

# Screening for liver disease in cystic fibrosis: analysis of clinical and genetic risk factors for its development

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**SUMMARY:** Fustik S, Trajkovska M, Jakovska T, Spirevska L, Josifovska T, Koceva S. Screening for liver disease in cystic fibrosis: analysis of clinical and genetic risk factors for its development. *Turk J Pediatr* 2008; 50: 526-532.

This study analyzes the prevalence and the role of possible clinical and genetic risk factors for the development of cystic fibrosis (CF)-related liver disease (LD) in a Macedonian CF population. All patients older than three years (n=52) were screened for LD. LD was defined by the finding of hepatomegaly and/or splenomegaly, significant and persistent increase of at least two serum liver enzyme levels, suggestive ultrasonographic abnormalities (score >4), and morphologic or functional scintigraphic abnormalities. According to predefined criteria, 18 patients (34.6%) were classified as having LD, three of them with portal hypertension. A male predominance was found in the group with LD (72%). There was no significant difference in the pulmonary function, nutritional status, and in the prevalence of meconium ileus. Genetic analysis showed higher frequency of  $\Delta F508$  mutation in the LD group (77.8%) vs. the no LD group (66.2%). All patients with LD had severe mutations:  $\Delta F508$ , G542X, N1303K, CFTRdel.21Kb, 1811+1G $\rightarrow$ C, and Y1092X.

*Key words:* cystic fibrosis, liver disease, screening, ultrasonography, hepatobiliary scintigraphy, genotype.

Cystic fibrosis (CF) is a complex inherited disorder classically presented with a clinical triad of: chronic pulmonary disease, malabsorption due to exocrine pancreatic dysfunction, and abnormally high concentrations of sweat chloride and sodium caused by defective sweat electrolyte reabsorption. Although liver cirrhosis was recognized as a complication of CF in Anderson's original description in 1938, until recently the importance of subclinical liver disease (LD) has been eclipsed by the more obvious respiratory and pancreatic signs and symptoms<sup>1</sup>. More effective management of these has resulted in greatly improved survival, and so LD has become important to a large number of CF patients and those involved in their care. The histopathologic feature characteristic of CF-related LD is focal biliary cirrhosis with inspissated, granular eosinophilic material within the bile ducts<sup>2</sup>. Focal lesions may progress to multilobular cirrhosis often

predisposing to portal hypertension. The patient may suffer from variceal bleeding and, rarely, liver failure requiring liver transplantation.

It has been suggested that early therapy may prevent progression of CF-related LD<sup>3</sup>. Unfortunately, early recognition of LD in CF is not easy because its onset is often insidious and the clinical, biochemical, and other indicators are relatively insensitive. Many patients with cirrhosis caused by CF are well compensated and completely asymptomatic and may even have normal liver blood tests. Therefore, regular screening for LD and identification of patients with liver involvement, as well as early therapeutic intervention, are of great importance in the management of CF patients.

The reported prevalence of LD among CF patients ranges between 9% and 37%, depending on the study and criteria for defining LD<sup>2,4</sup>. Because there are no sensitive diagnostic markers of

liver involvement in CF, the actual prevalence might be even higher. In that context, the postmortem autopsy studies of CF individuals reported that the histological findings of focal biliary cirrhosis were present in 27% of children and 72% of adults<sup>5,6</sup>.

The aim of this study was to define the prevalence of CF-related LD in a Macedonian CF population, and the role of possible clinical factors as well as specific mutations in the CFTR (cystic fibrosis transmembrane conductance regulator) gene in the development of liver involvement.

### Material and Methods

All CF patients older than three years (n=52) who regularly attended the CF Center at the Pediatric Clinic in Skopje were included in the study. We retrospectively reviewed reports from the sequential screening procedures for LD obtained between 2000-2005.

#### Screening for Liver Disease

Patients were screened for LD through physical examination, liver biochemistry studies, echographic examination of the upper abdomen, and hepatobiliary scintigraphic assessment.

#### Physical examination

Careful examination of the liver and spleen was performed at each clinical visit. Hepatomegaly was considered to be present when the edge of the liver was palpable more than 2.5 cm below the right costal margin; splenomegaly was defined by the finding of palpable spleen below the left costal margin.

#### Liver biochemistry studies

The biochemical evaluation for liver injury and function included serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), and bilirubin level. Liver functional tests were obtained yearly in all CF patients. The results were considered abnormal if at least two serum liver enzyme levels were higher than 1.5 times the upper limit of normal: AST >52 IU/L, ALT >72 IU/L, GGT >78 IU/L, and ALP >400 IU/L. In those patients, the tests were repeated at shorter intervals (within 3-6 months). If levels remained elevated, without another explanation for the elevation, they were indicative of liver involvement due to CF.

### Echographic examination

Ultrasonography of the liver, biliary tract, gallbladder, spleen and hepatic vasculature was performed yearly in all CF patients older than three years. An ultrasound scoring system suggested by Williams et al.<sup>7</sup> was used to provide a grading system for the presence or absence of underlying LD. The scoring system depends upon the detection of three ultrasound characteristics: parenchymal irregularity, periportal fibrosis (increased periportal echoes) and irregularity of the liver edge consistent with the macronodular pattern of cirrhosis (Table I). A total score of 3 was consistent with an entirely normal liver (no LD group), while all subjects with a score of 4 or more were allocated in the LD group. The authors suggested that the scoring is able to identify a group of patients with evidence of LD but in pre-cirrhotic phase (scores 4-7) and patients with established cirrhosis (score 8 or 9). Doppler ultrasound analysis was used to detect dilatation and flow patterns of hepatic vasculature. Portal hypertension was suggested by enlarged spleen, distended portal vein and abnormal portal venous flow (decreased portal venous flow velocities or reversal of flow).

**Table I.** The Ultrasound Scoring System by Williams et al.<sup>7</sup>

Score	1	2	3
Characteristic			
Hepatic parenchyma	Normal	Coarse	Irregular
Liver edge	Smooth	-	Nodular
Periportal fibrosis	None	Moderate	Severe

### Hepatobiliary scintigraphy

Hepatobiliary scintigraphy was performed with brome iminodiacetic acid derivate labeled with technetium-99m (99mTc mebrofenin). After intravenous administration, the derivate is cleared from circulation by hepatocytes, excreted into the canaliculus and bile ducts, stored in the gallbladder, and excreted into the duodenum. 99mTc mebrofenin was injected intravenously as a bolus. Serial analogue images were taken per frame every 10 seconds for 30 minutes by gamma camera to estimate the dynamic of bile flow, followed by static images at 60 and 90 minutes. If the gallbladder was seen, the patient was given a high-fat meal and was subsequently scanned to assess emptying of the gallbladder. If the gallbladder

was not seen, additional images were obtained hourly for up to three hours. Morphological scintigraphic abnormalities suggestive of biliary tract obstruction (cholestasis) were: stasis of the tracer in the intrahepatic bile ducts (visualization of secondary and tertiary intrahepatic bile ducts was indicative of intrahepatic delayed excretion); retention of the tracer in the extrahepatic biliary tree; an irregular tapering (segmental narrowing) of the common bile duct; and enlarged gallbladder with delayed emptying. Microgallbladder and non-visualized gallbladder were relatively frequent scintigraphic findings, but are not indicative of biliary tract obstruction. Functional scintigraphic abnormalities suggestive of biliary tract obstruction were: prolonged time of visualization of the intestine (>30 minutes) and prolonged half-time of hepatic wash out (normal value:  $T/2 < 23$  minutes).

Using the above-mentioned methods, LD was defined by finding some of the following abnormalities: hepatomegaly and/or splenomegaly; significant and persistent increase of at least two serum liver enzyme levels; suggestive ultrasonographic abnormalities (score >4); and morphologic or functional scintigraphic abnormalities.

### Clinical Assessment

The following data from patients' medical history were collected: current age of patient, sex, history of meconium ileus, history of distal intestinal obstruction syndrome (DIOS), pancreatic functional status, current pulmonary function [forced vital capacity (FVC) and forced expiratory volume in the 1st second ( $FEV_1$ )], and current nutritional status expressed as standard deviation score for height (Z-score/height), standard deviation score for weight (Z-score/weight), and body mass index (BMI) for the patients older than nine years.

### Genetic Analysis

Genetic analysis for CFTR mutations was carried out in all CF patients. The initial screening of all samples included polyacrylamide gel electrophoresis of polymerase chain reaction (PCR)-amplified fragments for the detection of the  $\Delta F508$  mutation. The other common CFTR mutations were screened in the PCR products by allele-specific oligonucleotide

(ASO) hybridization and/or by restriction fragment length polymorphism (RFLP) analysis<sup>8</sup>. Screening for unknown mutations was performed by radioactive SSCP (single-strand conformational polymorphism) analysis and by DGGE (denaturing gradient gel electrophoresis) analysis. Samples showing an abnormal pattern on SSCP or DGGE were further analyzed by direct sequence analysis of PCR-amplified fragments<sup>9</sup>.

### Statistical Analysis

The data were analyzed by the Excel Version 9.0 (Microsoft, IL, USA). Variables are reported as means  $\pm$  SD, unless otherwise stated. Frequency distributions and contingency tables were created for clinical and demographic characteristics of the patients (gender, pancreatic status, meconium ileus or its equivalent), liver variables, and CFTR mutations. Statistical comparisons between two groups of patients with and without LD were performed using Student's t test. The difference was considered significant at  $p \leq 0.05$ .

### Results

Of the 52 CF patients older than three years who were regularly followed at the CF Center at the Pediatric Clinic in Skopje, 18 patients (34.6%) were classified as having CF-related LD according to predefined criteria. Among them, 6 (33.3%) had clinical signs of LD, 12 (66.6%) had abnormal liver function tests, 13 (72.2%) had scintigraphic abnormalities and all had ultrasonographic changes (Table II). Abnormal imagings on hepatobiliary scintigraphy were seen in 12 patients and functional scintigraphic abnormalities suggested delayed biliary flow in 3 patients with LD. Echographic evidence

**Table II.** Hepatic Abnormalities in CF Patients with Liver Disease

Abnormalities	No.	%
Hepatomegaly	6	33.3
Splenomegaly	3	16.6
Biochemical abnormalities	12	66.6
Ultrasonographic abnormalities	18	100
– ultrasound score 4-7	11	61.1
– ultrasound score 8-9 (cirrhosis)	7	38.9
– portal hypertension	3	16.6
Scintigraphic abnormalities	13	72.2
– morphologic	12	66.6
– functional	3	16.6

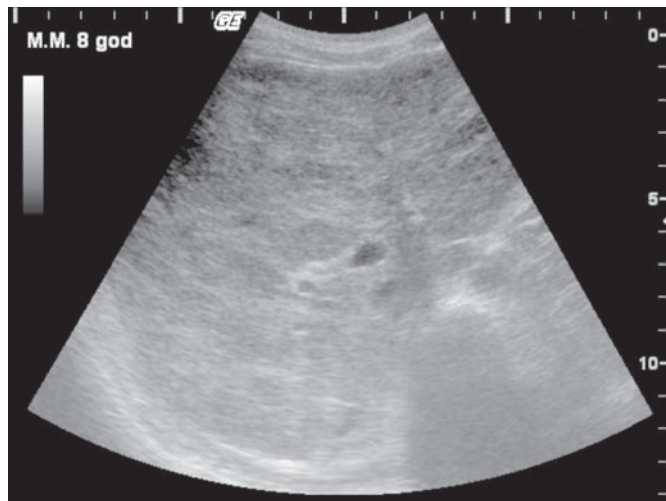
of hepatic cirrhosis (coarsely nodular liver; score 8 or 9) was found in 7 patients, 3 of them with portal hypertension (Fig. 1). The diagnosis of cirrhosis was confirmed by liver biopsy findings in 4 patients.

Clinical characteristics of the patients with and without LD are reported in Table III. The prevalence of LD increased with age: average age of  $13.2 \pm 5.3$  years in the LD group vs.  $9.9 \pm 5.6$  years in the no LD group. A male predominance was found in the group with LD (72%). Pancreatic insufficiency was present in all patients with LD. CF patients with LD tended to have milder pulmonary disease, but the differences in pulmonary function were not significant. All except 1 patient had relatively preserved lung function. There was also no significant difference in the nutritional status or in the prevalence of meconium ileus or DIOS between the two groups.

Genetic analysis showed higher frequency of  $\Delta F508$  mutation in the LD group (77.8%) in comparison with the no LD group (66.2%), although not statistically significant. Molecular basis of CF in patients with LD is shown in Table IV. All CF patients with LD had severe mutations associated with pancreatic insufficiency and a more severe clinical course of the disease.

**Table IV.** Genotype Frequency in CF Patients with Liver Disease

Genotype	No.	%
$\Delta F508/\Delta F508$	11	61
$\Delta F508/G542X$	2	11
$\Delta F508/CFTR\delta 21$	1	5.5
$\Delta F508/Y1092X$	1	5.5
$\Delta F508/1811+1G \rightarrow C$	1	5.5
$G542X/N1303K$	1	5.5
$\Delta F508/unknown$	1	5.5



**Fig. 1.** Hepatic ultrasound scan showing marked nodularity (cirrhosis).

**Table III.** Comparison of Clinical Variables Between Patients With and Without Liver Disease

Variables	LD	no LD	p
n (%)	18 (34.6)	34 (65.4)	
Male/female (%)	13/5 (72/28)	19/15 (56/44)	
Current age, years	$13.7 \pm 5.34$	$9.91 \pm 5.56$	<0.05
Pancreatic insufficiency (%)	18 (100)	31 (91)	
Meconium ileus/DIOS (%)	0 (0)/1 (5.5)	2 (5.9)/3 (8.8)	
BMI ( $\text{kg}/\text{m}^2$ )	$17.6 \pm 1.72$	$16.4 \pm 2.58$	>0.05
Z-score /Weight	$-0.83 \pm 1.04$	$-0.61 \pm 0.95$	>0.05
Z-score/Height	$-0.98 \pm 1.44$	$-0.54 \pm 1.15$	>0.05
FVC (% predicted)	$98.9 \pm 14.10$	$90.38 \pm 18.30$	>0.05
FEV <sub>1</sub> (% predicted)	$96.9 \pm 14.83$	$87.93 \pm 22.09$	>0.05

LD: Liver disease. DIOS: Distal intestinal obstruction syndrome. BMI: Body mass index. FVC: Forced vital capacity. FEV<sub>1</sub>: Forced expiratory volume in the 1<sup>st</sup> second.

## Discussion

With improved survival of CF patients, the relative importance of LD has increased and it now represents the second commonest cause of mortality from CF<sup>10</sup>.

The pathogenesis of CF-associated LD is considered to be a secondary effect of the basic defect. The CFTR gene in the normal human liver is expressed in the epithelia of the intrahepatic and extrahepatic bile ducts and gallbladder, and the CFTR protein is localized to the apical domain of these cells<sup>11</sup>. CFTR chloride channel dysfunction caused by CFTR mutations leads to impaired or abolished chloride secretion, and concomitant reduction in water and sodium movement into bile. Altered bile composition (hyperviscosity of bile) and decreased bile flow cause obstruction of biliary ducts that may induce collagen synthesis by stellate cells and its deposition in portal tracts in several ways<sup>1</sup>. The progression from cholestasis (decreased bile flow) to focal biliary cirrhosis and to multilobular cirrhosis takes years in some CF patients, or never occurs in others. Thus, some patients have progressive LD and develop portal hypertension and liver insufficiency, while a sizeable proportion of patients may have only slight changes that remain stable life-long. What causes the progression of CF-associated LD to an end-stage cirrhosis in some patients but not in others remains an enigma.

Several studies aimed at identifying possible risk factors for the development of chronic LD and liver cirrhosis have been published<sup>2,12-16</sup>. Colombo et al.<sup>12,13</sup> reported that patients with CF and a history of meconium ileus or its equivalent (DIOS) are at increased risk for the development of LD. We did not find that these clinical presentations of CF occurred more frequently in patients with LD. Other data from the literature also fail to support that a history of meconium ileus carries an increased risk for the development of LD<sup>2,17</sup>. There has been some evidence to suggest that compression/stenosis of the common bile duct as it passes through the diseased pancreas in CF may contribute to the pathogenesis of hepatic fibrosis<sup>18</sup>. Subsequent reports indicate that this phenomenon is rare<sup>19,20</sup>.

The possible association of chronic LD with a specific gene defect has been investigated but not identified<sup>2,12,21</sup>. We found higher frequency

of  $\Delta F508$  mutation in the LD group (77.8%) in comparison with the no LD group (66.2%) and slight dominance for homozygosity for  $\Delta F508$  in the LD group. Although our data, as well as the data from previous reports, failed to reveal any relationship with specific CFTR mutations, LD occurred only in patients harboring severe mutations, which are associated with complete loss of CFTR function. All patients with LD were pancreatic insufficient, which indicated that LD together with pancreatic insufficiency is a component of the severe CF phenotype.

The variable onset and severity of LD suggest that there are other modifying genes outside of the CF locus or possibly environmental factors (nutritional deficiencies, drug hepatotoxicity, infections) that may be involved in the pathogenesis and progression of LD in CF. Our study has suggested an almost 3:1 male to female bias in those with LD. Moreover, all patients with frank cirrhotic liver changes (scores 8, 9) were males. Therefore, male sex is an independent risk factor for CF-related LD. A similar association was found in a few other studies<sup>4,12,13,22</sup>, but not confirmed by others<sup>2,17</sup>. Further evidence of a genetic influence has come from HLA studies, which have shown that the HLA haplotype B7-DR15-DQ6 was associated with an increased risk of chronic LD in male patients with CF, implicating a possible immune pathogenesis of hepatobiliary injury in addition to the CFTR defect<sup>1,23</sup>. It is likely that such immune mechanisms are a secondary phenomenon of the obstructive biliary lesions, but may be important in determining the extent of damage observed<sup>24</sup>.

Liver disease in patients with CF begins insidiously, making it difficult to determine the onset accurately. Five of our patients had advanced LD at entry in the study. In two of them, the diagnosis of CF was established in early adolescent age, after the development of portal hypertension and hypersplenism. It seems that the LD usually develops during the first decade and may progress silently, only manifesting as end-stage LD and portal hypertension. It is likely that active follow-up evaluation in our study facilitated more frequent detection of fibrosis/cirrhosis at the initial stage (11 patients with ultrasound score 4-7).

The diagnosis of CF-related LD can be established by the presence of one or more of the following: hepatomegaly; splenomegaly; a small, hard liver

(indicative of cirrhosis); persistently elevated liver enzymes; abnormal liver histology; or signs of portal hypertension (varices, ascites)<sup>25</sup>. Screening for LD should include an annual physical examination and evaluation of serum liver enzymes, as recommended by the Cystic Fibrosis Foundation Hepatobiliary Disease Consensus Group<sup>1</sup>. Recent data suggest that ultrasound is useful in initial diagnosis as well as in following the progression of CF-related LD and should be added in annual screening procedures<sup>7,13,15,26</sup>. Ultrasound is likely to remain the most practical and frequently used imaging modality for assessing the hepatobiliary system. Hepatobiliary scintigraphy has limited clinical utility compared with ultrasound, but has been advocated by some authors for detecting CF-related LD<sup>12,27</sup>. Liver biopsy may be useful to confirm the presence of LD and determine the extent of portal fibrosis or cirrhosis. However, not all clinicians think that liver biopsy is indicated in investigating LD in CF because there is no definitive therapy, and because patients may be at a higher risk of complications<sup>1,25</sup>.

At present, oral ursodeoxycholic acid (UDCA) therapy, aimed at improving biliary secretion in terms of bile viscosity and bile composition, is the only available therapeutic approach in CF-related LD. UDCA, a naturally occurring hydrophilic bile acid, has in many studies been shown to normalize liver enzymes, improve hepatobiliary scintigraphy and suggest an improvement in liver morphology<sup>3,28-30</sup>, but so far an effect of UDCA on the ultimate outcome of the disease has not yet been proven<sup>25</sup>. However, recent evidence suggests that the use of UDCA at an early stage may abort or inhibit the development of LD in CF, and thus may favorably affect the natural course of the focal biliary fibrosis/cirrhosis<sup>29,30</sup>.

Our study suggested that subclinical LD is common among patients with CF. The overall prevalence of LD among our CF patients older than three years was 34.6%. However, investigators from different countries have reported markedly variable rates of LD among their CF patients<sup>2,4,12-16,23</sup>. There are a number of possible causes for these differences, including the fact that criteria used for the diagnosis of CF-related LD have varied widely in the published literature. The criteria for LD in our study were clinical, biochemical,

ultrasonographic and radionuclide imaging test results. The physical examination by palpation and measurement of the liver and spleen span below the costal margins should be performed regularly for the defining of usually asymptomatic hepatomegaly and/or splenomegaly. Biochemical assessment of liver function would be expected to be the first-line investigation. However, previous studies have suggested a low sensitivity for biochemical indices in the detection of LD in CF<sup>26,31</sup>. In our study, 66% of patients in the LD group had evidence of persistent and significant increased liver enzymes during the study period. Our data clearly highlight the value of ultrasound as the most sensitive tool for detection and follow-up of patients with LD. Ultrasonographic abnormalities of the liver appearance were more frequent than biochemical abnormalities, and once present were more likely to be persistent. The ultrasound scoring system suggested by Williams et al.<sup>7</sup> is, we believe, simple and useful for detecting, grading and monitoring the progression of LD. For better assessment of hepatic status, hepatobiliary scintigraphy was performed in patients with suggestive ultrasonographic abnormalities. We found that radionuclide imaging test provides useful information about liver function and biliary secretion and should be added in the screening methods for identification of LD and for monitoring the response to therapy. Liver biopsy and histologic assessment of the liver was not included in the study protocol. Needle biopsies of the liver were performed in several patients. Liver biopsy may be the gold standard for diagnosis of LD in CF patients, but percutaneous biopsy may miss the patchy earlier lesions<sup>2,31</sup>. The widespread use of an invasive technique, with an attendant risk of hemorrhage, is probably not acceptable, especially within the pediatric population.

Implementation of a screening program for LD in all CF patients older than three years is an important step in targeting a population for early intervention and prophylactic treatment. The policy of examining only patients with abnormal liver function test results or those who are symptomatic fails to detect a number of young patients who clearly have abnormal echo texture of the liver, some of whom may progress to more severe LD. UDCA therapy may be of the greatest benefit if initiated

early in the course of the disease, prior to the development of focal biliary cirrhosis and multilobular cirrhosis, again emphasizing the need for sensitive and specific markers for early detection of CF-LD. Patients identified by screening procedures are expected to progress at a slower rate than patients who first come to attention as a result of symptoms. Therefore, active follow-up evaluation directed at detection of LD should be started early in life, preferentially in male patients, who are pancreatic insufficient and with severe CFTR mutations, who are exposed to a higher risk for developing this complication of CF.

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