LIVER DISEASE IN CHILDREN WITH CYSTIC FIBROSIS

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ABSTRACT

Liver disease is an early complication in children with cystic fibrosis (CF).The clinical manifestations in hepatobiliary disease of CF include neonatal cholestasis, liver steatosis, liver fibrosis, biliary lithiasis, focal biliary cirrhosis and multilobular cirrhosis, with or without portal hypertension. Changes in the tests evaluating the liver function are inconsistent and are not correlated with the severity of the liver lesions. The diagnosis of liver disease in CF requires the presence of at least two of the following four diagnosis criteria:clinical manifestations, pathologic liver function tests, ultrasound and histologic changes.The annual follow-up to evaluate the liver function is s recommended for diagnosis of asymptomatic liver disease and early initiation of treatment with ursodeoxycholic acid.The improvement of the liver function influences life quality and increases the survival rate in patients with CF.

Keywords: cystic fibrosis, liver disease, ursodeoxycholic acid, children

Cystic fibrosis (CF) is the most frequent autosomal recessive disorder, potentially lethal, found in the Caucasian population. The lung implication is the main cause of morbidity and mortality, but the phenotypic expression of the CF is extremely polymorphic through multi-organ impairment: pancreatic, salivary glands, intestinal, liver, reproductive system. The CF-associated liver disease (CFLD) is the third cause of mortality after the lung disease and post-transplant complications (1). The hepatobiliary disease has been recognized as a complication in CF from the Andersen's original description from 1938 (2).

In the absence of specific diagnosis markers, the real incidence of CFLD is hard to establish. The literature data show that the CFLD incidence in children varies between 27 and 35%, decreasing after 18 years old (3). CFLD is frequent in children and adolescents, decreasing with age. According to the prospective study done by Lindblad et al., 25% of the children over 4 years old presented biochemical markers of liver disease, with an evolution to multilobular cirrhosis in their first life decade in 5 to 10% of the cases (4,5). Therefore, the liver disease is an early and frequent complication of CF. In the longitudinal prevalence study done by Lamireauet al. on a group of 241 patients with CF, they demonstrated that the liver disease develops mainly during the first ten years of life, with a prevalence of 41% in 12-year olds, 7.8% of patients had liver cirrhosis and 5 patients benefitted from liver transplant (6). Liver failure is occasionally found in pediatric ages, being found rather in adults with CF (7).

The liver disease as an initial manifestation of CF was found in 1.5% of the patients with CF. Therefore, the sweat test must be part of the initial protocol for the investigation of the liver disease of unspecified etiology (8).

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The mortality rate due to the liver disease in patients with CF is of 2.5%, which is much higher than the mortality rate in patients with CF without liver disease (9).

Physiopathology of the CF-associated liver disease

CF is due to mutations in the gene located on the long arm of chromosome VII in position 7q31.Normally, the gene codifies the synthesis of a protein made of 1480 amino acids, called the "cystic fibrosis transmembrane conductance regulator" (CFTR) protein, which works like a regulator of the chloride channels. Until now, more than 150 different mutations have been described, the most frequent of which, being characterized by the depletion of phenylalanine in position 508 (ΔF508 – Phe-508del), is found in 30-80% of the patients with CF, depending on the ethnic group (10). The CFTR gene mutations are grouped in 6 different classes, in regard to functional consequences at cellular level (1).

The wide range of hepatobiliary diseases in patients with CF is the consequence of specific changes due to the defective CFTR channel (neonatal cholestasis, sclerosing cholangitis, microlithiasis, vesical lithiasis, focal or multilobular biliary cirrhosis, portal hypertension), to lesions of iatrogenic origin (drug-induced hepatotoxicity, liver steatosis), as well as to the effects of the extrahepatic diseases (liver stasis due to heart failure, common bile duct stenosis) (3).

The liver lesion typical to CF, related to the CFTR gene defect in the cholangiocytes, is focal biliary cirrhosis, secondary to bile duct obstruction and progressive periportal fibrosis. This hypothesis consists of the accumulation of toxic bile acids in the liver, the depletion of hepatic antioxidants, which affect the liver. Subsequently, the activation of the hepatic stellate cells increases the synthesis of TGF-β profibrogenic cytokines determining liver fibrosis and cirrhosis in some cases (1). Subsequent extension of the fibrogenesis process, which is initially focal, leads later on to multilobular biliary cirrhosis, portal hypertension and associated complications (11).

CFLD has not been associated with mutations specific to the CFTR. Hepatobiliary manifestations are frequently found in patients with severe mutations classes I, II, III, and IV, which affect the synthesis, processing or adjustment of the CFTR, without any correlation between the phenotype and the mutations specific to CFTR (12). The hepatic phenotype of patients with CF with the same CFTR genotype is variable, which suggests that environmental factors or modifying genes are involved in the development of CFLD. Bartlet et al. described the association between 5 non-CFTR genes and severe liver disease with portal hypertension: α1-antitripsina (SERPINA1), conversion enzyme inhibitors (CEI), glutathione S-transferase (GSTP1), mannose-binding lectin 2 (MBL2)and transforming growth factor β1 (TGFB1). Of all these genes involved, only allele SERPINA1 Z proved to be strongly associated to CFLD and portal hypertension (13).Pereira et al. (2012) have suggested a genetic predisposition independent of CFTR gene involved in the pathogenesis of CFLD. It was demonstrated different expression of several genes associated with liver fibrogenesis including those involved in the synthesis of matrix metalloproteinases, the collagen and chemokines in patients with CFLD. In the control group (CF without liver disease) showed reduced expression of tissue remodeling genes, including tissue inhibitor of metalloproteinase-1 and plasminogen activation inhibitor-1 (14).Additional informations arenecessary to confirm this hypothesis.

Steatosis is a common liver lesion associated with CF that does not seem to be directly related to the defective secretion in CF, though. Massive steatosis has become rare due to early diagnosis and optimum nutritional support. Minor steatosis is more frequent and it has been associated to selective nutritional deficits, especially in essential fatty acids, and to changes in the phospholipid metabolism (3). CF steatosis has been considered a benign condition, without being able to demonstrate any relation to the subsequent development of cirrhosis. Despite this, the available data regarding the role of non-alcoholic steatohepatitis as a cause for cirrhosis in adults may lead to reconsidering this issue in patients with CF (15).

The changes in the intrahepatic bile ducts similar to sclerosing cholangitis have been described both in children and adults with CFLD. These lesions are the consequence of the inflammatory process in the bile ducts, of the accumulation of proteins and mucus, as well as of the narrowing of the intrahepatic bile ducts due to fibrosis. Current data show that CFLD is the consequence of bile duct obstruction due to the CFTR cholangiocyte defect, the retention of toxic substances leading to peribiliary fibrosis and the increase in the "sludge" amount in the bile duct, which generates microlithiasis (3).

Clinical presentation of the liver disease in CF

The clinical manifestations in CFLD reflect underlying lesions: include neonatal cholestasis, liver steatosis, liver fibrosis, focal biliary cirrhosis, and multilobular cirrhosis, with or without portal hypertension. Hepatic complication may be asymptomatic, manifesting only cytolysis.

In CF, liver disease is early, manifesting itself in childhood, and most times it is initially asymptomatic. Clinically, the patient has hepatomegaly, accompanied or not by changes in the laboratory tests, depending on the progression of the liver lesions (16). The ultrasound examination and the anatomopathological examination reveal liver steatosis or fibrosis lesions.

Jaundice is present in infants with neonatal cholestasis or in patients with multilobular biliary cirrhosis in terminal phase (16). Although CF is the cause for neonatal cholestasis in only 1% of cases, the sweat test is recommended in all infants with the cholestasis syndrome (1).

Multilobular cirrhosis manifests clinically mostly at the end of the first life decade, associating signs of portal hypertension: firm liver, with a nodular structure (lesions located especially in the left liver lobe), with or without splenomegaly generating discomfort or abdominal pain, extrahepatic signs of chronic hepatitis (palm erythema, clubbing, jaundice, limb edema, abdominal collateral circulation, ascites). All these clinical manifestations are tardy in the liver disease evolution. As there is no sensitive and specific test for the assessment of the biliary cell function, periodical monitoring of the liver function is necessary through periodical clinical examination with the purpose of identifying the hepato-splenomegaly and through annual biochemical and imaging tests.

Portal hypertension manifests clinically through splenomegaly, hypersplenism, upper digestive hemorrhage secondary to the bleeding of esophageal varices. The evolution of focal biliary cirrhosis to progressive multilobular cirrhosis varies in time, but the emergence of multilobular cirrhosis and portal hypertension are signs of reserved prognostic (17).

Steatosis is the most frequent liver lesion associated to CF, found in up to 67% of patients, regardless of age. Liver steatosis has been described in patients with pancreatic failure and severe malnutrition, being much more rarely associated to the early diagnosis and good nutritional status. Colombo *et al* (2004) considered that the liver is an "innocent spectator" as regards fatty infiltration (18). Nutritional deficits (deficit in essential fatty acids, carnitine, minerals and dietary elements) associated to antibiotherapy are involved in the liver steatosis pathogeny.

Liver disease in CF is found in 5% to 33% of the cases and manifests anomalies of the intra- and extrahepatic bile ducts, thickening of the gall bladder walls with motility disorders, microlithiasis and biliary lithiasis (19).

Paraclinical investigations in CF-associated liver disease

Stages of the liver disease in CF:

1. Stage I – biochemical changes – change in the liver enzymes
2. Sage II – fatty infiltration of the liver revealed by the ultrasound examination
3. Stage III – decompensated liver disease – portal hypertensionm hypoalbuminemia, ascites, coagulation disorders.

Liver function tests

Changes in the tests evaluating the liver function are inconsistent and are not correlated with the severity of the liver lesions (20). 20-30% of the children with CF have increased liver enzymes during the evolution of the disease (21). Transitory increase in the liver enzymes is correlated to the hypoxemia, infection and antibiotic treatment during pulmonary exacerbations. Also, other causes for hepatic cytolysis must be excluded: viral infections (A, B, C, D hepatitis virus, cytomegalovirus, Epstein Barr virus), alpha1 anti-tripsine deficit, the celiac disease, autoimmune hepatitis, Wilson disease, nutritional causes (malnutrition, obesity, diabetes mellitus) (3).

It is mandatory to do periodical evaluations of the liver enzymes originated in the biliary epithelium (gamma-glutamyl transferase - GGT, 5-nucleotidaseand alkaline phosphatase-AP), whose change is much more specific to CFLD in comparison to ALT and AST. Many times, patients with CF and multilobular biliary cirrhosis have normal liver tests (3). The biochemical and ultrasonographic evaluation reflects different stages of the disease progression (Williams et al, 2002). Establishing the degree of liver damage in CF involves the correlation of several tests. The Cystic Fibrosis Foundation in the United States recommends suspecting the liver disease if any liver enzyme is higher by 1.5 in comparison to the upper limit of the normal values in two successive evaluations six months apart (11).

The isolated increase in the transaminases values associated to normal concentrations of the cho-

lestasis enzymes (GGT and FA) raises suspicion on steatosis, while the isolated increase in the FA associated to normal values of the GGT and transaminases is not specific to liver disease in growing children. In addition, over 59% of the children with CF have changes in their liver tests during the first year after being diagnosed, with complete normalization under substitution treatment and correction of nutritional deficits during the following 2-3 years. In CF, the causes of neonatal cholestasis are multiple: meconium ileus, abdominal surgeries, total parenteral nutrition. In these cases, the cholestasis spontaneously disappears during the first months of life and has no impact on the subsequent development of the liver disease (22).

The use of serum markersof liver fibrogenesis (e.g. collagen VI, prolyl hydroxilase) for diagnosis and prognostic is still under evaluation (23).

Hepatobiliary ultrasound examination is a non-invasive accessible method of diagnosis and monitoring of the hepatobiliary disease in CF. In children, it is a multiple-advantage screening method: it does not irradiate, it is well tolerated, it slows a good evaluation of the liver and bile duct sizes and structure. In the attempt of establishing a correlation between the ultrasound and the biochemical tests, it has been noticed that the changes revealed by the ultrasound examination precede the clinical and biological changes in patients with CFLD. The ultrasound examination allows the identification of patients with focal liver lesions but with normal liver function, who proved to respond to the early administration of the ursodeoxicolic acid treatment (UDCA) (3). On the other hand, the predictive positive value of the ultrasound of normal aspect is of 33%, with sensitivity higher than 57%, which is why the ultrasound examination is always interpreted on the clinical and biological background (3,24). An ultrasound score has been proposed for the liver evaluation in the pre-cirrhosis stage and monitoring, based on the change in the liver parenchyma structure, the aspect of the liver margin, the increase in the periportal echogeneity (25).

EchoDoppler measurements of the portal vein evaluate its size and the vascular blood flow in order to evaluate portal hypertension (3). The decrease in the portal venous flow speed and the reversal of the flow in the portal vein are signs of portal hypertension.

Liver elastography (FibroScan)

FibroScan is a quick non-invasive and reproducible method of quantification of the liver fibrosis based on the analysis of elastic shock waves travel propagation in liver tissues. Studies are being done to establish its usefulness for the diagnosis of CFLD and the evaluation of the disease progression in patients with CF (3).

Hepatobiliary scintigraphy

Hepatobiliary scintigraphy with iminodacetic acid derivatives highlights the dilation of the intra- and extrahepatic bile ducts, the delay of the bile secretion of the tracer in the bowel (3). The scintigraphy is a method complementary to the hepatic ultrasound for the monitoring of the liver lesion progression and of the evolution of the treatment with AUDC (17).

Computed tomography (CT) and magnetic resonance imaging (MRI)

CT and MRI are non-invasive imaging methods used to differentiate the liver fibrosis from liver steatosis on the one hand, and on the other hand to distinguish them from other types of focal lesions (for instance, focal steatosis, hemangioma, focal nodular hyperplasia, hepatocellular carcinoma). In children, the MRI tends to replace the CT as it does not expose the patient to radiation and enables the visualization of the biliary tree.

The cholangio-MRI is not usually done in CF, but it could be useful to detect early the damage of the intrahepatic bile duct, which can contribute to the development of biliary cirrhosis, especially in children with abdominal symptoms (for example jaundice, abdominal pain) or signs (for instance, dilation of the bile ducts in the ultrasound) suggestive for sclerosing cholangitis, distal stenosis of the common bile duct or choledoch lithiasis (19). Thus, cholangio-MRI could substitute endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangiography, which are invasive methods not recommended as screening and diagnosis methods in CFLD (3).

Liver biopsy

Though controversial, percutaneous liver biopsy enables the anatomo-pathologic evaluation of the liver, representing the "golden standard" for the diagnosis and monitoring of the chronic liver disease. The initial lesions being focal, the liver biopsy may not catch the changes of the liver structure, underestimating the severity of the lesions (3). The anatomo-pathologic examination provides important information on the type of lesion (focal biliary steatosis or cirrhosis), the degree of the portal fibrosis, the progression rate and the response to treatment (18).

CFLD diagnosis criteria

According to the current CF diagnosis and management guides, CFLD needs the presence of at least two of the following 4 diagnosis criteria (3):

1. Clinical manifestations:

– Hepatomegaly – the enlargement of the liver over the sizes specific to the age, or the palpation of the lower edged of the liver 2 cmunder the costal margin, on the midclavicular line and confirmed by the ultrasound examination. Furthermore, the palpation of the left liver lobe in the epigastrium suggests multilobular cirrhosis.

– Splenomegaly – associated or not to hepatomegaly.

2. Changes in the liver function test: increase in the transaminase values (AST and ALT) and GGT value above upper limit, at least in 3 consecutive determinations during 12 months and after excluding other causes for liver disease.

3. Ultrasound changes

– Hepatic: increase and/or heterogeneity of the liver echogenicity, irregular liver margins, liver nodules) or a sign of portal hypertension (splenomegaly, increase in the thickness of the small epiploon, splenorenal anastomoses, collateral venous circulation, ascites)

– Anomalies of the bile ducts (bile duct dilation)

4. Anatomo-pathologic changes highlighted after the liver biopsy, in case of uncertain diagnosis.

Figure 1. represents the CFLD investigation and follow-up algorithm (3).

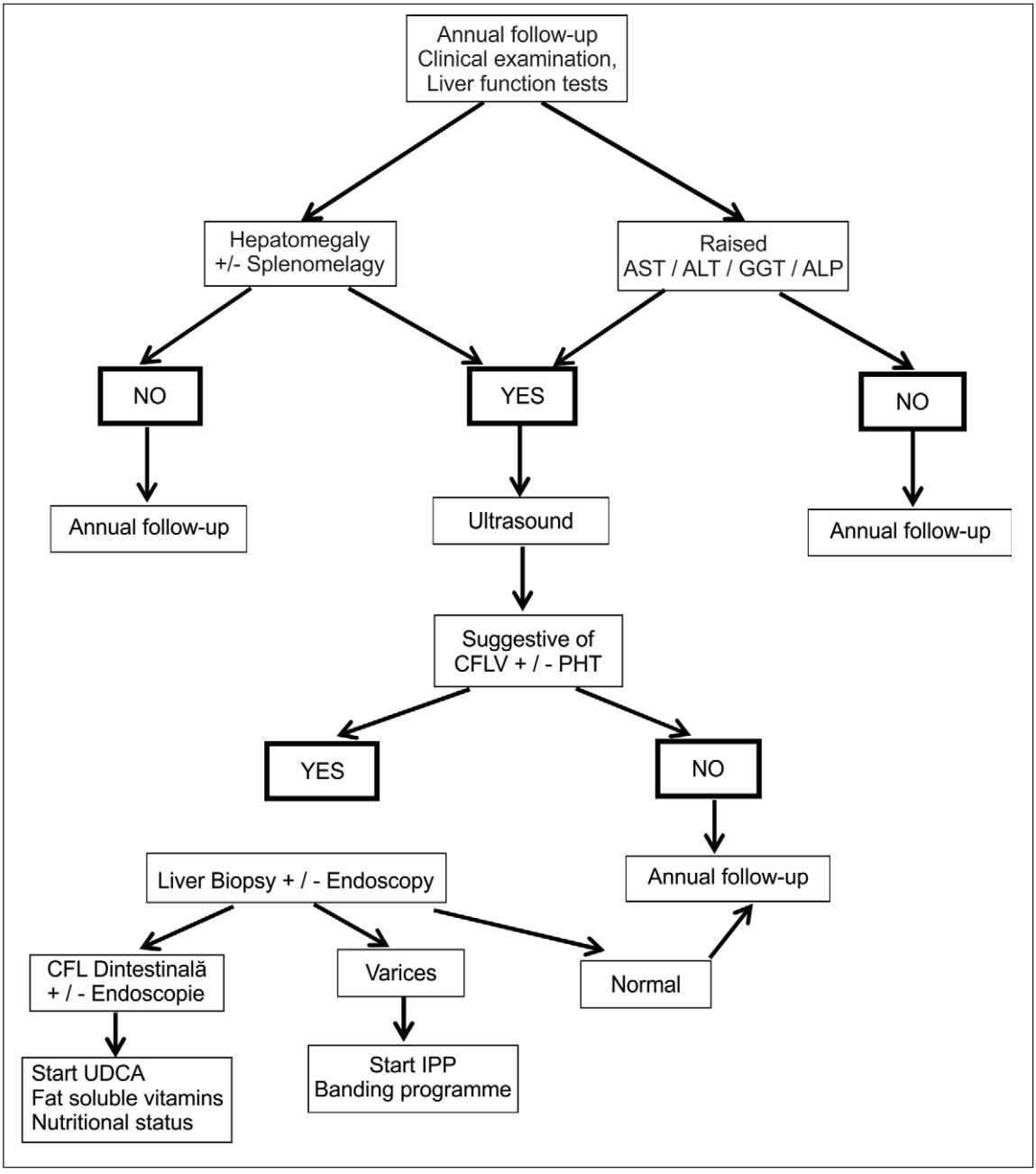

FIGURE 1. *Algorithm for investigation and follow-up of patients with AHFC (3)*

The protocol for annual follow-up patients with CF for the identification of CFLD includes: clinical examination, liver function investigation (AST, ALT, GGT, ALP, prothrombin time, platelets), abdominal ultrasound +/- CT or MRI for the identification of liver lesions or changes in the bile duct.

Patients with CFLD must be annually follow-up by the gastroenterologist or hepatologist in order to evaluate the disease progression to cirrhosis, and screening for the portal hypertension development:

– Patients with cirrhosis must be monitored through upper digestive endoscopy for esophageal varices every 2-3 years. After the development of esophageal varices, upper digestive endoscopy is recommended annually.

– The hepatopulmonary syndrome is checked by measuring the oxygen saturation in orthostatism and clinostatism conditions: a significant decrease of the oxygen saturation (> 5%) (calledorthodeoxia) suggests the diagnosis.

– Pulmonary arterial hypertension (for instance the portopulmonary syndrome) is monitored by means of periodic echocardiography

– Thrombocytopenia and leukopenia secondary to hypersplenism impose platelet concentrate transfusions only in case of bleeding invasive procedures or surgical procedures and the use of granulocyte colony stimulating factors in case of severe leukopenia.

– In order to identify the early signs of liver failure, the (at least) annual determination of the prothrombin time (PT) and the coagulation factors is necessary. The PT prolongation (over 13.5 seconds or the decrease in the PT activity under 70% of the normal value) and the decrease in the cofactors are non-specific markersfor liver failure. The important decrease in the coagulation factor V, in comparison to other cofactors is observed in patients with important splenomegaly, probably as a consequence of the intrasplenic consumption. The isolated decrease of cofactors VII, X and II suggests vitamin K deficit, which calls for the oral or intramuscular administration of vitamin K.

– In patients with cirrhosis (adolescents and adults) the liver ultrasound as well as the determination of the alpha-phetoprotein (risk of hepatocellular carcinoma) are recommended annually (3).

Treatment for the CF-associated liver disease

1. Nutritional recommendations

CFLD maintains malnutrition by intensifying lipid malabsorption and hypoproteinemia.

Nutritional recommendations in patients with CFLD:

– Increase in the caloric intake to 150% in comparison to the recommended daily necessary amounts by increasing mainly the lipid intake and rarely adding carbohydrates (glucose polymers), due to the risk of developing CF-related diabetes mellitus.

– The increase in the lipid intake by 40-50% to the daily caloric intake, by adding medium-chain triglycerides and carefully adding polyunsaturated fatty acids.

– Supplementing the protein intake to ensure 3g/kg/day in patients with no signs of liver failure.

– Ensuring the optimum dose of pancreatic enzymes to enable optimum absorption on long-chain triglycerides and essential fatty acids.

– Avoiding supplementing salt in patients with CF and cirrhosis and/or PHT to prevent ascites.

– Administrating liposoluble vitamins (with plasmatic monitoring to prevent toxicity or deficits):

- vitamin A: 5000-15000 UI/day per os
- vitamin E: alpha tocopherol 100-500 mg/day
- vitamin D – alpha calcidiol 50 ng/kg up to1 μg
- Vitamin K (inconsistent) 1-10 mg/day) (3).

In children with CF and anorexia, enteral nutrition is recommended. Gastrostoma contraindications are: esophageal varices or portal hypertensive gastropathy due to the risk of upper digestive bleeding.

2. Medical treatment

Treatment with ursodeoxicolic acid (UDCA)

The treatment with UDCA in children with CF must be initiated early after diagnosing liver damage to prevent the disease progression (Fig. 1). UDCA is a natural hydrophilic bile acid that increases the bile flow, moves the toxic hydrophobic bile acids, stimulates the bicarbonate secretion in the bile and has a general cytoprotective and immunomodulating effect on cholangiocytes. The daily dose recommended at first is of 20 mg/kg, divided in two or three sub-doses, having the possibility of increasing the dose subsequently due to intestinal malabsorption (3). The hepatic cytolytic and cholestatic markers must be tested after 3 and 6 months from the beginning of the treatment to test the efficacy of the dose, having the possibility of increasing it, if necessary. A prospective study done on patients with CF for 10 years highlighted the role of UDCA in stopping the progression of the early focal biliary cirrhosis lesions. Liver function tests had normal results after one year and no case of upper digestive hemorrhage has been reported (26).

The study done by Smith JL et al. revealed the protective role of the UDCA in patients with CF

without liver disease (27). Another study, did by Siano et al. demonstrated higher prevalence of CFLD in patients who presented meconium ileus and received treatment with UDCA at the debut of the liver disease, in comparison to patients with CF and meconium ileus who received UDCA as a prophylaxis measure and who developed the liver disease to a smaller degree. These data suggest that early treatment with UDCA may have an important role in preventing the emergence of liver damage in patients who had meconium ileus (28).

Portal hypertension treatment

In patients with CFLD, severe portal hypertension and hypersplenism raise problems when it comes to the treatment. The efficacy of the treatment with alpha-blockers has not been evaluated in CF due to the side effects of the alpha-blockers on the reactivity of the airways.

Variceal bleeding in patients with PHT needs sclerotherapy or endoscopic ligature. In some patients, variceal bleeding or portal hypertensive gastropathy need multiple therapeutic interventions. In a study done by Debray et al., 50 % of the children with CF who developed esophageal varices bled early in their second life decade (3). The initial intervention was upper digestive endoscopy with sclerotherapy or ligaturing (29). Prophylactic sclerotherapy is not beneficial in CF (30). In older children and adults with esophageal varices, ligaturing has replaced sclerotherapy as a first line treatment in preventing bleeding (29). Elastic bands are placed around the varices using a device attached to the end of the endoscope.

The transjugular intrahepatic portosystemic shunt (TIPS) is indicated in patients with refractory digestive hemorrhage, both as a long-term treatment for PHT and as a treatment while waiting for a liver transplant (29). Complications include TIPS dysfunction manifested through stenosis, occlusion or thrombosis. The technique and complications in children are comparable with the adults' (31).

Splenectomy or partial splenectomy (when the upper pole of the spleen is kept) have been done in patients with CF and variceal hemorrhage and/or important splenomegaly (3).

3. Liver transplant

The selection criteria for liver transplant in patients with CF take into account, besides liver failure and the progressive deterioration of the nutrition status, of the lung function, recurrent multi-resistant bacterial infections with multiple admissions. Therefore, the liver transplant indications in patients with CF are:

- Progressive liver dysfunction (albumin < 30 g/l, coagulation disorders corrected by administrating vitamin K) unresponsive to standard treatment
- Ascites and jaundice
- Variceal bleeding
- Presence of the hepatopulmonary and portopulmonary syndrome
- Severe malnutrition unresponsive to intensive nutritional support
- Deterioration of the life quality related to the liver disease
- Deterioration of the lung function (FEV1/FVC <50%) (3).

Before doing the transplant, the lung and heart functions must be evaluated in order to establish the necessity of the liver transplant or of the heart-lung-liver transplant. Still, the current data do not show satisfying results, the survival rates after one year and five years from the combined heart-lung-liver transplant were of 69% and 49% respectively (32).

CONCLUSIONS

Precocious liver complication in children with CF requires iontophoresis test in all patients with liver disease.The pathogenesis of the liver disease is multi-factor, with variable contributions by the environmental factors as well as possible individual genetic features. The improvement of the liver function influences life quality and increases the survival rate in patients with cystic fibrosis. The appropriate clinical management enables the early identification of the liver disease and consequently the introduction of the treatment with UDCA, which is currently the only accepted treatment. New therapeutic strategies are necessary to prevent cystic fibrosis and its progression before the development of its connected complications.

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