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# Cystic Fibrosis-related liver disease: clinical presentations, diagnostic and monitoring approaches in the era of CFTR modulator therapies

## Authors

Jérémy Dana<sup>1,2,3,4</sup>, Dominique Debray<sup>5,6</sup>, Aurélie Beaufrère<sup>7</sup>, Sophie Hillaire<sup>8</sup>, Monique Fabre<sup>9</sup>,  
Caroline Reinhold<sup>10,11</sup>, Thomas Baumert<sup>2,3,4</sup>, Laureline Berteloot<sup>12</sup>, Valérie Vilgrain<sup>1</sup>

## Affiliations

1 Department of Radiology, Beaujon Hospital, Université de Paris, Clichy, France

2 Université de Strasbourg, Strasbourg, France.

3 Inserm, U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, Strasbourg, France.

4 IHU-Strasbourg (Institut Hospitalo-Universitaire), Pôle Hépato-digestif, Nouvel Hôpital Civil, Strasbourg, France.

5 Department of Pediatric Hepatology, Necker-Enfants Malades Hospital, Assistance Publique - Hôpitaux de Paris, Université de Paris, Paris, France.

6 Sorbonne Université, INSERM, Centre de recherche Saint-Antoine (CRSA), Institute of Cardiometabolism and Nutrition (ICAN), F-75012 Paris, France.;7 Department of Pathology, Beaujon Hospital, Université de Paris Clichy, France.

8 Department of Hepatology, Foch Hospital, Suresnes, France

9 Department of Pathology, Necker-Enfants Malades Hospital, Assistance Publique des Hôpitaux de Paris, Université de Paris, Paris, France

10 Department of Diagnostic Radiology, McGill University, Montreal, Canada

11 Augmented Intelligence & Precision Health Laboratory of the Research Institute of McGill University Health Centre, Montreal, Canada

12 Department of Pediatric Radiology, Necker-Enfants Malades Hospital, Assistance Publique des Hôpitaux de Paris, Université de Paris, Paris, France

1  
2 **Corresponding Author**

3  
4 Jérémy Dana, M.D.

5  
6 Department of Radiology, Beaujon Hospital, Clichy, France

7  
8 Telephone number: +33 1 40 87 53 58

9  
10 [jeremy.dana@etu.unistra.fr](mailto:jeremy.dana@etu.unistra.fr)

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invasive Diagnostic; Radio-pathologic correlations

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34  
35 Jérémy Dana wrote the original draft of the article under the supervision of Valérie Vilgrain and  
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Dominique Debray.

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Dominique Debray provided her expertise in pediatric hepatology and cystic fibrosis related liver  
disease.

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Sophie Hillaire and Thomas Baumert provided their expertise in adult hepatology.

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Aurélie Beaufrère and Monique Fabre provided their expertise in adult and pediatric liver pathology,  
respectively.

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Caroline Reinhold and Valérie Vilgrain provided their expertise in adult liver imaging and Laureline  
Berteloot in pediatric liver imaging.

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## **Abstract**

1 Cystic fibrosis (CF) is the most common autosomal recessive disease in the Caucasian population.  
2  
3 Cystic Fibrosis-related Liver Disease (CFLD) is defined by the pathogenesis related to the underlying  
4  
5 CFTR defect in biliary epithelial cells. CFLD needs to be distinguished from other liver  
6  
7 manifestations that may not have any pathological significance. The clinical and histological  
8  
9 presentation as well as the severity of CFLD varies. The main histological presentation of CFLD is  
10  
11 focal biliary fibrosis, which is usually asymptomatic. Portal hypertension develops in a minority of  
12  
13 cases (about 10%) and may require specific management including liver transplantation for end-stage  
14  
15 liver disease. Portal hypertension is usually the result of the progression of focal biliary fibrosis to  
16  
17 multilobular cirrhosis during childhood. Nevertheless, non-cirrhotic portal hypertension as a result of  
18  
19 porto-sinusoidal vascular disease is now identified increasingly more frequently in these patients,  
20  
21 mainly in young adults. To evaluate the effect of new CFTR modulator therapies on the liver, the  
22  
23 spectrum of hepatobiliary involvement must first be precisely classified. This paper discusses the  
24  
25 phenotypical features of CFLD, its underlying physiopathology and relevant diagnostic and follow-  
26  
27 up approaches with a special focus on imaging.  
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## **Key Points**

- 38 - Cystic Fibrosis-related Liver Disease, related to the underlying CFTR defect, includes two main,  
39  
40 sometimes coexisting, manifestations: focal biliary fibrosis and porto-sinusoidal vascular disease.  
41  
42
- 43 - Portal hypertension, either as a direct complication of biliary cirrhosis or secondary to porto-  
44  
45 sinusoidal vascular disease, should be carefully assessed because it may develop in the absence of  
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47 cirrhosis and requires careful management, in some cases including liver transplantation for end-stage  
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49 disease.  
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- 52 - Ultrasound of the liver is a key test for the diagnosis and follow-up of this entity and can provide a  
53  
54 complete evaluation of the liver parenchyma (normal, homogeneous or heterogeneous  
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56 hyperechogenicity, nodular pattern) and portal hypertension.  
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- Liver elastography is of interest to detect early-stage fibrosis and could play a role in long-term follow-up.

- Non-cirrhotic portal hypertension should be carefully investigated and may require liver biopsy and/or hemodynamic measurements of the hepatic venous pressure gradient in the presence of “red-flag” imaging features: 1) the presence of portal hypertension as a primary feature with the absence of liver parenchyma abnormalities 2) liver stiffness values below the threshold for cirrhosis with clinically significant portal hypertension and, 3) a coarse macronodular pattern suggesting regenerative nodular hyperplasia.

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## 1 **Introduction**

2 Cystic fibrosis (CF) is the most common autosomal recessive disease in the Caucasian population. It  
3  
4 3 is caused by pathogenic variants in the gene encoding the cystic fibrosis transmembrane conductance  
5  
6 4 regulator (*CFTR*), a chloride channel expressed in epithelial cells. Liver involvement, which is  
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9 5 reported in up to approximately 40% of CF patients [1] includes all hepatic manifestations including  
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11 6 clinical or biochemical liver abnormalities. These may be related to epiphenomena such as infectious  
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14 7 episodes or drug hepatotoxicity, steatosis from diverse origins (diabetes, chronic diarrhea, pancreatic  
15  
16 8 insufficiency, malnutrition, obesity...) or hepatic congestion of cardio-pulmonary origin. [2,3]. Thus,  
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19 9 liver involvement in these patients is not always CF-related liver disease (CFLD) which only includes  
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21 10 disease due to the underlying *CFTR* defect. Until the pathogenic mechanisms of CFLD are better  
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23  
24 11 understood, a phenotypical approach seems to be the most appropriate way to analyze and report  
25  
26 12 outcomes in these cases. The lexicon should follow the North American and European Societies for  
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28  
29 13 Paediatric Gastroenterology Hepatology and Nutrition which recommend the following phenotypic  
30  
31 14 characterization: multilobular cirrhosis with or without portal hypertension or liver failure, liver  
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33 15 involvement with or without portal hypertension but without cirrhosis, or no evidence of liver  
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36 16 involvement [4].  
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38 17 This review article discusses the spectrum of CFLD and provides their radiological-pathological  
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40  
41 18 correlations.

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## **Epidemiology**

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48 21 The absence of a precise definition of CFLD and increasingly frequent reports of cases of non-  
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51 22 cirrhotic portal hypertension explain the significant epidemiological variations in the scientific  
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53 23 literature [2,5]. European criteria to define CFLD were historically based on the presence of at least  
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56 24 two of the three following variables: abnormal physical examination, abnormal liver tests and  
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58 25 abnormal liver ultrasound suggesting fibrosis or cirrhosis (excluding steatosis) [6]. These criteria  
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61 26 were used to perform most epidemiological studies. The cumulative incidence of CFLD was found  
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1 to reach a plateau around the age of 18 with an incidence of 1.8% in CF patients [5]. As patient  
2 management has improved (including for pulmonary involvement) and with the recent increase in  
3 life expectancy of CF patients, studies have begun reporting liver disease in adults [1,7]. A large  
4 prospective cohort study including 3328 CF patients recently reported an incidence of CFLD that  
5 increased by approximately 1% every year, to reach 32% by age 25 and an incidence of severe CFLD  
6 (i.e. cirrhosis or portal hypertension) that was increasing after the age of 5 to reach 10% by age 30  
7 [1].

### 8 **Cystic Fibrosis Liver Disease**

9 Portal hypertension, either as a direct complication of biliary cirrhosis and/or due to porto-sinusoidal  
10 vascular disease, plays a central role in CFLD because it is the main cause of morbidity  
11 (undernutrition, ascites, infection and variceal bleeding) and one of the leading causes of death after  
12 lung failure and lung transplantation [2,5,8] in these patients. In a large series of 905 CF patients with  
13 cirrhosis [9], the estimated 10-year cumulative rate of all liver-related adverse events (variceal  
14 hemorrhage, liver transplantation and death due to liver failure) was 20.4%. In another cohort  
15 including 561 patients with cirrhosis, CFLD-related complications appeared to be limited to patients  
16 with portal hypertension [10]. A study from the European Liver Transplant Registry reported that  
17 before liver transplantation, 89% of the CF patients presented with esophageal varices with at least  
18 one episode of gastrointestinal bleeding in 42% of cases, ascites in 44% and encephalopathy in 13%  
19 [11]. Although the progression of liver disease to liver failure is unpredictable, it rarely occurs during  
20 childhood [12].

### 21 **Pathogenesis of CFLD**

22 The pathogenesis of CFLD remains poorly understood. In the liver, CFTR chloride channel  
23 expression is restricted to the apical membrane of cholangiocytes lining the biliary ducts [13]. CFTR  
24 is essential to maintain pH regulation and biliary  $\text{HCO}_3^-$  secretion. A proper alkaline balance,  
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26



1 described by Beuers et al as the 'bicarbonate umbrella', provides a protective shield to cholangiocytes  
2 against hydrophobic bile acids [14].

3  
4 3 The primary hypothesis for the development of biliary fibrosis is based on a decreased or absent  
5  
6 4 CFTR function in the bile duct, causing decreased transport of chloride, bicarbonate, and osmotically  
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8  
9 5 coupled water into the bile, leading to increased viscosity, reduced bile flow, and biliary obstruction  
10  
11 6 with bile and mucoid secretions. This would cause retention of endogenous hydrophobic bile acids,  
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14 7 cell membrane injury, peribiliary inflammation, and focal biliary fibrosis [15]. However, this  
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16 8 mechanism is not fully supported by relatively rare findings of inspissated bile secretions [16] and is  
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19 9 probably not the only cause. In addition, in CFTR KO mice models, a causal relationship has been  
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21 10 identified between intestinal inflammation, which is a frequent finding in CF patients, and biliary  
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23  
24 11 damage [17]. The innate immunity/gut dysbiosis axis could help further explain the pathogenesis of  
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26 12 CFLD. Indeed, CFTR has been shown to play a role in regulating the innate immunity signalling  
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29 13 pathways in cholangiocytes [18]. More precisely, functional CFTR keeps inactivated Rous sarcoma  
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31 14 oncogene cellular homolog (Src) tyrosine kinases at the apical membrane of cholangiocytes [19].  
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33 15 Thus, in the presence of a defective CFTR, Src tyrosine kinases can self-activate and lead to an  
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36 16 inflammatory process by the phosphorylation of toll-like receptor 4 (TLR4), activation of nuclear  
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38 17 factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) and the production of pro-  
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41 18 inflammatory cytokines [20]. Gut dysbiosis, which is frequently reported in CF patients, could trigger  
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43 19 this dysregulated innate immunity [21,22]. Several explanations have been proposed for gut  
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46 20 dysbiosis: prolonged intestinal transit, which is thought to favor bacterial overgrowth in the small  
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48 21 intestine [21,23], frequent exposure to antibiotics, pancreatic enzymes and/or proton pump inhibitors  
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51 22 [24]. Altered microbiota is thought to be responsible for intestinal inflammation, damaging the tight  
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53 23 and adherence junctions of epithelial intestinal cells and increasing intestinal permeability [25–27].  
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55 24 Thus, the increased intestinal permeability associated with the more pathogenic strains of bacteria,  
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58 25 which are also the result of altered microbiota [21,28], could lead to the translocation of bacteria and  
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60 26 bacterial products and pathogen-associated molecular patterns (PAMPs) [29,30]. Furthermore,  
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1 altered microbiota also leads to disturbed bile acid homeostasis by the disruption of the farnesoid X  
2 receptor (FXR) - fibroblast growth factor 19 (FGF19) activation pathway with more toxic bile acids.  
3  
4 3 Indeed, FXR is expressed in enterocytes and activates FGF19 synthesis, which circulates to the liver  
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6 4 to act as a negative feedback regulator of cholesterol 7 $\alpha$ -hydroxylase (Cyp7a1), encoding the first  
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9 5 enzyme of the bile acid synthesis pathway [31]. Impaired bile acid homeostasis and the translocation  
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11 6 of bacterial endotoxins and PAMPs into the portal venous system and to the liver may trigger  
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14 7 uncontrolled biliary inflammation by activating the dysregulated Src/NF- $\kappa$ B signalling pathway in  
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16 8 CFTR-defective cholangiocytes. In addition, CFTR-deficient mice models have demonstrated a  
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19 9 defect in peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) function whose stimulation could  
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21 10 downregulate NF- $\kappa$ B mediated inflammation [32,33].  
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23  
24 11 On the other hand, the pathogenesis of porto-sinusoidal vascular disease has not been clarified. The  
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26 12 anatomical proximity of the portal system to the bile ducts and the pro-inflammatory state resulting  
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29 13 from CFTR-defective cholangiocytes have not been explored. One hypothesis is the involvement of  
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31 14 the intra-hepatic portal system secondary to the pro-inflammatory state centered on the bile ducts.  
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34 15 There are two approaches to the proposed pathogenesis: micro-thrombotic phenomena due to platelet  
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36 16 activation and CFTR defect-related endothelial lesion damage. Indeed, CFTR is both functional and  
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39 17 expressed in cell types other than cholangiocytes including platelets, endothelial cells and smooth  
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41 18 muscle cells [34,35]. Recent studies have suggested that the CFTR defect promotes platelet  
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44 19 activation, resulting in increased platelet aggregation [36]. Moreover, platelet function, which plays  
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46 20 a key role in the down-regulation of inflammation, [37] also seems to be affected by the CFTR defect  
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49 21 which might lead to a pro-inflammatory state [38–40]. The role of CFTR in endothelial dysfunction  
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51 22 is not well understood [41]. The CFTR defect leads to decreased nitric oxide [35], decreased  
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54 23 endothelium-dependent relaxation and increased arterial stiffness (increased vasoconstriction in  
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56 24 response to vasoactive agents and impaired cAMP-dependent vasorelaxation due to the altered  
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58 25 mechanical properties of smooth muscle cells) [42,43]. It could also lead to increased oxidative stress  
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61 26 (reactive oxygen species) [44], a pro-inflammatory state (VCAM-1, ICAM-1, pro-inflammatory  
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1 interleukins, TGF- $\beta$ , CD40-L,...) [41,45–47], increased membrane permeability (due to altered  
2 distribution of transmembrane protein Vascular Endothelial cadherin and p120-catenin leading to  
3 impaired intercellular adherence junctions) [48] and a pro-coagulant state [49,50]. Once again,  
4 bacterial endotoxins in the gut could play a role in the pathogenesis of endothelial lesions.

5 However, it is not clear why CFLD only develops in a minority of patients (about one third), with  
6 varying degrees of severity. The progression from focal biliary fibrosis to multilobular cirrhosis is a  
7 slow and unpredictable process [5,51–53]. The severity of histological lesions does not appear to be  
8 correlated to age or progression over time [16,54]. While the presence of CFLD appears to be limited  
9 to severe genotypes (class I, II or III mutations in both alleles, resulting in a lack of synthesis or total  
10 loss of CFTR function [1,5,55–57]), no correlation has been established with specific mutations [5].  
11 Furthermore, while the heterogeneity of the manifestations of CFLD in siblings suggest that factors  
12 in addition to CFTR play an important role, the major impact of environmental factors can also be  
13 excluded [58,59]. Thus modulatory genetic (called "modifier genes") and/or extrinsic (therapeutic  
14 and iatrogenic) factors probably play a decisive role in the heterogeneity of the disease. SERPINA-  
15 1, encoding the  $\alpha$ -1antitrypsin protein, is the only modifier gene identified to date [60]. Indeed, CF  
16 patients carrying the SERPINA1 Z allele have a higher risk of developing CFLD and related  
17 complications than noncarriers [61,62]. The accumulation of the Z variant of  $\alpha$ 1-antitrypsin in  
18 cholangiocytes could induce stress in the endoplasmic reticulum and promote biliary damage from  
19 bacterial endotoxins and hydrophobic bile acids. Nevertheless, the much lower proportion (2.7%) of  
20 CF patients carrying the Z allele compared to the proportion (30.5%) of patients who develop CFLD,  
21 suggests that other modifier genes also play a role [60,62]. Finally, several studies have reported a  
22 higher incidence of CFLD in male patients [5,10,12]. This is also true for patients with a history of  
23 meconium ileus, although this remains controversial [2,5,8,12].

## 24 Diagnostic Techniques

1 Because liver disease progresses insidiously and is rarely symptomatic during the early stages, and  
2 portal hypertension can remain silent until the development of life-threatening complications,  
3  
4 European guidelines recommend annual screening for liver involvement by clinical examination  
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6 (hepatomegaly, splenomegaly), liver tests, and abdominal ultrasound (Figure 1). However, the  
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8 accuracy of liver tests and ultrasound is low for the diagnosis of liver fibrosis, especially in the early  
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10 stages. Liver tests abnormalities are found in more than 50% of CF infants, which then normalize in  
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12 most cases before the age of 2-3 years old [54]. Only persistently elevated gammaglutamyl transferase  
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14 (GGT) has been associated with the development of cirrhosis [63,64]. Despite the significant intra-  
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16 and inter-reader variability [65], liver ultrasound remains a key examination. It provides complete  
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18 evaluation of the liver parenchyma (normal, homogeneous or heterogeneous hyperechogenicity,  
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20 nodular) (Figure 2), liver contours, gallbladder and biliary tree (i.e., focal dilatation, cholelithiasis,  
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22 micro-gallbladder or gallbladder dyskinesia) [66–68]. Portal hypertension may also be directly  
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24 (porto-systemic shunts, decreased or reversed portal flow, dilated portal vein) or indirectly  
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26 (splenomegaly, ascites and, in children, increased thickness of the lesser omentum  $> 1.5$  times the  
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28 diameter of the aorta) evaluated. Ultrasound patterns (normal, homogeneous or heterogeneous  
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30 hyperechogenicity, nodular) seem to be correlated to the biomarkers of liver fibrosis (APRI and Fib-  
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32 4 scores) [68]. A heterogeneous hyperechogenic pattern has also been associated with a risk of  
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34 progression to a nodular pattern, suggesting the development of cirrhosis [66,69]. One long-term  
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36 study with a 4-year follow-up reported a 9-fold increased risk of developing a nodular ultrasound  
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38 pattern in patients with an initially heterogeneous pattern, compared to those with a normal pattern  
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40 [66]. However, it should be noted that the progression to a nodular pattern was only found in 13/55  
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42 patients (23%). Moreover, a nodular pattern is not specific for cirrhosis but may also indicate nodular  
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44 regenerative hyperplasia during porto-sinusoidal vascular disease. Finally, a heterogeneous pattern  
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46 does not systematically indicate liver fibrosis, but may be represent patchy steatosis [70]. Additional  
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48 ultrasound tools can be helpful in identifying steatosis, but must be validated in CF in particular, the  
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50 attenuation coefficient based on the ultrasound attenuation of the echo wave by the steatotic liver  
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1 (like the controlled attenuation coefficient in transient elastography (TE)) as well as the hepatorenal  
2 B-mode ratio, defined as the ratio of the echogenicity of the liver parenchyma to the renal cortex  
3 [71,72]. Moreover, MRI can provide non-invasive quantification of steatosis in the entire liver. One  
4 method called Proton Density Fat Fraction (PDFF), defined as the fraction of mobile protons (H1)  
5 linked to the triglyceride relative to those of water, is achieved by application of multi-echo Dixon  
6 and compensates for multiple confounders including T2\* relaxation effects and the spectral  
7 complexity of fat [73].

8 Liver biopsy is a decisive examination that can provide specific features of CFLD phenotypes. It is  
9 rarely performed in children with CF because it is invasive, there is a risk of under- or overestimating  
10 the lesions due to the focal nature of the fibrosis and treatment options may be limited [74–76]. The  
11 risk of under- or overestimation can be reduced by a dual-pass biopsy [70]. Liver biopsy can be  
12 performed by the transjugular route in association with hemodynamic measurements of the hepatic  
13 venous pressure gradient when porto-sinusoidal vascular disease is suspected, especially when  
14 evaluating the clinical consequences and therapeutic options of a liver- or lung transplantation [3,77].

15 Liver stiffness, a biomarker based on the elasticity of the liver, has been found to be valuable for even  
16 early stage diagnosis, as well as for monitoring liver fibrosis in CF in children [78] and adults [79].

17 Different modalities are available including vibration controlled TE or ultrasound-guided  
18 elastography such as point ShearWave Elastography (pSWE) and 2D ShearWave Elastography (2D  
19 SWE) [80], as well as Magnetic Resonance Elastography (MRE), but none has yet been shown to be  
20 better than the other methods to detect liver fibrosis in CF patients. It is difficult to identify cut-off  
21 values because of the many different techniques. One-dimensional TE estimates liver stiffness by  
22 measuring the velocity of an elastic shear wave propagating through the liver parenchyma using  
23 pulsed echo ultrasound acquisitions. This technique appears to be as effective as 2D SWE [79,81–  
24 83]. Ultrasound-guided elastography techniques (pSWE and 2D SWE) use ultrasound focused beams  
25 to generate shear waves. A quantitative estimation of tissue elasticity is obtained by measurement of  
26 shear wave velocity. These techniques are accurate and reproducible and targeted measurements can

1 be obtained by guided B-mode ultrasound [53,84]. Unlike pSWE, which only provides focal  
2 measurement of liver stiffness, 2D SWE provides real-time liver stiffness 2D color mapping. TE and  
3  
4 2D SWE can be easily performed in children. MRE is also a promising technique in children and has  
5  
6 become the new non-invasive gold-standard for the assessment of liver stiffness in adults [85]. A  
7  
8 complete map of liver stiffness is obtained using shear waves generated by an external acoustic driver  
9  
10 that is especially useful in patients with focal fibrosis [86]. This technique can be performed in the  
11  
12 presence of ascites and steatosis does not affect liver stiffness measurements [87]. However, several  
13  
14 challenges remain in children. First, spin-echo echo-planar imaging should be performed instead of  
15  
16 gradient-recalled echo, as it only requires a single breath hold, which can be critical in CF patients  
17  
18 with lung involvement [88]. The focal nature of fibrosis may also require volumetric segmentation  
19  
20 using regions of interest-based segmentation thus reinforcing the interest of 3D stiffness color  
21  
22 mapping [89]. The most commonly used techniques are TE, pSWE and 2D SWE. They have been  
23  
24 found to be reliable in longitudinal studies [78,79,84,90,91] but none of them has been shown to be  
25  
26 effective for the longitudinal monitoring of the progression of liver fibrosis in CF patients. The many  
27  
28 different techniques as well the absence of a precise definition of CFLD and the absence of several  
29  
30 studies validated by histological examination of the liver make it difficult to identify reliable cut-off  
31  
32 values.

33  
34 Finally, portal hypertension should be carefully looked for and not underestimated because it may  
35  
36 develop independently of cirrhosis (i.e., porto-sinusoidal vascular disease). In addition to ultrasound,  
37  
38 upper gastrointestinal endoscopy and hemodynamic measurements of the hepatic venous pressure  
39  
40 gradient may be indicated to accurately assess and characterize the cause of portal hypertension. Also,  
41  
42 in the presence of discrepant diagnoses, spleen elastography has been shown to accurately predict  
43  
44 portal hypertension and its complications such as high-risk esophageal varices, in other chronic liver  
45  
46 diseases both in adults and children [92,93]. The only study performed in CF children reported higher  
47  
48 spleen stiffness values than in healthy controls [94]. Thus, this approach has not yet been fully  
49  
50 validated for the assessment of portal hypertension in CF. On the other hand, like in other chronic  
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1 liver diseases [92] it has been suggested that liver elastography could be of added-value to predict  
 2 clinically significant portal hypertension in CF [95].

### 7 Biliary Fibrosis

9 Historically, the hepatic histological feature in these cases is focal biliary fibrosis, characterized by  
 10 heterogenous portal fibrosis that can gradually progress by forming bridges between the portal spaces,  
 11 12 associated with bile duct proliferation and biliary plugs (Figure 3) [96].

13  
 14 15 Patients are asymptomatic, and in the absence of advanced fibrosis there is usually no sign of portal  
 16 17 hypertension. Increased heterogeneous echogenicity of the liver may be identified on ultrasound,  
 18 19 suggesting focal fibrosis with or without associated steatosis, a frequent associated finding in CF  
 20 21 patients. Liver stiffness measurements may be particularly helpful in these cases to identify focal  
 22 23 fibrosis over isolated patchy steatosis. Indeed, elastography can improve or clarify misleading  
 24 25 ultrasound patterns by identifying liver fibrosis. Most studies on liver elastography have included  
 26 27 children with CFLD defined by clinical, biological or ultrasound findings [5,6,97]. Because of the  
 28 29 high prevalence of biliary fibrosis in children with no evidence of portal hypertension, the following  
 30 31 results can safely be applied to biliary fibrosis. These studies have proposed cut-off values from 5.5  
 32 33 to 7.1 kPa to predict CFLD using Transient Elastography (TE), [78,82,98]. The discrepancies may be  
 34 35 related to different definitions of CFLD and rare pathological examinations [5,6,97]. The only study  
 36 37 including a pathological examination of the liver by Lewindon et al. showed a good correlation  
 38 39 between liver stiffness measurements and the METAVIR stages of fibrosis (Spearman rank = 0.67)  
 40 41 [91]. The proposed cut-off value to predict fibrosis stage  $\geq$  F3 with TE was 8.7 kPa, which is  
 42 43 comparable to chronic B and C viral hepatitis [99,100]. Two studies evaluated Acoustic Radiation-  
 44 45 force Impulse (ARFI) elastography with cut-off values of 1.28 [101] and 1.45 m/s to predict CFLD  
 46 47 [98]. In the study by Karlas et al. [101] the diagnostic value was similar between ARFI and TE in the  
 48 49 same population. 2D SWE was evaluated by one study with a cut-off value of 6.85 kPa to predict  
 50 51 CFLD with a sensitivity and specificity of 75% and 71%, respectively [53]. Once again, it is important  
 52 53 54 55 56 57 58 59 60 61 62 63 64 65

1 to mention that the stiffness and cut-off values of these different elastography techniques are not  
2 comparable. Thus, liver stiffness should be monitored with the same elastography technique from the  
3 same manufacturer in the same patient. MRI can provide additional information such as a patchy  
4 increased signal on T2- and diffusion weighted imaging [102]. This can be completed with  
5 elastography to identify focal increases in liver stiffness on a complete color mapping of the liver.  
6 (Figure 4). Morphological changes (hypertrophy of the caudate lobe, atrophy of segment IV,  
7 hypertrophy of the left lobe and finally atrophy of the liver) and irregular contours suggest advanced  
8 fibrosis or cirrhosis.

#### 9 Porto-sinusoidal vascular disease

10 Porto-sinusoidal vascular disease, secondary to obliterative portal venopathy and/or nodular  
11 regenerative hyperplasia, is another presentation of CFLD. This entity has been more frequently  
12 reported in adults than in children [3,77,103]. Although the prevalence of non-cirrhotic portal  
13 hypertension in adults is important when deciding on the diagnostic approach, this should not exclude  
14 the presence of non-cirrhotic portal hypertension in children. In a single center series of 10 young  
15 adults who underwent liver transplantation for severe portal hypertension, 8 had features of non-  
16 cirrhotic portal hypertension with METAVIR stage 2 fibrosis in 3 livers and focal areas of stage 3 in  
17 5 livers [3]. Only one of these 8 patients had co-existing focal biliary fibrosis. The two remaining  
18 patients in this series had biliary cirrhosis. It is interesting to note that in a study by Pereira's et al in  
19 15 children with CFLD and portal hypertension between 6.7 and 16 years old, only 4 (27%) had stage  
20 4 fibrosis on pathological examination and 3 (20%) had stage 0 or 1 fibrosis [104]. However, this  
21 analysis was based on biopsy specimens, thus, there was a risk of under- or overestimating fibrosis.  
22 Specific characterization of the CFLD phenotypes was not provided in this study. Another series  
23 including 40 children with suspected CFLD who underwent liver biopsy [70], supported these results.  
24 Nine of them had portal hypertension but only 2 had stage 4 fibrosis (2 had stage 2 and 5 had stage 3  
25 fibrosis). The diagnosis of porto-sinusoidal vascular disease is difficult on imaging and can co-exist



1 with biliary fibrosis (Figure 5). Certain distinct imaging features should suggest this diagnosis. Liver  
2 biopsy and hemodynamic measurements of the hepatic venous pressure gradient may then be needed.  
3  
4 3 These “red-flag” imaging features include the presence of portal hypertension associated with an  
5  
6 4 absence of liver parenchyma abnormalities. The spleen is usually markedly enlarged [105] and porto-  
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9 5 systemic shunts are significant as seen in Figure 6. In children, the thickness of the lesser omentum  
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11 6 should be carefully evaluated compared to the aorta as a sign of portal hypertension. The caliber of  
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14 7 the intra-hepatic portal branches are reduced, while the portal vein is usually enlarged, but thrombosis  
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16 8 and mural calcifications may also be present in both [3,77,106] (Figures 6 and 7). Peripheral perfusion  
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19 9 disorders may be observed (Figure 6) and the hepatic artery may also be enlarged [106]. Unlike biliary  
20  
21 10 fibrosis, morphological changes characterized by hypertrophy of segment IV may indicate  
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23  
24 11 obliterative portal venopathy. Another “red-flag” finding is a coarse macronodular pattern of the liver.  
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26 12 This is highly suggestive of nodular regenerative hyperplasia as shown in Figures 7 and 8.  
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28  
29 13 Liver stiffness can play a key role in the diagnosis of porto-sinusoidal vascular disease. Indeed, in the  
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31 14 presence of typical clinically significant cirrhotic portal hypertension, liver stiffness values are  
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34 15 increased and higher than validated thresholds. In non-cirrhotic portal hypertension on porto-  
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36 16 sinusoidal vascular disease, liver stiffness values are lower because of the absence of the cirrhotic  
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39 17 process. In patients with porto-sinusoidal vascular disease, the discordance between liver stiffness  
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41 18 values that are lower than those expected in cirrhotic portal hypertension is the third “red-flag”  
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44 19 imaging feature. However, it should also be noted that liver stiffness values may still be increased  
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46 20 because porto-sinusoidal vascular disease is often associated with grade 2 or 3 fibrosis.  
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49 21 The results of liver biopsy will show signs of porto-sinusoidal vascular disease: obliterative portal  
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51 22 venopathy (intrahepatic portal vein wall thickening, portal venule narrowing, disappearance, or  
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53 23 sclerosis), nodular regenerative hyperplasia, incomplete septal fibrosis, portal tract abnormalities  
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56 24 (arterialization, periportal vessels, aberrant portal vessels), architectural disorganization (irregular  
57  
58 25 portal tracts and centrilobular vein distribution), non zonal sinusoidal dilatation and mild  
59  
60 26 perisinusoidal fibrosis [3,107,108]. However, the histological diagnosis of porto-sinusoidal vascular  
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1 disease can be complex because the characteristic lesions may be unevenly distributed and not always  
2 concurrent. Reticulin staining and a systematic analysis of the microcirculation on liver biopsy,  
3  
4 including vascular markers of sinusoids, portal venules and arteries, are essential for a diagnosis of  
5  
6 porto-sinusoidal vascular disease [109]. Besides the pathological features of porto-sinusoidal vascular  
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8 disease, bile duct dilation and mucosal biliary plugs are usually absent, except in the presence co-  
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10  
11 existent biliary fibrosis.  
12

13  
14 The hepatic venous pressure gradient often confirms the diagnosis of porto-sinusoidal vascular  
15  
16 disease, because it is clearly below the threshold for clinically significant portal hypertension (10  
17  
18 mmHg in adults) [77,110]. Indeed, hemodynamic measurements of the hepatic venous pressure  
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20  
21 gradient only reflect hepatic sinusoidal pressure and not portal pressure. Because porto-sinusoidal  
22  
23 vascular disease represents pre-sinusoidal involvement, the hepatic venous pressure gradient is falsely  
24  
25 low which is highly informative for the diagnosis of porto-sinusoidal vascular disease. This procedure  
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27  
28 is performed by transjugular route, allowing a liver biopsy during the same procedure.  
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### 31 32 33 Biliary Injury 34

35  
36 Biliary injury is thought to be a result of abnormal bile acidification. Neonatal cholestasis, caused  
37  
38 by viscous and inspissated bile secretions, can sometimes be the first sign, mimicking biliary atresia  
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40  
41 [111,112]. Biliary involvement can present as lesions from sclerosing cholangitis with single or  
42  
43 multiple focal stenoses, micro-gallbladder, gallbladder dysfunction or pigmentary cholelithiasis  
44  
45 [113]. MR cholangiography can identify irregularities of the intrahepatic bile ducts with alternating  
46  
47 dilatations and strictures [113].  
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51 In a prospective study including 26 adult CF patients, intra-hepatic biliary injury was present in 18  
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53 (69%) including 10 (38%) with sclerosing cholangitis-like lesions. Eighteen of these patients had no  
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55 signs of liver involvement [114]. Micro-gallbladder, defined by a cut-off volume of 2 cm<sup>3</sup>, was  
56  
57 reported in up to one third of the patients [115,116]. Gallbladder dysfunction is frequently reported,  
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60 characterized by hypokinesia and resulting in impaired gallbladder emptying. However, Santamaria  
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1 et al. did not find any difference in gallbladder emptying (gallbladder volume after an overnight fast  
 2 and 30 minutes after a standardized meal) between 57 children with CF and 25 healthy controls  
 3  
 4 [115]. Cholelithiasis is reported in 0.2 to 0.6% of cases (CF Foundation Registry) [117].  
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6 Finally, a large cohort study over 20 years reported an increased risk of gallbladder carcinoma and  
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### **Steatosis**

Hepatic steatosis, most often moderate, is reported in up to 70% of cases in histological series  
 [70,119]. Steatosis is hypothesized not to be part of CFLD. There are numerous physiopathological  
 explanations of this event, including nutritional (essential fatty acid deficiency [120,121],  
 phospholipid metabolism abnormalities [122], malnutrition [119,123], or obesity), pancreatic  
 insufficiency, choline depletion [124–126], diabetes and prolonged antibiotic therapy. While  
 malnutrition has long been a subject of debate [127], this cannot be the only explanation, as patients  
 with good nutritional status can present with steatosis [128]. On the other hand, the increasing  
 prevalence of overweight and obese patients in the CF population (31.4% in 2019 versus 12.8% in  
 1999 [129]) is of concern because of the association of this entity with metabolic dysfunction-  
 associated fatty liver disease [130]. The progression to steatohepatitis and cirrhosis is still uncertain  
 in CF for the moment.

### **Differential diagnosis: non-CF related liver involvement**

Although the detailed pathological mechanisms of CFLD must still be clarified, the previously  
 discussed phenotypic involvements (biliary fibrosis, porto-sinusoidal vascular disease, and biliary  
 injuries) have been shown to be induced by the CFTR mutation. Other hepatic manifestations may be  
 epiphenomena or secondary to iatrogenic events (drug toxicity), extra-hepatic events (hepatic  
 congestion on heart failure and advanced lung damage), bacterial or viral infections or to associated

1 non-CF related liver diseases that should always be excluded, such as chronic hepatitis B or C,  
2 autoimmune hepatitis, alpha1 antitrypsin deficiency, Wilson disease, celiac disease or genetic  
3  
4 3 hemochromatosis [6].

5  
6 4 Hepatic congestion on heart failure may result in dilated inferior vena cava and hepatic veins, loss of  
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9 5 normal triphasic flow pattern on doppler ultrasound or a mosaic parenchymal pattern on contrast-  
10  
11 6 enhanced CT or MRI.

## 16 8 **Treatment**

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19 9 Although ursodeoxycholic acid (UDCA) has long been the proposed treatment of CF patients with  
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21 10 liver involvement to prevent or halt the progression of fibrosis to cirrhosis [6], its efficacy remains  
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23  
24 11 controversial. A Cochrane review including 3 randomised controlled trials on the use of  
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26 12 ursodeoxycholic acid for at least three months compared to placebo or no additional treatment ( $n =$   
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28  
29 13 118), reported no benefit and did not recommend UDCA for routine use in CF [131]. Colombo et al  
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31 14 also reported no evidence of any benefit on the occurrence of portal hypertension [132]. On the other  
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33  
34 15 hand, a large longitudinal cohort study ( $n = 3417$ ) suggested that UDCA had a positive effect on  
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36 16 survival in patients with mild CFLD [7]. Recently, a synthetic side-chain-shortened derivative of  
37  
38 17 UDCA, NorUrsodeoxycholic acid (norUDCA), was developed and was shown to improve cholestasis  
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40  
41 18 in primary sclerosing cholangitis in a phase II clinical study [133]. Unlike UDCA, which increases  
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43 19 bile acid secretion and the risk of cholangiocyte damage, norUDCA increases bile flow by stimulating  
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46 20 bicarbonate secretion. In addition, norUDCA is credited with a direct anti-inflammatory action by  
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48 21 interfering with the NF- $\kappa$ B pathway [134]. There is still no study in CF patients.

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51 22 Current management is based on symptomatic treatment, including nutritional support, management  
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53 23 of the complications of portal hypertension with variceal band ligation and liver transplantation in  
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56 24 patients who progress to end-stage liver disease [135]. Although the complications of portal  
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58 25 hypertension and hepatocellular insufficiency are commonly accepted indications for liver  
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60 26 transplantation in CF, there are no consensus guidelines because the natural history of CFLD has not  
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1 been completely clarified and phenotypical characterization is difficult. The survival rates of liver  
2 transplantation in children and adults are acceptable (5-year survival rate 85.8 and 72.7%,  
3 respectively in a large series of 203 patients) and the long-term outcomes are influenced by the  
4 involvement of other organs [136]. Porto-systemic shunt procedures such as distal splenorenal or  
5 portocaval shunts in patients with symptomatic portal hypertension (recurrent bleeding or refractory  
6 ascites) can be considered to be an alternative to liver transplantation, [12,137] while transjugular  
7 intrahepatic portosystemic shunts are a bridge to liver transplantation [138]. Portal hypertension on  
8 porto-sinusoidal vascular disease was the main indication for transplantation in a recent series of liver  
9 transplantation in CF patients [3,77]. CFTR modulator therapies (CFTR correctors, i.e. correcting  
10 CFTR misprocessing/folding allowing an increased surface cell expression of the protein, and CFTR  
11 potentiators, i.e. potentiating the opening of the protein channels) may be a shift in therapeutic  
12 management [139]. This approach is already extensively used in clinical practice for lung  
13 involvement, and correction of the F508del processing defect has resulted in improved lung function,  
14 including pulmonary exacerbations and nutritional status [139–142]. Initially dual  
15 (lumacaftor/ivacaftor), therapies including more recent CFTR correctors and potentiators have been  
16 used (tezacaftor/ivacaftor, elexacaftor/tezacaftor/ivacaftor) with fewer sides effects, drugs  
17 interactions as well as with more extensive indications in F508del heterozygous patients [143–145].  
18 CFTR modulator therapies are expected to prevent or stop the progression of CFLD by correcting  
19 and potentiating CFTR in cholangiocytes, endothelial cells and platelets. Nevertheless, data on the  
20 efficacy of these treatments on the course of CFLD remain limited, because advanced liver disease  
21 was an exclusion criterion in CFTR modulator clinical trials [139,140]. These randomized controlled  
22 clinical trials have reported increases in serum alanine transaminase (ALT) and aspartate  
23 transaminase (AST) levels in 5-10% of treated patients which returned to baseline in almost all cases  
24 once treatment was discontinued. Conversely, two recent studies reported decreased AST and GGT  
25 levels during the year following treatment and no worsening of liver involvement, even in patients  
26 with multinodular livers suggesting cirrhosis [146,147]. Interestingly, Drummond et al also reported

1 persistently decreased bilirubin levels and APRI scores 12 months after initiation of treatment and a  
2 significant increase in CFTR activity in patients with the most “responsive” biological profiles [147].  
3  
4 3 Another study reported an improvement in liver steatosis with lumacaftor/ivacaftor [148]. Finally the  
5  
6 4 European Medicines Agency reported post-marketing cases of liver failure leading to death in CF  
7  
8 5 patients with pre-existing cirrhosis and portal hypertension who were treated with  
9  
10 lumacaftor/ivacaftor [149]. Better understanding of the safety of CFTR modulator therapies is needed  
11  
12 6 in relation to the severity of CFLD to identify those at the highest risk of serious adverse events as  
13  
14 7 well as to select patients with the best risk-benefit ratio. Rather than liver tests with a low sensitivity  
15  
16 8 and specificity, liver stiffness may be monitored during CFTR modulator therapy to improve early  
17  
18 9 detection of a response or progression of CFLD. In association with CFTR modulator therapies, there  
19  
20 10 is ongoing research to identify new therapies. Fiorotto et al showed that inhibition of Src tyrosine  
21  
22 11 kinases targeting the TLR4 mediated inflammatory process resulted in the reduction of the pro-  
23  
24 12 inflammatory state in human CF cholangiocytes, paving the way for potential therapies [150]. FXR  
25  
26 13 agonists (e.g., obeticholic acid) could restore bile acid homeostasis. Although obeticholic acid was  
27  
28 14 found to be beneficial in patients with other chronic liver diseases, it was associated with serious  
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30 15 adverse events [151]. FGF19 analogs are also a potential candidates [152] and other fibroblast growth  
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32 16 factors such as FGF1, which is a PPAR $\gamma$  target, are potent potential therapeutic agents [153,154]. It  
33  
34 17 has been suggested that vitamin D may alter gut microbiota, downregulate pro-inflammatory  
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36 18 cytokines and IL-8 production, and prevent liver fibrosis by inhibiting the TGF $\beta$ 1/SMAD signaling  
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38 19 pathway [155–158].  
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## 53 Conclusion

54 Portal hypertension, either as a direct complication of biliary cirrhosis or secondary to porto-  
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56 24 sinusoidal vascular disease, is the main complication of CFLD, influences patient management and  
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58 25 may require liver transplantation. Screening for liver involvement and fibrosis should include an  
59  
60 26 annual clinical examination, liver tests, and abdominal ultrasound. We also suggest liver stiffness  
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1 measurements. Because characterization of CFLD can be difficult on imaging, certain “red-flag”  
2 features should suggest the diagnosis of non-cirrhotic portal hypertension. In these cases,  
3  
4 3 hemodynamic measurements of the hepatic venous pressure gradient and liver biopsy may be  
5  
6 4 indicated allowing evaluation of the liver parenchyma. To evaluate the efficacy of CFTR modulator  
7  
8 5 therapies to prevent the progression of liver disease or adverse events, precise characterization of  
9  
10 6 liver involvement is needed. Thus, the triptych of liver tests, ultrasound and elastography could be  
11  
12 7 used for early detection of the response to treatment or of the progression of CFLD.  
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**Abbreviations**

1 Acoustic Radiation-force Impulse (ARFI)  
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4 Aspartate transaminase to Platelet Ratio Index (APRI)  
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7 Cystic fibrosis (CF)  
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10 Cystic fibrosis transmembrane conductance regulator (CFTR)  
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13 CF liver-related disease (CFLD)  
14  
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16 Farnesoid X receptor (FXR)  
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19 Fibroblast growth factor (FGF)  
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22 Gammaglutamyltransferase (GGT)  
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25 Magnetic Resonance Elastography (MRE)  
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28 Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B)  
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31 Pathogen-associated molecular patterns (PAMPs)  
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34 Point ShearWave elastography (pSWE)  
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37 Rous sarcoma oncogene cellular homolog (Src)  
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40 Toll-like receptor 4 (TLR4)  
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43 Transient Elastography (TE)  
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46 Two-dimensional ShearWave Elastography (2D SWE)  
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## References

- 1 [1] Boëlle P-Y, Debray D, Guillot L, Clement A, Corvol H. Cystic Fibrosis Liver Disease: Outcomes  
2 and Risk Factors in a Large Cohort of French Patients. *Hepatology* 2019;69:1648–56.  
3 <https://doi.org/10.1002/hep.30148>.
- 4 [2] Lamireau T, Monnereau S, Martin S, Marcotte J-E, Winnock M, Alvarez F. Epidemiology of  
5 liver disease in cystic fibrosis: a longitudinal study. *J Hepatol* 2004;41:920–5.  
6 <https://doi.org/10.1016/j.jhep.2004.08.006>.
- 7 [3] Hillaire S, Cazals-Hatem D, Bruno O, Miranda S de, Grenet D, Poté N, et al. Liver  
8 transplantation in adult cystic fibrosis: Clinical, imaging, and pathological evidence of  
9 obliterative portal venopathy. *Liver Transplantation* 2017;23:1342–7.  
10 <https://doi.org/10.1002/lt.24842>.
- 11 [4] Debray D, Narkewicz MR, Bodewes FAJA, Colombo C, Housset C, de Jonge HR, et al. Cystic  
12 Fibrosis-related Liver Disease: Research Challenges and Future Perspectives. *J Pediatr*  
13 *Gastroenterol Nutr* 2017;65:443–8. <https://doi.org/10.1097/MPG.0000000000001676>.
- 14 [5] Colombo C, Battezzati PM, Crosignani A, Morabito A, Costantini D, Padoan R, et al. Liver  
15 disease in cystic fibrosis: A prospective study on incidence, risk factors, and outcome.  
16 *Hepatology* 2002;36:1374–82. <https://doi.org/10.1053/jhep.2002.37136>.
- 17 [6] Debray D, Kelly D, Houwen R, Strandvik B, Colombo C. Best practice guidance for the  
18 diagnosis and management of cystic fibrosis-associated liver disease. *J Cyst Fibros* 2011;10  
19 *Suppl 2*:S29-36. [https://doi.org/10.1016/S1569-1993\(11\)60006-4](https://doi.org/10.1016/S1569-1993(11)60006-4).
- 20 [7] Toledano MB, Mukherjee SK, Howell J, Westaby D, Khan SA, Bilton D, et al. The emerging  
21 burden of liver disease in cystic fibrosis patients: A UK nationwide study. *PLOS ONE*  
22 2019;14:e0212779. <https://doi.org/10.1371/journal.pone.0212779>.
- 23 [8] Chrystostalis A, Hubert D, Coste J, Kanaan R, Burgel P-R, Desmazes-Dufeu N, et al. Liver  
24 disease in adult patients with cystic fibrosis: A frequent and independent prognostic factor  
25 associated with death or lung transplantation. *Journal of Hepatology* 2011;55:1377–82.  
26 <https://doi.org/10.1016/j.jhep.2011.03.028>.
- 27 [9] Ye W, Narkewicz MR, Leung DH, Karnsakul W, Murray KF, Alonso EM, et al. Variceal  
28 Hemorrhage and Adverse Liver Outcomes in Patients with Cystic Fibrosis Cirrhosis. *J Pediatr*  
29 *Gastroenterol Nutr* 2018;66:122–7. <https://doi.org/10.1097/MPG.0000000000001728>.
- 30 [10] Stonebraker JR, Ooi CY, Pace RG, Corvol H, Knowles MR, Durie PR, et al. Features of Severe  
31 Liver Disease With Portal Hypertension in Patients With Cystic Fibrosis. *Clin Gastroenterol*  
32 *Hepatol* 2016;14:1207-1215.e3. <https://doi.org/10.1016/j.cgh.2016.03.041>.
- 33 [11] Melzi ML, Kelly DA, Colombo C, Jara P, Manzanares J, Colledan M, et al. Liver transplant in  
34 cystic fibrosis: a poll among European centers. A study from the European Liver Transplant  
35 Registry. *Transplant International* 2006;19:726–31. <https://doi.org/10.1111/j.1432-2277.2006.00344.x>.
- 36 [12] Debray D, Lykavieris P, Gauthier F, Dousset B, Sardet A, Munck A, et al. Outcome of cystic  
37 fibrosis-associated liver cirrhosis: management of portal hypertension. *J Hepatol* 1999;31:77–  
38 83.
- 39 [13] Cohn JA, Strong TV, Picciotto MR, Nairn AC, Collins FS, Fitz JG. Localization of the cystic  
40 fibrosis transmembrane conductance regulator in human bile duct epithelial cells.  
41 *Gastroenterology* 1993;105:1857–64. [https://doi.org/10.1016/0016-5085\(93\)91085-v](https://doi.org/10.1016/0016-5085(93)91085-v).
- 42 [14] Beuers U, Hohenester S, de Buy Wenniger LJM, Kremer AE, Jansen PLM, Elferink RPJO. The  
43 biliary HCO<sub>3</sub>(-)-umbrella: a unifying hypothesis on pathogenetic and therapeutic aspects of  
44 fibrosing cholangiopathies. *Hepatology* 2010;52:1489–96.  
45 <https://doi.org/10.1002/hep.23810>.
- 46
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60  
61  
62  
63  
64  
65
- [15] Feranchak AP, Sokol RJ. Cholangiocyte biology and cystic fibrosis liver disease. *Semin Liver Dis* 2001;21:471–88. <https://doi.org/10.1055/s-2001-19030>.
  - [16] Potter CJ, Fishbein M, Hammond S, McCoy K, Qualman S. Can the histologic changes of cystic fibrosis-associated hepatobiliary disease be predicted by clinical criteria? *J Pediatr Gastroenterol Nutr* 1997;25:32–6.
  - [17] Debray D, Mourabit HE, Merabtene F, Brot L, Ulveling D, Chrétien Y, et al. Diet-Induced Dysbiosis and Genetic Background Synergize With Cystic Fibrosis Transmembrane Conductance Regulator Deficiency to Promote Cholangiopathy in Mice. *Hepatology Communications* 2018;2:1533–49. <https://doi.org/10.1002/hep4.1266>.
  - [18] Fiorotto R, Strazzabosco M. Pathophysiology of Cystic Fibrosis Liver Disease: A Channelopathy Leading to Alterations in Innate Immunity and in Microbiota. *Cell Mol Gastroenterol Hepatol* 2019;8:197–207. <https://doi.org/10.1016/j.jcmgh.2019.04.013>.
  - [19] Fiorotto R, Villani A, Kourtidis A, Scirpo R, Amenduni M, Geibel PJ, et al. The cystic fibrosis transmembrane conductance regulator controls biliary epithelial inflammation and permeability by regulating Src tyrosine kinase activity. *Hepatology* 2016;64:2118–34. <https://doi.org/10.1002/hep.28817>.
  - [20] Fiorotto R, Scirpo R, Trauner M, Fabris L, Hoque R, Spirli C, et al. Loss of CFTR affects biliary epithelium innate immunity and causes TLR4-NF- $\kappa$ B-mediated inflammatory response in mice. *Gastroenterology* 2011;141:1498–508, 1508.e1-5. <https://doi.org/10.1053/j.gastro.2011.06.052>.
  - [21] Flass T, Tong S, Frank DN, Wagner BD, Robertson CE, Kotter CV, et al. Intestinal lesions are associated with altered intestinal microbiome and are more frequent in children and young adults with cystic fibrosis and cirrhosis. *PLoS ONE* 2015;10:e0116967. <https://doi.org/10.1371/journal.pone.0116967>.
  - [22] Albillos A, de Gottardi A, Rescigno M. The gut-liver axis in liver disease: Pathophysiological basis for therapy. *Journal of Hepatology* 2020;72:558–77. <https://doi.org/10.1016/j.jhep.2019.10.003>.
  - [23] Lisowska A, Wójtowicz J, Walkowiak J. Small intestine bacterial overgrowth is frequent in cystic fibrosis: combined hydrogen and methane measurements are required for its detection. *Acta Biochim Pol* 2009;56:631–4.
  - [24] Li L, Somers S. The clinical significance of the gut microbiota in cystic fibrosis and the potential for dietary therapies. *Clinical Nutrition* 2014;33:571–80. <https://doi.org/10.1016/j.clnu.2014.04.004>.
  - [25] Bruzzese E, Raia V, Gaudiello G, Polito G, Buccigrossi V, Formicola V, et al. Intestinal inflammation is a frequent feature of cystic fibrosis and is reduced by probiotic administration. *Alimentary Pharmacology & Therapeutics* 2004;20:813–9. <https://doi.org/10.1111/j.1365-2036.2004.02174.x>.
  - [26] Werlin SL, Benuri-Silbiger I, Kerem E, Adler SN, Goldin E, Zimmerman J, et al. Evidence of intestinal inflammation in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 2010;51:304–8. <https://doi.org/10.1097/MPG.0b013e3181d1b013>.
  - [27] De Lisle RC. Disrupted tight junctions in the small intestine of cystic fibrosis mice. *Cell Tissue Res* 2014;355:131–42. <https://doi.org/10.1007/s00441-013-1734-3>.
  - [28] Scanlan PD, Buckling A, Kong W, Wild Y, Lynch SV, Harrison F. Gut dysbiosis in cystic fibrosis. *Journal of Cystic Fibrosis* 2012;11:454–5. <https://doi.org/10.1016/j.jcf.2012.03.007>.
  - [29] Werlin SL, Benuri-Silbiger I, Kerem E, Adler SN, Goldin E, Zimmerman J, et al. Evidence of Intestinal Inflammation in Patients With Cystic Fibrosis. *Journal of Pediatric Gastroenterology and Nutrition* 2010;51:304–8. <https://doi.org/10.1097/MPG.0b013e3181d1b013>.
  - [30] Hallberg K, Grzegorzczak A, Larson G, Strandvik B. Intestinal Permeability in Cystic Fibrosis in Relation to Genotype. *Journal of Pediatric Gastroenterology and Nutrition* 1997;25:290–5.

- [31] Kliewer SA, Mangelsdorf DJ. Bile Acids as Hormones: The FXR-FGF15/19 Pathway. *DDI* 2015;33:327–31. <https://doi.org/10.1159/000371670>.
- [32] Scirpo R, Fiorotto R, Villani A, Amenduni M, Spirli C, Strazzabosco M. Stimulation of nuclear receptor peroxisome proliferator-activated receptor- $\gamma$  limits NF- $\kappa$ B-dependent inflammation in mouse cystic fibrosis biliary epithelium. *Hepatology* 2015;62:1551–62. <https://doi.org/10.1002/hep.28000>.
- [33] Harmon GS, Dumlao DS, Ng DT, Barrett KE, Dennis EA, Dong H, et al. Pharmacological correction of a defect in PPAR- $\gamma$  signaling ameliorates disease severity in Cftr-deficient mice. *Nat Med* 2010;16:313–8. <https://doi.org/10.1038/nm.2101>.
- [34] Tousson A, Van Tine BA, Naren AP, Shaw GM, Schwiebert LM. Characterization of CFTR expression and chloride channel activity in human endothelia. *Am J Physiol* 1998;275:C1555-1564. <https://doi.org/10.1152/ajpcell.1998.275.6.C1555>.
- [35] Poore S, Berry B, Eidson D, McKie KT, Harris RA. Evidence of Vascular Endothelial Dysfunction in Young Patients With Cystic Fibrosis. *Chest* 2013;143:939–45. <https://doi.org/10.1378/chest.12-1934>.
- [36] Lindberg U, Svensson L, Hellmark T, Segelmark M, Shannon O. Increased platelet activation occurs in cystic fibrosis patients and correlates to clinical status. *Thrombosis Research* 2018;162:32–7. <https://doi.org/10.1016/j.thromres.2017.12.012>.
- [37] von Hundelshausen P, Weber C. Platelets as Immune Cells. *Circulation Research* 2007;100:27–40. <https://doi.org/10.1161/01.RES.0000252802.25497.b7>.
- [38] Ortiz-Muñoz G, Yu MA, Lefrançois E, Mallavia B, Valet C, Tian JJ, et al. Cystic fibrosis transmembrane conductance regulator dysfunction in platelets drives lung hyperinflammation. *J Clin Invest* 2020;130:2041–53. <https://doi.org/10.1172/JCI129635>.
- [39] O’Sullivan BP, Michelson AD. The Inflammatory Role of Platelets in Cystic Fibrosis. *Am J Respir Crit Care Med* 2006;173:483–90. <https://doi.org/10.1164/rccm.200508-1243PP>.
- [40] Mattoscio D, Evangelista V, Cristofaro RD, Recchiuti A, Pandolfi A, Silvestre SD, et al. Cystic fibrosis transmembrane conductance regulator (CFTR) expression in human platelets: impact on mediators and mechanisms of the inflammatory response. *The FASEB Journal* 2010;24:3970–80. <https://doi.org/10.1096/fj.10-159921>.
- [41] Declercq M, Zeeuw P de, Conchinha NV, Geldhof V, Ramalho AS, García-Caballero M, et al. Transcriptomic analysis of CFTR-impaired endothelial cells reveals a pro-inflammatory phenotype. *European Respiratory Journal* 2021;57. <https://doi.org/10.1183/13993003.00261-2020>.
- [42] Buehler T, Steinmann M, Singer F, Regamey N, Casaulta C, Schoeni MH, et al. Increased arterial stiffness in children with cystic fibrosis. *European Respiratory Journal* 2012;39:1536–7. <https://doi.org/10.1183/09031936.00212511>.
- [43] Robert R, Norez C, Becq F. Disruption of CFTR chloride channel alters mechanical properties and cAMP-dependent Cl<sup>-</sup> transport of mouse aortic smooth muscle cells. *The Journal of Physiology* 2005;568:483–95. <https://doi.org/10.1113/jphysiol.2005.085019>.
- [44] Khalaf M, Scott-Ward T, Causer A, Saynor Z, Shepherd A, Górecki D, et al. Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) in Human Lung Microvascular Endothelial Cells Controls Oxidative Stress, Reactive Oxygen-Mediated Cell Signaling and Inflammatory Responses. *Frontiers in Physiology* 2020;11:879. <https://doi.org/10.3389/fphys.2020.00879>.
- [45] Nowak JK, Wojsyk-Banaszak I, Mądry E, Wykrętowicz A, Krzyżanowska P, Drzymala-Czyż S, et al. Increased Soluble VCAM-1 and Normal P-Selectin in Cystic Fibrosis: a Cross-Sectional Study. *Lung* 2017;195:445–53. <https://doi.org/10.1007/s00408-017-0029-y>.
- [46] Sprague AH, Khalil RA. Inflammatory cytokines in vascular dysfunction and vascular disease. *Biochem Pharmacol* 2009;78:539–52. <https://doi.org/10.1016/j.bcp.2009.04.029>.

- [47] Falco A, Romano M, Iapichino L, Collura M, Davì G. Increased soluble CD40 ligand levels in cystic fibrosis. *Journal of Thrombosis and Haemostasis* 2004;2:557–60. <https://doi.org/10.1111/j.1538-7836.2004.00683.x>.
- [48] Totani L, Plebani R, Piccoli A, Di Silvestre S, Lanuti P, Recchiuti A, et al. Mechanisms of endothelial cell dysfunction in cystic fibrosis. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 2017;1863:3243–53. <https://doi.org/10.1016/j.bbadis.2017.08.011>.
- [49] Romano M, Collura M, Iapichino L, Pardo F, Falco A, Chiesa PL, et al. Endothelial Perturbation in Cystic Fibrosis. *Thromb Haemost* 2001;86:1363–7. <https://doi.org/10.1055/s-0037-1616736>.
- [50] Serisier DJ, Carroll MP. Catheter-related thrombosis associated with elevated factor VIII levels in cystic fibrosis. *Journal of Cystic Fibrosis* 2006;5:201–4. <https://doi.org/10.1016/j.jcf.2006.02.003>.
- [51] Lamireau T, Monnereau S, Martin S, Marcotte J-E, Winnock M, Alvarez F. Epidemiology of liver disease in cystic fibrosis: a longitudinal study. *Journal of Hepatology* 2004;41:920–5. <https://doi.org/10.1016/j.jhep.2004.08.006>.
- [52] van de Peppel IP, Bertolini A, Jonker JW, Bodewes FAJA, Verkade HJ. Diagnosis, follow-up and treatment of cystic fibrosis-related liver disease. *Curr Opin Pulm Med* 2017;23:562–9. <https://doi.org/10.1097/MCP.0000000000000428>.
- [53] Calvopina DA, Noble C, Weis A, Hartel GF, Ramm LE, Balouch F, et al. Supersonic shear-wave elastography and APRI for the detection and staging of liver disease in pediatric cystic fibrosis. *J Cyst Fibros* 2019. <https://doi.org/10.1016/j.jcf.2019.06.017>.
- [54] Lindblad A, Glaumann H, Strandvik B. Natural history of liver disease in cystic fibrosis. *Hepatology* 1999;30:1151–8. <https://doi.org/10.1002/hep.510300527>.
- [55] Rowe SM, Miller S, Sorscher EJ. Cystic Fibrosis. *New England Journal of Medicine* 2005;352:1992–2001. <https://doi.org/10.1056/NEJMra043184>.
- [56] Zeitlin PL. Pharmacologic restoration of  $\alpha\delta F508$  CFTR-mediated chloride current. *Kidney International* 2000;57:832–7. <https://doi.org/10.1046/j.1523-1755.2000.00922.x>.
- [57] Slieker MG, Deckers-Kocken JM, Uiterwaal CSPM, Ent CK van der, Houwen RHJ. Risk factors for the development of cystic fibrosis related liver disease. *Hepatology* 2003;38:775–6. <https://doi.org/10.1053/jhep.2003.50403>.
- [58] Terlizzi V, Lucarelli M, Salvatore D, Angioni A, Bisogno A, Braggion C, et al. Clinical expression of cystic fibrosis in a large cohort of Italian siblings. *BMC Pulm Med* 2018;18:1–8. <https://doi.org/10.1186/s12890-018-0766-6>.
- [59] Castaldo G, Fuccio A, Salvatore D, Raia V, Santostasi T, Leonardi S, et al. Liver expression in cystic fibrosis could be modulated by genetic factors different from the cystic fibrosis transmembrane regulator genotype. *Am J Med Genet* 2001;98:294–7. [https://doi.org/10.1002/1096-8628\(20010201\)98:4<294::aid-ajmg1097>3.0.co;2-k](https://doi.org/10.1002/1096-8628(20010201)98:4<294::aid-ajmg1097>3.0.co;2-k).
- [60] Debray D, Corvol H, Housset C. Modifier genes in cystic fibrosis-related liver disease. *Curr Opin Gastroenterol* 2019;35:88–92. <https://doi.org/10.1097/MOG.0000000000000508>.
- [61] Bartlett JR, Friedman KJ, Ling SC, Pace RG, Bell SC, Bourke B, et al. Genetic modifiers of liver disease in cystic fibrosis. *JAMA* 2009;302:1076–83. <https://doi.org/10.1001/jama.2009.1295>.
- [62] Boëlle P-Y, Debray D, Guillot L, Corvol H, French CF Modifier Gene Study Investigators. SERPINA1 Z allele is associated with cystic fibrosis liver disease. *Genet Med* 2019;21:2151–5. <https://doi.org/10.1038/s41436-019-0449-6>.
- [63] Woodruff SA, Sontag MK, Accurso FJ, Sokol RJ, Narkewicz MR. Prevalence of elevated liver enzymes in children with cystic fibrosis diagnosed by newborn screen. *Journal of Cystic Fibrosis* 2017;16:139–45. <https://doi.org/10.1016/j.jcf.2016.08.002>.
- [64] Bodewes FAJA, van der Doef HPJ, Houwen RHJ, Verkade HJ. Increase of Serum  $\gamma$ -Glutamyltransferase Associated With Development of Cirrhotic Cystic Fibrosis Liver Disease. *J*

Pediatr Gastroenterol Nutr 2015;61:113–8.

<https://doi.org/10.1097/MPG.0000000000000758>.

- [65] Mueller-Abt PR, Frawley KJ, Greer RM, Lewindon PJ. Comparison of ultrasound and biopsy findings in children with cystic fibrosis related liver disease. *J Cyst Fibros* 2008;7:215–21. <https://doi.org/10.1016/j.jcf.2007.08.001>.
- [66] Siegel MJ, Freeman AJ, Ye W, Palermo JJ, Molleston JP, Paranjape SM, et al. Heterogeneous Liver on Research Ultrasound Identifies Children with Cystic Fibrosis at High Risk of Advanced Liver Disease: Interim Results of a Prospective Observational Case-Controlled Study. *The Journal of Pediatrics* 2020;219:62-69.e4. <https://doi.org/10.1016/j.jpeds.2019.12.033>.
- [67] Leung DH, Ye W, Molleston JP, Weymann A, Ling S, Paranjape SM, et al. Baseline Ultrasound and Clinical Correlates in Children with Cystic Fibrosis. *The Journal of Pediatrics* 2015;167:862-868.e2. <https://doi.org/10.1016/j.jpeds.2015.06.062>.
- [68] Ling SC, Ye W, Leung DH, Navarro OM, Weymann A, Karnsakul W, et al. Liver Ultrasound Patterns in Children With Cystic Fibrosis Correlate With Noninvasive Tests of Liver Disease. *Journal of Pediatric Gastroenterology and Nutrition* 2019;69:351–7. <https://doi.org/10.1097/MPG.0000000000002413>.
- [69] Lenaerts C, Lapierre C, Patriquin H, Bureau N, Lepage G, Harel F, et al. Surveillance for cystic fibrosis-associated hepatobiliary disease: early ultrasound changes and predisposing factors. *The Journal of Pediatrics* 2003;143:343–50. [https://doi.org/10.1067/S0022-3476\(03\)00329-9](https://doi.org/10.1067/S0022-3476(03)00329-9).
- [70] Lewindon PJ, Shepherd RW, Walsh MJ, Greer RM, Williamson R, Pereira TN, et al. Importance of hepatic fibrosis in cystic fibrosis and the predictive value of liver biopsy. *Hepatology* 2011;53:193–201. <https://doi.org/10.1002/hep.24014>.
- [71] Dioguardi Burgio M, Ronot M, Reizine E, Rautou P-E, Castera L, Paradis V, et al. Quantification of hepatic steatosis with ultrasound: promising role of attenuation imaging coefficient in a biopsy-proven cohort. *Eur Radiol* 2020;30:2293–301. <https://doi.org/10.1007/s00330-019-06480-6>.
- [72] Moret A, Boursier J, Debry PH, Riou J, Crouan A, Dubois M, et al. Evaluation of the Hepatorenal B-Mode Ratio and the “Controlled Attenuation Parameter” for the Detection and Grading of Steatosis. *Ultraschall Med* 2020. <https://doi.org/10.1055/a-1233-2290>.
- [73] Tang A, Tan J, Sun M, Hamilton G, Bydder M, Wolfson T, et al. Nonalcoholic Fatty Liver Disease: MR Imaging of Liver Proton Density Fat Fraction to Assess Hepatic Steatosis. *Radiology* 2013;267:422–31. <https://doi.org/10.1148/radiol.12120896>.
- [74] Lachaux A, Le Gall C, Chambon M, Regnier F, Loras-Duclaux I, Bouvier R, et al. Complications of percutaneous liver biopsy in infants and children. *Eur J Pediatr* 1995;154:621–3.
- [75] Rowland M, Bourke B. Liver disease in cystic fibrosis. *Curr Opin Pulm Med* 2011;17:461–6. <https://doi.org/10.1097/MCP.0b013e32834b7f51>.
- [76] Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pylsopoulos NT, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002;97:2614–8. <https://doi.org/10.1111/j.1572-0241.2002.06038.x>.
- [77] Witters P, Libbrecht L, Roskams T, De Boeck K, Dupont L, Proesmans M, et al. Liver disease in cystic fibrosis presents as non-cirrhotic portal hypertension. *Journal of Cystic Fibrosis* 2017;16:e11–3. <https://doi.org/10.1016/j.jcf.2017.03.006>.
- [78] Klotter V, Gunchick C, Siemers E, Rath T, Hudel H, Naehrlich L, et al. Assessment of pathologic increase in liver stiffness enables earlier diagnosis of CFLD: Results from a prospective longitudinal cohort study. *PLoS ONE* 2017;12:e0178784. <https://doi.org/10.1371/journal.pone.0178784>.
- [79] Sadler MD, Crotty P, Fatovich L, Wilson S, Rabin HR, Myers RP. Noninvasive methods, including transient elastography, for the detection of liver disease in adults with cystic fibrosis. *Can J Gastroenterol Hepatol* 2015;29:139–44.

- 1 [80] Sigrist RMS, Liau J, Kaffas AE, Chammas MC, Willmann JK. Ultrasound Elastography: Review of  
2 Techniques and Clinical Applications. *Theranostics* 2017;7:1303–29.  
3 <https://doi.org/10.7150/thno.18650>.
- 4 [81] Lewindon PJ, Puertolas-Lopez MV, Ramm LE, Noble C, Pereira TN, Wixey JA, et al. Accuracy of  
5 Transient Elastography Data Combined With APRI in Detection and Staging of Liver Disease in  
6 Pediatric Patients With Cystic Fibrosis. *Clin Gastroenterol Hepatol* 2019;17:2561-2569.e5.  
7 <https://doi.org/10.1016/j.cgh.2019.03.015>.
- 8 [82] Kitson MT, Kemp WW, Iser DM, Paul E, Wilson JW, Roberts SK. Utility of transient  
9 elastography in the non-invasive evaluation of cystic fibrosis liver disease. *Liver Int*  
10 2013;33:698–705. <https://doi.org/10.1111/liv.12113>.
- 11 [83] Aqul A, Jonas MM, Harney S, Raza R, Sawicki GS, Mitchell PD, et al. Correlation of Transient  
12 Elastography With Severity of Cystic Fibrosis-related Liver Disease. *J Pediatr Gastroenterol*  
13 *Nutr* 2017;64:505–11. <https://doi.org/10.1097/MPG.0000000000001448>.
- 14 [84] Enaud R, Frison E, Missonnier S, Fischer A, de Ledinghen V, Perez P, et al. Cystic fibrosis and  
15 noninvasive liver fibrosis assessment methods in children. *Pediatric Research* 2021:1–7.  
16 <https://doi.org/10.1038/s41390-021-01427-4>.
- 17 [85] Hayes D, Krishnamurthy R, Hu HH. Magnetic resonance elastography demonstrates elevated  
18 liver stiffness in cystic fibrosis patients. *J Cyst Fibros* 2018;17:e54–6.  
19 <https://doi.org/10.1016/j.jcf.2018.08.010>.
- 20 [86] Dana J, Girard M, Debray D. Hepatic manifestations of cystic fibrosis. *Curr Opin Gastroenterol*  
21 2020. <https://doi.org/10.1097/MOG.0000000000000624>.
- 22 [87] Venkatesh SK, Yin M, Ehman RL. Magnetic resonance elastography of liver: Technique,  
23 analysis, and clinical applications. *Journal of Magnetic Resonance Imaging* 2013;37:544–55.  
24 <https://doi.org/10.1002/jmri.23731>.
- 25 [88] Serai SD, Dillman JR, Trout AT. Spin-echo Echo-planar Imaging MR Elastography versus  
26 Gradient-echo MR Elastography for Assessment of Liver Stiffness in Children and Young  
27 Adults Suspected of Having Liver Disease. *Radiology* 2016;282:761–70.  
28 <https://doi.org/10.1148/radiol.2016160589>.
- 29 [89] Rezvani Habibabadi R, Khoshpouri P, Ghadimi M, Shaghaghi M, Ameli S, Hazhirkarzar B, et al.  
30 Comparison between ROI-based and volumetric measurements in quantifying heterogeneity  
31 of liver stiffness using MR elastography. *Eur Radiol* 2020;30:1609–15.  
32 <https://doi.org/10.1007/s00330-019-06478-0>.
- 33 [90] Van Biervliet S, Verdievel H, Vande Velde S, De Bruyne R, De Looze D, Verhelst X, et al.  
34 Longitudinal Transient Elastography Measurements Used in Follow-up for Patients with Cystic  
35 Fibrosis. *Ultrasound in Medicine & Biology* 2016;42:848–54.  
36 <https://doi.org/10.1016/j.ultrasmedbio.2015.11.011>.
- 37 [91] Lewindon PJ, Puertolas-Lopez MV, Ramm LE, Noble C, Pereira TN, Wixey JA, et al. Accuracy of  
38 Transient Elastography Data Combined With APRI in Detection and Staging of Liver Disease in  
39 Pediatric Patients With Cystic Fibrosis. *Clinical Gastroenterology and Hepatology*  
40 2019;17:2561-2569.e5. <https://doi.org/10.1016/j.cgh.2019.03.015>.
- 41 [92] Elkrief L, Ronot M, Andrade F, Dioguardi Burgio M, Issoufaly T, Zappa M, et al. Non-invasive  
42 evaluation of portal hypertension using shear-wave elastography: analysis of two algorithms  
43 combining liver and spleen stiffness in 191 patients with cirrhosis. *Aliment Pharmacol Ther*  
44 2018;47:621–30. <https://doi.org/10.1111/apt.14488>.
- 45 [93] Tomita H, Ohkuma K, Masugi Y, Hosoe N, Hoshino K, Fuchimoto Y, et al. Diagnosing native  
46 liver fibrosis and esophageal varices using liver and spleen stiffness measurements in biliary  
47 atresia: a pilot study. *Pediatr Radiol* 2016;46:1409–17. <https://doi.org/10.1007/s00247-016-3637-4>.
- 48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- [94] Cañas T, Maciá A, Muñoz-Codoceo RA, Fontanilla T, González-Rios P, Miralles M, et al. Hepatic and Splenic Acoustic Radiation Force Impulse Shear Wave Velocity Elastography in Children with Liver Disease Associated with Cystic Fibrosis. *BioMed Research International* 2015;2015:e517369. <https://doi.org/10.1155/2015/517369>.
- [95] Malbrunot-Wagner AC, Bridoux L, Nousbaum JB, Riou C, Dirou A, Ginies JL, et al. Transient elastography and portal hypertension in pediatric patients with cystic fibrosis Transient elastography and cystic fibrosis. *J Cyst Fibros* 2011;10:338–42. <https://doi.org/10.1016/j.jcf.2011.04.004>.
- [96] Oppenheimer EH, Esterly JR. Pathology of cystic fibrosis review of the literature and comparison with 146 autopsied cases. *Perspect Pediatr Pathol* 1975;2:241–78.
- [97] Koh C, Sakiani S, Surana P, Zhao X, Eccleston J, Kleiner DE, et al. Adult-onset cystic fibrosis liver disease: Diagnosis and characterization of an underappreciated entity. *Hepatology* 2017;66:591–601. <https://doi.org/10.1002/hep.29217>.
- [98] Friedrich-Rust M, Schlueter N, Smaczny C, Eickmeier O, Rosewich M, Feifel K, et al. Non-invasive measurement of liver and pancreas fibrosis in patients with cystic fibrosis. *Journal of Cystic Fibrosis* 2013;12:431–9. <https://doi.org/10.1016/j.jcf.2012.12.013>.
- [99] Ziol M, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005;41:48–54. <https://doi.org/10.1002/hep.20506>.
- [100] Chon YE, Choi EH, Song KJ, Park JY, Kim DY, Han K-H, et al. Performance of Transient Elastography for the Staging of Liver Fibrosis in Patients with Chronic Hepatitis B: A Meta-Analysis. *PLoS One* 2012;7:e44930. <https://doi.org/10.1371/journal.pone.0044930>.
- [101] Karlas T, Neuschulz M, Oltmanns A, Güttler A, Petroff D, Wirtz H, et al. Non-invasive evaluation of cystic fibrosis related liver disease in adults with ARFI, transient elastography and different fibrosis scores. *PLoS ONE* 2012;7:e42139. <https://doi.org/10.1371/journal.pone.0042139>.
- [102] Poetter-Lang S, Staufer K, Baltzer P, Tamandl D, Muin D, Bastati N, et al. The Efficacy of MRI in the diagnostic workup of cystic fibrosis-associated liver disease: A clinical observational cohort study. *Eur Radiol* 2019;29:1048–58. <https://doi.org/10.1007/s00330-018-5650-5>.
- [103] Wu H, Vu M, Dhingra S, Ackah R, Goss JA, Rana A, et al. Obliterative Portal Venopathy Without Cirrhosis Is Prevalent in Pediatric Cystic Fibrosis Liver Disease With Portal Hypertension. *Clin Gastroenterol Hepatol* 2019;17:2134–6. <https://doi.org/10.1016/j.cgh.2018.10.046>.
- [104] Pereira TN, Lewindon PJ, Greer RM, Hoskins AC, Williamson RM, Shepherd RW, et al. Transcriptional Basis for Hepatic Fibrosis in Cystic Fibrosis-associated Liver Disease. *Journal of Pediatric Gastroenterology and Nutrition* 2012;54:328–35. <https://doi.org/10.1097/MPG.0b013e3182432034>.
- [105] Chow KU, Luxembourg B, Seifried E, Bonig H. Spleen Size Is Significantly Influenced by Body Height and Sex: Establishment of Normal Values for Spleen Size at US with a Cohort of 1200 Healthy Individuals. *Radiology* 2016;279:306–13. <https://doi.org/10.1148/radiol.2015150887>.
- [106] Glatard A-S, Hillaire S, d'Assignies G, Cazals-Hatem D, Plessier A, Valla DC, et al. Obliterative portal venopathy: findings at CT imaging. *Radiology* 2012;263:741–50. <https://doi.org/10.1148/radiol.12111785>.
- [107] Guido M, Alves VAF, Balabaud C, Bathal PS, Bioulac-Sage P, Colombari R, et al. Histology of portal vascular changes associated with idiopathic non-cirrhotic portal hypertension: nomenclature and definition. *Histopathology* 2019;74:219–26. <https://doi.org/10.1111/his.13738>.
- [108] De Gottardi A, Rautou P-E, Schouten J, Rubbia-Brandt L, Leebeek F, Trebicka J, et al. Porto-sinusoidal vascular disease: proposal and description of a novel entity. *The Lancet*

Gastroenterology & Hepatology 2019;4:399–411. [https://doi.org/10.1016/S2468-1253\(19\)30047-0](https://doi.org/10.1016/S2468-1253(19)30047-0).

- [109] Bakshi N, Gulati N, Rastogi A, Chougule A, Bihari C, Jindal A. Nodular regenerative hyperplasia – An under-recognized vascular disorder of liver. *Pathology - Research and Practice* 2020;216:152833. <https://doi.org/10.1016/j.prp.2020.152833>.
- [110] de Franchis R. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. *Journal of Hepatology* 2015;63:743–52. <https://doi.org/10.1016/j.jhep.2015.05.022>.
- [111] Padoan R, Cirilli N, Falchetti D, Cesana BM. Risk factors for adverse outcome in infancy in meconium ileus cystic fibrosis infants: A multicentre Italian study. *Journal of Cystic Fibrosis* 2019;18:863–8. <https://doi.org/10.1016/j.jcf.2019.07.003>.
- [112] Lykavieris P, Bernard O, Hadchouel M. Neonatal cholestasis as the presenting feature in cystic fibrosis. *Arch Dis Child* 1996;75:67–70. <https://doi.org/10.1136/adc.75.1.67>.
- [113] Assis DN, Debray D. Gallbladder and bile duct disease in Cystic Fibrosis. *Journal of Cystic Fibrosis* 2017;16:S62–9. <https://doi.org/10.1016/j.jcf.2017.07.006>.
- [114] Durieu I, Pellet O, Simonot L, Durupt S, Bellon G, Durand DV, et al. Sclerosing cholangitis in adults with cystic fibrosis: a magnetic resonance cholangiographic prospective study. *Journal of Hepatology* 1999;30:1052–6. [https://doi.org/10.1016/S0168-8278\(99\)80259-1](https://doi.org/10.1016/S0168-8278(99)80259-1).
- [115] Santamaria F, Vajro P, Oggero V, Greco L, Angelillo M, Carrillo F, et al. Volume and emptying of the gallbladder in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1990;10:303–6. <https://doi.org/10.1097/00005176-199004000-00006>.
- [116] Curry MP, Hegarty JE. The gallbladder and biliary tract in cystic fibrosis. *Curr Gastroenterol Rep* 2005;7:147–53. <https://doi.org/10.1007/s11894-005-0053-6>.
- [117] Cystic Fibrosis Foundation Registry - Annual Data Report n.d.
- [118] 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. <https://doi.org/10.1093/jnci/djs481>.
- [119] Craig J, Haddad H, Shwachman H. The Pathological Changes in the Liver in Cystic Fibrosis of the Pancreas. *AMA Journal of Diseases of Children* 1957;93:357–69. <https://doi.org/10.1001/archpedi.1957.02060040359002>.
- [120] Strandvik B, Hultcrantz R. Liver function and morphology during long-term fatty acid supplementation in cystic fibrosis. *Liver* 1994;14:32–6.
- [121] Werner A, Havinga R, Bos T, Bloks VW, Kuipers F, Verkade HJ. Essential fatty acid deficiency in mice is associated with hepatic steatosis and secretion of large VLDL particles. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 2005;288:G1150–8. <https://doi.org/10.1152/ajpgi.00456.2004>.
- [122] Kneeman JM, Misdraji J, Corey KE. Secondary causes of nonalcoholic fatty liver disease. *Therap Adv Gastroenterol* 2012;5:199–207. <https://doi.org/10.1177/1756283X11430859>.
- [123] Isenberg JN. Cystic Fibrosis: Its Influence on the Liver, Biliary Tree, and Bile Salt Metabolism. *Semin Liver Dis* 1982;2:302–13. <https://doi.org/10.1055/s-2008-1040717>.
- [124] Bernhard W. Choline in cystic fibrosis: relations to pancreas insufficiency, enterohepatic cycle, PEMT and intestinal microbiota. *Eur J Nutr* 2021;60:1737–59. <https://doi.org/10.1007/s00394-020-02358-2>.
- [125] Bernhard W, Lange R, Graepler-Mainka U, Engel C, Machann J, Hund V, et al. Choline Supplementation in Cystic Fibrosis—The Metabolic and Clinical Impact. *Nutrients* 2019;11:656. <https://doi.org/10.3390/nu11030656>.
- [126] Chen AH, Innis SM, Davidson AGF, James SJ. Phosphatidylcholine and lysophosphatidylcholine excretion is increased in children with cystic fibrosis and is

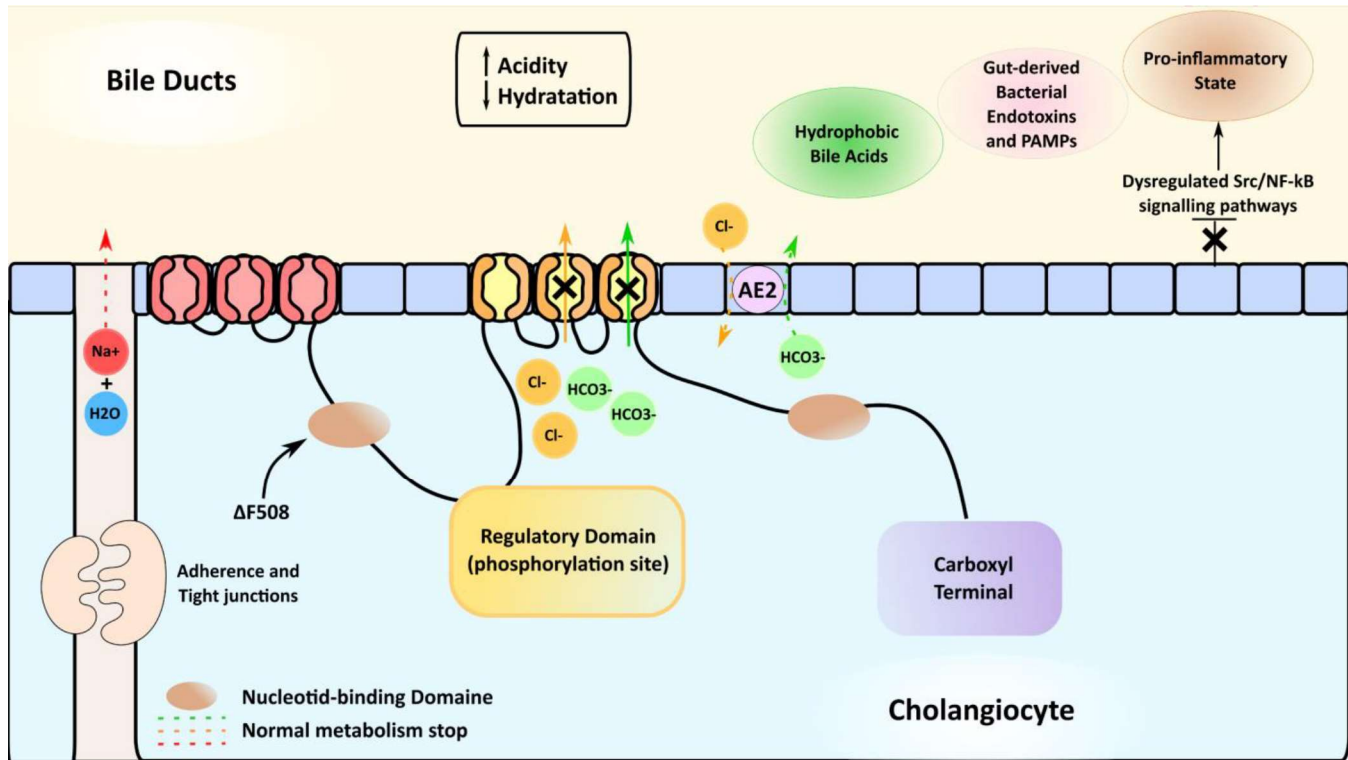


associated with plasma homocysteine, S-adenosylhomocysteine, and S-adenosylmethionine. *Am J Clin Nutr* 2005;81:686–91. <https://doi.org/10.1093/ajcn/81.3.686>.

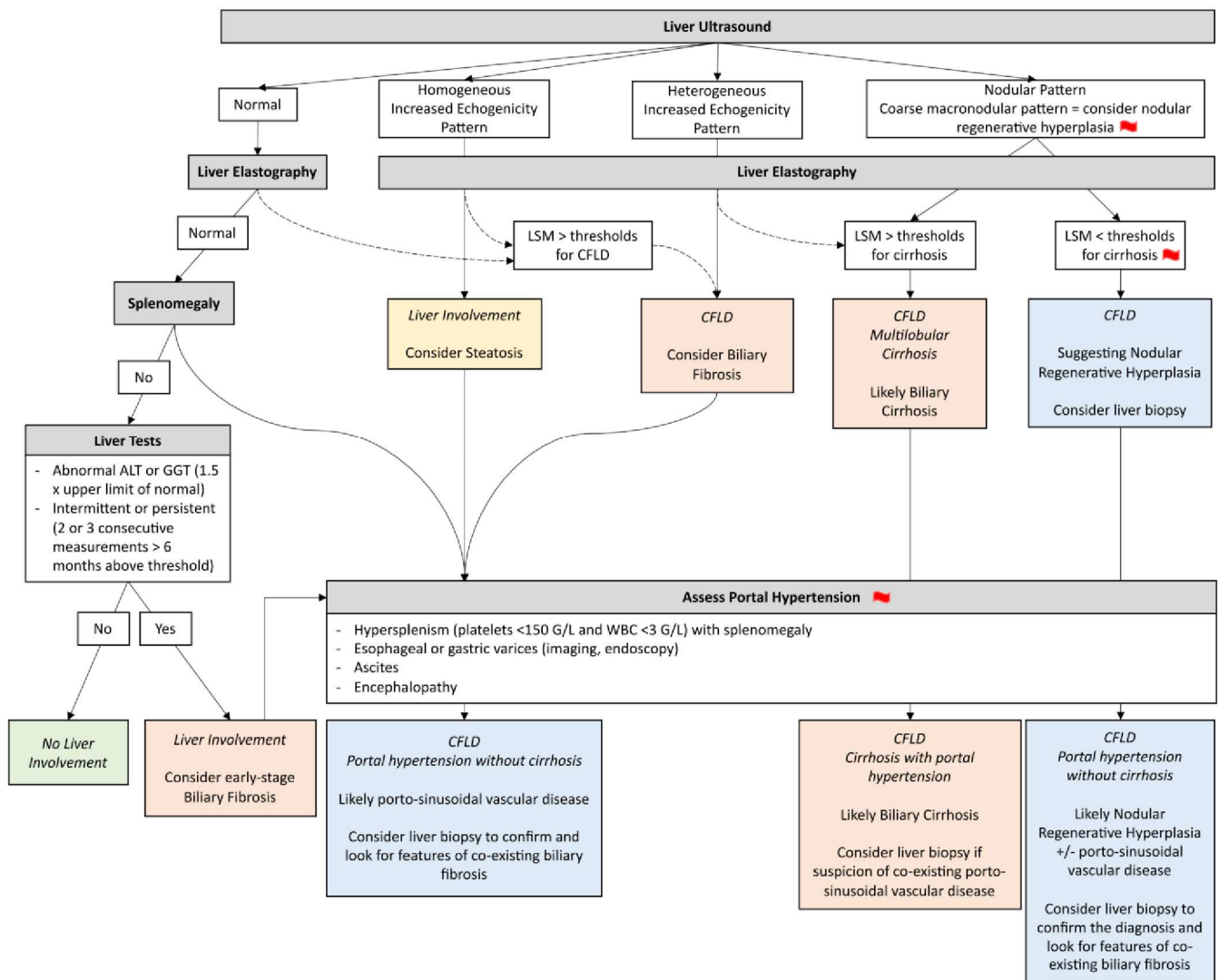
- [127] Gobato AO, Vasques ACJ, Ribeiro AF, Yamada RM, Hessel G. Prevalence of hepatic steatosis among children and adolescents with cystic fibrosis and its association with nutritional status. *Rev Paul Pediatr* 2019;37:435–41. <https://doi.org/10.1590/1984-0462/;2019;37;4;00007>.
- [128] Lindblad A, Hultcrantz R, Strandvik B. Bile-duct destruction and collagen deposition: a prominent ultrastructural feature of the liver in cystic fibrosis. *Hepatology* 1992;16:372–81. <https://doi.org/10.1002/hep.1840160215>.
- [129] Cystic Fibrosis Foundation. 2019 Patient Registry Annual Data Report 2019:92.
- [130] Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *Journal of Hepatology* 2020;73:202–9. <https://doi.org/10.1016/j.jhep.2020.03.039>.
- [131] Cheng K, Ashby D, Smyth RL. Ursodeoxycholic acid for cystic fibrosis-related liver disease. *Cochrane Database of Systematic Reviews* 2017. <https://doi.org/10.1002/14651858.CD000222.pub4>.
- [132] Colombo C, Alicandro G, Oliver M, Lewindon PJ, Ramm GA, Ooi CY, et al. Ursodeoxycholic acid and liver disease associated with cystic fibrosis: A multicenter cohort study. *Journal of Cystic Fibrosis* 2021. <https://doi.org/10.1016/j.jcf.2021.03.014>.
- [133] Fickert P, Hirschfield GM, Denk G, Marschall H-U, Altorjay I, Färkkilä M, et al. norUrsodeoxycholic acid improves cholestasis in primary sclerosing cholangitis. *Journal of Hepatology* 2017;67:549–58. <https://doi.org/10.1016/j.jhep.2017.05.009>.
- [134] Halilbasic E, Steinacher D, Trauner M. Nor-Ursodeoxycholic Acid as a Novel Therapeutic Approach for Cholestatic and Metabolic Liver Diseases. *DDI* 2017;35:288–92. <https://doi.org/10.1159/000454904>.
- [135] Freeman AJ, Sellers ZM, Mazariegos G, Kelly A, Saiman L, Mallory G, et al. A Multidisciplinary Approach to Pretransplant and Posttransplant Management of Cystic Fibrosis–Associated Liver Disease. *Liver Transplantation* 2019;25:640–57. <https://doi.org/10.1002/lt.25421>.
- [136] Mendizabal M, Reddy KR, Cassuto J, Olthoff KM, Faust TW, Makar GA, et al. Liver transplantation in patients with cystic fibrosis: Analysis of united network for organ sharing data. *Liver Transplantation* 2011;17:243–50. <https://doi.org/10.1002/lt.22240>.
- [137] Lemoine C, Lokar J, McColley SA, Alonso EM, Superina R. Cystic fibrosis and portal hypertension: Distal splenorenal shunt can prevent the need for future liver transplant. *Journal of Pediatric Surgery* 2019;54:1076–82. <https://doi.org/10.1016/j.jpedsurg.2019.01.035>.
- [138] Lupi A, Barbiero G, Battistel M, Ferrarese A, Loy M, Feltracco P, et al. Transjugular intrahepatic portosystemic shunt in non-cirrhotic portal hypertension related to cystic fibrosis in a lung transplant patient. *Journal of Cystic Fibrosis* 2020;19:e45–7. <https://doi.org/10.1016/j.jcf.2020.06.019>.
- [139] Wainwright CE, Elborn JS, Ramsey BW, Marigowda G, Huang X, Cipolli M, et al. Lumacaftor-ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del CFTR. *N Engl J Med* 2015;373:220–31. <https://doi.org/10.1056/NEJMoa1409547>.
- [140] Ratjen F, Hug C, Marigowda G, Tian S, Huang X, Stanojevic S, et al. Efficacy and safety of lumacaftor and ivacaftor in patients aged 6-11 years with cystic fibrosis homozygous for F508del-CFTR: a randomised, placebo-controlled phase 3 trial. *Lancet Respir Med* 2017;5:557–67. [https://doi.org/10.1016/S2213-2600\(17\)30215-1](https://doi.org/10.1016/S2213-2600(17)30215-1).

- 1 [141] Burgel P-R, Munck A, Durieu I, Chiron R, Mely L, Prevotat A, et al. Real-Life Safety and  
2 Effectiveness of Lumacaftor–Ivacaftor in Patients with Cystic Fibrosis. *Am J Respir Crit Care*  
3 *Med* 2020;201:188–97. <https://doi.org/10.1164/rccm.201906-1227OC>.
- 4 [142] Masson A, Schneider-Futschik EK, Baatallah N, Nguyen-Khoa T, Girodon E, Hatton A, et al.  
5 Predictive factors for lumacaftor/ivacaftor clinical response. *Journal of Cystic Fibrosis*  
6 2019;18:368–74. <https://doi.org/10.1016/j.jcf.2018.12.011>.
- 7 [143] Rowe SM, Daines C, Ringshausen FC, Kerem E, Wilson J, Tullis E, et al. Tezacaftor-Ivacaftor  
8 in Residual-Function Heterozygotes with Cystic Fibrosis. *N Engl J Med* 2017;377:2024–35.  
9 <https://doi.org/10.1056/NEJMoa1709847>.
- 10 [144] Heijerman HGM, McKone EF, Downey DG, Van Braeckel E, Rowe SM, Tullis E, et al. Efficacy  
11 and safety of the elexacaftor plus tezacaftor plus ivacaftor combination regimen in people  
12 with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase  
13 3 trial. *The Lancet* 2019;394:1940–8. [https://doi.org/10.1016/S0140-6736\(19\)32597-8](https://doi.org/10.1016/S0140-6736(19)32597-8).
- 14 [145] Middleton PG, Mall MA, Dřevínek P, Lands LC, McKone EF, Polineni D, et al. Elexacaftor–  
15 Tezacaftor–Ivacaftor for Cystic Fibrosis with a Single Phe508del Allele. *New England Journal*  
16 *of Medicine* 2019;381:1809–19. <https://doi.org/10.1056/NEJMoa1908639>.
- 17 [146] Paluck F, Power N, Lynch C, Cox D, McNally P, Rowland M, et al. Liver function tests in  
18 F508del homozygous paediatric patients with cystic fibrosis taking lumacaftor/ivacaftor  
19 combination therapy. *Irish Medical Journal* 2021;114(2), P259.
- 20 [147] Drummond D, Dana J, Berteloot L, Schneider-Futschik EK, Chedevergne F, Bailly-Botuha C,  
21 et al. Lumacaftor-ivacaftor effects on cystic fibrosis-related liver involvement in adolescents  
22 with homozygous F508 del-CFTR. *Journal of Cystic Fibrosis* 2021.  
23 <https://doi.org/10.1016/j.jcf.2021.07.018>.
- 24 [148] Kutney K, Donnola SB, Flask CA, Gubitosi-Klug R, O’Riordan M, McBennett K, et al.  
25 Lumacaftor/ivacaftor therapy is associated with reduced hepatic steatosis in cystic fibrosis  
26 patients. *World Journal of Hepatology* 2019;11:761–72.  
27 <https://doi.org/10.4254/wjh.v11.i12.761>.
- 28 [149] Orkambi, summary of product characteristics. European Medicines Agency 2018.  
29 <https://www.ema.europa.eu/en/medicines/human/EPAR/orkambi>.
- 30 [150] Fiorotto R, Amenduni M, Mariotti V, Fabris L, Spirli C, Strazzabosco M. Src kinase inhibition  
31 reduces inflammatory and cytoskeletal changes in  $\Delta$ F508 human cholangiocytes and  
32 improves cystic fibrosis transmembrane conductance regulator correctors efficacy.  
33 *Hepatology* 2018;67:972–88. <https://doi.org/10.1002/hep.29400>.
- 34 [151] Nevens F, Andreone P, Mazzella G, Strasser SI, Bowlus C, Invernizzi P, et al. A Placebo-  
35 Controlled Trial of Obeticholic Acid in Primary Biliary Cholangitis. *New England Journal of*  
36 *Medicine* 2016;375:631–43. <https://doi.org/10.1056/NEJMoa1509840>.
- 37 [152] Hirschfield GM, Chazouillères O, Drenth JP, Thorburn D, Harrison SA, Landis CS, et al. Effect  
38 of NGM282, an FGF19 analogue, in primary sclerosing cholangitis: A multicenter, randomized,  
39 double-blind, placebo-controlled phase II trial. *Journal of Hepatology* 2019;70:483–93.  
40 <https://doi.org/10.1016/j.jhep.2018.10.035>.
- 41 [153] Lin H, Zhou C, Hou Y, Li Q, Qiao G, Wang Y, et al. Paracrine Fibroblast Growth Factor 1  
42 Functions as Potent Therapeutic Agent for Intrahepatic Cholestasis by Downregulating  
43 Synthesis of Bile Acid. *Frontiers in Pharmacology* 2019;10:1515.  
44 <https://doi.org/10.3389/fphar.2019.01515>.
- 45 [154] Jonker JW, Suh JM, Atkins AR, Ahmadian M, Li P, Whyte J, et al. A PPAR $\gamma$ –FGF1 axis is  
46 required for adaptive adipose remodelling and metabolic homeostasis. *Nature*  
47 2012;485:391–4. <https://doi.org/10.1038/nature10998>.
- 48  
49  
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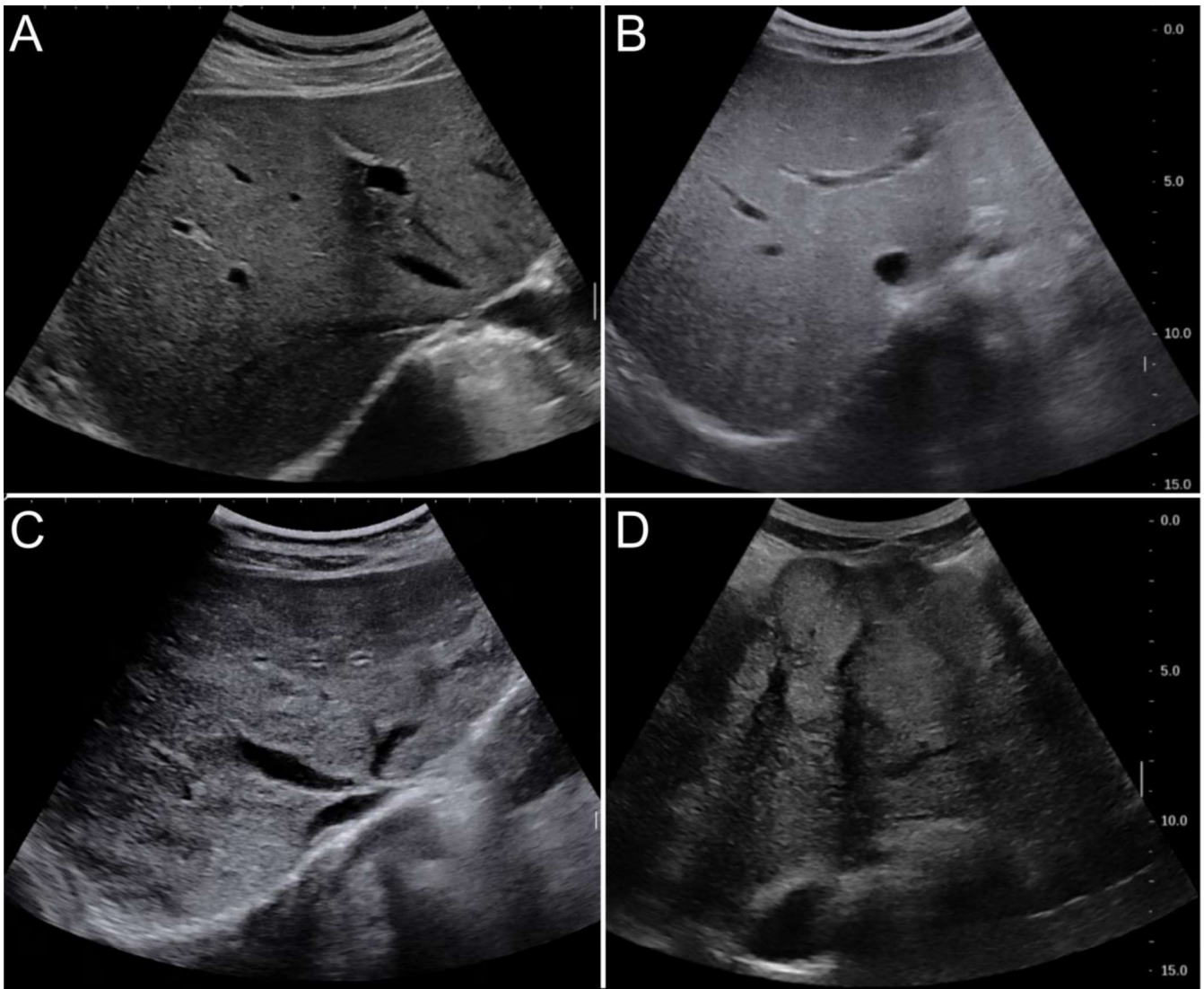
- 1 [155] Kanhere M, Chassaing B, Gewirtz AT, Tangpricha V. Role of vitamin D on gut microbiota in  
2 cystic fibrosis. *The Journal of Steroid Biochemistry and Molecular Biology* 2018;175:82–7.  
3 <https://doi.org/10.1016/j.jsbmb.2016.11.001>.
- 4 [156] McNally P, Coughlan C, Bergsson G, Doyle M, Taggart C, Adorini L, et al. Vitamin D receptor  
5 agonists inhibit pro-inflammatory cytokine production from the respiratory epithelium in  
6 cystic fibrosis. *Journal of Cystic Fibrosis* 2011;10:428–34.  
7 <https://doi.org/10.1016/j.jcf.2011.06.013>.
- 8 [157] Dauletbaev N, Herscovitch K, Das M, Chen H, Bernier J, Matouk E, et al. Down-regulation of  
9 IL-8 by high-dose vitamin D is specific to hyperinflammatory macrophages and involves  
10 mechanisms beyond up-regulation of DUSP1. *British Journal of Pharmacology*  
11 2015;172:4757–71. <https://doi.org/10.1111/bph.13249>.
- 12 [158] Ding N, Yu RT, Subramaniam N, Sherman MH, Wilson C, Rao R, et al. A vitamin D  
13 receptor/SMAD genomic circuit gates hepatic fibrotic response. *Cell* 2013;153:601–13.  
14 <https://doi.org/10.1016/j.cell.2013.03.028>.
- 15  
16  
17  
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**Graphical Abstract. Schematic structure of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein, its functioning, and its impact on ionic exchanges within the cholangiocyte.** The extra-cellular transport of Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> by CFTR indirectly allows the transport of water coupled with sodium by the hydric and electrolytic equilibrium of HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> by the exchanger AE2. A pro-inflammatory state, mediated by dysregulated Src/NF-κB signalling pathways, associated with gut-derived bacterial endotoxins and pathogen-associated molecular patterns (PAMPs), secondary to gut dysbiosis, intestinal inflammation and increased intestinal permeability, contribute to the pathogenesis of biliary fibrosis.

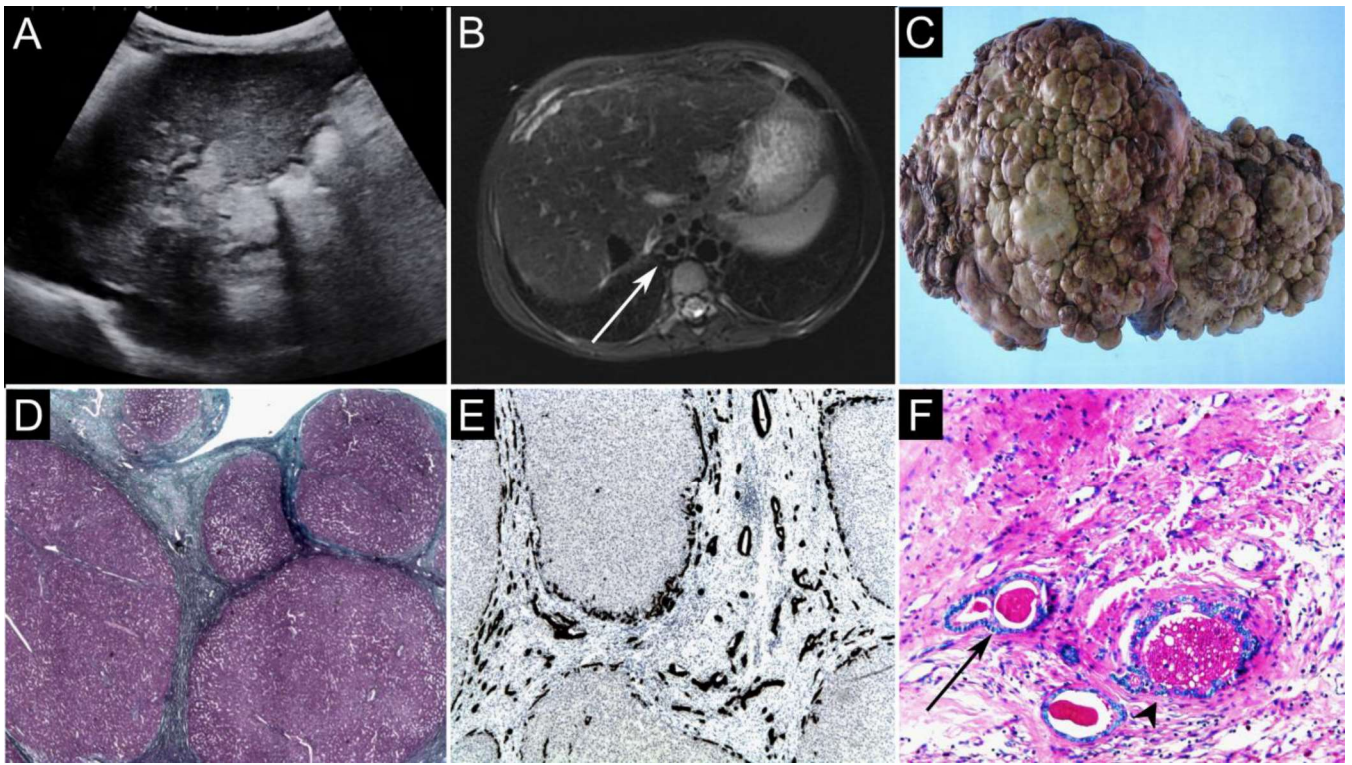


**Figure 1. Diagnostic approach to Liver Involvement and Cystic Fibrosis Liver Disease based on Debray et al [4].** Cystic Fibrosis Liver Disease (CFLD) is defined by the CFTR defect while liver involvement includes all hepatic manifestations including epiphenomena. In the presence of “red-flag” signs, porto-sinusoidal vascular disease should be investigated and liver biopsy and/or hemodynamic measurements of the hepatic venous pressure gradient should be considered.

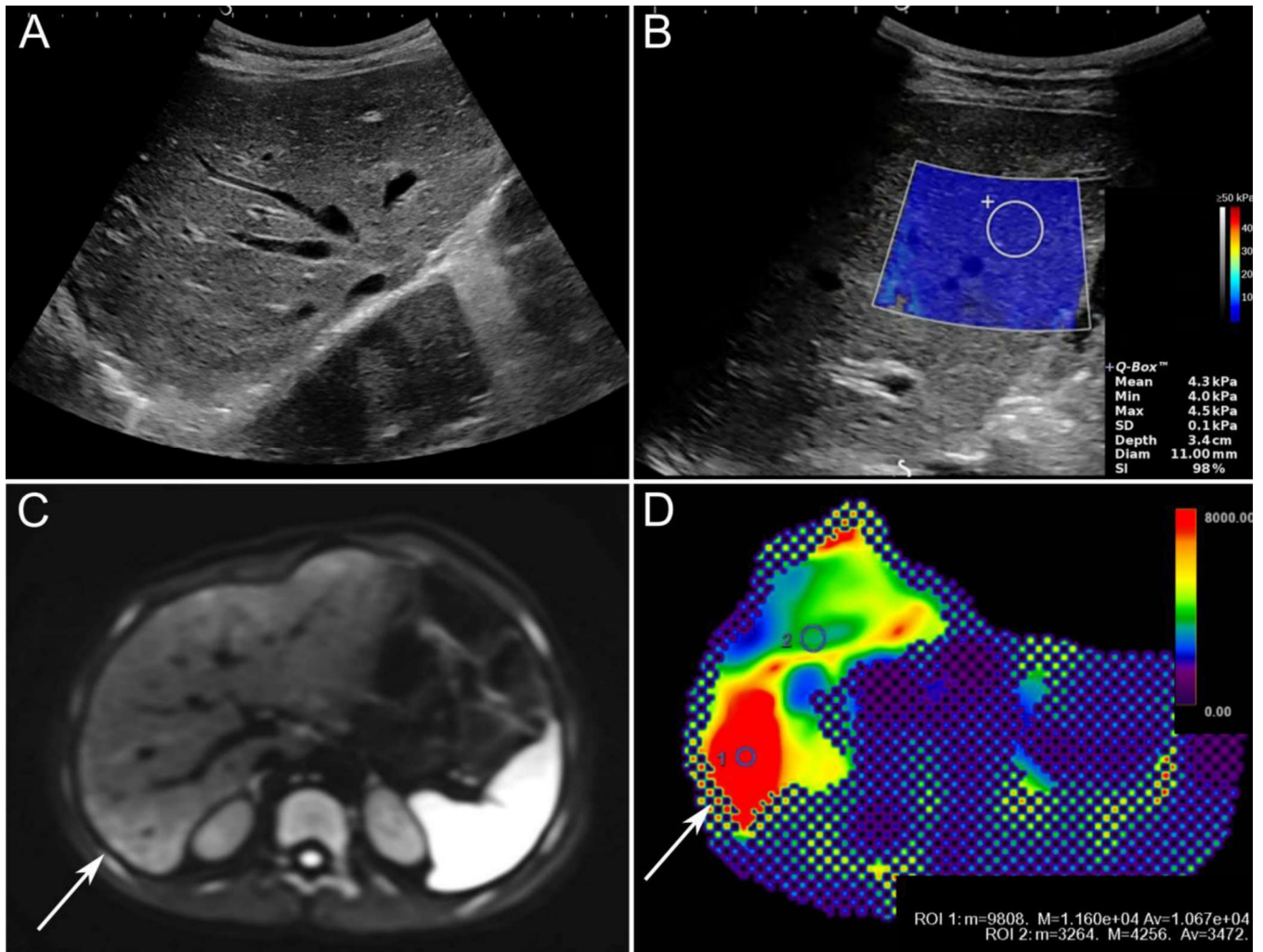


**Figure 2. Ultrasound Patterns in Patients with Cystic Fibrosis.** (A) Normal. (B) Homogeneous hyperechogenicity. (C) Heterogeneous hyperechogenicity. (D) Nodular.



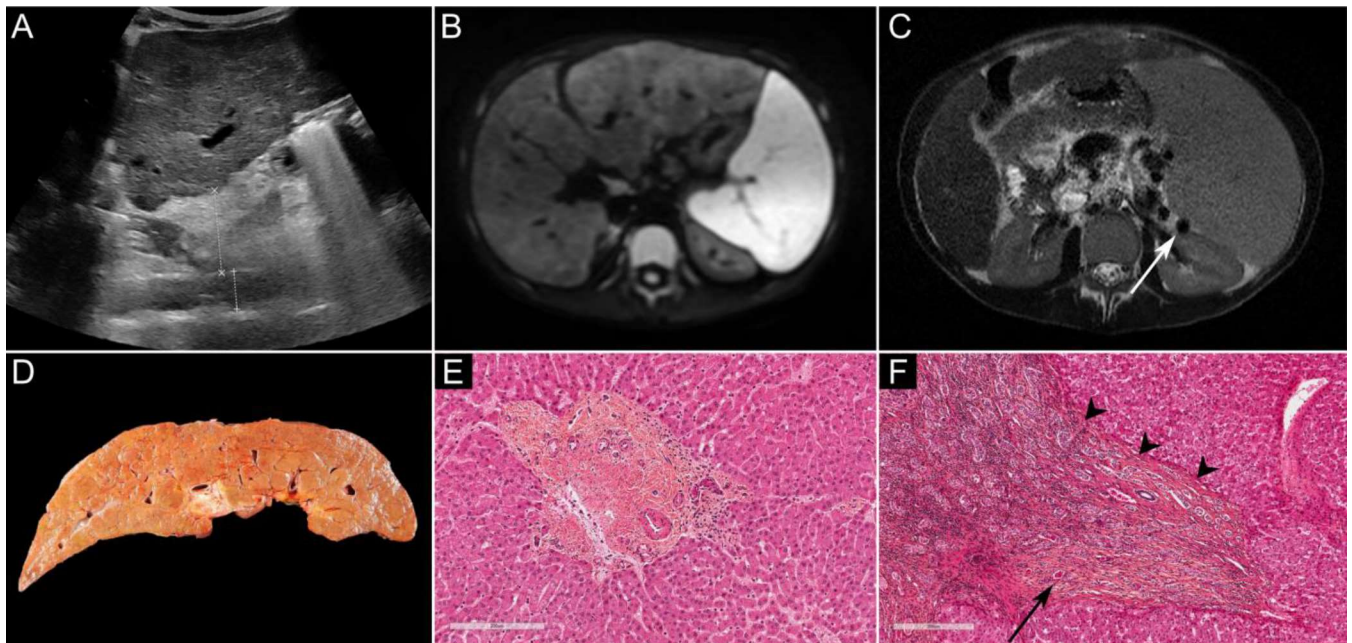


**Figure 3. Biliary Cirrhosis with portal hypertension in an 8-year-old girl.** Median liver stiffness measured by Transient Elastography, 2D ShearWave Elastography and Magnetic Resonance Elastography was increased to 71 kPa, 75.1 kPa and 10.6 kPa, respectively. Liver ultrasound (A) and axial fat suppressed-T2 weighted MRI showed morphological changes, nodular contours and esophageal varices (white arrow). Patient underwent liver transplantation. Explant liver pathology confirmed the diagnosis of biliary cirrhosis. Macroscopic examination (C) showed nodular contours. Microscopic examination showed regenerative nodules on Masson's trichrome staining (D), bile duct proliferation on cytokeratin 7 staining (E) and biliary plugs (black arrow) including a foamy and vacuolized plug (black arrowhead) on periodic acid schiff staining (F).

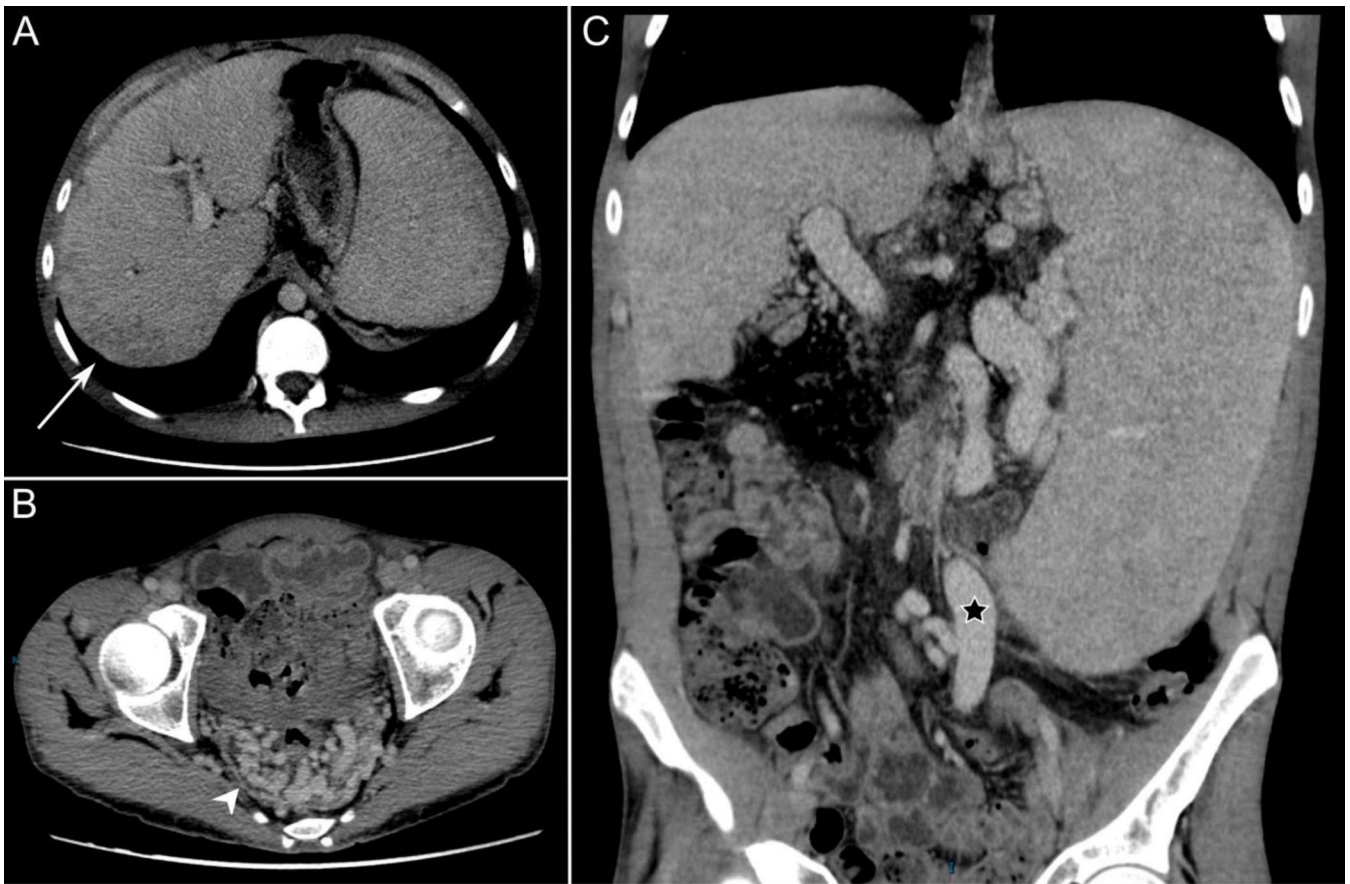


**Figure 4. Focal Biliary Fibrosis without portal hypertension in an 8-year-old boy.** Liver ultrasound (A) shows a slightly heterogeneous hyperechogenic pattern with smooth liver contours and no signs of portal hypertension. Liver stiffness on 2D ShearWave Elastography (B) was normal (4.3 kPa). Axial diffusion (B=800) (C) weighted MRI shows an abnormal increased signal in segment VII (arrow). Focal increase in liver stiffness (arrow) on Magnetic Resonance Elastography (D) was consistent with the diagnosis of focal biliary fibrosis.

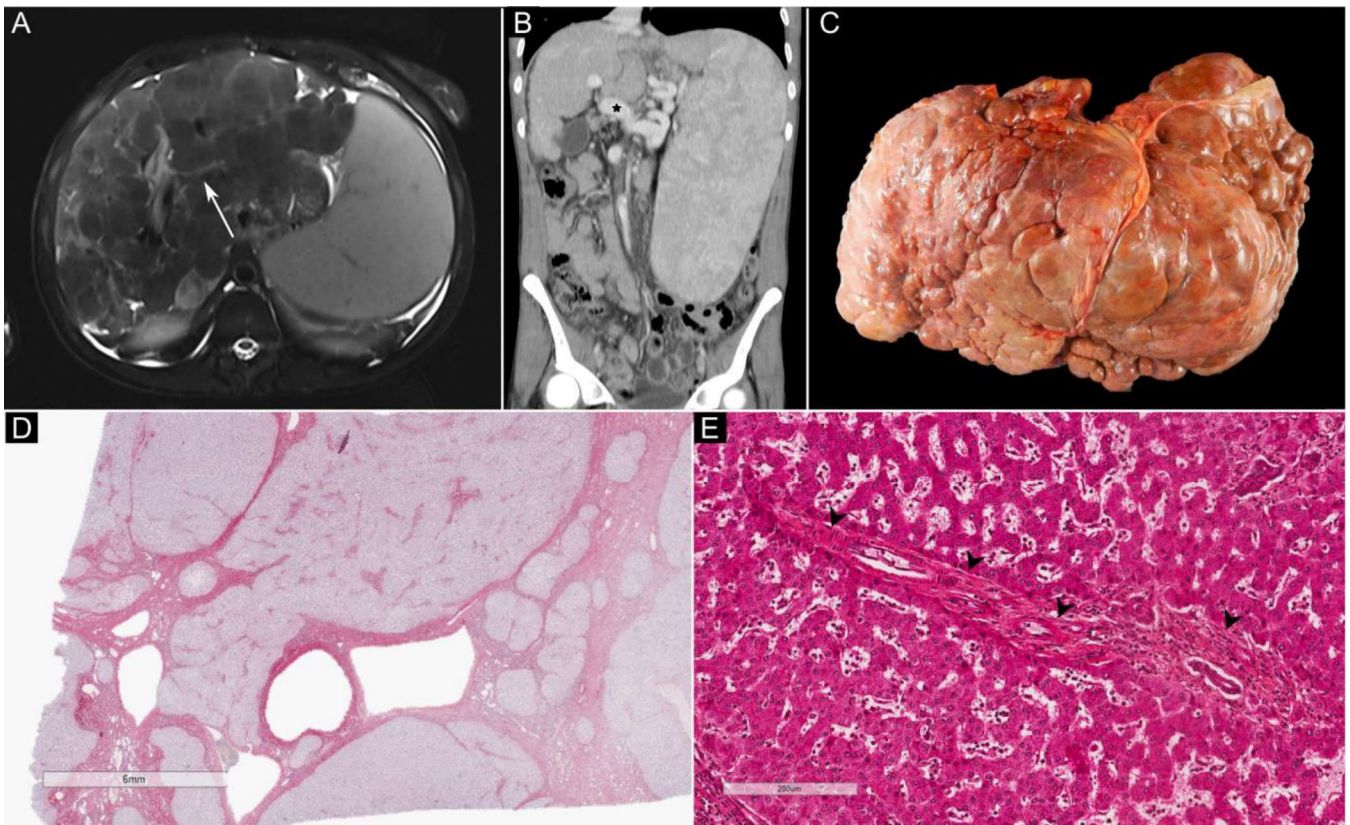




**Figure 5. Cirrhosis with portal hypertension on co-existing biliary and vascular injuries in a 16-year-old man.** Liver stiffness on Transient Elastography, ultrasound-guided elastography and Magnetic Resonance Elastography was consistent with advanced fibrosis (> F2 stage). (A) Liver ultrasound showed a heterogeneous hyperechogenic liver pattern with nodular contours and enlargement of the lesser omentum compared to the aorta. Axial diffusion (B) and fat-suppressed T2- (C) weighted-MRI showed morphological changes, nodular contours and a patchy increased signal. Large splenomegaly and porto-systemic shunts (splenorenal - arrowhead) were consistent with portal hypertension. The patient underwent liver transplantation. (D) Macroscopic examination showed macronodular cirrhosis. Microscopic examination using hematoxylin and eosin staining demonstrated (E) vascular lesions with obliterative portal venopathy and portal tract arterialization and (F) biliary lesions with ductular proliferation (arrowheads) in the fibrous septa as well as certain ductules containing inspissated granular eosinophilic material (arrow).

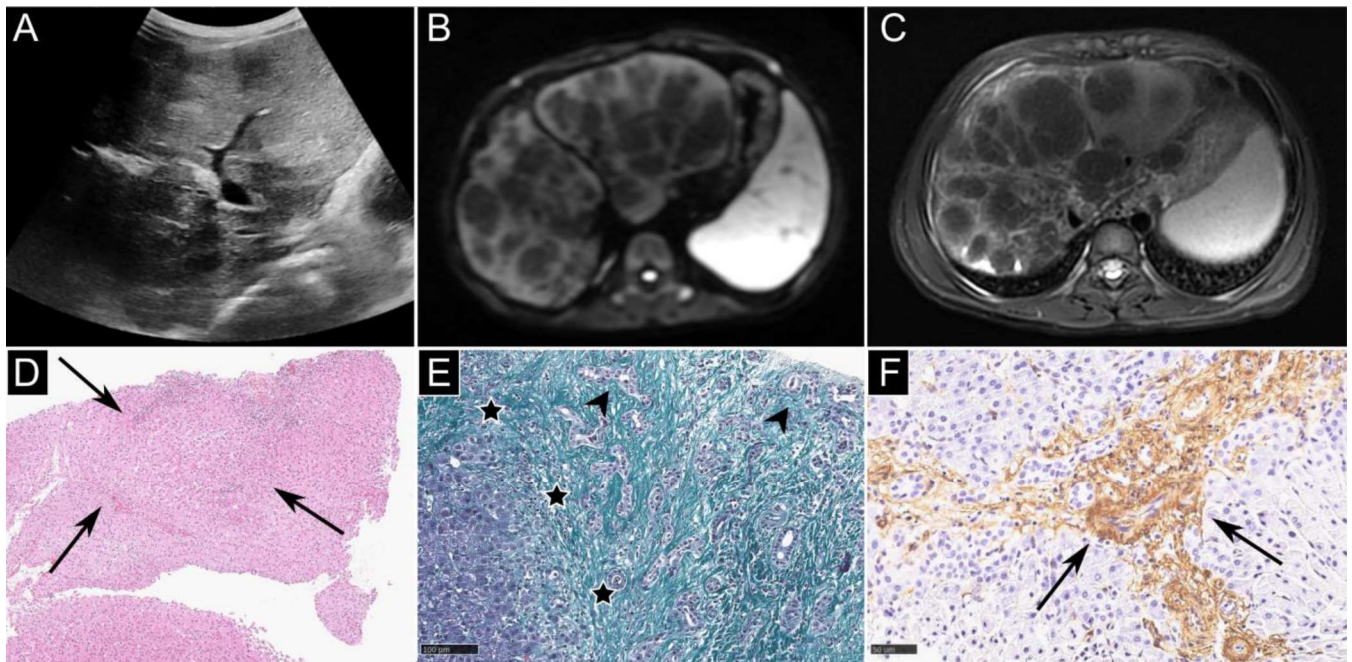


**Figure 6. Porto-sinusoidal vascular disease in a 21-year-old man.** Liver stiffness on Transient Elastography was increased to 10.1 kPa. Axial (A, B) and coronal (C) portal venous CT-scan showed severe portal hypertension – rectal varices (arrowhead), portal vein dilation and inferior mesenteric vein dilation (star) – while the liver showed mild morphological changes with smooth contours and peripheral heterogeneous enhancement (arrow). The caliber of intra-hepatic portal branches was reduced. Explant liver pathology confirmed the diagnosis of porto-sinusoidal vascular disease with obliterative portal venopathy and regenerative nodular hyperplasia. No biliary abnormalities were noted.



**Figure 7. Macronodular cirrhosis with portal hypertension in a context of vascular lesions in a 30-year-old woman.** (A) Macronodular cirrhosis on axial fat-suppressed T2 weighted MRI. The caliber of intra-hepatic portal branches was reduced (white arrow). (B) Large splenomegaly and porto-systemic shunts on coronal portal-venous phase CT-scan were consistent with portal hypertension. The portal vein was enlarged (18 mm, star). (C) Macroscopic examination of the liver explant and (D) microscopic examination using picrosirius staining confirmed the macronodular aspect of cirrhosis. (E) Hematoxylin and eosin staining demonstrated residual portal tracts without the portal vein and with numerous arterial sections (arrowheads).





**Figure 8. Macronodular cirrhosis with portal hypertension on co-existing vascular and biliary injuries in a 9-year-old girl.** (A) Liver ultrasound showed morphological changes with a nodular pattern. (B, C) Axial fat-suppressed T2- and diffusion (B=800) weighted MRI showed large liver nodules with a rim in increased T2 and diffusion signal, splenomegaly, and esophageal varices (confirmed by gastroscopy). Microscopic examination of liver biopsy showed (D – hematoxylin and eosin staining) regenerative nodules (arrows), (E – trichrome staining) biliary fibrosis (stars) with bile duct proliferation (arrowhead) and (F – actin staining) fibrous intimal thickening of the portal vein (arrows), consistent with a diagnosis of porto-sinusoidal vascular disease in association with biliary injuries.