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Hepatobiliary involvement in cystic fibrosis

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Abstract

The improved life expectancy of cystic fibrosis (CF) patients has increased the prevalence of extra-pulmonary complications. Liver disease is present in about one-third of CF patients and presents in various forms. The clinically most relevant forms are cirrhosis, possibly arising from the pathognomonic focal biliary fibrosis, and non-cirrhotic portal hypertension. Various forms of liver disease may present in different age groups, including childhood. Cirrhosis mostly presents in the first or second decade of life. The pathogenesis of focal biliary fibrosis and cirrhosis in CF is incompletely understood, although the most accepted hypothesis envisions fibrosis as a response to stasis of inspissated bile, possibly with a second hit provided by increased pro-inflammatory responses of CF bile ducts to gut-derived microbial byproducts. Since the disease is often subclinical until complications develop, and because serum liver enzymes are often non-specifically elevated in CF, annual screening for structural liver disease is recommended. Once the diagnosis is established, treatment with ursodeoxycholate is often advised, although evidence for its efficacy remains a subject of discussion. The efficacy of CFTR modulators in preventing or reversing liver disease progression is not yet established. Complications of severe liver disease are related to portal hypertension, functional hepatic decompensation including exacerbation of malnutrition and secondary complications of cirrhosis in other organs, and may warrant liver transplantation. CF liver disease confers poorer survival and accounts for 2-3% of mortality among CF patients. Current research is aimed at diagnosing early, progressive disease non-invasively, as well as at the discovery of novel therapies.

The improved management of respiratory complications and malnutrition in CF over the past decades has led to an increase in median life expectancy, which is now 44 years of age in the US (1). With increased life expectancy, the clinical relevance of extra-pulmonary complications of CF, such as liver disease, has increased, leading to a new need for preventative, diagnostic and therapeutic tools.

Definition and classification

Most patients with CF have evidence of hepatobiliary involvement, although with no clinical consequences for the vast majority. CF patients may present with a variety of hepatobiliary signs, symptoms, and clinical disease patterns, ranging from isolated elevated liver enzymes, biliary disease, steatosis, to fibrosis and cirrhosis (**Table 1**). Although clinically relevant hepatobiliary involvement in CF is frequently gathered under the umbrella term Cystic Fibrosis Liver Disease (CFLD), the definition of CFLD is not clearly demarcated. Likewise, there is no definite classification of hepatobiliary involvement in CF. Precise and functional definitions and classifications are important for use in establishing the natural course of the disease, for selecting patients in clinical trials and for use in patient registries.

The most commonly used definition of CFLD was described by Debray and Colombo (2), in which CFLD diagnosis is based on a combination of the presence of at least two conditions among 1) hepatomegaly, 2) persistent elevation of plasma AST, ALT and GGT and 3) abnormalities on liver ultrasound (**Table 2**). This classification has been frequently used to select patient that may be eligible for treatment with ursodeoxycholic acid. Although well-established in clinical practice, the Debray-Colombo criteria for CFLD diagnosis present some shortcomings. Whereas it is unknown whether hepatomegaly reflects clinically relevant liver involvement in CF, elevations in liver enzymes during routine follow-up, in particular the transaminases AST and ALT, are a very frequent observation in CF patients (3). However, abnormal liver enzymes levels are not demonstrated to universally precede more severe forms of liver involvement in CF, in particular cirrhosis, and may often be due to other causes, such as antibiotics usage (4). Persistent increases in serum GGT, on the other hand, was found to be a predictor for the development of cirrhosis and portal hypertension (5). It is not clear whether the heterogeneous phenotypes diagnosed as CFLD by these criteria relate to clinically relevant cirrhosis or portal hypertension.

Another approach to classify hepatobiliary involvement in CF is based on the various phenotypical presentations, as shown in **table 1.** This classification does not imply any causal relation between the various phenotypes. However, it includes a complete spectrum of currently described clinical presentations, separated based on clinical consequences and severity.

Table 1: Hepatobiliary involvement in CF: classification based on phenotypical presentations

A	Persistent increase in liver enzymes - GGT, ALT and AST
B	Liver parenchyma ultrasound abnormalities - increased echogenicity (associated with steatosis) - inhomogeneous (associated with fibrosis) - nodular deformation (associated with cirrhosis)
C	Portal hypertension (splenomegaly, variceal bleeding) - cirrhotic - non cirrhotic
D	Cholangiopathies and biliary disease - bile duct stones, gallstones - micro-gallbladder - bile duct stenosis
E	Malignancies - hepatocellular carcinoma - biliary tract cancer

Table 2: 2011 "Debray-Colombo" criteria for CFLD diagnosis (2)

CF patients are considered to have developed CFLD if at least 2 of the following conditions are present:
(1) hepatomegaly (increase in liver span relative to age, or liver edge palpable more than 2 cm below the costal margin on the mid-clavicular line), confirmed by ultrasonography
(2) elevated serum liver enzyme levels, consisting of elevation above the upper normal limits of 2 of the following: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and y-glutamyltransferase (GGT), on at least 3 consecutive occasions over 12 months, after excluding other causes of liver disease
(3) ultrasound abnormalities other than hepatomegaly (i.e., increased, heterogeneous echogenicity, nodularity, irregular margins, biliary abnormalities, splenomegaly) A liver biopsy is indicated in case of diagnostic doubt.

Epidemiology

Within the CF population, CFLD prevalence ranges between 10-41% (6–12), and between 4-28% of patients are reported to have *severe* CFLD (6,7,11–14). This great variation depends on the population studied and the diagnostic criteria utilized.

The mean age at diagnosis ranges between 10 and 35 years across studies (9–12,15–18), with most studies reporting disease onset at the end of the first decade of life,

but the recent recognition that a second wave of CFLD onset may occur later in life, possibly due to distinct pathophysiological mechanisms.

Risk factors

The most frequently reported risk factors for the development of clinically severe CFLD are male gender (6,11,12,18–20), meconium ileus (6,8,11,12,17–19) and severe genotype (6,12,16,17,19,21–23). Why only a minority of patients with the same severe (class I, II and III) CFTR mutations progresses to clinically significant liver disease is unclear. It has been hypothesized that inheritance of non-CF modifier genes, such as polymorphisms in genes that upregulate inflammation, fibrosis or oxidative stress, confers an increased susceptibility. In a multinational gene modifier study of different candidate genes, the SERPINA 1 Z allele of alpha-1 antitrypsin was found to be strongly associated with severe CFLD (20). This supports the hypothesis that severe CFLD is a result of a double-hit pathogenesis, however, only a minority of CF patients are carrier. Hitherto undefined environmental factors are also likely to be involved.

Etiology and pathogenesis

The pathogenesis of liver disease in CF is not yet completely understood. Besides the basic CFTR secretory defect, the multi-organ complexity of CF disease offers numerous factors that could contribute to damage and dysfunction of the hepatobiliary tract. A multifactorial pathophysiology, combined with the role of modifier genes and environmental factors, could explain the heterogeneous epidemiology and spectrum of disease that is observed in this patient population.

In the normal hepatobiliary tract, CFTR is expressed on the apical membrane of cholangiocytes lining both intrahepatic and extrahepatic bile ducts (24), as well as in the gallbladder (25) and is not found in hepatocytes or other types of liver cells. In the bile ducts, CFTR fulfils a role in bile flow. Along with other chloride channels such as anoctamin-1 (ANO-1 or TMEM16A), CFTR exports chloride ions to the lumen to drive the secretion of water and bicarbonate into bile (26). In addition to regulating epithelial efflux of chloride, bicarbonate and water, CFTR is also known to regulate sodium absorption, glutathione transport, as well as mucin secretion and maturation, via incompletely understood pathways.

Fibrosis and cirrhosis

The mechanisms leading to fibrosis and cirrhosis are of major interest, as these lesions represent the most clinically relevant manifestations of CF liver involvement.

The oldest and most widely accepted theory directly blames defective CFTR secretory function in cholangiocytes to result in inspissated bile. As deducible from the functional role of CFTR in biliary epithelia, loss of CFTR is expected to result in less hydrated and less alkalinized bile. These characteristics could also impede proper mucin maturation (27). The resulting inspissated bile would then reduce bile flow, causing more or less severe ductal cholestasis and exposing cholangiocytes to the cytotoxic effects of bile acids. Inflammation resulting from cytotoxicity would then progress to focal biliary fibrosis mediated by activated stellate cells (28). Bridging of multiple fibrotic foci could then eventually lead to multilobular cirrhosis and its related complications (29). Supporting this theory, excessive mucus was found in intrahepatic ducts of some infants with CF, although it was only occasionally associated with peri-portal changes or cholestasis (30). Other studies reported that bile inspissation is found infrequently in patients (31,32) and in mice with CFLD (33). Furthermore, the highly variable presentation of fibrosis and cirrhosis across patients is not fully elucidated by this theory.

Hepatic stellate cell activation could also arise as a consequence of increased cytotoxicity of bile in CF due to a more hydrophobic composition of biliary bile acids (34,35), linked or not with inspissation of bile and thus prolonged exposure to hydrophobic bile acids. Studies in CF patients and mice have found that their bile contains a higher proportion of primary, more hydrophobic bile acids (36). This shift in biliary bile acid composition could stem from the yet unexplained disruption of the enterohepatic circulation of bile acids in CF (37), which could deplete bile of secondary bile acids and stimulate hepatic production of primary bile acids. Deficiency of glutathione and fat-soluble antioxidants in CF could worsen oxidative stress in response to cytotoxicity (38). However, a study in CF mice found that the development of liver disease was unrelated to the hydrophobicity of bile, which was in fact decreased in this study (39). A higher biliary bile acid-to-phospholipid ratio was found in CF mice and was proposed to promote cell damage in combination with a more hydrophobic bile (40), however these findings were not confirmed in subsequent studies (39,41).

A series of studies reported that CFTR deficiency in cholangiocytes promotes inflammation in response to gut-derived bacterial endotoxins, suggesting a role of CFTR in the regulation of inflammatory responses resulting in prolonged and more vigorous TLR4 responses (42–44). Patients with primary sclerosing cholangitis (PSC), a rare liver disease with histological findings similar to CFLD, have an increased probability to carry CFTR polymorphisms (45). PSC is often accompanied by

inflammatory bowel disease (IBD) (46). Both CF and IBD patients have an intestinal phenotype that features inflammation, dysbiosis and increased permeability (46,47), characteristics that increase the likelihood of intermittent translocation of bacterial products from the gut to the liver (portal bacteremia). This gut-liver axis hypothesis in which the CF gut promotes translocation of bacterial by-products to the liver (48) was supported by the increased finding of macroscopic intestinal lesions in CF patients with cirrhosis compared to those without liver disease (49), as well as a different gut microbiota composition among the two groups. Unfortunately, this study was unable to determine causality, as intestinal lesions and altered microbiota could also arise after the development of cirrhosis and portal hypertension. Furthermore, differences in intestinal permeability or calprotectin were not found between patients with cirrhosis and without liver disease (49). It is however likely that increased portal bacteremia could play a role in the development or progression of CFLD.

Finally, because the development of portal hypertension in CF patients has been frequently observed before the onset of overt cirrhosis (50–52), vascular changes, namely obliteration of portal vein branches with fibrosis, has been observed and suggested to lead to portal hypertension in this patient population (50,52–54). However, such vascular changes were not observed in another study in patients with non-cirrhotic portal hypertension (55).

Steatosis

The pathophysiological mechanisms leading to the increased prevalence of steatosis in CF are still unclear and are likely distinct from those leading to fibrosis and cirrhosis. Whether steatosis in CF progresses to cirrhosis is not clear, and although steatosis in CF, especially when mild, has long been considered a benign condition, the discovery that non-alcoholic steatohepatitis can progress to cirrhosis has led to reconsideration of the risk.

Several pathophysiological mechanisms have been proposed. Deficiencies of essential fatty acids, which are common in CF (56,57), are associated with hepatic steatosis (58,59) and were reported to correlate with steatosis in CF patients (60). However, in one study, essential fatty acids supplementation did not prevent steatosis progression in patients (61), whereas in another, administration of docosahexaenoic acid was observed to reduce peri-portal inflammation in CF mice, however no effect on steatosis was reported (62). It must be noted that CF mice are pancreatic-sufficient and only develop steatosis upon feeding of lipid-rich (liquid) diets. Interestingly, they may be more prone to developing steatosis than wildtype mice upon this diet (63). This finding may suggest that dietary factors are important in the development of steatosis in CF, as they are for the general population, especially in the light of the high-fat, often unbalanced diet followed by CF patients (64). An association between

overweight in CF and hepato-steatosis has been reported (65). The prevalence of overweight and obesity in CF are respectively 6-15% and 1-8% (66–68).

Other dietary deficiencies suggested to contribute to steatosis in CF are those of carnitine (69), choline (70) and trace elements (19). Administration of choline did not decrease liver lipid levels in CF (71).

Low-grade chronic systemic inflammation, which is present in CF (72), was suggested to contribute to steatosis in non-CF patients (73). However, chronic pseudomonas colonization was not associated with hepatic steatosis in a study (65). Although hepato-steatosis is associated with insulin resistance and type 2 diabetes in the general population (74), no association between steatosis and CF-related diabetes was found (65), likely because the pancreatic component predominates over insulin resistance in CFRD. Dysregulations in bile acid metabolism have been recently implicated in the development of NAFLD (75); NAFLD patients exhibit a deficiency in fibroblast growth factor 19 (FGF19) (76), which is implicated in lipids homeostasis. Interestingly, reduced serum levels of FGF19 were also found in CF patients (77) and are thought to be linked to intestinal bile acid malabsorption. FGF19 was shown to ameliorate hepato-steatosis and fibrosis in animal models of NAFLD (78). The role of extrahepatic factors in the development of liver steatosis in CF is supported by the finding that steatosis is common in allograft liver biopsies of liver transplants of CF patients, solely in patients with preexisting steatosis in their explant. Of note, steatohepatitis was also common following liver transplant (79).

Further studies will be needed to elucidate the pathophysiology, as well as the clinical relevance of hepato-steatosis in CF.

Clinical presentation

CF liver involvement with fibrosis and cirrhosis

Liver involvement with fibrosis and cirrhosis is the most severe form of CF-related liver involvement. The pathological hepatic feature is focal biliary cirrhosis (**Figure 1**). There are often no clinical features before the development of portal hypertension. Complications are discussed below.

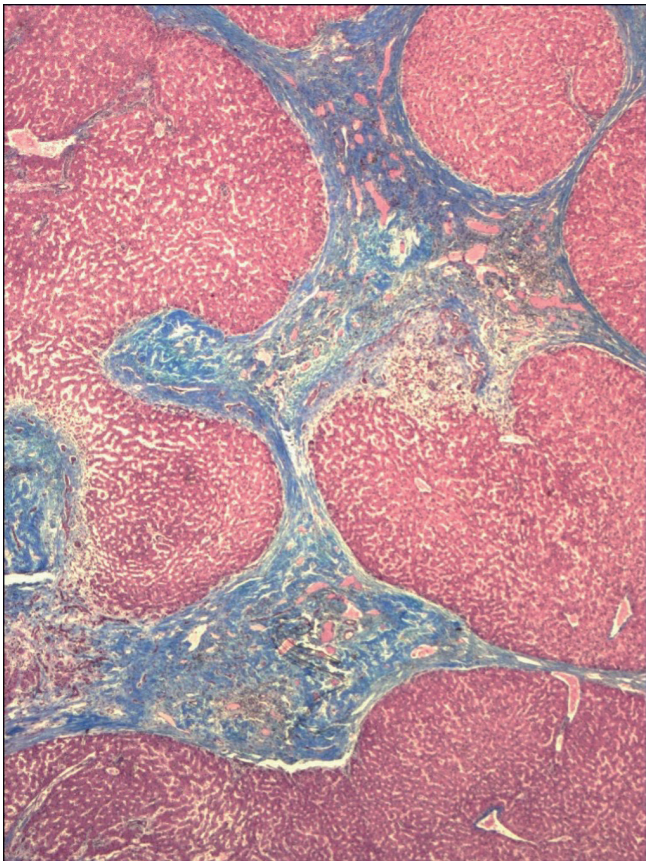

Figure 1: Histology (Masson trichrome staining) of explanted liver of a 12 year old CF patient transplanted for cirrhosis-related hepatopulmonary syndrome, clearly demonstrating biliary nodular cirrhotic pattern.

Non-cirrhotic portal hypertension in CF

Recent publications reported that portal hypertension in CF may also develop independently of extensive fibrosis or cirrhosis (50,54). These patients had all the classical features of portal hypertension, such as splenomegaly, thrombocytopenia and esophageal varices. This form of non-cirrhotic portal hypertension (80) has been defined as a disease of uncertain etiology characterized by peri-portal fibrosis and involvement of small and medium branches of the portal vein, resulting in the development of portal hypertension.

Biliary tract disease

There have been numerous reports of a variety of intrahepatic and extrahepatic abnormalities of the biliary tree in CF, although the incidence of these findings is controversial. These findings include irregularities and tapering of the small

intrahepatic ducts, as well as stricturing, beading and segmental dilation of the larger extrahepatic ducts, similar to primary sclerosing cholangitis (81). There is also an increased risk of biliary tract cancer in CF, also before transplantation. The risk increased even more after solid organ transplantation and the consequent need for immunosuppressive therapy. Based on these findings, it was recommended to screen CF patients over 40 years old for biliary tract cancer using abdominal ultrasound, magnetic resonance cholangiopancreatography, or endoscopic ultrasonography (82).

Cholestasis develops rarely in CF infants and may be the presenting feature, often together with meconium ileus (83). Gallbladder abnormalities have been frequently reported and include either microgallbladders or distended gallbladders featuring defective function and require no treatment. Of clinical relevance, an increased incidence of gallbladder and intrahepatic stones was reported (21).

Diagnosis and follow-up

Diagnosis

The evidence of liver disease in CF is often subclinical until complications develop. When clinical signs are present, physical examination is directed at the detection of hepato- and splenomegaly and other signs of chronic liver disease. Serum biomarkers are useful in detecting complications, for instance persistent thrombocytopenia in combination with splenomegaly suggests portal hypertension. However, as elevated serum liver enzymes in CF patients are common, nonspecific and may reflect different underlying etiologies, as discussed above, routine biochemistry is generally not helpful in reliably identifying patients with multilobular cirrhosis or in predicting the development of end-stage liver disease (2,51). Persistently elevated GGT predicted cirrhosis (5), and portal hypertension was predicted accurately by various indexes that combine demographic values with plasma biomarkers such as Hepascore, AST to Platelet Ratio Index (APRI) and Forns index (Forns index combines age, GGT, cholesterol and platelet count), although cutoff scores are variable, and more studies are needed for validation (reviewed in (84)).

The major challenge in the diagnosis of liver involvement in CF is distinguishing patients who will progress to clinically relevant complications such as cirrhosis and portal hypertension. Since this is not clear, although practice varies among institutions, international guidelines recommend screening every CF patient every year with a hepatobiliary ultrasound (1), including Doppler measurements of flow in the portal vein. However, the positive predictive value of a normal scan and sensitivity are low (85). The gold standard for diagnosing fibrosis and cirrhosis in CF is liver biopsy, including two biopsies rather than one large biopsy (51) to circumvent the focal nature of disease. Because liver biopsy is an invasive procedure with inherent

risks, numerous studies are being performed to identify non-invasive methods to detect early CF liver involvement which may clinically progress. MRI is valuable (86), but costs are a concern, and it is reserved, as CT, in cases of diagnostic doubt. Since the degree of histological liver fibrosis can predict the development of portal hypertension (51), non-invasive tests such as transient elastography (TE, Fibroscan R) and acoustic radiation force impulse (ARFI), both of which measure liver stiffness as a surrogate measure for fibrosis, are being studied in CF patients. Although these tests had low sensitivities in detecting earlier liver changes, they seem fairly accurate in detecting portal hypertension and cirrhosis (84) and may become implemented in the screening and follow-up of CF liver involvement, once cutoff values are defined, and likely in combination with other tests. MRI, scintigraphy of the hepatobiliary tract, and magnetic resonance cholangiography are useful for visualizing bile duct and gallbladder disease. Because the clinical relevance of biliary tract abnormalities in CF is unclear, these tests do not find a place in the annual screening, which instead usually includes clinical examination, abdominal ultrasound and serum biochemistry.

Differential diagnosis of liver involvement in CF

In CF patients that present with signs of liver involvement, e.g. persistent elevation of liver enzymes, it is imperative to consider a general differential diagnosis including e.g. hepatitis (viral or autoimmune), alpha-1 antitrypsin deficiency, celiac disease and Wilson disease. In the case of steatosis, other causes of steatosis like malnutrition, diabetes mellitus need to be considered. Interestingly, a subgroup of patients with primary sclerosing cholangitis may have electrophysiological and genetic similarities with CF patients (45). In the work of liver involvement liver biopsy may have to considered to diagnose or exclude other cause of liver disease besides CF.

Follow-up

It is useful to follow-up patients with early, subclinical liver involvement annually to screen for development of cirrhosis and/or portal hypertension. Patients with cirrhosis and/or portal hypertension are followed up to assess treatment efficacy and to prevent complications such as variceal bleeding (**table 3**). In these patients, screening for hepatocellular carcinoma is recommended with ultrasound and plasma alpha-fetoprotein levels (2).

Management: prevention and treatment

UDCA

Debray et al (2) recommended that treatment with the bile acid ursodeoxychlolic acid (UDCA) should be initiated at the time of CFLD diagnosis (according to the Debray-Colombo criteria, **table 1**) at a daily dose of 20 mg/kg/day to delay progression. UDCA aims to induce a bicarbonate-rich bile that flows more easily, as well as to

reduce the hydrophobicity of the bile acid pool (87). However, a Cochrane review revealed that there is insufficient evidence to support the use of UDCA in CF (88). UDCA treatment was associated with slight improvements in serum liver enzymes in the short term (89–93). Moreover, long-term studies suggested benefits of UDCA on CFLD progression as assessed by ultrasonography; unfortunately, a control group was missing (94,95). Long-term controlled trials showing effectiveness of UDCA in delaying CFLD progression, preventing cirrhosis, liver transplantation or death are lacking. Furthermore, UDCA treatment and in particular the recommended dose has been questioned after a trial of high-dose UDCA in primary sclerosing cholangitis was terminated due to increased risk of severe side effects (96). These limitations of UDCA therapy in CFLD have highlighted the need of novel preventative and therapeutic options.

CFTR modulators

The emergence of novel targeted agents that directly modulate CFTR folding or function has led to new treatment opportunities for patients with class II (misfolded protein) and III (reduced protein function) mutations. Bicarbonate secretion, intestinal inflammation, as well as other factors that may be contributing to CFLD may be, at least partially, improved by CFTR modulators. It should be possible to reliably follow their effect on liver fibrosis by transient elastography. These studies are still lacking, but are expected to be included in further clinical trials. Ivacaftor treatment was reported to reverse hepatic steatosis, a liver complication frequently seen in CF patients, in one patient (97). On the other hand, there have been reports of raised liver enzymes in some trials, but no detrimental clinical effect (98). Monitoring of liver function upon CFTR modulators is warranted, especially in patients with raised liver enzymes.

Other targets

A number of investigational avenues targeting different pathophysiological aspects of liver disease are being explored:

1. Bile acid analogues: norUDCA (a side chain–shortened homologue of UDCA with one less methylene group) undergoes cholehepatic shunting leading to a bicarbonate-rich hyperchloresis. This drug has direct anti-inflammatory, anti-fibrotic and anti-proliferative properties, and stimulates alternative bile acid detoxification and elimination routes. It has shown encouraging effects improving serum liver tests in primary sclerosing cholangitis, an immune-mediated liver disease with biliary morphological similarities to CFLD (99), although it did not decrease biliary injury in a CF mouse model with liver pathology prompted by inducing colitis (43).

2. Farnesoid X receptor (FXR) agonists: activation of FXR, a nuclear receptor, suppresses bile acid synthesis via fibroblast growth factor 19 (FGF19), stimulates bile acid and bicarbonate secretion, modulates fibrosis, and regulates lipid and glucose metabolism (100).
3. Fibroblast growth factor 1 (FGF1) is a peroxisome proliferator-activated receptor gamma (PPARγ) target in visceral adipose tissue and is critical to adipose remodeling (101). FGF1 improved hepatic inflammation, steatosis and damage in leptin-deficient ob/ob and choline-deficient mice, two etiologically different NAFLD models (102).
4. Vitamin D receptors also offer potential for treatment intervention with their activation implicated in preventing hepatic fibrosis involving transforming growth factor beta 1 (TGFβ1) signaling via pro-fibrotic genes (103).

Moreover, supplementation of docosahexaenoic acid, stimulation of peroxisome proliferator-activated receptor, as well as Src inhibition have been used with success in a mouse model of CF, where they reduced bile duct injury (44,104,105). However, these treatments are in an early experimental stage and, besides effectiveness in patients, their safety is to be explored.

Treatment of complications of cirrhosis

The management of GI varices in CF is according to general guidelines (106). However, prophylactic treatment of variceal bleeding with non-selective beta blockers may be contraindicated in CF due to pulmonary side effects. The treatment of choice for bleeding varices is injection sclerotherapy or band ligation. Both transjugular intrahepatic portosystemic shunt (TIPS) and surgical portosystemic shunts are used in CF patients to relieve endoscopic uncontrollable GI bleeding due to portal hypertension. However, these procedures may present with a high rate of complications due to shunt obstruction and hepatic encephalopathy. Therefore, it is advisable that, in this specific complicated clinical scenario, liver transplantation is also considered as a treatment option (107).

Liver transplantation

End-stage liver disease in CF or otherwise not treatable life-threatening complications of liver disease (e.g. hepatopulmonary syndrome) in CF are accepted indications for liver transplantation. However, CF is an exceptional indication for liver transplantation, as it is a multi-organ disease including, lung, pancreas, and gut complications. Disease presentation in these organs will not be cured by a single organ liver transplantation. The latter may be the reason why liver transplantation in cystic fibrosis is associated with poorer long-term patient survival compared to non-cystic fibrosis patients (108). The need for immunosuppression following liver transplantation, which increases the risk for pulmonary infections, should also be taken into consideration while considering and planning liver transplantation.

Outcomes

Liver involvement in CF is regarded as relatively benign, with low prevalence of complications and progression (10,14,109), although it accounts for 2.1% of the mortality of CF patients in Europe, representing the 3rd CF-related leading cause of death, and 3.4% in North America (1,7). Furthermore, CFLD is an independent risk factor for mortality (8,110).

The most clinically prominent features and complications of CF liver disease with portal hypertension are related to severe portal hypertension (**Table 3**). The splenomegaly can be striking and may cause mechanical and abdominal complaints. The secondary thrombocytopenia may be associated with bleeding and epistaxis. CF patients with portal hypertension are at risk for variceal bleeding in the gastrointestinal tract. A recent report based on the US CF Foundation Patient Registry found that variceal bleeding episodes occur in ~6% of CF patients with cirrhosis, although without increasing mortality (111). Moreover, there have been reports of hepatopulmonary syndrome (112).

Comparable to cirrhotic disease in other patient populations, CF patient with cirrhosis may develop hepatocellular carcinoma (113–115). Accordingly, CF patient with cirrhosis should be screened for hepatocellular carcinoma by periodical liver ultrasound and alpha fetoprotein testing.

Although CF patients with cirrhosis seldom develop end stage liver disease (109), adverse liver outcomes are frequent after cirrhosis has been diagnosed in CF patients (111). Additionally, CF patients with cirrhosis reportedly have a poorer survival (116).

Table 3. Complications of CF liver involvement with cirrhosis

Complications of portal hypertension - GI tract variceal bleeding - Ascites - Spontaneous bacterial peritonitis (SPB) - Hepatopulmonary syndrome (HPS), - Porto-pulmonary hypertension (PPH)
Signs and symptoms of functional liver decompensation - Hepatic protein synthesis defects (e.g. hypoalbuminemia) - Vitamin K-independent coagulopathy - Liver detoxification defects - Jaundice - Hypoglycemia
Hepatocellular carcinoma

Whether lung function is influenced by CFLD is a matter of debate, with studies reporting worsened (6,109,117,118), unchanged (19,22,119) and even improved (120,121) lung function. Severe CF-related diabetes (CFRD) was reported to occur more frequently in CFLD patients than in the general CF population (109). CFLD did not seem associated with bone mineralization abnormalities in a study (117).

Research tools and directions

Clinical studies

There are currently no therapeutic clinical trials directly targeting liver disease in CF. When considering clinical trials for CFLD, there are two potential directions.

First, the effects of the relatively new CFTR protein modulators on CFLD progression could be studied. In the currently performed trials for e.g. Ivacaftor, Ivacaftor/Lumacaftor or tezacaftor–ivacaftor combination therapies, CFLD patients, in particular those with cirrhosis/or portal hypertension, were excluded. Furthermore, liver function was not reported as an outcome parameter. On the other hand, liver enzymes are monitored in clinical trials to evaluated liver toxicity and adverse events. It would be of great clinical and scientific interest to include CFLD-related outcome measures in future clinical trials with CFTR protein modulators, such as elastography and liver function tests including AST, ALT, alkaline phosphatase, bilirubin and GTT, to gather information on whether these drugs are able to prevent the evolution to liver fibrosis or to reverse disease if present. In case patients with liver cirrhosis are included, markers of liver protein synthesis (e.g. albumin, clotting factors) or liver detoxification (total bilirubin, ammonia) as biomarkers of liver function could be added. Additionally, new outcome measures for liver function are currently evaluated. For example, in a recent study is was demonstrated that the change in 7α-hydroxy-4-cholesten-3-one (C4), that is used as a surrogate marker for BA synthesis, can be used to monitor the effect of CFTR modulation on hepatic bile acid synthesis in CF (77,122).

A second direction for clinical trials in CFLD may include new anti-fibrotic agents or novel therapies that target bile acid receptors and metabolism to improve liver disease. Clinical trials for these new drugs are currently performed in non-CF related liver disease, including primary sclerosing cholangitis, primary biliary cholangitis, viral hepatitis and NASH. If proven successful or effective, these new treatment options could also be considered for CFLD patients.

An international agreement on the definition, classification and diagnostic criteria of CF liver involvement and CFLD is urgently needed to consistently select patients for clinical trials and for use in patient registries.

Animal models

Since 1992, a great number of animal models of CF have been created utilizing small animals such as the mouse, rat, ferret, and rabbit, as well as large animals such as the pig and the sheep. In each animal model, the most prominent manifestation of disease is a severe intestinal phenotype characterized by meconium ileus and/or post-weaning intestinal obstruction. The extent and nature of liver disease varies across animal models, being milder in small and severer in large animal models.

Several mouse models of CF are available, on different genetic backgrounds, which cover CFTR defects class I (including various knockouts with none or residual CFTR function and a conditional knockout), II (including models carrying the F508del or G480C mutation) III (G551D mutation) and IV (R117H mutation) (reviewed in (123,124)). The intestinal phenotype is particularly severe in *Cftr*-null strains, which show high rates of intestinal obstruction at weaning. CF mice do not usually develop spontaneous pulmonary nor pancreatic disease, although reports of these conditions in *Cftr*-null mice exist (33). Reports of absent liver pathology in the various CF mouse models predominate. However, 20% of $Cftr^{G551D}$ mice show hyperplasia of the bile duct epithelium (125). *Cftr*-null mice were reported to have focal biliary and even lobular cirrhosis (33,39), and about half of aged $Cftr^{F508del}$ mice exhibited mild patchy cholangiopathy in a study (40). Induction of colitis in *Cftr*-null mice leads to more severe bile duct injury compared with wild-type mice (126). The liver phenotype of CF mice may to some extent depend on environmental factors, such as the diet administered. CF mouse strains at high risk for intestinal obstruction have been maintained on either a liquid diet or chow with added polyethylene glycol in drinking water. The development of steatosis was observed in CF mice to a greater extent than wild-type mice upon liquid diet (33,63) and an 11% fat diet (40), as that of cholangiopathy (127). The gallbladder was reported to be enlarged and sometimes filled with black bile in some strains (125,128,129).

In a CF rat model, about 70% of *Cftr*-null rats developed intestinal obstruction around weaning, but no histological liver abnormalities were noted (130).

Besides CF mice and rats, a *Cftr*-null ferret was created by Sun et al. (131) to take advantage of fast breeding and more similar lung physiology of the ferret to humans. The *Cftr*-null kits displayed a severe intestinal phenotype, as about 75% developed meconium ileus, as well as pancreatic disease and failure to thrive. Despite no evidence for other hepatic histological changes indicating cholestasis or focal biliary cirrhosis, *Cftr*-null kits with or without MI had increased plasma levels of alanine amino- transferase (ALT) and direct and indirect bilirubin, similarly to human CF infants (60), which improved upon treatment with ursodeoxycholic acid (UDCA) in combination with an osmotic laxative and antibiotics. This treatment regimen did not improve the nutritional status, which was instead improved by proton pump

inhibitor treatment in combination with a liquid elemental diet and pancreatic enzyme replacement. The authors additionally attempted to improve the intestinal phenotype by expressing the human CFTR in the intestine only. Only one such transgenic kit with the highest CFTR protein expression did not develop MI and survived. Liver function tests were not reported for the gut-corrected kits.

In addition to the *Cftr*-null ferret model, Sun et al. also created *Cftr*-null rabbits and is currently developing rabbits with the F508del mutation. The intestinal and liver phenotype of CF rabbits has not been characterized yet.

Both the *Cftr*-null (132) and F508del (133) pig models have meconium ileus with 100% penetrance and develop pancreatic insufficiency. The liver showed infrequent histological changes consistent with early focal biliary cirrhosis. As in some patient studies (21,134), the piglets displayed a micro-gallbladder, which was often filled with congealed bile, as were the bile ducts. Uc et al. reported that the baseline bile volume was unchanged in newborn *Cftr*-null piglets, however upon stimulation with secretin, CF pigs failed to increase bile secretion. Bile was thick, contained more proteins and the pH was somewhat lower in CF piglets than in wild types (135). Transgenic expression of porcine CFTR restricted to the intestine alleviated meconium ileus, but did not ameliorate the liver and gallbladder phenotype (136,137). By relieving meconium ileus with an ileostomy or cecostomy, the pigs aged to develop hepatic steatosis (133) and lung disease (138) by 2-3 months of age.

Like the CF pig, recently created *Cftr*-null sheep showed 100% penetrance of meconium ileus. The liver phenotype was severe, although distinct form other animal models. Most lambs had (neonatal) intrahepatic cholestasis, which is rare in humans (139), with biliary and periportal fibrosis, and excessive hepatic glycogen accumulation. The gallbladder was hypoplastic and often empty.

Animal models of CF have been valuable in understanding the role of CFTR in cholangiocytes. Moreover, they have permitted the study of changes in biliary physiology in CF and the dysregulated response to inflammation of CF cholangiocytes. However, an animal model that reliably mirrors human CFLD is still lacking.

Summary

Due to the improved management of nutritional and pulmonary issues and increased life expectancy, the relevance and prevalence of liver disease in CF have increased. About one third of CF patients have evidence of liver involvement, although only a minority develops complications, which are mostly related to portal hypertension. Definite diagnostic criteria that are linked to clinical outcomes are lacking. Because pathophysiological understanding is incomplete, effective treatment is not yet available. Although treatment commencement with ursodeoxycholic acid at diagnosis is recommended, its efficacy is not proven. The management of complications of portal hypertension and liver function failure are generally similar to non-CF patients. Scientific efforts are needed to elucidate the etiology and pathogenesis and to establish safe and effective prevention and treatment.

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