Cystic fibrosis liver disease in children – A review of our current understanding

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ABSTRACT

Cystic fibrosis is the second most common genetic disease in infancy. It is the result of a mutated channel protein, the CFTR, which secretes chloride ions, fluidifying secretions.

Recent improvements in the treatment have increased life expectancy in these patients. Nevertheless, liver involvement remains the third cause of death. Unfortunately, our understating of the physiopathology is still deficient.

Biliary obstruction secondary to the presence of thick secretions is considered to lead to cirrhosis. However, treatment with ursodeoxycolic acid has not changed the natural history. Furthermore, the presence of portal hypertension in the absence of cirrhosis cannot be explained.

Recently, the role of CFTR as modulator of immune tolerance has been proposed, which could explain the presence of a persistent portal inflammation leading to fibrosis, and the gut-liver axis would also have a role in disease presentation and progression.

Key words: liver disease; cystic fibrosis; cystic fibrosis transmembrane conductance regulator; portal vein/pathology.

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INTRODUCTION

Cystic fibrosis (CF) is the second most common genetic affecting 1 in 2500 living births. Complex, heterogeneous and multiorganic, it affects every secretory epithelium, predominantly the lungs, pancreas and the gastrointestinal tract.[1]

It is the consequence of mutations in the cystic fibrosis transmembrane regulator (*CFTR*) gene in chromosome 7, a chloride ion transporting channel and bicarbonate exchanger.[2-5]

Pulmonary and pancreatic compromise are the main responsibles for morbidity and mortality, but cystic fibrosis liver diseases (CFLD) is the third cause of death. Novel therapies have improved life expectancy, rising from 20 years in 1980 to more than 45 years by 2017. However, these changes came with new challenges like liver disease.[6,7]

Much is yet to be learned about CFLD, and we intend to highlight some of the current knowledge on its pathophysiology.

CFTR IN THE LIVER

High levels of CFTR expression is reported in the apical membranes of bile duct epithelial cells. Decrease in bicarbonate secretion by cholangiocytes exposes them to bile acid toxicity, and the lesion of these cells could be one of the mechanisms of liver disease observed in CF patients.[8]

In the last two decades, new evidence supports a more ubiquitous distribution of CFTR.[9-12] In a recent paper, researchers used 3D reconstruction to demonstrate the presence of CFTR in the autophagosomes of human macrophages using labeled LC3, a microtubule-associated protein.[13] Although the function of CFTR in macrophages is unknown, given that 80% of resident macrophages are found in the liver, its mere presence in these cells is important. Considering that macrophages play a central role in immune responses, debris clearance and collagen deposit, it is possible that *CFTR* mutations alter their capacity to adapt and respond to different stimuli or cause them to abnormally respond to otherwise non-pathogenic stimuli.[2,3,14,15]

CYSTIC FIBROSIS LIVER DISEASE (CFLD)

Liver involvement in CF is heterogenous, defined in current guidelines as either cirrhosis or portal hypertension, liver involvement with persistently or intermittently abnormal liver enzymes, steatosis, fibrosis, cholangiopathy or ultrasound abnormalities.[7,16,17] European guidelines consider the presence of CFLD when at least two of the following findigns are found: 1) hepatomegaly or splenomegaly; 2) abnormal serum liver enzymes; 3) liver involvement at ultrasound (heterogeneous liver, nodularity, portal hypertension, or biliary abnormalities).[7,18-20] It is worth noting that less than 50% show clinical and biochemical signs, and only 25% has clinical and ultrasonographic findings, or biochemical and ultrasound abnormalities.[20]

Using the European approach, Boëlle et al.,[20] found that CFLD is the third cause of mortality in CF accounting for 3% of deaths. Severe CFLD is usually present before puberty, with around 40% of diagnosis made before 12 years of age. Incidence increases until the third decade and stabilizes at around 25 years old.[20]

Neonatal cholestasis

Even if infrequent, CFLD is part of the differential diagnosis of neonatal cholestasis, accounting for less than 2% of cases, being a more common finding among CF patients with meconium ileus.[1,3,21] However, there are no differences in the development of liver cirrhosis between those with a history of cholestasis and those without.[22-24] Furthermore, most patients with an uneventful CF neonatal cholestasis have a good long-term prognosis and complete resolution of symptoms is the norm.[23,25-27]

Focal biliary cirrhosis

Focal biliary cirrhosis (FBC) is characterized by the presence of scattered areas of thickened periportal tissues with inflammation and fibrosis, cholestasis and ductular reaction, and the presence of an eosinophilic material plugging the affected bile ducts.[1,27]

Unfortunately, FBC is a histologic diagnosis found in 11% of infants, 26% of children at 1 year and up to 70% of adults; and even though MRI may show compatible findings, FBC is silent in most patients, not even revealed by biochemical tests.[1,7,20,27] FBC is believed to be the first step in disease progression towards the always symptomatic multilobular cirrhosis. However, even though there have not been many prospective studies, the best estimates put this risk between 0.8-8%.[7,23,27]

Multilobular cirrhosis

Multilobular cirrhosis is present in about 10% of patients and is different from FBC. While the latter is usually a histological finding with

few, if any, other findings, multilobular cirrhosis can be detected on physical examination as an enlarged, hard and nodular liver, or in latter stages with evidence of portal hypertension and its complications.[3,27,28] However, unlike in other forms of cirrhosis, there is a remarkable absence of clinical findings before the apparition of its complications, and it is not unusual for variceal bleeding to be the first sign of advanced liver disease.[20,29] Histology shows diffuse collagen deposits extending between expanded portal tracts, delimitating multiples regeneration nodules with healthy parenchyma in-between. Unfortunately, liver biopsy has its limitations, owing to the diffuse and patchy nature of the disease.[1,22,30] Furthermore, even with histology it is usually difficult to differentiate this complication from nodular regenerative hyperplasia.

Unlike FBC, multilobular cirrhosis seems to be predominantly diagnosed in pediatric patients, with most cases between the first and second decade and without increase in prevalence later in life.[30,31] Unfortunately, liver function tests remain normal or only slightly altered, suggesting a low sensitivity for screening. Nonetheless, splenomegaly and hypersplenism are almost universal findings.[22,31-33]

Multilobular cirrhosis can be detected in imaging studies as a liver with heterogenous parenchyma and attenuation of ultrasound transmission. Nodularity, also compatible with nodular regeneration, is also a common finding.[22,33] Although multilobular cirrhosis is currently considered the last stage of liver disease, progression to liver failure is relatively infrequent, estimated around 0.4%.[34] Although variceal hemorrhage has a mortality rate of 39% ten years after initial presentation, it remains an uncommon occurrence when compared to other types of cirrhosis, with a 10-year cumulative risk of 6.6%. Furthermore, bleeding happens almost exclusively in adolescents, being extremely rare before 9 or after 30 years old.[29]

Portal hypertension without cirrhosis

The development of portal hypertension may be secondary to cirrhosis progression, but what appears to be more common, precede it or even being present without cirrhosis.[1] There is enough evidence suggesting the presence of a portal venopathy, central to its development. Study of whole liver explants showed portal vein abnormalities, with smooth muscle surrounding portal veins of diminished size.[35,36]

Some authors believe that this presentation is more prevalent in adult patients while multilobular cirrhosis would be the predominantly pediatric complication.[36-38] In a study of 8 patients with CFLD and portal hypertension, the hepatic venous portal gradient was measured in 4 of them and was compatible with presinusoidal origin. They all had clinically apparent portal hypertension with normal bilirubin, while none of them showed signs of end-stage liver disease. Biopsies did not show bile duct obstruction, but instead structural abnormalities of the portal branches. These vascular changes were present in all the samples (including five explants), with dense fibrosis around the portal tracts and almost complete disappearance of vascular structures.[39]

We have revised the histology of 5 patients with CFLD transplanted at our institution with a mean of 16.5 years. Everyone had clinical signs of cirrhosis, portal hypertension and liver failure. However, we found similar characteristics to those described previously: portal tract expansion with thick layers of smooth muscle surrounding portal vein branches and thin fibrous septa delimiting nodules and dysmorphic neoducts[40] (*Figure 1*).

Hepatic steatosis

Although difficult to establish, hepatic steatosis may be the most prevalent finding of CFLD, the prevalence ranges from 14 to 75% in patients of all ages and is reported in 70% of liver biopsies of children with CFLD.[1,18,41] While some hypothesize that it is the result of nutritional deficiencies,[24,36] other have found it in patients with adequate nutritional status.[27,42] Some possible explanations proposed are essential fatty-acid deficiency, carnitine deficiency, oxidative stress, and even peripheral insulin resistance.[43,44] Hepatic steatosis has not been related neither to disease progression, nor to higher rates of cirrhosis.[27] Recent data suggest that it might be a frequent complication after liver transplantation in CF patients. In a study of 13 post-transplantation liver biopsies in CFLD patients, 6 of them presented steatosis in as early as two years post-transplantation.[45]

Normally a silent condition, it may appear as a smoothly enlarged liver on physical examination and without signs of portal hypertension. Ultrasound usually reveals a homogeneous hyper-echogenicity, but pseudo-masses of up to 2 cm have been reported.[46] Lastly, most patients with ultrasound changes compatible with steatosis, will also have abnormal liver function tests.[22,33,47]

FIGURE 1. Liver explants of CF patients showing abnormal portal branches
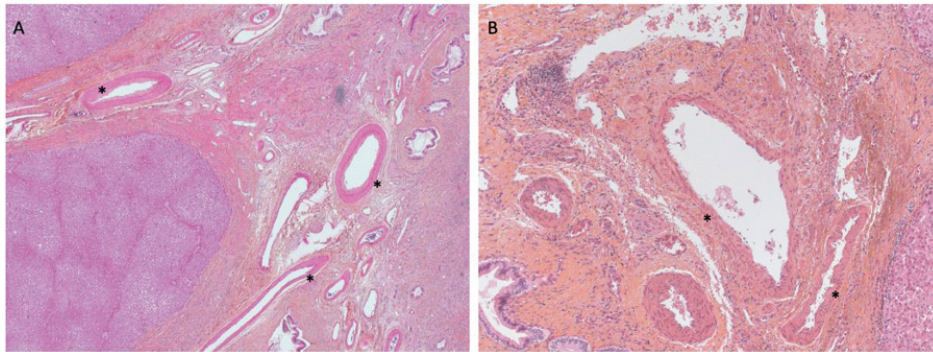
Portal tract expansion with collagen deposit. Portal branches surrounded by a thick layer of smooth muscle (*) in both pictures. Dysmorphic bile ducts are evident throughout de portal tract. (1.A 100x, 1.B 250x).

PATHOPHYSIOLOGY OF CFLD

CFTR gene mutations cause a decrease in the function of the protein, either by decreasing its synthesis, modifying its cellular location or its functioning. At the hepatic level, the excretion of chlorine and bicarbonate is decreased in the cholangiolar secretion, as is the coupled transport of water. Bile viscosity increases and bile flow decreases, with retention of bile salts in the ducts. Added to the changes in alkalinity, the detergent effect of the retained bile causes damage to the membranes and eventually fibrosis and cirrhosis.[2,3,7] This explanation of the pathophysiology of CFLD derives from the extrapolation of findings in the lung, pancreas and intestines.

However, histological findings of cholestasis are not always present, observed in less than 7% of biopsies with no direct correlation to the degree of fibrosis.[7,32,48] This may be due to the highly heterogeneous nature of liver involvement and the segmentary compromise of bile ducts, which may make biopsy findings somewhat random. This heterogeneity was evident in a series of 44 CF patients who underwent a scintigraphy, published in 1996; while most of them showed a homogeneous hepatic activity, focal intrahepatic retention was seen after 45 minutes. Similar findings were later reported by others.[49-51]

More recently, 17 patients who underwent liver transplantation for CFLD were studied and no explant was found to have bile duct dilation nor plugs of eosinophilic material, but small septa of fibrous tissue was noted in almost all of them. All showed nodular regenerative hyperplasia without cirrhosis, and most had diminished portal vein diameters within portal tracts.[35]

When reviewing the biopsies of our patients, we found that some explants showed evidence of multilobular cirrhosis with thin fibrous septa delimiting nodules and ductular reaction with neoducts formation (*Figure 2*).

Figure 2. Liver explants of CF patients showing nodular cirrhosis and bile duct dysmorphism
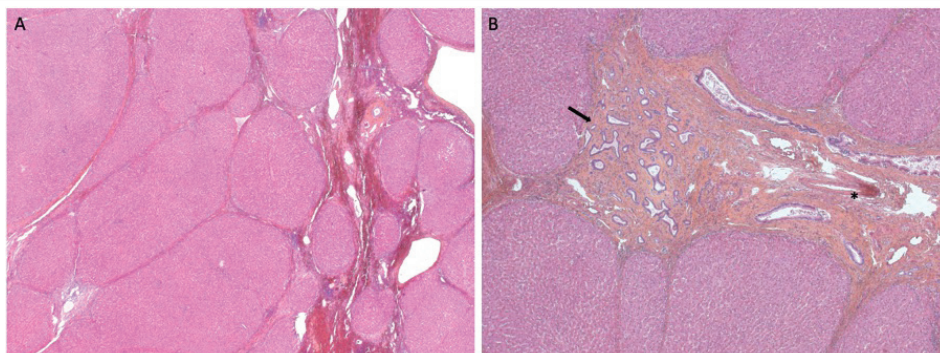
Multinodular cirrhosis with thin septa in both pictures. In 2.B portal tract expansion with a portal branch surrounded by smooth muscle, and ductular reaction with neoducts (arrow). (2.A 100x, 2.B 100x).

Treatment with ursodeoxycolic acid (UDCA) has shown to improve cholestasis when present and to normalize liver function tests, particularly γGT and alkaline phosphatase.[33, 34] However, it has shown to have no effect on the natural history of the disease, with no differences between the patients treated earlier or later.[9,20,39] These findings suggest that cholestasis and bile thickening are present at some degree in CF patients, but are not enough to explain the liver disease, rather being part of wider mechanisms of injury that have yet to be elucidated. Therefore, although UDCA improves bile flow and lessens the possible damage secondary to retained bile salts, it doesn't act upon the underlying mechanisms and the disease progresses.[20,37,49,52,53]

The possibility that CFTR may play a role in regulating immune tolerance, particularly in response to changes in gut microbiome, has been recently studied.[6,54] CFTR interacts and stabilizes proteins like Csk (C-terminal Src kinase) and Cbp (Csk binding protein) which keep Src kinases (tyrosine kinase) in an inactive state, but in the absence of CFTR they lose the ability to negatively regulate it. The active kinase will then phosphorylate the Toll-like receptor 4 (TLR4), a process necessary for immunological signaling.[6,27,55-57] In animal models, TLR4 phosphorylation was absent in physiological states in healthy cholangiocytes, but not in CFTR-KO (CFTR knock-out) mice's cholangiocytes. In this model, Src kinases inhibitors significantly lessened liver damage after the administration of dextran sodium sulphate (DSS). As DSS does not cause direct liver damage, but is rather used to induce an inflammatory colitis, liver damage must be explained by changes in the gut environment which is rendered more pro-inflammatory.[57,58]

Furthermore, peroxisome proliferator-activated receptor γ (PPAR-γ) in cholangiocytes seem to prevent the transcription of proinflammatory genes and was found to be upregulated in CFTR-KO mice, The use of agonist like rosiglitazone caused inhibition of gene expressions. Mice receiving this medication had attenuated liver damage when exposed to DSS. In this model, endogenous stimuli, as an imbalance between ω-3 and ω-6 polyunsaturated fatty, lessened the activation of this receptor and led to persistent inflammation.[58]

This immunological hypothesis could explain why disease activity appears to be predominantly found in portal branches, as these are the areas that receive the largest amounts of toxins coming from the intestins.[24,32,35,48] In turn, this would justify

the development of obliterative portal venopathy and non-cirrhotic hypertension, as the loss of immunotolerance causes a persistent low-grade inflammation.[1,32,35]

Furthermore, it recognizes the role that the gut-liver axis plays in disease progression. CF patients are exposed to long and repeated courses of antibiotics, undergo multiple hospitalizations from a young age, and suffer from nutritional deficits; all of which are known factors that disrupt the normal gut microbiome development.[6,7] It should also be taken in consideration that CF patients have an increased fecal loss of bile acid at rates similar of patients with terminal ileum resections, and to compensate these losses, the liver increases primary bile acids synthesis. While these changes have failed to show an increase in bile cytotoxicity in mice models, they may very well play a role in modifying the microbiome.[7,22,27,32,58,59] Recent findings highlight the importance of the gut-liver axis, as a significant reduction in intestinal inflammation markers was noted after treatment with lumacaftor (which increases the number of proteins CFTR in the cell surface) /Ivacaftor (which favors the opening of the chloride channel).[59,-64] Other studies have shown that diet-induced intestinal dysbiosis plays an integral role in the pathology of CF cholangiopathy in mice, which seems to be synergistic with genetic background and degree of CFTR function.[65] Microbiome participation may help explain the differences in liver disease presentation and progression between patients, as each of them is affected by different events during their lives.

A combined explanation of both bile thickening and vascular disease is plausible, accounting for the histological differences between patients. Peripheral portal tracts are more susceptible to inflammatory changes and vasculitis may further damage them.[54] This would give rise to a heterogeneous compromise, where pseudo-regenerative nodules are formed with fibrosis replacing most signs of cholestasis and leaving only ductular and vascular changes evident, and a degree of portal hypertension that is abnormally severe for the amount of fibrosis.[48] This would also explain why UDCA improves biochemical tests without having much effect on disease progression and mortality.

CONCLUSSIONS

The physiopathology of CFLD is complex, becoming ever more evident that bile thickening

fails to explain the whole picture. Furthermore, the existence of a non-cirrhotic portal hypertension can't be explained by this mechanism. Some authors have proposed an immunologic regulator role of the CFTR, and a pro-inflammatory state when mutated.

The combination of both explications seems attractive, where some degree of bile duct obstruction might be present but a "second hit" (vascular inflammation) is needed for progression. Some patients have a slow progression of portal venopathy developing non-cirrhotic portal hypertension later in life.

These hypotheses open a new field of research and the use of combinations of CFTR correctors and enhancers in CF treatment offer an opportunity to understand liver complications. ∎

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