syndrome, and constipation. *Curr. Gastroenterol. Rep.* 2011; **13**: 265–70.
111 Smith VV, Schappi MG, Bisset WM *et al*. Lymphocytic leiomyositis and myenteric ganglionitis are intrinsic features of cystic fibrosis: studies in distal intestinal obstruction syndrome and meconium ileus. *J. Pediatr. Gastroenterol. Nutr.* 2009; **49**: 42–51.
112 Blackman SM, Deering-Brose R, McWilliams R *et al*. Relative contribution of genetic and nongenetic modifiers to intestinal obstruction in cystic fibrosis. *Gastroenterology* 2006; **131**: 1030–9.
113 Karimi A, Gorter RR, Sleeboom C, Kneepkens CM, Heij HA. Issues in the management of simple and complex meconium ileus. *Pediatr. Surg. Int.* 2011; **27**: 963–8.
114 Efrati O, Nir J, Fraser D *et al*. Meconium ileus in patients with cystic fibrosis is not a risk factor for clinical deterioration and survival: the Israeli Multicenter Study. *J. Pediatr. Gastroenterol. Nutr.* 2010; **50**: 173–8.
115 Colombo C, Ellemunter H, Houwen R, Munck A, Taylor C, Wilschanski M. Guidelines for the diagnosis and management of distal intestinal obstruction syndrome in cystic fibrosis patients. *J. Cyst. Fibros.* 2011; **10**: S24–8.
116 van der Doef HP, Kokke FT, Beek FJ, Woestenenk JW, Froeling SP, Houwen RH. Constipation in pediatric cystic fibrosis patients: an underestimated medical condition. *J. Cyst. Fibros.* 2010; **9**: 59–63.
117 Robertson MB, Choe KA, Joseph PM. Review of the abdominal manifestations of cystic fibrosis in the adult patient. *Radiographics* 2006; **26**: 679–90.
118 Chaudry G, Navarro OM, Levine DS, Oudjhane K. Abdominal manifestations of cystic fibrosis in children. *Pediatr. Radiol.* 2006; **36**: 233–40.
119 Wilschanski M, Durie PR. Patterns of GI disease in adulthood associated with mutations in the CFTR gene. *Gut* 2007; **56**: 1153–63.
120 Maisonneuve P, Marshall BC, Knapp EA, Lowenfels AB. Cancer risk in cystic fibrosis: a 20-year nationwide study from the United States. *J. Natl Cancer Inst.* 2013; **105**: 122–9.
121 Neglia JP, FitzSimmons SC, Maisonneuve P *et al*. The risk of cancer among patients with cystic fibrosis. Cystic Fibrosis and Cancer Study Group. *N. Engl. J. Med.* 1995; **332**: 494–9.
122 Mehta A. Cystic fibrosis as a bowel cancer syndrome and the potential role of CK2. *Mol. Cell. Biochem.* 2008; **316**: 169–75.
123 Zhang JT, Jiang XH, Xie C *et al*. Downregulation of CFTR promotes epithelial-to-mesenchymal transition and is associated with poor prognosis of breast cancer. *Biochim. Biophys. Acta* 2013; **1833**: 2961–9.