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The non-invasive evaluation of liver involvement in patients with cystic fibrosis: A prospective study

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Abstract

Background and Aims: Porto-sinusoidal vascular disease (PSVD) has been described as the prominent pathology in liver explants of patients with cystic fibrosis (CF), but data outside the transplant setting are lacking. We aimed to investigate the prevalence of portal hypertension (PH) in CF-associated liver disease (CFLD) and develop an algorithm to classify liver involvement in CF patients.

Methods: This is a cross-sectional study of consecutive paediatric and adult patients in a tertiary centre between 2018 and 2019, who underwent ultrasound, liver (LSM) and spleen stiffness (SSM) measurement. CFLD was defined according to physical examination, liver tests and ultrasound findings. PSVD was likely if there were PH signs in the absence of advanced chronic liver disease (CF-ACLD, LSM <10 kPa). A historical cohort was used to validate the prognostic significance of the new definitions.

Results: Fifty (27.5%) patients met CFLD criteria. At least one sign of PH was found in 47 (26%) patients, but most (81%) had LSM <10 kPa and were likely to have PSVD;

Abbreviations: ACLD, advanced chronic liver disease; ALT, alanine transaminase; AST, aspartate transaminase; AUROC, area under ROC curve; BMI, body mass index; CF, cystic fibrosis; CFLD, cystic fibrosis-associated liver disease; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; GGT, gamma-glutamyl transferase; HVPG, hepatic venous pressure gradient; IC, interval of confidence; IQR, interquartile range; LSM, liver stiffness measurement; LT, liver transplantation; NITs, non-invasive tests; NRH, nodular regenerative hyperplasia; OR, odds ratio; PH, portal hypertension; PSVD, Porto-sinusoidal vascular disease; PV, portal vein; TE, transient elastography.

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only 9 (5%) had CF-ACLD. PSVD and CFLD (LSM <10 kPa) co-existed in most (23/36) cases. In the historical cohort ($n = 599$ patients), likely PSVD and CFLD+PH were independently associated with a 2-fold and 3.5-fold increase in mortality compared to patients without PH, respectively. In 34 patients with SSM, values <21 and >50 kPa accurately diagnosed specific signs of PH.

Conclusions: PSVD is the prevailing cause of PH in CF patients. We developed a new diagnostic algorithm based on clinical and elastosonography criteria to classify liver involvement in patients with CF.

KEYWORDS

CFLD, liver stiffness, liver transplantation, noncirrhotic portal hypertension, porto-sinusoidal vascular disease, spleen stiffness

1 | INTRODUCTION

Cystic fibrosis-associated liver disease (CFLD) is a significant cause of morbidity and mortality in this genetic disease.[1] The actual CFLD diagnostic criteria are based on the combination of clinical, laboratory and ultrasound findings, but in the last years, liver elastography has been proposed to be included in the so-called 'expanded criteria'.[2] Traditionally, CFLD has been considered a channelopathy,[3] where the epithelial damage due to bile stasis would lead to periportal fibrosis and eventually cirrhosis with portal hypertension (PH).[4] However, this explanation is not fully supported by experimental and clinical data,[5] and the underlying scenario seems more complex.[4]

In patients with cystic fibrosis (CF) and portal hypertension (PH) undergoing liver transplantation (LT), both in paediatric[6] and adult patients,[7,8] the predominant histological feature in explants was attributed to noncirrhotic portal hypertension (NCPH), and it was not related to biliary cirrhosis. For instance, Hillaire et al.[6] found nodular regenerative hyperplasia (NRH) in 94% of the explants, whereas only 35.3% of the patients presented focal biliary fibrosis.

Recently, a new entity called porto-sinusoidal vascular disease (PSVD), including different NCPH entities (such as NRH or obliterative portal venopathy), has been proposed.[9]

To the best of our knowledge, no study has systematically investigated the prevalence, characteristics and diagnosis of PSVD in CF patients outside the LT setting.[10,11] Moreover, defining PSVD in these patients is challenging, as no generally accepted criteria are available to date,[11] and the standard tests, such as liver biopsy or hepatic venous pressure gradient (HVPG), are both invasive for the patients and not validated in the specific context of CF.[12–14] For this purpose, non-invasive tests (NITs), such as liver (LSM) and spleen (SSM) stiffness measurement assessed by transient elastography (TE), have been widely validated as accurate surrogates of liver fibrosis, advanced chronic liver disease (ACLD)/cirrhosis and PH[15–17] and could even help distinguish cirrhosis from vascular disorders, such as PSVD.[18–22]

Key points

In cystic fibrosis, portal hypertension is not associated with advanced chronic liver disease but should be attributed to porto-sinusoidal vascular disease. The combination of biochemical tests, ultrasound, liver and spleen elastography can be used to non-invasively diagnose and classify the heterogenous forms of liver involvement in this context.

Thus, we aimed to (i) investigate the prevalence of portal hypertension signs and the presence of CFLD; (ii) evaluate the presence of vascular liver diseases in CF patients; (iii) propose a new algorithm to classify liver involvement in CF patients non-invasively; (iv) explore the role of SSM by TE in the diagnosis of PH in CF.

2 | MATERIALS AND METHODS

2.1 | Patients and data collection

This is a prospective cross-sectional study of both paediatric and adult CF patients followed in the tertiary centre of Verona University Hospital (Verona CF centre) and referred to the Gastroenterology Unit for liver investigations between January 2018 and August 2019, who consecutively underwent an ultrasound and TE evaluation. Data regarding CF mutations, ileus meconium, pancreas insufficiency, lung function tests and history of liver disease were collected for each subject. Eligible patients were negative for hepatitis B surface antigen, anti-hepatitis C virus antibodies and excluded if other causes of liver disease (autoimmune, hemochromatosis, alcohol consumption above recommended limits) were present. Patients with an interval >6 months between the TE and the last biochemical examinations and patients with insufficient clinical data were excluded from the analysis.

2.2 | Definitions

CFLD diagnosis was made when at least two of the following criteria were present[14,23]: (1) clinical hepatomegaly, confirmed by ultrasound; (2) persistent abnormal (above upper limit of normal) serum liver enzyme levels of at least two among aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT); (3) abnormalities findings at the ultrasound evaluation, that is nodular or heterogeneous pattern of liver parenchyma, irregular liver margins and splenomegaly. The 'expanded' CFLD criteria were met when either CFLD criteria were fulfilled or LSM values were >6.8 kPa.[24]

Portal hypertension was defined by the presence of any of the following signs[25,26]: splenomegaly (above >13 cm in adults, or according to age-appropriate cut-offs in paediatric patients[23,27]), dilated portal vein (PV) trunk (>12 mm), reduced PV velocity (<14 cm/s), presence of ascites, collaterals or thrombocytopenia (platelet count $<150\times10^9$/L).[14]

Advanced chronic liver disease was defined by LSM values >10 kPa (CF-ACLD), in agreement with current guidelines that suggest this cut-off to rule-out compensated ACLD.[15,20] Based on the paper by Elkrief et al.,[20] LSM >10 kPa showed a sensitivity of 96.7% to diagnose ACLD and a specificity of 65% to distinguish between ACLD and PSVD; negative predictive value (NPV) to rule-out ACLD was 85%, whereas positive predictive value (PPV) 91%. We also performed a sensitivity analysis using the disease-specific 8.7 kPa cut-off, previously shown to identify CF patients with F3-F4 fibrosis stage at liver biopsy.[2]

We suspected the presence of PSVD[11,20] in patients who presented signs of PH but did not have an advanced liver disease CF-ACLD (i.e. LSM <10 kPa).[2] We performed a preliminary analysis for different definitions of PH (with increasing specificity). We found a consistent rate of patients with low LSM (i.e. no ACLD) among patients with PH signs (likely PSVD), as shown in Supplemental Material 1.

2.2.1 | Validation cohort

We used a retrospective cohort of almost 600 CF patients followed in our centre between 2012 and 2017 (pre-elastography era) to validate the prognostic relevance of the newly proposed clinical definitions against a hard clinical outcome such as transplant-free survival. In this cohort, we used three risk groups: no portal hypertension, likely PSVD (portal hypertension in the absence of CFLD) and CFLD with portal hypertension. CFLD and portal hypertension were defined as above. For each category, we reported the crude rate of death/liver transplantation and the risk of death after adjustment for relevant prognostic factors, such as demographics, lung disease severity and genotype.

Given the lack of elastography data in this cohort, we could not further discriminate between patients with CF-ACLD and PH vs patients with mild CFLD and possible PSVD in the category of patients meeting the criteria for both CFLD and PH diagnosis.

2.3 | Non-invasive tests for liver fibrosis

After overnight fasting, the LSM and SSM values were assessed by TE with FibroScan®, 'M' probe (Echosens, Paris, France). The LSM reliability criteria were in agreement with recent guidelines.[28] LSM values >6.8 kPa were used to diagnose expanded CFLD, and >10 kPa were used to diagnose CF-ACLD.[2] In a subset of patients, the SSM was assessed the same day as LSM, as previously described.[29] We evaluated the performance of the Baveno VII recommended cut-offs of 21 and 50 kPa to respectively rule-out and rule-in clinically significant portal hypertension.[15,17] Abdominal ultrasound (US) was performed in the same TE assessment session. As previously described, we also evaluated serum markers for fibrosis, such as APRI and FIB-4 scores.[16]

2.4 | Statistical analysis

Categorical data were expressed as numbers (percentages) and continuous variables as medians (and values of the 25% and the 75% percentiles, interquartile range [IQR]). The chi-square, Mann–Whitney, Mc-Nemar and Kruskal–Wallis tests used group comparisons of categorical and continuous variables. The area under the ROC curve (AUROC) for CFLD diagnosis was reported for each non-invasive test; the DeLong test was performed to test the equality of the AUROCs. A multivariate time-dependent cox proportional hazards model was used to predict death or liver transplantation (transplant-free survival) according to the presence of CFLD and portal hypertension, after adjustment for demographic data, lung disease severity and CF genotype. All p-values referred to two-tailed tests of significance. $p<.05$ was considered significant. The statistical analysis was carried out using Stata/SE (Version 14.0; Stata Corp).

2.5 | Ethics

This study was conducted in compliance with the Declaration of Helsinki and approved by the local institutional review board. All patients, parents or legal guardians of study participants provided written informed consent.

3 | RESULTS

3.1 | Population

Of the 199 eligible patients evaluated at our centre, 17 (8.5%) patients were excluded due to insufficient data or suboptimal intervals between liver ultrasound and biochemical tests. Finally, 182 patients were included in the final analysis. The median age was 20 (11–30.4) years; 103 (57%) and 81 (45%) were adult patients and male, respectively. The most common CF genotype was ΔF508 heterozygosis (80, 44%), and most of the patients ($n=148$,

81%) had pancreatic insufficiency, of whom 16 (8.8%) were on insulin treatment. According to the liver ultrasound examination, 80 (44%) presented hepatomegaly, 67 (27%) liver steatosis and 35 (19%) splenomegaly.

The CFLD diagnosis has been reached in 50 (27.5%) patients. According to the CFLD status, patients' characteristics are summarized in Table 1.

3.2 | Non-invasive test for the diagnosis of CFLD

Patients with CFLD had higher values of LSM and APRI scores but not FIB-4. LSM presented excellent accuracy for the clinical diagnosis of 'classical' CFLD (AUROC 0.806, 95% CI: 0.715–0.896), significantly higher than that of APRI (AUROC 0.686, 95% CI: 0.580–0.793, $p = .043$; Supplemental Material 2A). However, among patients outside these clinical CFLD criteria, seven (5.3%) additional patients presented LSM values >6.8 kPa, and one had values compatible with CF-ACLD (13.8 kPa) (Supplemental Material 2B). The inclusion of LSM by TE in the 'expanded' criteria for liver disease would increase the rate of patients with CFLD and with CF-ACLD by 13% and 9%, respectively.

3.3 | Portal hypertension in CF patients

3.3.1 | Prevalence of PH and its causes

At least one sign of portal hypertension was found in 47 (26%) of the patients; the most common signs were splenomegaly (35, 19%), abnormal PV diameter (15, 8%) or velocity (9, 6%), and thrombocytopenia (8, 4%). Only 32 (68%) out of the 47 patients with PH met the clinical CFLD definition. Among the 32 patients with CFLD and PH, only 9 (28%) presented CF-ACLD; the majority ($n = 23$) presented LSM <10 kPa and were considered as having mild CFLD and, however, in the presence of PH, were also classified as possible PSVD. On the other side, 15 patients with PH but without CFLD were classified as likely PSVD; none of these patients had LSM values >6.8 kPa (range 2.6–5.3 kPa). Accordingly, PSVD was suspected in 21% (38/182) of the whole cohort of patients (Table 2 and Figure 1). Similar findings were found in a sensitivity analysis, where the disease-specific cut-off (8.7 kPa) was used to define ACLD in patients with CF (Supplemental Material 3). Among CF-ACLD patients, four patients presented with LSM >20 kPa, of whom three had signs of portal hypertension (two had collaterals).

Upper endoscopy was available in 10 (5.5%) patients. All these patients met CFLD criteria and presented splenomegaly, four had collaterals at ultrasound. Of note, 5 (50%) out of 10 patients had oesophageal varices (3 small F1 varices, 2 large F2 varices), of whom 3 had LSM >10 kPa (CF-ACLD) and 2 had LSM <10 kPa (7.8 and 8 kPa), as per possible PSVD.

Finally, patients' characteristics according to paediatric or adult status are reported in Supplemental Material 4.

Based on these data, we proposed a new stepwise algorithm for stratifying the liver involvement in patients with CF (Figure 2). PSVD was suspected to be the most prevalent cause of PH in CF patients, representing 81% (38/47) of the patients with PH, and it co-existed with a diagnosis of CFLD in most (23/36) cases. CF-ACLD with PH was diagnosed in 9 (5%) of the entire cohort cases. Patients' characteristics according to the liver involvement (no liver involvement, CFLD without PH, CF-ACLD with PH, likely PSVD) are summarized in Table 3. Of note, patients with likely PSVD were older, presented more frequently with normal liver enzymes, preserved liver function (serum albumin), regular/inhomogeneous liver pattern at the liver ultrasound and a severe pancreas insufficiency. The differences between patients with possible (concomitant CFLD) vs. likely (no CFLD) PSVD are reported in Supplemental Material 5.

3.4 | Prognostic role of PH and likely PSVD definition in a historical cohort

To validate the clinical definition of PH and likely PSVD used in our prospective cohort, we tested the ability of these criteria to predict survival in a historical cohort of 599 CF paediatric and adult patients in the pre-TE era. Patients' characteristics are reported in Supplementary material 6; briefly, median age was 26 (15–38) years, and 285 (48%) patients were male. During follow-up, 65 patients died, and 2 patients underwent liver transplantation. CFLD prevalence was 22% (129/599). At least one sign of PH was found in 105 (17.5%) patients yet only 63 of these patients met the CFLD criteria. We therefore identified three risk groups: patients without PH ($n = 494$), patients with PH signs but no CFLD, hence likely PSVD ($n = 42$), and patients with CFLD and PH signs ($n = 63$). Patients with likely PSVD (a category of patients outside the current criteria for liver disease in CF) showed mortality/transplantation rates (23.8%) significantly higher than those seen in patients without PH signs (8.3%) and comparable those seen in patients with CFLD and PH (25.4%) (Table 4). In multivariate analysis, likely PSVD was independently associated with a 2-fold increase in mortality (HR 2.137, 95%-CI: 1.062–4.298), whereas CFLD+PH was associated with a 3-fold increase (HR 3.311, 95%-CI: 1.795–6.109). Figure 3 depicts how transplantation-free survival progressively worsens among the three categories: patients with no PH, patients with likely PSVD and patients with CFLD and PH ($p < 0.0001$).

3.5 | Role of SSM in the diagnosis of PH and likely PSVD

SSM by TE was assessed in a subgroup of 34 patients. Overall, patients with signs of PH presented significantly higher values of SSM than patients without PH (17.8 kPa vs. 30 kPa, $p = .004$); SSM accuracy in diagnosing PH was excellent (AUROC 0.841; 95%-CI: 0.706–0.976). The performance of the Baveno VII SSM cut-offs to rule-out and rule-in any portal hypertension and clinically significant

TABLE 1 Patients' characteristics according to CFLD presence.

Variable	All patients ($n = 182$)	CFLD ($n = 50$)	No CFLD ($n = 132$)	p-value
General characteristics				
Age (years)	19.9 (11–30.4)	19.7 (11.8–26.2)	19.9 (10.6–30.5)	.895
Gender (male) (%)	81 (44.5)	21 (42)	60 (45.5)	.676
Height (cm)	161 (144–172)	165 (145–176)	160 (142–170)	.191
Weight (kg)	53 (36–62)	56 (36–67)	53 (35–62)	.271
BMI (kg/m^2)	19.6 (17.2–21.9)	19.4 (17.3–22.3)	19.9 (17.1–21.9)	.694
Cystic fibrosis characteristics				
Genotype—CFTR mutation				.816
ΔF508 homozygosis (%)	45 (24.7)	12 (24)	33 (25)	
ΔF508 heterozygosis (%)	80 (44)	20 (40)	60 (45.5)	
Other (%)	52 (28.6)	16 (32)	36 (27.3)	
Not available (%)	5 (2.7)	2 (4)	3 (2.3)	
Meconium ileus (%)	25 (13.7)	10 (20)	15 (11.4)	.131
Pancreatic insufficiency (%)	148 (81.3)	47 (94)	101 (76.5)	**.007**
Use of insulin (%)	16 (8.8)	9 (18)	7 (5.3)	**.007**
FEV1 (L)	2.05 (1.42–2.90)	2.07 (1.44–2.99)	2.01 (1.41–2.83)	*.523*
Predicted FEV1 (%)	80 (63–100)	81 (69–100)	80 (61–100)	.611
FVC (L)	3.09 (2.20–3.91)	3.15 (2.17–4.40)	3.08 (2.20–3.90)	*.526*
Predicted FVC (%)	94 (80–108)	98 (80–107)	94 (80–108)	.745
FEV1/FVC	0.74 (0.62–0.82)	0.75 (0.62–0.82)	0.73 (0.62–0.82)	.797
Biochemical values				
ALT (U/L)	30 (22–44)	43 (30–76)	28 (21–38)	**<.0001**
AST (U/L)	45 (21–50)	45 (23–50)	42 (20–50)	.195
GGT (U/L)	16 (10–27)	37 (22–101)	13 (9–20)	**<.0001**
Platelet count (10^9/L)	314 (269–370)	264 (187–332)	334 (286–395)	**<.0001**
Albumin (g/dL)	3.7 (3.3–4)	3.5 (3.1–3.9)	3.7 (3.3–4)	*.055*
Bilirubin (mg/dL)	0.4 (0.3–0.6)	0.4 (0.4–0.7)	0.4 (0.2–0.6)	.127
Creatinine (mg/dL)	0.63 (0.5–0.78)	0.66 (0.54–0.79)	0.63 (0.49–0.78)	.353
Ultrasound findings				
Hepatomegaly (%)	80 (44)	49 (98)	31 (23.5)	**<.0001**
Steatosis (%)				*.059*
No steatosis	115 (63.2)	26 (52)	89 (67.4)	
Mild	52 (28.6)	16 (32)	36 (27.3)	
Moderate	12 (6.6)	7 (14)	5 (3.8)	
Severe	3 (1.6)	1 (2)	2 (1.5)	
Liver pattern (%)				**<.0001**
Homogenous	143 (78.6)	16 (32)	127 (96.2)	
Inhomogeneous	27 (14.8)	22 (44)	5 (3.8)	
Nodular	12 (6.6)	12 (24)	0 (0)	
Splenomegaly (%)	35 (19.2)	30 (60)	5 (3.8)	**<.0001**
Dilated portal vein (%)	15 (8.3)	9 (18.8)	6 (4.6)	**.002**
Reduced portal vein velocity (%)	9 (5.5)	5 (10.9)	4 (3.4)	*.061*
Presence of collaterals (%)	6 (3.3)	4 (8)	2 (1.5)	**.029**
Endoscopy				
Upper endoscopy available	10 (5.5)	10 (20)	0 (0)	<.0001
Presence of varices	5 (50)	5 (50)	0 (0)	<.0001

TABLE 1 (Continued)

Variable	All patients ($n=182$)	CFLD ($n=50$)	No CFLD ($n=132$)	p-value
Non-invasive tests				
LSM (kPa)	4.5 (3.6–6.1)	6.6 (4.8–8.8)	4.2 (3.3–5.1)	**<.0001**
SSM (kPa) ($n=34$)	22.7 (16.6–43.5)	29 (20–62)	19 (15–38)	.197
APRI score	0.27 (0.15–0.37)	0.37 (0.18–0.52)	0.24 (0.15–0.33)	**.0004**
FIB-4 Index	0.33 (0.18–0.57)	0.42 (0.20–0.66)	0.30 (0.17–0.53)	.187

Note: Bold was used to highlight a statistically significant difference between the two groups (p-value < .05), whereas italics for borderline values (p > .05 but <.1). For FEV1 and FVC, it is erroneously formatted in italics.

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CF, cystic fibrosis; CFLD, cystic fibrosis liver disease; CFTR, cystic fibrosis transmembrane receptor; FEV1, forced expiratory volume; FVC, forced vital capacity; GGT, gamma–glutamyl transferase; LSM, liver stiffness measurement; SSM, spleen stiffness measurement.

TABLE 2 Prevalence of portal hypertension signs according to the presence of CFLD and advanced fibrosis according to LSM values.

Clinical scenario	No signs of portal hypertension ($n=135$)	Presence of portal hypertension ($n=47$)	Diagnosis
No CFLD ($n=132$)	117	15	**Suspected PSVD** Prevalence: 20.8% (38/182)
CFLD, LSM <10 kPa ($n=39$)	16	23	
CFLD, LSM ≥10 kPa ($n=11$)	2	9	**CF-ACLD with PH** Prevalence: 5% (9/182)

Abbreviations: CFLD, cystic fibrosis liver disease; LSM, liver stiffness measurement; PH, portal hypertension; PSVD, porto-sinusoidal vascular disease.

portal hypertension (presence of collaterals, varices) are reported in Supplementary Material 7. Of note, SSM <21 kPa showed a NPV of 100% to rule-out clinically significant portal hypertension and SSM >50 kPa a PPV of 100% for this condition.

We then explored whether the SSM/LSM ratio could play a role distinguishing between the two forms of portal hypertension (Figure 4 and Supplemental Material 8). When comparing patients with likely PSVD to those without PH, they presented significantly higher SSM values but comparable LSM and SSM/LSM ratio values. However, no patient without PH had an SSM/LSM ratio >4.7.

When comparing patients with likely PSVD to those with CF-ACLD+PH, LSM values were significantly lower in former group (5.2 vs. 12 kPa, $p<.0001$) as per definition, whereas no difference was found in SSM values (30 kPa vs. 34.3 kPa, $p=.797$). The SSM/LSM ratio between the two groups was significantly higher in the likely PSVD group (5.7 vs. 2, $p=.002$), with little overlapping between their values (Figure 4). In particular, the 10th percentile of the SSM/LSM ratio in suspected PSVD patients was 2.5, suggesting that values below this cut-off are suggestive of CF-ACLD, whereas the 90th percentile of SSM/LSM ratio in CF-ACLD patients was 6.4, suggesting that values above this cut-off should point versus likely PSVD.

4 | DISCUSSION

We have found that the prevalence of portal hypertension was substantial (26%) in patients with CF, but only a few patients had advanced chronic liver disease. Therefore, the presence of porto-sinusoidal vascular disease (PSVD) could be the most common cause (>70%) of PH among patients with CF. We have proposed a new algorithm based on liver ultrasound and liver and spleen elastography that could non-invasively classify the heterogeneous manifestations of liver involvement in CF patients. We used a historical cohort of almost 600 patients to validate the prognostic significance of the newly proposed classes.

Liver disease is a common complication in CF, as it develops in up to 30% of the patients by the age of 25[30] and is a major cause of morbidity and mortality.[10,31,32] The classical clinical diagnosis of CFLD[14] is based on the presence of abnormal physical examination (i.e. hepatomegaly), abnormal liver tests and abnormal ultrasound findings and has been recently challenged by the introduction of non-invasive tests that can accurately assess the presence of fibrosis in patients with chronic liver disease,[16] including the specific context of CF.[2,24,33–36] More importantly, reports showed that NCPH and not biliary cirrhosis is the underlying histological cause of PH in the overwhelming majority (80%–90%) of CF patients undergoing LT.[6–8]

Noncirrhotic portal hypertension has consistently been recognized as a possible manifestation of liver involvement in CF.[8,13,37] However, its clinical relevance has been greatly underestimated by (i) the lack of unique diagnostic criteria, (ii) difficulty to distinguish between NCPH and ACLD with PH based solely on ultrasound and biochemical data and (iii) paucity of studies on liver biopsy evaluation that could rule-out advanced fibrosis/cirrhosis in such patients.[10,11] To the best of our knowledge, no study to date has primarily evaluated the presence of PSVD in CF patients outside the LT context and

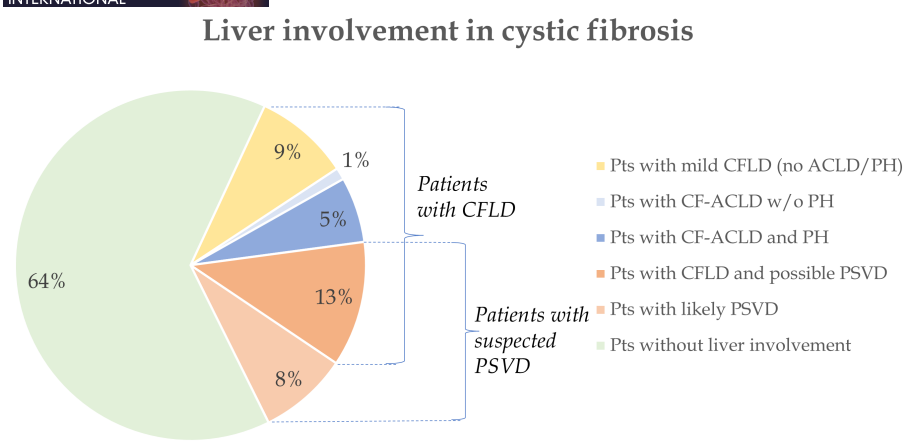

FIGURE 1 Liver diseases in patients with cystic fibrosis according to the presence of CF-associated liver disease, advanced fibrosis (LSM >10 kPa) and portal hypertension.

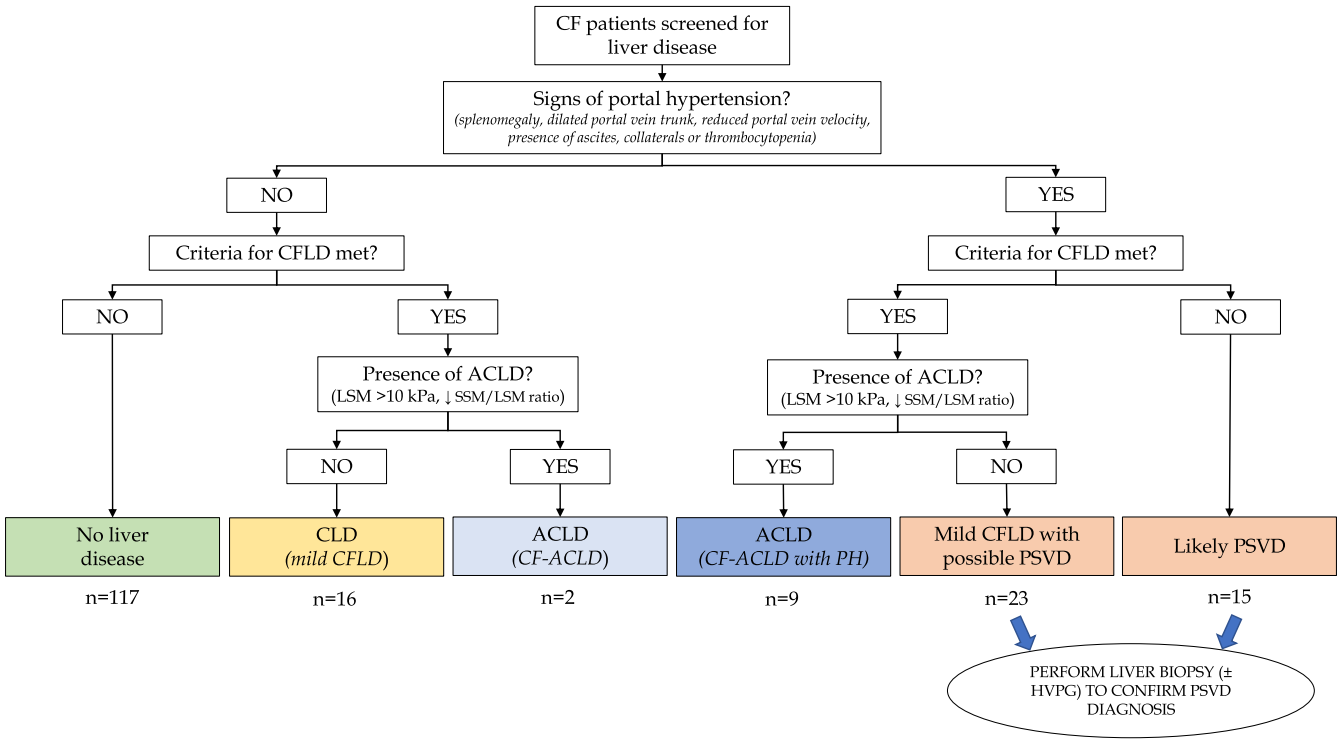

FIGURE 2 A new algorithm for classifying liver involvement in cystic fibrosis patients.

developed diagnostic criteria for this entity. In our study, we systematically and prospectively evaluated the presence of PH at abdomen ultrasound and biochemical tests (i.e. thrombocytopenia) and found that 47 (26%) out of 182 patients with CF presented with at least one sign of PH; however, CF-ACLD (LSM >10 kPa) was present in only 9 of these patients (5% of the entire cohort). The majority (81%) of the patients with PH had LSM <10 kPa and, therefore, could have PSVD (Figure 4 and Table 2), suggesting that PSVD could be the most prevalent cause of PH among patients with CF, just as previously shown in the transplant setting.[6] This finding was robust despite the criteria used to define portal hypertension.

We then developed a stepwise algorithm based on the presence of PH signs, CFLD clinical criteria, and elastography values to classify liver involvement in CF patients (Figure 4). PSVD was suspected in 21% of the patients that was identified in (i) those with only 'mild CFLD'—that could not justify the presence of PH -, and (ii) in those without CFLD criteria but with signs of PH. Interestingly, the latter group represents a non-negligible proportion of patients with portal hypertension that is excluded by the current definition of CFLD, despite being at risk of developing complications. This concept was clearly demonstrated in the historical cohort, as patients with PH outside CFLD criteria had an increased mortality compared to patients with no PH (Figure 3).

Patients with suspected PSVD were older and usually showed normal liver enzymes and normal/increased liver size with a regular liver pattern (or inhomogeneous pattern in 30% of the cases),

TABLE 3 Patients' characteristics according to the presence of CFLD, CF-ACLD and PSVD.

Variable	Patients with no CFLD nor PH ($n=117$)	Patients with CFLD, but no PH ($n=18$)	CF-ACLD patients with PH ($n=9$)	Suspected PSVD patients ($n=38$)	*p*-value
Age (years)	19 (9–30)	14 (6–22)	14 (11–21)	25 (17–41)	**.019**[a]
Gender (male) (%)	61 (52)	9 (50)	7 (78)	24 (63)	.325
BMI (kg/m^2)	20 (17–22)	18 (16–21)	18 (17–19)	20 (18–22)	.259
Meconium ileus (%)	14 (12)	4 (22)	0 (0)	7 (18)	.316
Pancreatic insufficiency (%)	88 (75)	16 (89)	9 (100)	35 (92)	**.036**
Use of Insulin (%)	6 (5)	1 (6)	1 (11)	8 (21)	**.025**
Predicted FEV1 (%)	81 (63–100)	81 (52–101)	87 (78–109)	74 (47–98)	.269
Predicted FVC (%)	95 (81–108)	85 (79–94)	110 (98–120)	97 (74–107)	.268
AST (U/L)	45 (20–50)	46 (245–50)	50 (45–53)	28 (18–50)	**.007**[a]
ALT (U/L)	29 (20–39)	45 (33–55)	94 (63–139)	29 (23–43)	**.0001**[a]
GGT (U/L)	14 (9–20)	38 (24–72)	107 (81–152)	19 (11–35)	**.0001**[a]
Albumin (g/dL)	3.7 (3.3–4)	3.5 (3.3–3.9)	3.3 (2.8–3.6)	3.7 (3.3–4)	.190[a]
Platelet count (10^9/L)	343 (288–396)	309 (267–346)	134 (68–185)	277 (233–320)	**.0001**[a]
Hepatomegaly	25 (21)	17 (94)	9 (100)	29 (76)	**<.0001**
Liver pattern					**<.0001**[a]
Inhomogeneous (%)	5 (4)	8 (44)	3 (33)	11 (29)	
Nodular (%)	0 (0)	4 (22)	6 (67)	2 (5)	
Steatosis	37 (32)	14 (78)	3 (33)	13 (34)	**.005**
Splenomegaly	0 (0)	0 (0)	8 (89)	27 (71)	**<.0001**
Collaterals	0 (0)	0 (0)	2 (22)	4 (11)	**<.0001**

Note: Bold was used to highlight a statistically significant difference between the two groups (*p*-value < .05).

Abbreviations: ACLD, advanced chronic liver disease; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CF, cystic fibrosis; CFLD, cystic fibrosis liver disease; FEV1, forced expiratory volume; FVC, forced vital capacity; GGT, gamma–glutamyl transferase; PSVD, porto-sinusoidal vascular disease.

[a]The difference between CF-ACLD with PH and PSVD patients for this variable was statistically significant.

TABLE 4 Prediction of transplant-free survival according to the presence of portal hypertension alone or in the context of CFLD in the historical cohort.

Risk group	Number of events	HR (95%-CI)[a]	*p*-value
No portal hypertension, $n=494$	41 (8.3%)	1 (reference)	
Portal hypertension, no CFLD (likely PSVD), $n=42$	10 (23.8%)	2.137 (1.062–4.298)	**.033**
Portal hypertension and CFLD, $n=63$	16 (25.4%)	3.311 (1.795–6.109)	**<.001**

Note: Bold was used to highlight a statistically significant difference between the two groups (*p*-value < .05).

Abbreviations: CFLD, cystic fibrosis liver disease; CI, confidence interval; HR, hazard ratio; PSVD, porto-sinusoidal vascular disease.

[a]Adjusted for age, gender, FEV1, genotype.

but with a disproportionate prevalence of PH signs. Interestingly, our findings are consistent with the recent review[10,11] that has suggested a higher prevalence of PSVD in adult CF patients, probably because the improved survival has revealed the emergence of different forms of manifestations that can contribute to the burden of liver disease in CF patients.

One of the main messages of our study is that LSM and SSM by transient elastography should play a central role in the assessment of liver complications in patients with CF. First, LSM showed superior accuracy in diagnosing the clinical diagnosis of CFLD, compared with serum-based NITs. Second, the inclusion of an LSM-based criterion (the so-called 'expanded') in the diagnosis of CFLD allowed identifying 7 additional patients with fibrotic liver disease, among whom 1 had CF-ACLD. Therefore, we support using the 'expanded' criteria for CFLD diagnosis.[24] Indeed, LSM leads to diagnosing liver disease in patients with fluctuating or normal liver enzymes but who still have an underlying chronic liver injury and fibrosis. More importantly, LSM was extremely helpful in evaluating patients with

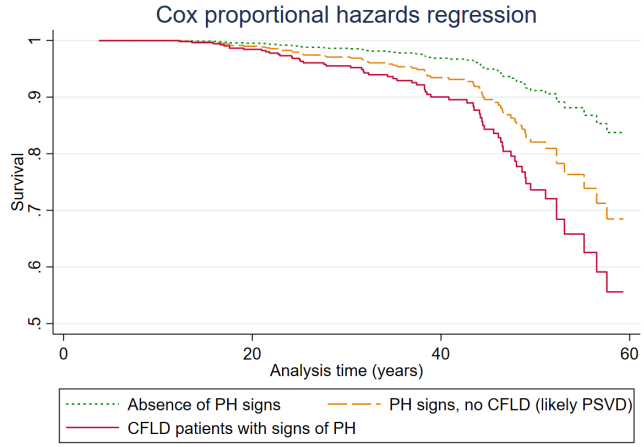

FIGURE 3 Survival in patients with likely porto-sinusoidal vascular disease and cystic fibrosis-associated liver disease with portal portal hypertension in a historical cohort.

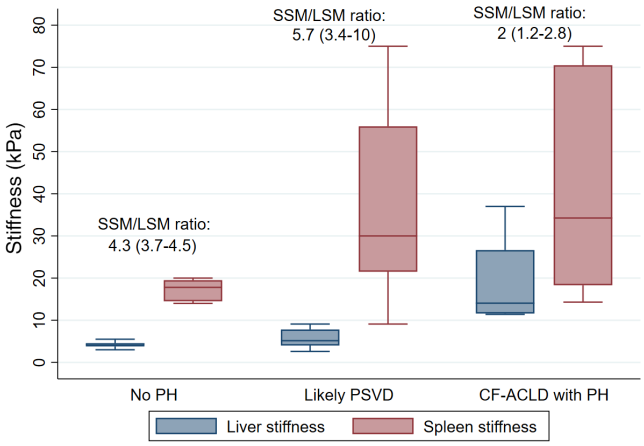

FIGURE 4 Liver stiffness measurement and spleen stiffness (SSM) measurement values in patients without portal hypertension, likely porto-sinusoidal vascular disease and CF-ACLD.

PH, as it could exclude advanced fibrosis/cirrhosis (i.e. CF-ACLD) and therefore identify patients at high risk for PSVD presence that should undergo liver biopsy to confirm this diagnosis. These findings align with a recent study by Elkrief et al.,[20] showing that LSM <10 kPa strongly suggested PSVD in patients with portal hypertension, with a specificity of 97% and a positive predictive value of 85%.

Finally, we described for the first time that SSM could play a role in diagnosing portal hypertension in CF patients and eventually suspect PSVD. SSM cut-offs of 21 and 50 kPa could accurately rule-out and rule-in, respectively, specific signs of portal hypertension such as collaterals at ultrasound or varices and endoscopy. Moreover, we trust that a higher SSM/LSM ratio could be a sensitive tool to distinguish between PSVD and CF-ACLD or even raise suspicion of PSVD in patients with apparently no liver disease.

From a clinical point of view, the application of our algorithm (Figure 3) and the differentiation between likely PSVD and CF-ACLD can have substantial implications for the management, treatment and prognosis of CF patients. In contrast to cirrhotic patients, PSVD patients usually have (i) a preserved liver function and (ii) an evident lower risk of developing hepatocellular carcinoma (described only in case reports).[38] Therefore, we should probably seek to ameliorate portal hypertension eventually in these patients by placing a transjugular intrahepatic portosystemic shunt (TIPS) when necessary, rather than referring to liver transplantation, which should be reserved for cases of hepatic failure or uncontrolled clinical manifestation despite the TIPS.

In our algorithm, PSVD should be highly suspected if portal hypertension is present in the absence of 'classical' or 'expanded' CFLD or in patients with portal hypertension meeting CFLD criteria but with low LSM values. Therefore, liver biopsy with eventual HVPG measurement should be performed to confirm its diagnosis in these patients. Although liver biopsy has not been actually recommended for the diagnosis of CFLD due to the risk of under/over-estimation of the biliary fibrosis degree (heterogeneous distribution), its role should be re-evaluated in the light of recent findings[11] to diagnose PSVD according to recent and well-accepted criteria.[9] Future studies should evaluate whether the severity and the natural history are different in patients with PSVD alone or mixed with CFLD.

The main limitation of our study is the lack of liver histology as the reference standard for diagnosing PSVD and the lack of HVPG measurement, endoscopy and cross-sectional imaging to diagnose portal hypertension (many of the signs used are not specific for clinically significant portal hypertension). Of note, the Baveno VII-recommend cut-off (10 kPa)[15] used to distinguish between ACLD and likely PSVD has only a 65% specificity for this purpose, albeit the PPV being >95%. However, (i) liver biopsy was not recommended by guidelines and is not actually routinely performed in CF patients; (ii) LSM has been proven to assess the presence of fibrosis reliably in many contexts[15,16]; (iii) HVPG measurements are often unreliable in PSVD due to the presinusoidal component of vascular resistance; (iv) we used a historical cohort to validate the definition of PH used against hard clinical outcomes, such as mortality. Finally, the study's prospective cross-sectional nature might have introduced a selection bias, even though the prevalence of CFLD in our cohort is in line with other extensive and contemporary studies.[24,30]

In conclusion, PH in the absence of advanced chronic liver disease is common in CF patients, therefore the PSVD diagnosis and liver biopsy should be strongly considered in these patients. We have proposed a new algorithm based on liver ultrasound, CFLD criteria and liver and spleen elastography to accurately classify the heterogeneous manifestations of liver involvement in patients with CF. Further studies are needed to validate our proposed algorithm in a larger, multicentre and prospective cohort of CF patients.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available upon reasonable request from the authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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