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Comparison of ultrasound and biopsy findings in children with cystic fibrosis related liver disease

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Abstract

Background: The objective of our study was to determine if hepatic ultrasound findings in paediatric patients with cystic fibrosis and suspected liver disease are related to histopathological results derived from liver biopsies.
Methods: A retrospective analysis of ultrasound and liver biopsy findings using published criteria was performed in 30 children with cystic fibrosis suspected as having liver disease on clinical, biochemical and ultrasonographic criteria. The results were correlated and assessed for intra- and interobserver agreement.
Results: A significant association was found for the prediction of fibrosis or cirrhosis on the basis of ultrasound ($p=0.03$). There was no significant association between normal or indeterminate ultrasound and histology results. A high intra- and interobserver variability was found in sonographic assessment of the hepatic echostructure.
Conclusions: The diagnosis of early liver disease in cystic fibrosis cannot reliably be made on the basis of ultrasound alone. A normal ultrasound does not preclude significant liver fibrosis in cystic fibrosis. An abnormal US that suggests cirrhosis predicts the presence of moderate to severe liver disease.

Keywords: Cystic fibrosis; Ultrasound; Liver disease; Histology

1. Introduction

The survival of patients with cystic fibrosis (CF) has improved considerably over the past two decades as a result of improved therapy. This has led to increased clinical relevance of diseases in non respiratory systems [1].

Liver disease (LD) is a significant cause of mortality in the CF population and was responsible for 5% of deaths before the advent of liver transplantation [2]. Cirrhosis of the liver occurs in up to 10% of the CF population and severe liver disease typically occurs during the second decade of life [2–4]. Because of the lack of specific and reliable non-invasive tests the prevalence of liver disease remains unknown with published reports ranging up to a maximum of 72% at autopsy [5].

The exact pathophysiology of LD in CF is not yet fully understood. The gene defect in CF encodes for the CF transmembrane conductance regulator (CFTR). In the liver CFTR is expressed only on the apical surface of the cells of the biliary epithelium. The defect leads to malhydration of bile with presumed obstruction of the intrahepatic biliary tree. Hepatic stellate cells, the principal drivers of hepatic fibrosis are activated and produce increased collagen with bile duct epithelium demonstrated as the principal source of the profibrogenic cytokine TGF-β [6]. This results in focal biliary fibrosis characterized by inflammatory infiltrates, bile duct proliferation and fibrosis [7–9]. Over several years this focal fibrosis may progress to diffuse cirrhosis with regenerative nodules and bridging fibrosis [8]. Hypotheses for the focal

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nature of the disease include inhomogeneous bile drainage throughout the liver and partial compensation for the defect by other mechanisms [8].

Despite the absence of agreed criteria for liver disease in CF early diagnosis is recommended [3], which permits opportunity for early intervention with ursodeoxycholic acid. It is thought that ursodeoxycholic acid may improve the natural history of CF related liver disease [10,11]. In the absence of reliable non-invasive parameters, diagnosis of CFLD is currently made on the basis of a combination of clinical, biochemical and US findings with needle biopsy used in equivocal cases.

Ultrasound (US) is a widely employed modality for the evaluation of liver diseases as it is readily available, non-invasive and does not require sedation in younger children. It is commonly used for the assessment of children with CF suspected as having liver disease [2,12–15]. Computed tomography has been used in correlation with US [16], but the use of ionizing radiation is a major disadvantage particularly with repeated screening in children and adolescents. MRI may be a useful tool for assessment of LD although routine use of MRI with CF has not yet been established [17]. In previous studies US findings in patients with CF were correlated with clinical and biochemical findings [13–15]. Limited data however is available comparing sonographic findings with the histology results of liver biopsy in patients with CF [2,10,14].

We wished to compare the findings on liver US with the biopsy results in children with CF who fulfilled published criteria for having liver disease [18] and to determine whether specific US findings can be identified indicating CF related liver disease (CFLD).

2. Material and methods

A retrospective analysis of ultrasound findings was performed in 30 CF-patients (13 girls, 17 boys) who underwent a liver biopsy and ultrasound between April 1997 and September 2003. The CF-patients undergoing liver biopsy were identified from the cystic fibrosis clinic database. Ethical approval of this study was granted by the institutional ethics committee as part of a wide study into liver fibrosis in cystic fibrosis.

Patients' age ranged from 11 months to 17 years, mean age 10 years. The time interval between biopsy and ultrasound was between 0 and 183 days (mean 42 days).

All patients were attending the CF clinic with positive sweat tests confirming CF. Patients underwent liver biopsy if two out of three of the following criteria were fulfilled: 1. evidence of biochemical liver disease (persistent elevation of ALT above upper limit of normal over a period of at least 6 months), 2. clinical hepatomegaly or hepatosplenomegaly, 3. sonographic evidence of liver disease. Informed consent was obtained from the parents for the biopsy.

Percutaneous liver biopsy was performed using ultrasound guidance. The ultrasound was used for biopsy guidance only and a detailed ultrasound assessment of the liver was not performed at the time of the biopsy. Two samples, to limit sampling error, were obtained from the right lobe using a triggered trucut to obtain 20 mm cores. No post biopsy complications were observed. In all specimens, at least 6 portal tracts were available for analysis and a Scheuer grading for fibrosis was allocated to each patient by a histopathologist blinded to US findings.

All sonograms were performed with one of the commercially available real-time scanners: Acuson Sequoia (Siemens Ltd. Medical, Erlangen, Germany) with 2.5–4 or 5.5–8.5 MHz-probes or ATL HDI 5000 (Philips Medical Systems, Best, The Netherlands) with 2–5 or 5–7 MHz-probes. US scans were obtained after a 4-hour fast in children under 2 years and a 6-hour fast in children over 2 years for gallbladder distension.

Sonographic images were independently reviewed two times on hardcopies by a pediatric radiology fellow (investigator 1) and an experienced paediatric radiologist (investigator 2). The reviewers were unaware of the clinical findings and previous interpretation and were blinded to the histology. After independent review a consensus result was reached in cases with differing interpretations for each of the ultrasound criteria evaluated.

US and Doppler parameters that have been reported to be associated with the presence of liver disease in CF were used [12,16,19]. The whole liver was evaluated for echotexture and contour. The echogenicity of the liver was compared with that of the kidney (none of the patients had evidence of renal disease). Hepatic echogenicity was considered abnormal if it was markedly increased compared to the renal cortex and similar in echogenicity to the renal sinus. Hepatic parenchyma was assessed as inhomogeneous if variable echogenicity and loss of definition of portal tracts was noted. Presence or absence of nodularity at the hepatic contour was assessed. Periportal echogenicity was called abnormal if its maximum diameter extended over 2 mm [16]. Attenuation was assessed as abnormal if a clear image of the right liver and kidney was obtained, but blurring of deep structures was noted with imaging of the liver alone. The diameter of the portal vein was regarded as normal within these limits: $8.5+/-2.7$ mm <10 years, $10+/-2$ mm >10 years [20]. Normal portal vein mean flow velocity was assessed as greater than 15 cm/s [21].

The gallbladder was assessed as abnormal in size if the length was below 2 cm after fasting [22]. A wall thickness greater than 3 mm was considered abnormal [23]. Presence or absence of gallstones was noted. The diameter of the common bile duct was assessed and considered as normal if smaller than 4 mm [23]. Spleen size was regarded normal if the spleen did not extend over the inferior margin of the left kidney [24]. Measurements of the splenic length were performed and correlated with normal values from the literature [25]. Presence or absence of varices and ascites was noted.

Table 1
Histologic grading after Scheuer [26]

Grade	Fibrosis
0	None
1	Enlarged, fibrotic portal tracts
2	Periportal or portal–portal septa but intact architecture
3	Fibrosis with architectural distortion but no obvious cirrhosis
4	Probable or definite cirrhosis

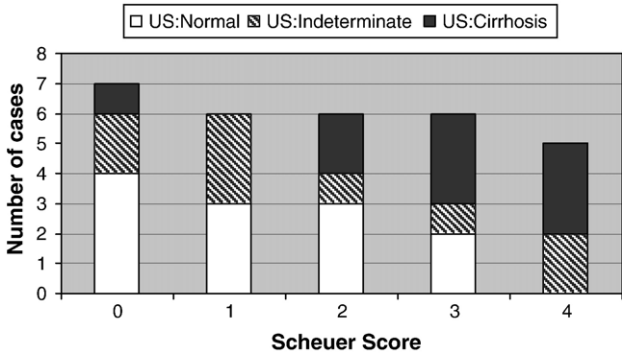

Fig. 1. Summary of the ultrasound and histology results.

A summary interpretation of the findings was performed by each reviewer. There were three categories: normal, indeterminate (suggestion of liver disease but no definite signs of cirrhosis) and cirrhosis.

Cases without liver abnormality were graded as normal. Increased hepatic echogenicity, heterogeneity and/or increased attenuation in the absence of nodularity of the liver surface were classified as indeterminate. Splenomegaly as an isolated finding was also regarded as indeterminate. All patients with nodularity of the liver surface were classified as cirrhosis.

2.1. Histological scoring system

The scoring system of Scheuer was used for histological grading [26] (see Table 1).

Scheuer-Score of 0 was regarded as normal, a score of 1–2 as mild to moderate reversible periportal changes and 3–4 was assessed as definite fibrosis/cirrhosis.

2.2. Statistical analysis

The association between US findings and histology results was evaluated using the chi-square test, or Fisher's exact test if cell frequencies were <5. Sensitivity and specificity of US for the diagnosis of 'normal' liver and for the diagnosis of 'fibrosis/cirrhosis' were calculated as the proportion of those with the condition of interest who were classified as positive on US, and the proportion of those without the condition of interest who were classified as negative on US, respectively, and reported with 95% confidence intervals. The Mantel–Haenzel chi-square test for trend was used to evaluate the association between frequency of diagnosis of cirrhosis on US and increasing Scheuer score. Ninety-five percent confidence intervals are given for test characteristics where appropriate. Intra- and interobserver agreement was estimated on the above criteria and US summary interpretation using the kappa statistics, where a

Table 2
Patients' details with clinical grading, individual Scheuer Scores, summary US interpretation and selected US finding

	Sex	Age (years)	ALT (U/l)	PHT	Scheuer	US	Echo	Hetero	Nodular	GB abn	Spleen
1	F	5	110	No	0	N				Yes	
2	M	6	58	No	0	N					
3	M	2	60	No	0	N				Yes	
4	F	8	44	No	0	N				Yes	
5	M	17	82	No	0	I	Yes				
6	F	15	35	No	0	I	Yes			Yes	
7	M	10	35	No	0	C		Yes	Yes	Yes	Yes
8	M	9	35	No	1	N					
9	F	2	41	No	1	N				Yes	
10	M	6	50	No	1	N					
11	F	16	64	No	1	I	Yes				
12	F	14	50	No	1	I	Yes	Yes			
13	F	11	65	No	1	I		Yes			
14	M	11	41	No	2	N					
15	F	10	17	No	2	N					
16	F	12	40	No	2	N					
17	M	15	35	No	2	I	Yes	Yes		Yes	
18	M	15	146	Yes	2	C		Yes	Yes		Yes
19	M	12	50	Yes	2	C		Yes	Yes		Yes
20	F	11	15	No	3	N					
21	M	10	84	Yes	3	N					
22	M	8	50	Yes	3	I	Yes	Yes			
23	F	5	60	Yes	3	C	Yes	Yes	Yes	Yes	Yes
24	F	14	132	Yes	3	C		Yes	Yes	Yes	Yes
25	M	3	35	No	3	C		Yes	Yes		
26	M	17	35	Yes	4	I					Yes
27	M	4	100	Yes	4	I	Yes				
28	F	15	86	Yes	4	C		Yes	Yes		Yes
29	M	10	36	Yes	4	C		Yes	Yes		Yes
30	F	11	20	Yes	4	C		Yes	Yes	Yes	Yes

ALT = Alanine aminotransferase. PHT = Clinical portal hypertension with splenomegaly or varices at endoscopy. Echo = Increased echogenicity. Hetero = Heterogeneity. Nodular = Nodularity. GB abn = Abnormality of gallbladder. Spleen = Splenomegaly. US findings: N = Normal, I = Indeterminate, C = Cirrhosis.

value of 0 indicates agreement attributable to chance alone and a value of 1 indicates perfect agreement. Data analysis was performed using SAS software (version 9.1, SAS Institute Inc, Cary, North Carolina).

3. Results

Out of our study group of 30 patients referred for evaluation of suspected liver disease, US was interpreted as normal in 12 cases. Of these 12 with normal US, four patients had normal histology six had mild to moderate fibrosis (Scheuer-Score 1 or 2) and two had advanced fibrosis (Scheuer Grade 3). A summary of the ultrasound and histology results is given in Fig. 1.

No significant association between normal US and histology classified as 'normal' (Scheuer-Score 0) was found ($\chi^2 = 1.12$, $p = 0.29$). The sensitivity of US to detect normal histology in our data was 0.57 (0.18–0.90) with a specificity of 0.65 (0.52–0.91). Corresponding positive predictive value was 0.33 (0.10–0.65) and negative predictive value 0.83 (0.58–0.96).

In nine patients US-results were indeterminate. Mild periportal changes on histology were noted in four of these patients and in two cases normal histology was found. In three patients, fibrosis or cirrhosis was found on histology, this included one patient with splenomegaly as the only pathologic finding at US who had cirrhosis on histology. No statistical significant relationship was found between indeterminate US and the histology results.

In nine patients US was interpreted as cirrhosis on the basis of nodularity of the liver surface. All nine patients demonstrated heterogeneity of the liver parenchyma and splenomegaly was found in all but one patient. Six of these were found to have advanced fibrosis on histology. Two had only moderate fibrosis (Scheuer Grade 2) and one patient had no fibrosis on biopsy. During follow up, both patients with only moderate fibrosis progressed to portal hypertension (PHT) with splenomegaly and both developed advanced fibrosis on repeat biopsy 2 years later. The patient with no fibrosis on biopsy demonstrated moderate fibrosis (Scheuer Grade 2) on biopsy 3 years later and a follow up ultrasound performed 4 months prior to the second biopsy (investigator 2) showed a heterogeneous nodular liver and splenomegaly in this patient.

There was a significant association between a summary finding of cirrhosis on the basis of US and liver histology ($p = 0.04$, Fisher's exact test). The sensitivity to detect fibrosis or cirrhosis with US was 0.55 (0.23–0.83) with a specificity of 0.84 (0.60–0.97). Corresponding positive predictive value was 0.67 (0.29–0.92) and negative predictive value 0.76 (0.52–0.92). There was also a significant association between cirrhosis

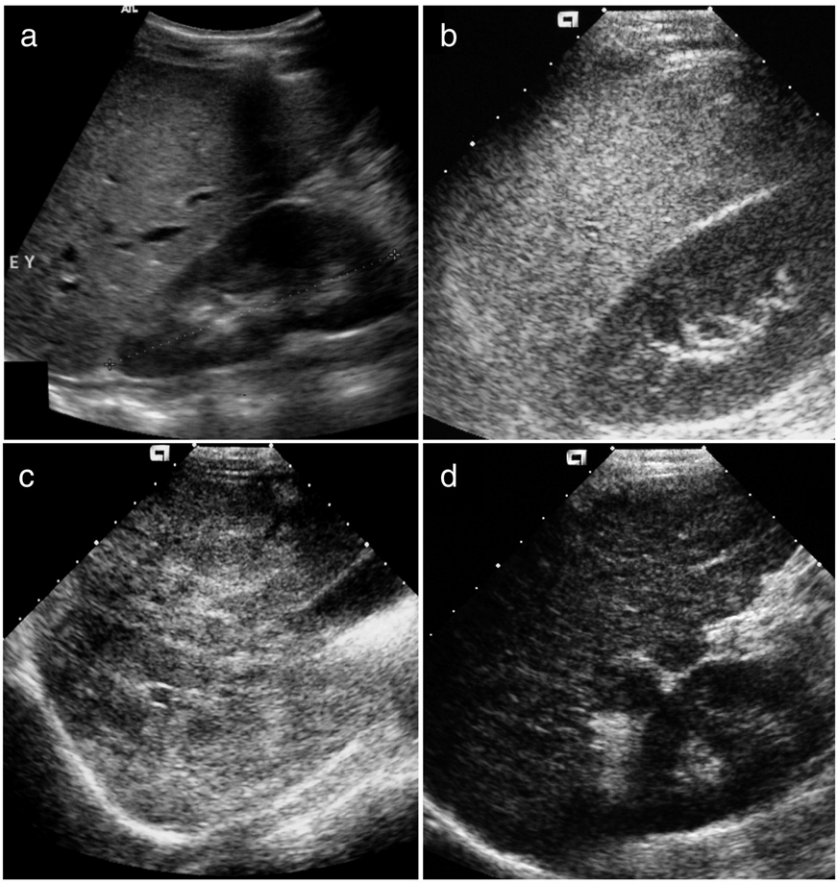

Fig. 2. Representative sonographic findings (findings on histology in parentheses): a. normal appearing liver (moderate periportal changes), b. echogenic, slightly inhomogeneous liver (mild periportal changes), c. distinct inhomogeneity of liver structure (fibrosis), d. nodular liver surface (cirrhosis).

Table 3
Intra- and interobserver agreement for selected US-criteria (kappa)

	Intraobserver agreement		Interobserver agreement	
	Investigator 1	Investigator 2	1st reading	2nd reading
Echogenicity	0.39	0.56	0.44	0.53
Inhomogeneity	0.45	0.63	0.45	0.45
Nodularity	0.76	0.76	0.62	0.65
Attenuation	0.79	0.72	0.55	0.50
Spleen size	0.60	0.94	0.69	0.95
Normal	0.70	0.79	0.56	0.51
Indeterminate	0.39	0.70	0.26	0.29
Cirrhosis	0.45	0.94	0.67	0.54

on biopsy and an ultrasound finding of hepatic nodularity ($p=0.04$, Fisher's exact test) and splenomegaly ($p=0.05$, Fisher's exact test).

A significant association was found between the US finding of a microgallbladder and non-fibrotic liver ($p=0.006$). In 24 patients where the gallbladder was visualised, all of the five patients with normal histology had a microgallbladder, whereas of the 19 patients with abnormal histology, only five had a microgallbladder and the remaining 14 had a 'normal' sized gall bladder.

Patient details including the clinical grading, individual Scheuer Scores, selected US findings and summary US interpretation are listed in Table 2.

Representative US findings are depicted in Fig. 2.

3.1. US findings: Inter-/intraobserver agreement

Highest kappa values for intraobserver agreement (0.76–0.94) were obtained for nodularity, attenuation and spleen size. Interobserver agreement demonstrated highest kappa values (0.62–0.95) for nodularity and spleen size.

It is noted that kappa values for hepatic homogeneity/coarseness were relatively low, indicating high variance in interpretation. Selected kappa values are shown in Table 3.

No abnormal findings were demonstrated in our series for the following US-criteria: periportal echogenicity, portal vein diameter and flow velocity, gallbladder wall thickness, gallstones, diameter of the common bile duct, varices and ascites.

These were therefore excluded from further analysis.

4. Discussion

We have demonstrated that the exclusion of early CF related liver disease is not reliably made with US. Specifically we have demonstrated the positive predictive value of a normal ultrasound is only 33% and a sensitivity of 57%. A significantly abnormal US however is a good predictor of advanced liver disease with a specificity of at least 84%, as there may be some variability with the liver biopsy. The specific findings of nodularity and splenomegaly seem to be reliable markers of advanced fibrosis.

Due to its low cost, US is the ideal tool for imaging these patients with CF, but awareness of the limitations of each modality in specific disorders is crucial for good clinical management.

The strongest association of hepatic US-criteria to histology results was obtained for nodularity of the liver surface. A statistically significant association was demonstrated between hepatic nodularity and fibrosis/cirrhosis. These findings are in keeping with results from previous studies relating US findings to liver function using biochemical and clinical parameters [12,27].

As with other forms of cirrhotic liver disease, splenomegaly appears to be a reliable indicator of liver cirrhosis and portal hypertension, confirmed in our series where splenomegaly was associated with hepatic fibrosis or cirrhosis. Other previously described indicators of PHT (collateral vessels, lesser omental thickening) [15] were not identified in our series.

Hepatic parenchymal inhomogeneity has been observed in several studies and is assumed to reflect liver disease, however without histopathologic confirmation of these findings [12,27]. In our series hepatic inhomogeneity was not a reliable criterion in comparison with histology. Inhomogeneity was observed in patients with and without histopathologic evidence of LD.

The prevalence of a microgallbladder is reported as 30% in all CF-patients [4] and this is in keeping with our results. Interestingly the association between normal gallbladder size and liver disease is statistically significant in our series. With the small number in our series however further investigation of this finding appears necessary.

US may be more sensitive for CFLD than clinical and biochemical abnormalities [15]. This conclusion is supported by the results of our series, where initial US findings detected pathological changes before histologic fibrosis was demonstrated. On the other hand, it has been shown that patients with normal US can have severe hepatic fibrosis [2,10] and that biochemical abnormalities can occur before sonographic changes are detected [28].

Our results are similar to the findings of Lindblad et al. who examined 41 patients with CF including adults. In their group with normal US, two out of 21 patients were found to have moderate to severe fibrosis [2]. The series reported by Spray et al. [10] consisted of 18 US/histology pairs. In their series three out of four patients with normal US demonstrated cholangitis, portal fibrosis or steatosis in histology.

The role of liver biopsy for diagnosing liver disease in CF has been controversial in light of sampling tissue from a focal disease [29,30]. These concerns are not confined to CF, but broadly apply to liver diseases particularly those involving biliary disorders such as sclerosing cholangitis.

At present, liver biopsy remains the only test for assessing fibrosis even with these acknowledged concerns of sampling reliability [2]. Support for biopsy is also given by other authors [29,30] who demonstrated similar morphology over time with repeated needle biopsies. Potter et al. [31] performed open wedge biopsies with simultaneous needle biopsy in five patients and found similar histology. Our observations suggest that liver biopsy can miss advanced fibrosis, as demonstrated by the patient with US findings of cirrhosis with a normal biopsy. A subsequent repeat biopsy 3 years later showed fibrosis. The ultrasound was consistently abnormal during this period.

Generally, we found fair to good inter- and intraobserver agreement in evaluating the echostructure of the liver. This is in keeping with the observation by Dietrich et al. [32], who found the interpretation of hepatic inhomogeneity to be largely subjective.

Williams et al. [13] in contrast found a high level of agreement in their study. This may partly be explained by less variation in image characteristics with US performed by a single observer. But even in this ideal setting the assessment of mild hepatic structural changes in our experience leads to varying results.

A limitation of this study is the small number of patients. Nevertheless, this is the largest cohort of CF-patients with comparison of liver histology and specific sonographic findings investigated to date. Another potential limitation of this study was that the ultrasound accuracy was measured against liver biopsy but liver biopsy itself may result in sampling errors [2]. At present, there is no gold standard for the diagnosis of cystic fibrosis liver disease but liver biopsy, with its recognised sampling limitations, remains the standard for assessing liver fibrosis [2]. As this is a retrospective study, US examinations and liver biopsies were not timed and a prolonged time interval could lead to discrepancies in observation. However there is evidence that CFLD is a slowly progressing disease [2] and a significant impact on the results therefore appears to be unlikely. The recently described high resolution sonography of the liver surface [33] was not part of our imaging protocol, however the focal character of CFLD may limit benefits of this technique in our patient population.

In summary our findings demonstrate that the diagnosis of early liver fibrosis cannot reliably be made on the basis of US alone. A normal US does not preclude liver fibrosis in patients with CF and may still require biopsy [2].

An abnormal US that suggests cirrhosis however does predict the presence of moderate to severe LD and liver biopsy in these patients may potentially be avoided. Hepatic nodularity and splenomegaly are good predictors of cirrhosis.

Identifying early LD in CF remains a challenging task and further research is required to define the individual value of clinical, biochemical and imaging findings.

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