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

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15  
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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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8  
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  
57  
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  
5  
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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60 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  
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30 studies validated by histological examination of the liver make it difficult to identify reliable cut-off  
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32 values.

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34 Finally, portal hypertension should be carefully looked for and not underestimated because it may  
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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  
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44 portal hypertension and its complications such as high-risk esophageal varices, in other chronic liver  
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46 diseases both in adults and children [92,93]. The only study performed in CF children reported higher  
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48 spleen stiffness values than in healthy controls [94]. Thus, this approach has not yet been fully  
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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].  
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#### 7 Biliary Fibrosis

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

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

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.  
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4 3 These “red-flag” imaging features include the presence of portal hypertension associated with an  
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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  
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21 10 fibrosis, morphological changes characterized by hypertrophy of segment IV may indicate  
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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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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  
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58 25 portal tracts and centrilobular vein distribution), non zonal sinusoidal dilatation and mild  
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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 including vascular markers of sinusoids, portal venules and arteries, are essential for a diagnosis of  
4 porto-sinusoidal vascular disease [109]. Besides the pathological features of porto-sinusoidal vascular  
5 disease, bile duct dilation and mucosal biliary plugs are usually absent, except in the presence co-  
6 existent biliary fibrosis.

7 The hepatic venous pressure gradient often confirms the diagnosis of porto-sinusoidal vascular  
8 disease, because it is clearly below the threshold for clinically significant portal hypertension (10  
9 mmHg in adults) [77,110]. Indeed, hemodynamic measurements of the hepatic venous pressure  
10 gradient only reflect hepatic sinusoidal pressure and not portal pressure. Because porto-sinusoidal  
11 vascular disease represents pre-sinusoidal involvement, the hepatic venous pressure gradient is falsely  
12 low which is highly informative for the diagnosis of porto-sinusoidal vascular disease. This procedure  
13 is performed by transjugular route, allowing a liver biopsy during the same procedure.

### 14 Biliary Injury

15 Biliary injury is thought to be a result of abnormal bile acidification. Neonatal cholestasis, caused  
16 by viscous and inspissated bile secretions, can sometimes be the first sign, mimicking biliary atresia  
17 [111,112]. Biliary involvement can present as lesions from sclerosing cholangitis with single or  
18 multiple focal stenoses, micro-gallbladder, gallbladder dysfunction or pigmentary cholelithiasis  
19 [113]. MR cholangiography can identify irregularities of the intrahepatic bile ducts with alternating  
20 dilatations and strictures [113].

21 In a prospective study including 26 adult CF patients, intra-hepatic biliary injury was present in 18  
22 (69%) including 10 (38%) with sclerosing cholangitis-like lesions. Eighteen of these patients had no  
23 signs of liver involvement [114]. Micro-gallbladder, defined by a cut-off volume of 2 cm<sup>3</sup>, was  
24 reported in up to one third of the patients [115,116]. Gallbladder dysfunction is frequently reported,  
25 characterized by hypokinesia and resulting in impaired gallbladder emptying. However, Santamaria  
26



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].

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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-  
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12 6 enhanced CT or MRI.

## 13 14 7 **Treatment**

15  
16 8 Although ursodeoxycholic acid (UDCA) has long been the proposed treatment of CF patients with  
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19 9 liver involvement to prevent or halt the progression of fibrosis to cirrhosis [6], its efficacy remains  
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21  
22 10 controversial. A Cochrane review including 3 randomised controlled trials on the use of  
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24 11 ursodeoxycholic acid for at least three months compared to placebo or no additional treatment ( $n =$   
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26 12 118), reported no benefit and did not recommend UDCA for routine use in CF [131]. Colombo et al  
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29 13 also reported no evidence of any benefit on the occurrence of portal hypertension [132]. On the other  
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32 14 hand, a large longitudinal cohort study ( $n = 3417$ ) suggested that UDCA had a positive effect on  
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34 15 survival in patients with mild CFLD [7]. Recently, a synthetic side-chain-shortened derivative of  
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36 16 UDCA, NorUrsodeoxycholic acid (norUDCA), was developed and was shown to improve cholestasis  
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39 17 in primary sclerosing cholangitis in a phase II clinical study [133]. Unlike UDCA, which increases  
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41  
42 18 bile acid secretion and the risk of cholangiocyte damage, norUDCA increases bile flow by stimulating  
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44 19 bicarbonate secretion. In addition, norUDCA is credited with a direct anti-inflammatory action by  
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47 20 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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54 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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59 25 hypertension and hepatocellular insufficiency are commonly accepted indications for liver  
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61 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].  
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4 3 Another study reported an improvement in liver steatosis with lumacaftor/ivacaftor [148]. Finally the  
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6 4 European Medicines Agency reported post-marketing cases of liver failure leading to death in CF  
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9 5 patients with pre-existing cirrhosis and portal hypertension who were treated with  
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11 6 lumacaftor/ivacaftor [149]. Better understanding of the safety of CFTR modulator therapies is needed  
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14 7 in relation to the severity of CFLD to identify those at the highest risk of serious adverse events as  
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16 8 well as to select patients with the best risk-benefit ratio. Rather than liver tests with a low sensitivity  
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19 9 and specificity, liver stiffness may be monitored during CFTR modulator therapy to improve early  
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21 10 detection of a response or progression of CFLD. In association with CFTR modulator therapies, there  
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24 11 is ongoing research to identify new therapies. Fiorotto et al showed that inhibition of Src tyrosine  
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26 12 kinases targeting the TLR4 mediated inflammatory process resulted in the reduction of the pro-  
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29 13 inflammatory state in human CF cholangiocytes, paving the way for potential therapies [150]. FXR  
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31 14 agonists (e.g., obeticholic acid) could restore bile acid homeostasis. Although obeticholic acid was  
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33 15 found to be beneficial in patients with other chronic liver diseases, it was associated with serious  
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36 16 adverse events [151]. FGF19 analogs are also a potential candidates [152] and other fibroblast growth  
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38 17 factors such as FGF1, which is a PPAR $\gamma$  target, are potent potential therapeutic agents [153,154]. It  
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41 18 has been suggested that vitamin D may alter gut microbiota, downregulate pro-inflammatory  
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43 19 cytokines and IL-8 production, and prevent liver fibrosis by inhibiting the TGF $\beta$ 1/SMAD signaling  
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45 20 pathway [155–158].  
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## 22 Conclusion

23 Portal hypertension, either as a direct complication of biliary cirrhosis or secondary to porto-  
24 sinusoidal vascular disease, is the main complication of CFLD, influences patient management and  
25 may require liver transplantation. Screening for liver involvement and fibrosis should include an  
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  
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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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9 Cystic fibrosis transmembrane conductance regulator (CFTR)  
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12 CF liver-related disease (CFLD)  
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14 Farnesoid X receptor (FXR)  
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16 Fibroblast growth factor (FGF)  
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19 Gammaglutamyltransferase (GGT)  
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22 Magnetic Resonance Elastography (MRE)  
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24 Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B)  
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27 Pathogen-associated molecular patterns (PAMPs)  
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29 Point ShearWave elastography (pSWE)  
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32 Rous sarcoma oncogene cellular homolog (Src)  
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34 Toll-like receptor 4 (TLR4)  
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37 Transient Elastography (TE)  
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39 Two-dimensional ShearWave Elastography (2D SWE)  
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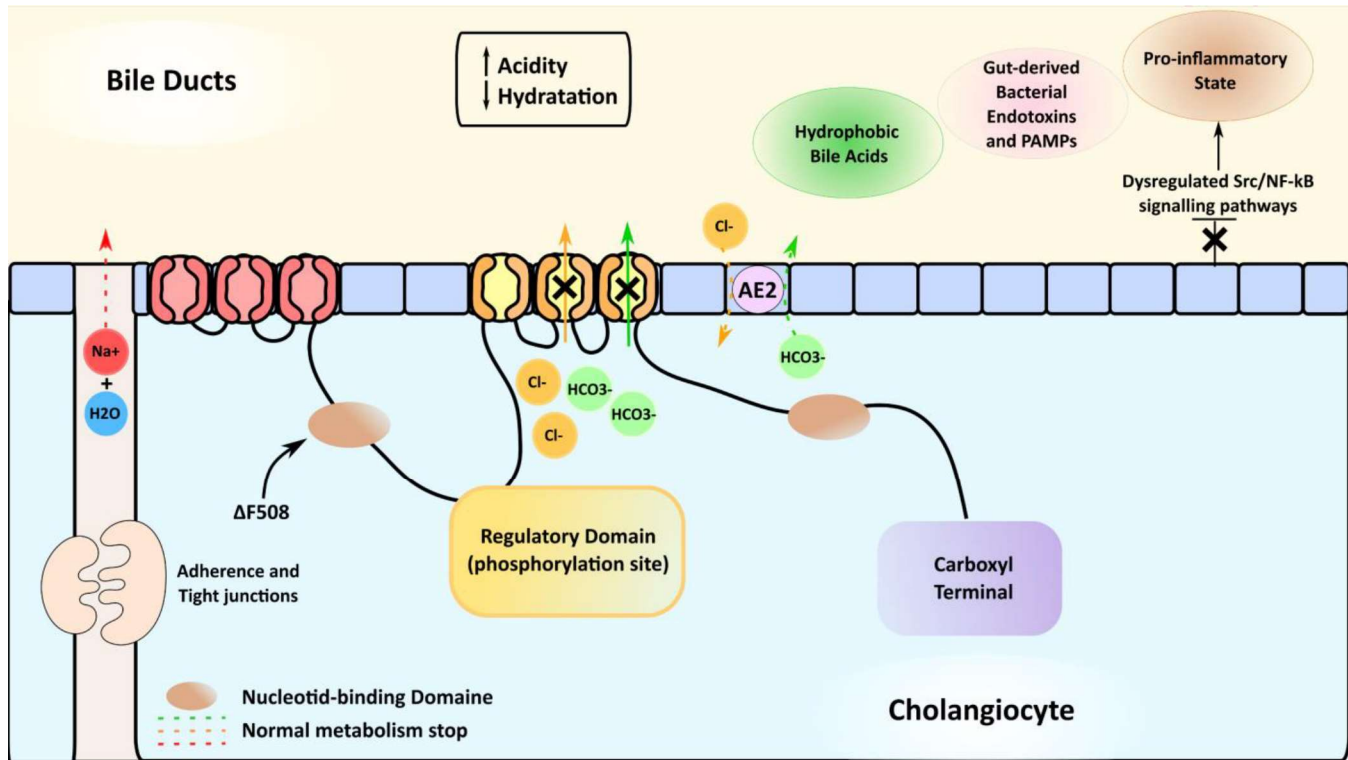


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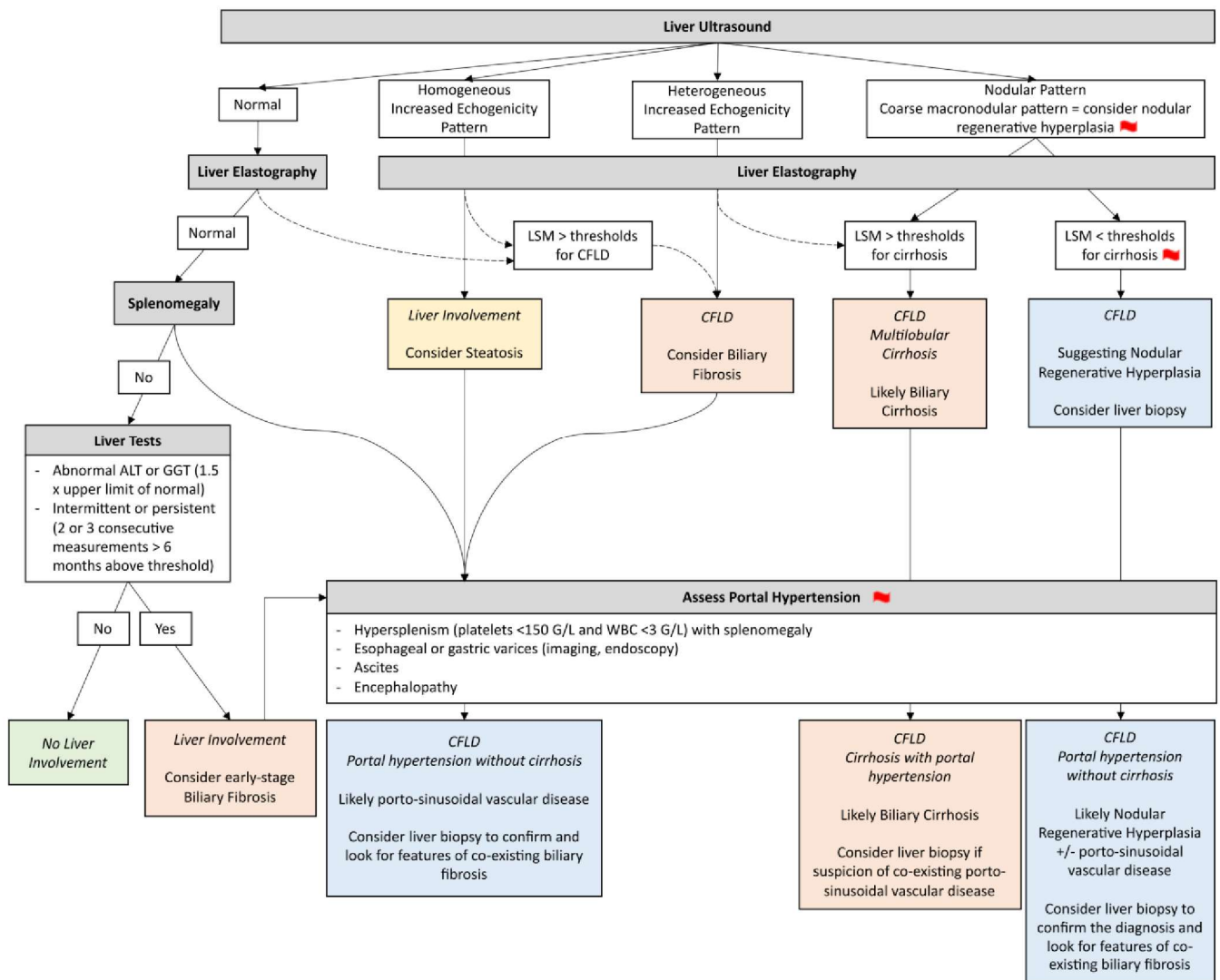
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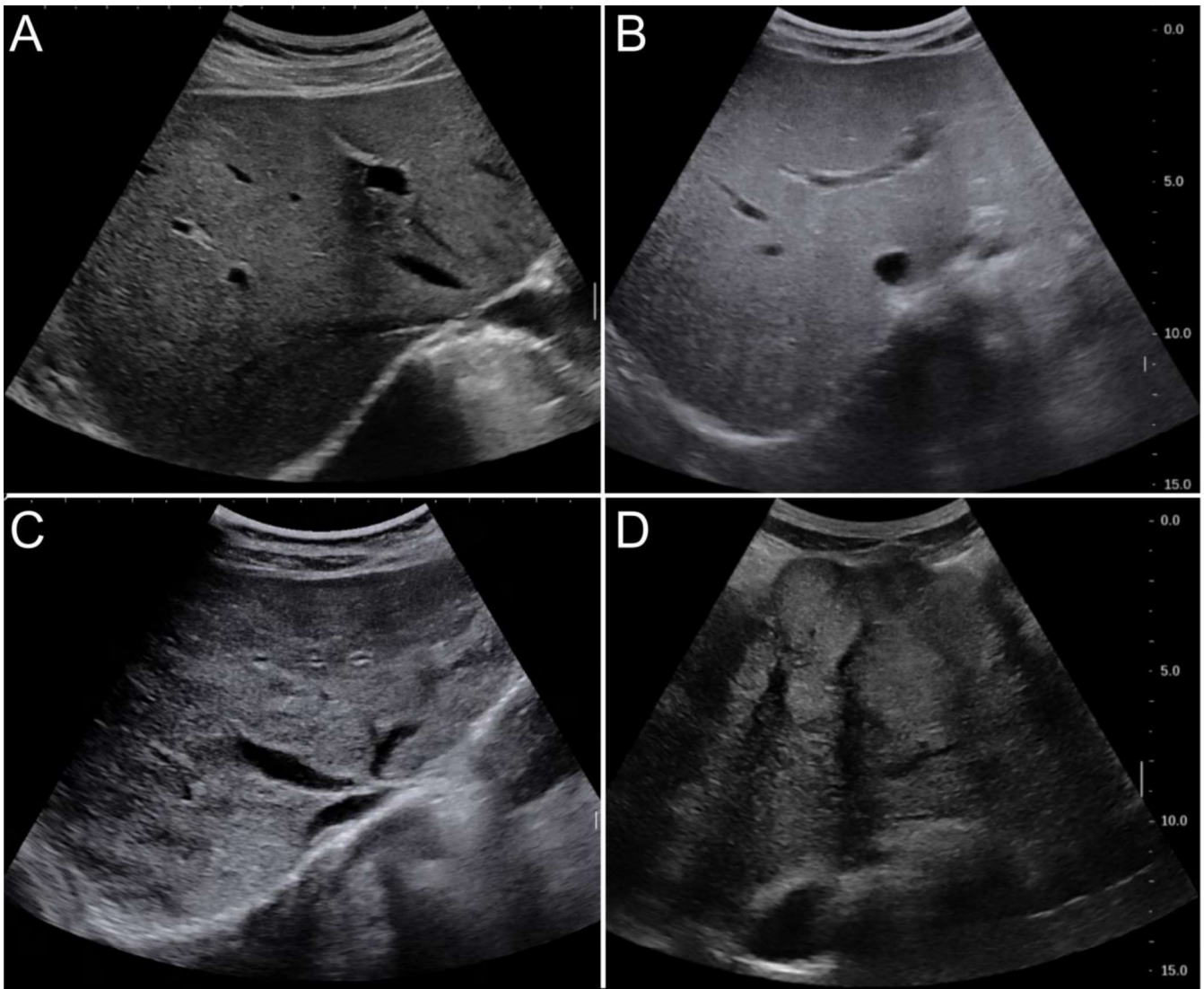
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**Graphical Abstract. Schematic structure of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein, its functioning, and its impact on ionic exchanges within the cholangiocyte.** The extra-cellular transport of Cl<sup>-</sup> and HCO<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.

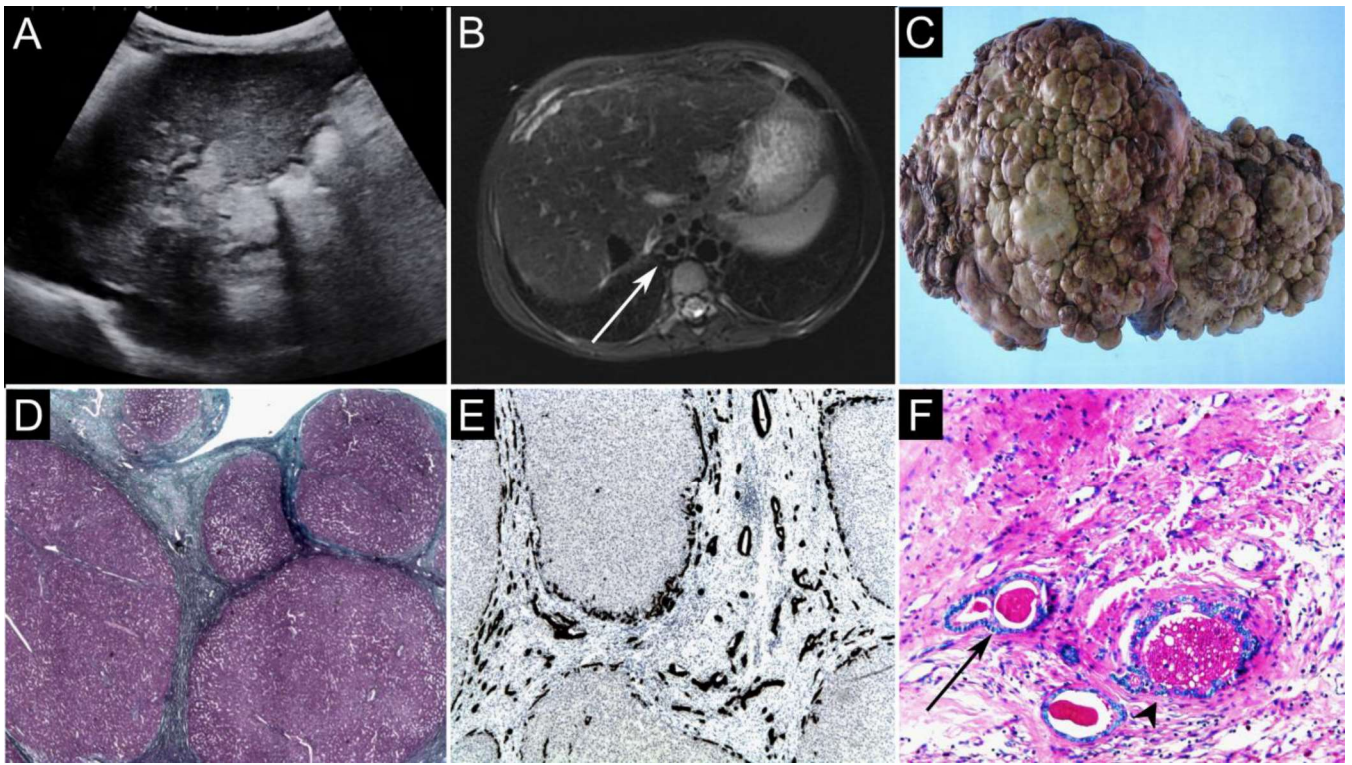


**Figure 1. Diagnostic approach to Liver Involvement and Cystic Fibrosis Liver Disease based on Debray et al [4].** Cystic Fibrosis Liver Disease (CFLD) is defined by the CFTR defect while liver involvement includes all hepatic manifestations including epiphenomena. In the presence of “red-flag” signs,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

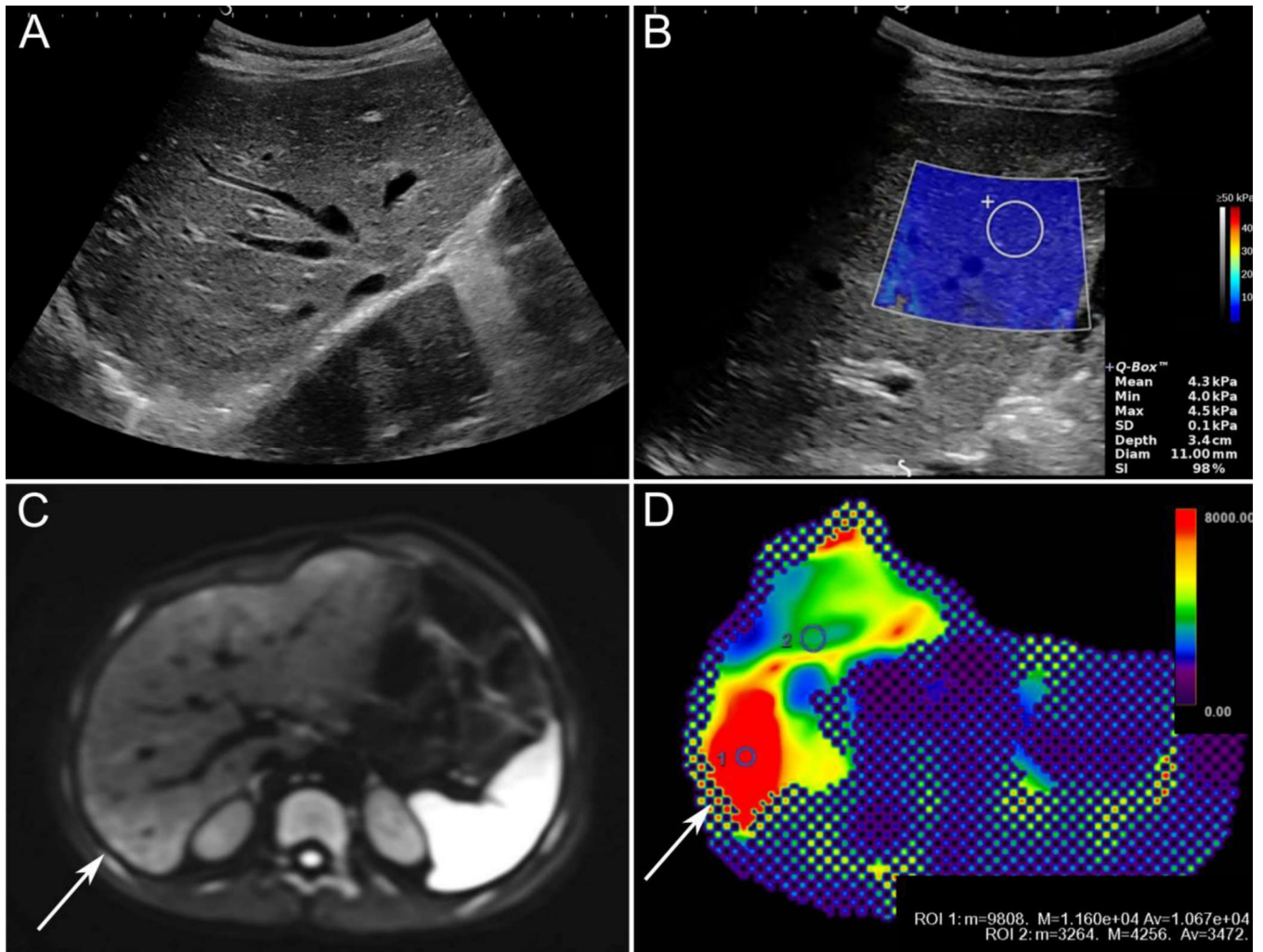


**Figure 2. Ultrasound Patterns in Patients with Cystic Fibrosis. (A) Normal. (B) Homogeneous hyperechogenicity. (C) Heterogeneous hyperechogenicity. (D) Nodular.**



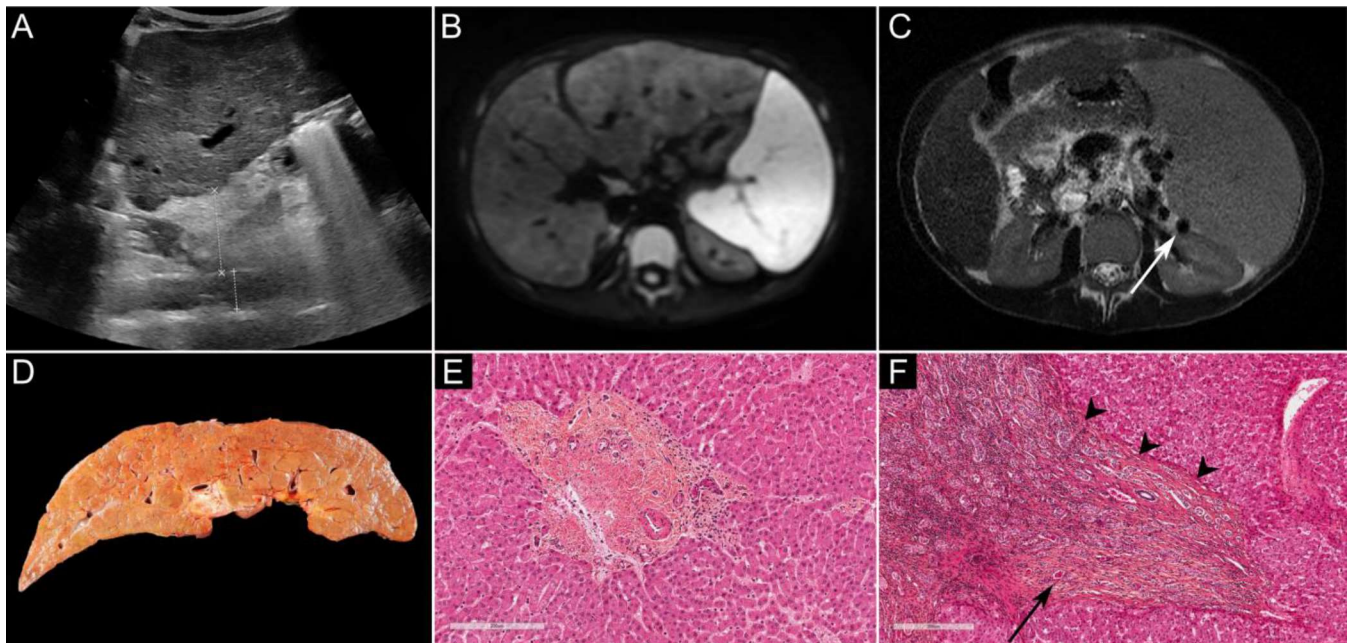


**Figure 3. Biliary Cirrhosis with portal hypertension in an 8-year-old girl.** Median liver stiffness measured by Transient Elastography, 2D ShearWave Elastography and Magnetic Resonance Elastography was increased to 71 kPa, 75.1 kPa and 10.6 kPa, respectively. Liver ultrasound (A) and axial fat suppressed-T2 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).

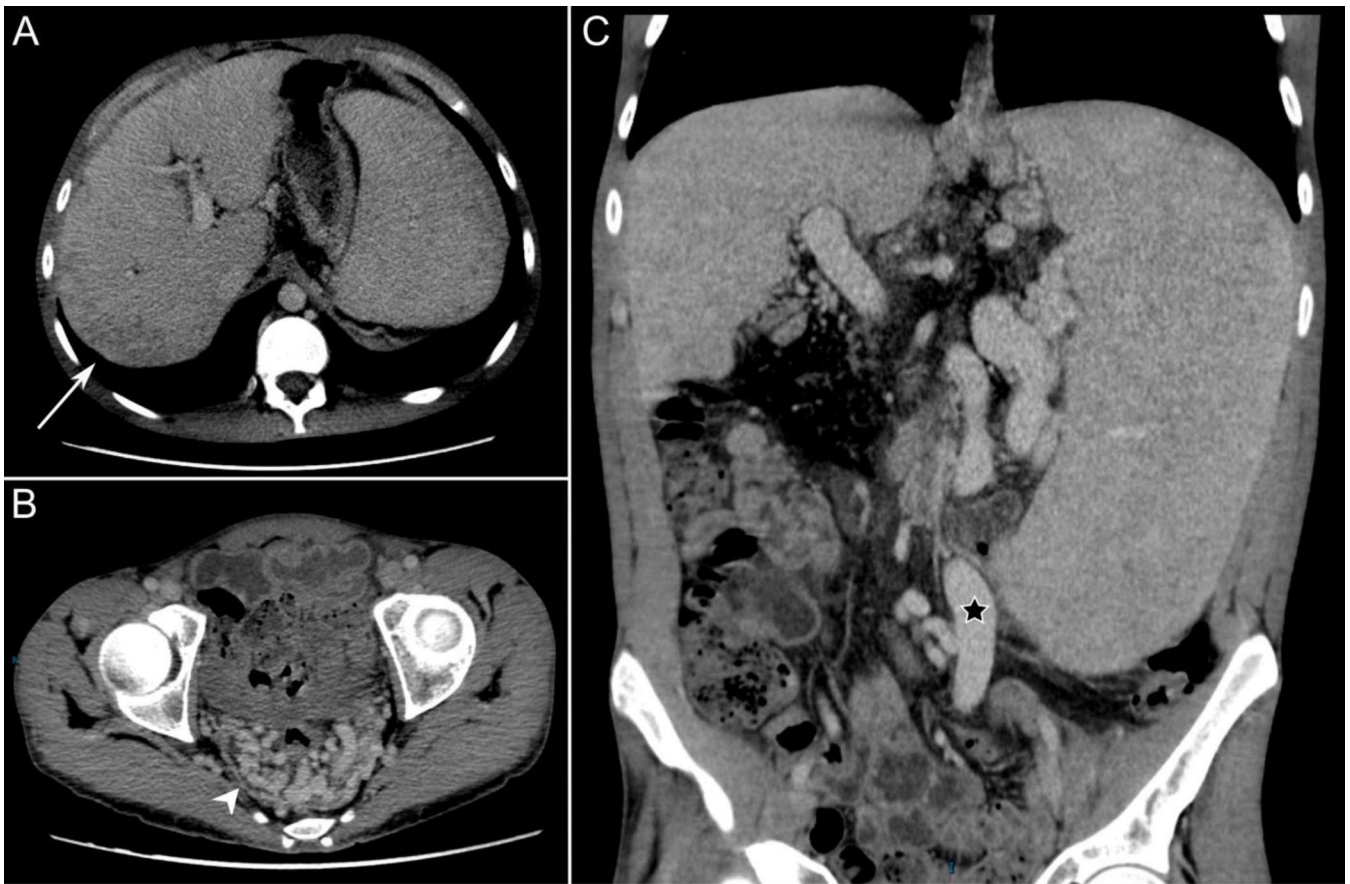


**Figure 4. Focal Biliary Fibrosis without portal hypertension in an 8-year-old boy.** Liver ultrasound (A) shows a slightly heterogeneous hyperechogenic pattern with smooth liver contours and no signs of portal hypertension. Liver stiffness on 2D ShearWave Elastography (B) was normal (4.3 kPa). Axial 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.

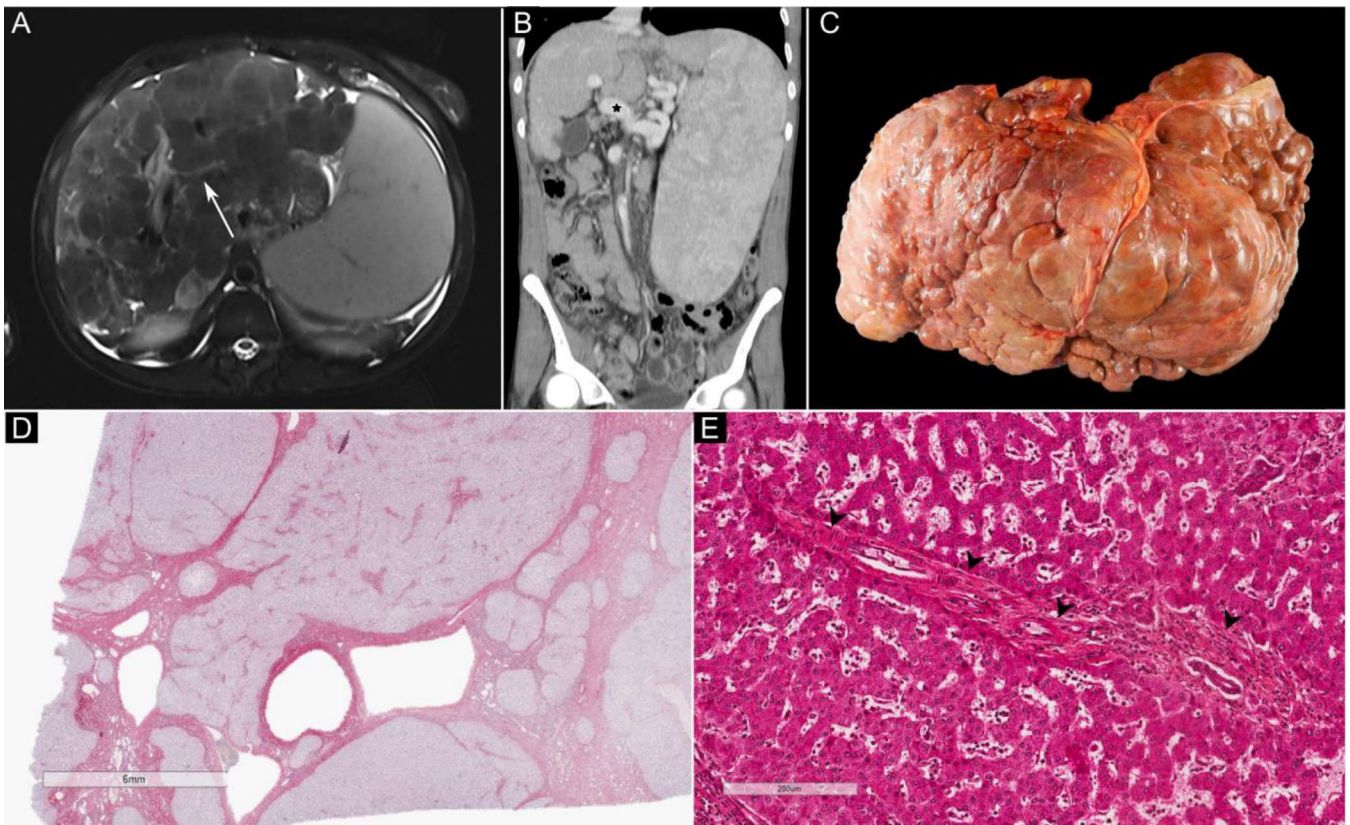




**Figure 5. Cirrhosis with portal hypertension on co-existing biliary and vascular injuries in a 16-year-old man.** Liver stiffness on Transient Elastography, ultrasound-guided elastography and Magnetic Resonance Elastography was consistent with advanced fibrosis (> F2 stage). (A) Liver ultrasound showed 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).

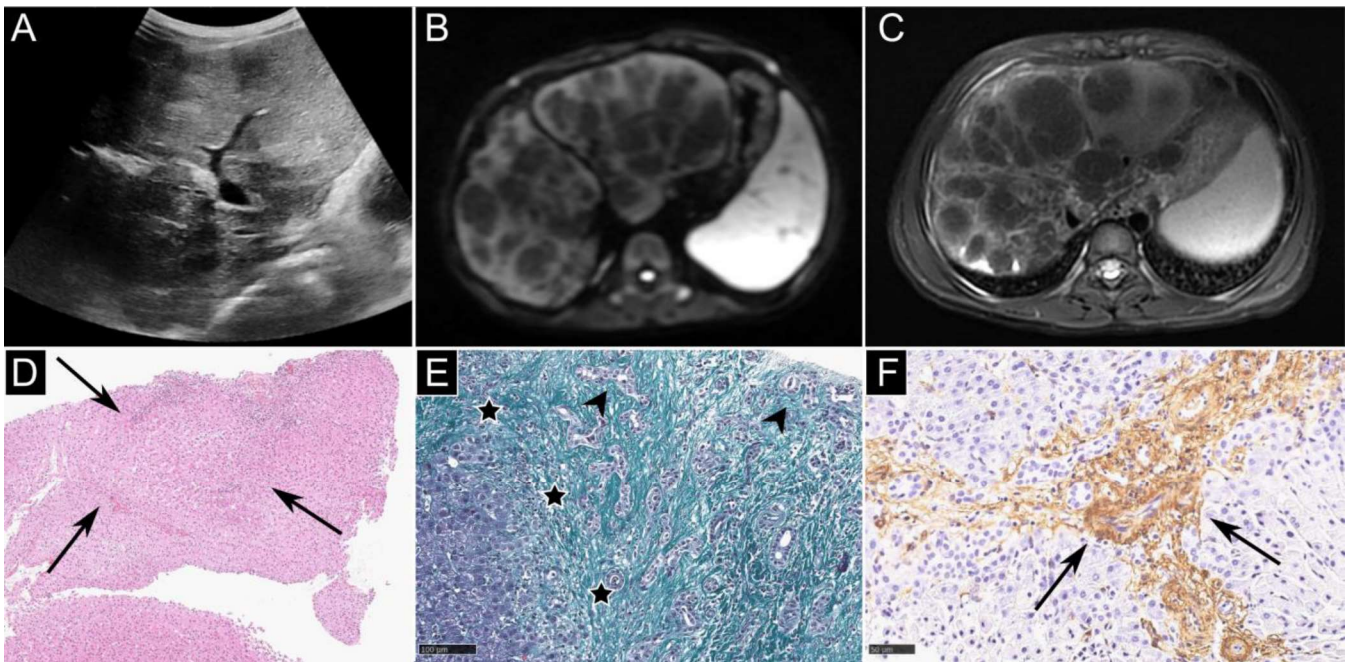


**Figure 6. Porto-sinusoidal vascular disease in a 21-year-old man.** Liver stiffness on Transient Elastography was increased to 10.1 kPa. Axial (A, B) and coronal (C) portal venous CT-scan showed severe portal hypertension – rectal varices (arrowhead), portal vein dilation and inferior mesenteric vein 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.