Early Detection of Hepatobiliary Involvement in Cystic Fibrosis: Biomarkers, Elastography and Genetic Influences

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August 17, 2023

Abstract

Cystic fibrosis-related hepatobiliary involvement (CFHBI) is a term used to describe a spectrum of hepatobiliary involvement ranging from benign elevation of transaminase levels to advanced cystic fibrosis associated liver disease (aCFLD). While CFHBI is common among people with cystic fibrosis (PwCF), aCFLD is rare impacting only approximately 5-10% of the CF population. Importantly though, aCFLD is the third leading cause of death among PwCF, is an independent predictor of all-cause mortality and is associated with significant morbidity. Despite this recognition, our ability to predict those patients at greatest risk for aCFLD, identify early aCFLD and monitor incremental progression of CFHBI is lacking. Here we review the strengths and weaknesses of the common biomarkers and imaging modalities used in the evaluation and monitoring of CFHBI, as well as the current understanding of genetic modifiers related to aCFLD.

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Senthilkumar Sankararaman has no conflicts to disclose

A. Jay Freeman has received grant funding from National Institute of Health and the Cystic Fibrosis Foundation. Has done advisory work Abbvie and Takeda.

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Abstract:

Cystic fibrosis-related hepatobiliary involvement (CFHBI) is a term used to describe a spectrum of hepatobiliary involvement ranging from benign elevation of transaminase levels to advanced cystic fibrosis associated liver disease (aCFLD). While CFHBI is common among people with cystic fibrosis (PwCF), aCFLD is rare impacting only approximately 5-10% of the CF population. Importantly though, aCFLD is the third leading cause of death among PwCF, is an independent predictor of all-cause mortality and is associated with significant morbidity. Despite this recognition, our ability to predict those patients at greatest risk for aCFLD, identify early aCFLD and monitor incremental progression of CFHBI is lacking. Here we review the strengths and weaknesses of the common biomarkers and imaging modalities used in the evaluation and monitoring of CFHBI, as well as the current understanding of genetic modifiers related to aCFLD.

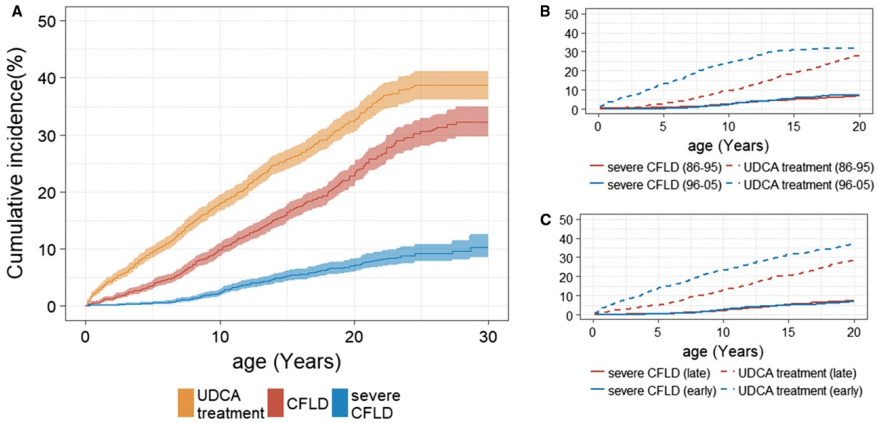
Keyworlds – CF, cystic fibrosis, CFTR, liver disease, portal hypertension

Introduction:

Cystic fibrosis-related hepatobiliary involvement (CFHBI) is common in people with cystic fibrosis (PwCF) with a varying severity. A wide spectrum of hepatic manifestations are noted in CF including but not limited to steatosis, intermittent or persistent elevated liver enzymes, abnormalities noted on radiological imaging and end-stage liver disease. The pathophysiology of CFHBI is poorly understood but likely multifactorial including non-specific and related extrahepatic causes such as malnutrition, drug-related toxicities, viral/bacterial infections, and hepatic congestion, but also could be due to the effects of CFTR dysfunction in the biliary epithelia [1] or vascular abnormalities including obliterative portal venopathy or non-cirrhotic portal hypertension [2,3]. The clinically significant pathognomonic finding of focal biliary fibrosis is noted in approximately 30% of PwCF with a varying degree of severity[4-9]. In autopsy studies, focal biliary fibrosis has been documented up to 70% of PwCF [10]. However, only about 5-10% of PwCF progress to the severe form of fibrosis namely the multilobular cirrhosis usually by the end of the first decade of life[4-9]. Along with multilobular cirrhosis (with or without portal hypertension), the other phenotypes of advanced CF-related liver disease (aCFLD) include one (or more) of the following: nodular liver, advanced hepatic fibrosis (F4 of the METAVIR staging) and non-cirrhotic portal hypertension (obliterative portal venopathy).

The prevalence of clinically defined CFHBI varies but is generally felt to occur in 30-40% of PwCF in most studies depending on the definition utilized [7,11,12]. To minimize confusion, experts proposed an unified approach for the classification of CFHBI[11,13]. According to the Cystic Fibrosis Foundation 2021 Annual Registry, aCFLD accounts for the third leading cause of mortality in PwCF accounting for 2.6% of the deaths after respiratory and transplant-related complications [14]. Even though this is relatively small portion of the population, aCFLD may adversely impact lung function and nutritional status [7,15], and is strongly associated with CF related diabetes (CFRD)[16], all of which may further impact morbidity and mortality. This is supported by a review of the French CF Registry finding that liver involvement at baseline was an independent risk factor for lung transplant or death [17] and a review of the United Kingdom CF registry concluding that aCFLD also has significantly increased all-cause mortality with a hazard ratio (HR) of 1.54 [18].

Historically, CFHBI was viewed as a complication impacting PwCF almost exclusively prior to 20 years of age [5]. However, as survival for PwCF improves, liver involvement among adults with CF is increasingly recognized. A report by the National Institutes of Health (NIH) proposed a modified criteria to evaluate CFHBI among adults that revealed 47% of their population had some degree of hepatic involvement and that CHHBI was more common among adults than previously reported[19]. Importantly though, aCFLD remains a rare finding among adults with single-center studies concluding that adult CFHBI is a largely a benign condition [20-23] and well-illustrated by the French CF Modifier Gene Study [7] (see**Figure 1**)



The primary challenge facing the CF clinician is early identification of those patients that will progress to aCFLD compared to those with more benign forms of CFHBI. Initial strategies focused on early screening for hepatic fibrosis. Liver fibrosis is often utilized as the surrogate endpoint for clinical trials and considered an early marker of liver disease progression, yet this strategy has been largely underwhelming to date likely due to a number of a factors. Although histological assessment is considered the gold standard method in the evaluation of liver fibrosis, it has a number of limitations in evaluating CFHBI. It is both invasive and expensive which precludes its use as a screening test, and requires general anesthesia/sedation in the pediatric population. Despite double-pass liver sampling, liver biopsy is still prone to sampling errors (both underestimation or overestimation are possible based on the site of sampling) as CFHBI is felt to have a patchy distribution [24,25]. Liver sampling may not predict the exact severity of the disease process based on the area sampled [4,26]. Also, the pathologists' histopathological assessment is prone to intra- and interobserver variabilities, which may be further complicated by unusual findings such as non-obliterative portal venopathy or non-cirrhotic portal hypertension.

An ideal screening test for evaluating CFHBI should be noninvasive, broadly available, reliable, reproducible, and cost-effective for both evaluating and follow-up of CFHBI. Specifically for CF, screening modalities should be able to capture various forms of hepatic involvement. The currently available screening tests can be broadly classified as laboratory biomarkers (primarily from blood) and radiological methods utilizing greyscale ultrasound and/or elastography technologies. Often these tests are utilized together for diagnostic precision of liver fibrosis [13,27,28]. Unfortunately, current screening methods are best at identifying those without aCFLD, or those unlikely to advance to aCFLD. While more work is required to improve early detection of aCFLD, here we review the widely available screening methods currently employed.

BioMarkers:

Indirect laboratory biomarkers for screening hepatobiliary involvement in PwCF:

Conventional markers of liver disease, such as aspartate transferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) along with platelet levels are obtained annually for PwCF. These commonly employed indirect blood tests used independently or in combination (AST to platelet ratio index (APRI), fibrosis index (FIB-4) based on four factors (age, AST, ALT, and platelets), GGT to platelet ratio

(GPR), and AST/ALT ratio) have been widely studied.[29-34]. These tests have been extensively utilized and validated in other chronic liver diseases such as viral hepatitis (hepatitis B and hepatitis C), and nonalcoholic fatty liver disease in both children and adults [28,35,36]. The tests are based on the general principle that AST remains stable or increases, ALT typically increases with worsening fibrosis, and platelet levels decrease with the development of portal hypertension, with or without cirrhosis [37,38].

A number of studies have reported statistically different values among these conventional markers of liver disease among PwCF with aCFLD compared to those without liver disease [39-44]. Among these, GPR ([?]0.28), APRI ([?]0.425) have the diagnostic precision with the highest AURUC's, generally between 0.75 to 0.9[29,31,32]. Importantly though, these tests have shown differences between disease classifications (e.g. normal and advanced liver disease) but their utility in detecting early disease, identifying small, incremental disease progression, or predicting the development of aCFLD are not clear.

Even though these tests are simple, noninvasive, and cost-effective, they have a number of major drawbacks. Non-specific elevation of liver enzymes in PwCF is not uncommon due to reasons such as intercurrent infections and medications (CFTR modulator therapies, and antibiotics)[45,46]. In a prospective CF study, by age 21 years, at least one increased liver enzyme (ALT, AST, and GGT) was noted in all participants, and most elevations were noted in the early years of life [4,45]. Therefore, persistently elevated liver enzymes are generally more valuable in the predicting liver disease [4,45]. Also, in people with aCFLD, liver enzymes can be near normal with advanced stages of fibrosis[5]. Standalone, they demonstrate poor sensitivity and specificity but can be effectively incorporated into the algorithmic approach of liver involvement evaluation [27]. Another challenge in utilizing these tests is the wide range of values that extend between disease category and determining the definition of the upper limit of normal [32]. Additionally, the lack of adjustment for confounding variables such as age, sex, and modulator status may further complicate the interpretation of these markers among PwCF. All non-invasive tests utilized in CFHBI are summarized in

Table 1.

Direct serum biomarkers of fibrosis:

In hepatic fibrosis, there is excessive extracellular matrix deposition which occurs due to a homeostatic imbalance between matrix deposition and removal [47]. Several biomarkers involved either in fibrogenesis or fibrolysis have been utilized in screening fibrosis. Tissue inhibitors of metalloproteinase (TIMPs), collagen-IV, matrix metalloproteinase (MMP)-2, hyaluronic acid, and prolyl hydroxylase have been explored in CFHBI [48-50]. MicroRNAs (miRNAs) are short RNA molecules that modulate gene expression at the post-transcriptional level which are increased in chronic liver diseases and have also been investigated in CFHBI [24,51,52]. However, these tests are expensive, not widely available for clinical settings, results can also be confounded by other fibrotic processes seen CF [53-55], and have not resulted in improved diagnostic accuracy compared to conventional markers outlined above[56,57].

Biomarker conclusions:

Taken together, direct and indirect serum biomarkers have yet to show the ability to predict the progression to aCFLD. Normal liver indices are very sensitive (i.e. exclude aCFLD) but specificity and predictability remain poor. Clinically, intraindividual changes over time are likely more significant and should be followed closely rather adhering to strict cut-offs for any individual marker.

Radiological methods:

Ultrasound in evaluation of CFHBI:

Ultrasound (US) is a relatively inexpensive and widely available tool to assess the liver. The initial role of US was established in a single-center study involving a cohort of 106 children with CF followed for approximately 10 years. In this study, a heterogeneous echogenic pattern on liver US was utilized to evaluate children at risk for aCFLD (defined as a nodular liver in US) with or without portal hypertension[58]. The authors demonstrated that children with CF with a heterogeneous echogenic appearance had a 5.2-fold increased

incidence of a nodular appearance and a 6.1-fold increased incidence of portal hypertension compared with participants with a normal echogenic pattern [58]. These results were confirmed by the Prospective Study of Ultrasound to Predict Hepatic Cirrhosis in CF (PUSH study) which showed the heterogenous pattern had 9.5 times increased incidence of the nodular pattern when compared to participants with normal liver appearance over a six-year period [59].

In addition to showing an association between a heterogenous US pattern and the development of a nodular pattern on US, the PUSH study also demonstrated significant differences between US groups (normal, heterogenous, homogenous and nodular) for spleen size, and non-invasive hepatic markers depicting the ability of a research ultrasound in the evaluation of CFHBI and aCFLD [44]. FIB-4, GPR, and APRI were significantly increased, and platelets decreased in participants with a heterogeneous pattern when compared with PwCF with a normal liver appearance [60]. In another study with longitudinal follow up, the same group of PUSH study investigators noted that in approximately 6.3 years of average follow-up, six participants with nodular liver appearance developed esophageal varices, two had bleeding related to varices, and two had liver transplants [61]. The control population had no liver-related adverse events highlighting the feasibility of research US in discriminating the progression of aCFLD from the individuals with normal liver appearance.

While the data regarding US in CF is encouraging, there remain significant clinical concerns. US lacks the ability to detect early stages of aCFLD reliably and a normal US does not preclude significant liver fibrosis [12]. Another major limitation is a high percentage of interpretation variabilities (both intra- and interobserver) between sonologists specifically in clinical settings[12], an important point to note given the PUSH study was conducted by trained and experienced radiologist within a research protocol. US is likely best utilized clinically to compliment serum biomarkers. This is highlighted by the proposed algorithm by Sellars et al integrating conventional liver indices to guide when to perform a liver ultrasound (US). They utilized GGT as an initial screening test and further utilized APRI and GPR to determine when an abdominal ultrasound is needed [27]. This is supported by the final PUSH multivariate analysis showing that the combination of initial US pattern, age and GPR produced the best AUC in predicting the development of a Nodular US [60].

Elastography in evaluating CFHBI:

With progressive fibrosis, the stiffness of the liver increases with decreasing elasticity which can be utilized for screening using several elastography techniques [38]. A high cutoff for LSM increases the specificity of diagnosis (ruling in) advanced liver fibrosis and conversely a low cut-off provides a relatively higher sensitivity for the evaluation of early stages of (minimal or no) fibrosis. Common radiological methods include vibration-controlled transient elastography (TE), ultrasound-guided elastography such as point shear wave elastography (SWE) or 2D-shear wave elastography, and magnetic resonance elastography (MRE) have been studied.

TE (FibroScan®) has been utilized in the early evaluation and monitoring of liver fibrosis in both children and adults with CF[39-42] Lewindon et al demonstrated that liver stiffness measured by TE was significantly higher in children with CFHBI (10.7 kPa, SD 2.4) when compared with children without CFHBI (4.6, SD 0.1) and healthy control population (4.1 kPa, SD 0.1)[41]. Using 5.5 kPa as a screening cut-off for CFHBI, the liver stiffness measurement (LSM) identified children with CFHBI with an AUROC of 0.82, 70% sensitivity and 82% specificity ($P < 0.0001$). A higher cutoff of 8.7 kPa improved the specificity of the diagnosis and differentiated children with aCFLD (diagnosis of aCFLD based on ultrasound findings or by liver biopsy, stages F3-F4) vs patients with early fibrosis (stages F1-F2), with an AUROC, 0.87; 75% sensitivity, 100% specificity [41]. Also, the combined utility of APRI and LSM identified children with CFHBI with higher diagnostic accuracy (AUROC of 0.89, 87% sensitivity, and 74% specificity). Also, the combination of APRI and LSM improved the differentiation of patients with severe fibrosis vs mild fibrosis [41]. Lam and colleagues did a meta-analysis by pooling results from six studies (605 participants) and demonstrated an ideal cut-off for LSM by TE and APRI as [?]5.95 kPa and [?]0.329 respectively [62] The sensitivity, specificity, and AUROC were 55%, 87%, 0.76 and 52%, 93%, and 0.84 for LSM and APRI respectively. When both parameters were combined, the sensitivity, specificity, PPV, and NPV were 43%, 99%, 92%, and 87% with a diagnostic odds ratio of 75.

Although encouraging, LSM by TE suffers from poor intra- and interindividual variation. The poor repeatability of TE was highlighted by Rowland and colleagues in healthy children (normal range of LSM was as 2.88–6.52 kPa) across various age categories [63]. A difference of >1kPa between paired measurements was noted in approximately 26% of the participants [63]. Therefore well agreed standard cut-off values for TE in the evaluation of CFHBI are urgent needed.

Calvopina and colleagues demonstrated that LSM by supersonic SWE showed good diagnostic accuracy in children with CFHBI [64]. LSM was significantly higher in CFHBI (8.1 kPa, IQR = 6.7–11.9) versus without liver involvement (6.2 kPa, IQR = 5.6–7.0; $P < 0.0001$) and control population (5.3 kPa, IQR=4.9–5.8; $P < 0.0001$) [64]. The diagnostic accuracy improved when supersonic SWE was combined with APRI.

In a single-center study, both TE using FibroScan$^{(r)}$ and point SWE (Virtual Touch^{TM} Quantification) were compared in children with CF for their repeatability and reproducibility[34]. The performance of both techniques was good and investigators concluded that either one of the two methods can be utilized for evaluating CFHBI [34]. However, the intraclass correlation was superior for TE than SWE for both intra- and interobserver agreements [28]. Unlike TE, the LSM measurements between studies may not be comparable in difference studies involving SWE as the results may vary based on the technology utilized (point SWE vs 2D-SWE, probe and frequency (6Hz vs 9 Hz) differences, and availability of different commercial brands [65].

MRE is the most accurate elastographic technique but its availability is limited to research settings in tertiary care centers. MRE is expensive and also poses technical challenges in young children, people with severe obesity, and advanced lung disease [66]. In a single-center prospective study involving 55 percipients, all three (TE, SWE, and MRE) elastography modalities were compared[67]. PwCF aged 6-18 years were included and based on US findings, participants were grouped into three groups (aCFLD, heterogenous increased echogenicity, and normal/homogeneous echogenicity). All tests were done on the same day. LSM on all elastography methods was significantly higher compared to the other two groups. TE and SWE were highly correlated (r=0.9) and concordant in identifying aCFLD (Cohen's k=0.84) while MRE was moderately correlated and concordant with TE (r=0.41; k=36) and SWE (r=0.5; k=0.50)[67]. Even though these elastography techniques have shown good reliability in the evaluation of early diagnosis of significant liver fibrosis, their effect on longitudinal monitoring of disease progression is lacking, and further studies are awaited[1].

Spleen elastography:

Increased spleen stiffness measured by elastography methods has been utilized in both children and adults with advanced liver disease and may help to predict the severity of portal hypertension[68,69]. In a single-center study involving children, higher swear wave velocity was noted in CF than in control population.[70]. But no differences in swear wave velocity were noted in CF children with or without liver involvement and further studies are needed to explore its utilization in the early stages of aCFLD [70].

Radiological methods conclusions:

Greyscale US is often clinically utilized in evaluation of aCFLD due to wide availability and relatively low cost. However, it lacks the sensitivity to detect early stages of aCFLD reliably and also has high percentage of interpretation variabilities (both intra- and interobserver) among radiologists. US-based elastographies (TE and SWE) are relatively readily available, technically easier to perform, and cost effective. LSM obtained by various elastography techniques (TE, SWE, and MRE) are modality-dependent, and cannot be compared directly to one another. Also, these radiological tests are best utilized to complement the serum biomarkers in the evaluation of CFHBI. Finally, spleen elastography may be advantageous to evaluate portal hypertension, with or without cirrhosis, but its availability is limited and additional CF-specific studies are needed.

Genetics in the evaluation of CFHBI :

While CFHBI has been associated with pancreatic insufficiency, history of meconium ileus and CFRD, no clear genotype association has been established [7]. While some studies have shown F508del homozygosity was associated with aCFLD, it is unclear if that reflects the frequency of the mutation rather a true risk

factor. It is generally believed that severe mutations (class 1-3) are associated with aCFLD[71], but given the relatively low incidence rate among patients with two severe mutations, it has long been believed that additional genetic modifiers contribute to aCFLD.

To date, only the SERPINA1 Z allele has been associated with the development of aCFLD [72,73], but only accounts for a small portion of those with aCFLD. However, SERPINA1 has since been shown to be an independent risk factor for advanced liver disease in a number of chronic liver conditions [74], and thus unlikely to be a CF-specific modifier. In silico modeling has also supported the idea that additional genetic modifiers beyond SERPINA1 Z may contribute to the development of aCFLD [75]Currently, both the PUSH study team and researchers for the CF Foundation are conducting studies to better understand the role of genetic modifiers in CFLD (Clinical Trials NCT01144507 and NCT00804583).

Genetic modifiers conclusion:

To date, only the SERPINA1 Z allele has been associated with aCFLD but only accounts for a small portion of the population, and is unlikely to be CF-specific. Additional work is underway to better understand the potential genetic modifiers associated with CFHBI and aCFLD.

Conclusions:

Many questions remain as to how best to predict the onset of aCFLD. It is unlikely a single test will reliably detect or diagnose CFHBI, but rather a combination of tests with an algorithmic approach (e.g. blood tests + radiological studies) relying on changes over time rather than strict cut-offs. Elastography methods are currently gaining rapid momentum and soon will likely be incorporated in routine clinical settings. Future methods also include the incorporation of newer biomarkers and other upcoming modalities such as multi-omics, and artificial intelligence (AI)-driven protocols[38,70,76].

Table 1 – Summary of non-invasive tests utilized in cystic fibrosis-related hepatobiliary involvement

Tests
Blood tests – AST, ALT, GGT, AST to platelet ratio index (APRI), fibrosis index-4 (FIB-4), GGT to platelet ratio, and A
Direct biomarkers
US
TE
SWE
MRE
Liver biopsy

aCFLD - advanced cystic fibrosis associated liver disease; US – ultrasound; TE - transient elastography; SWE - shear wave elastography; MRE - magnetic resonance elastography

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