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Cystic fibrosis related liver disease: Research challenges and future perspectives

Dominique Debray[1], Michael R. Narkewicz[2], Frank A.J.A. Bodewes[3], Carla Colombo[4], Chantal Housset[5], Hugo R. de Jonge[6], Johan W. Jonker[7], Deirdre A. Kelly[8], Simon C Ling[9], Thierry Poynard[10], Philippe Sogni[11], Michael Trauner[12], Peter Witters[13], Ulrich Baumann[14], Michael Wilschanski[15], Henkjan J Verkade[3].

[1] Pediatric Hepatology Unit, APHP-Hôpital Necker and UPMC Univ Paris 06, INSERM, UMR_S 938, Centre de Recherche Saint-Antoine, Paris, France

[2] Digestive Health Institute, Children's Hospital Colorado and Section of Pediatric Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, University of Colorado School of Medicine Aurora, CO, USA

[3] Pediatric Gastroenterology and Hepatology, Dept. Pediatrics, Beatrix Children's Hospital / University Medical Center Groningen, University of Groningen, PO Box 30001, 9700 RB Groningen, The Netherlands

[4] CF Center, Fondazione IRCCS Ca' Granda; Ospedale Maggiore Policlinico, University of Milan, Italy

[5] Sorbonne Universités, UPMC Univ Paris 06, INSERM, UMR_S 938, Centre de Recherche Saint-Antoine, Institute of Cardiometabolism and Nutrition. Institut Hospitalo-Universitaire & Assistance Publique-Hôpitaux de Paris, Hôpital Saint-Antoine, Centre de Référence Maladies Rares des Maladies Inflammatoires des Voies Biliaires, Service d'Hépatologie, Paris, France

[6] Department of Gastroenterology & Hepatology, Erasmus University Medical Center, Rotterdam, The Netherlands

[7] Center for Liver, Digestive and Metabolic Diseases, Department of Pediatrics, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands

[8] The Liver Unit, Birmingham Children's Hospital, Birmingham, UK

[9] Division of Gastroenterology, Hepatology & Nutrition, The Hospital for Sick Children, Department of Paediatrics, University of Toronto, Toronto, Ontario, Canada

[10] Sorbonne Universités, UPMC Univ Paris 06, INSERM, UMR_S 938, Centre de Recherche Saint-Antoine, Institute of Cardiometabolism and Nutrition Institut Hospitalo-Universitaire & Assistance Publique-Hôpitaux de Paris, Hôpital Pitié-Salpêtrière, Paris, France

[11] Paris-Descartes, Sorbonne Paris-Cité University, INSERM U-1223 – Pasteur Institute, and Hepatology Unit, Assistance Publique – Hôpitaux de Paris, Cochin Hospital, Paris, France

[12] Division of Gastroenterology and Hepatology, Dept of Internal Medicine III, Medical University of Vienna, Austria

[13] Department of Paediatric Gastroenterology, Hepatology and Nutrition University Hospitals Leuven, Leuven, Belgium

[14] Dept of Paediatric Kidney, Liver and Metabolic Diseases, Division of Paediatric Gastroenterology and Hepatology, Hannover Medical School, Hannover, Germany

[15] Pediatric Gastroenterology, Hadassah Hebrew University Medical Center, Jerusalem, Israel

Address correspondence and reprint requests:

Dominique Debray, MD, PhD

Pediatric Surgery Department- Pediatric Liver Unit- Hôpital Necker-Enfants Malades

149 rue de Sèvres, 75015 Paris, France

Tel: +33 1 44 49 42 54 ; Fax: +33 1 44 49 42 00

E-mail: dominique.debray@aphp.fr

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List of abbreviations: CF, cystic fibrosis ; CFLD, CF-related liver disease; AST, aspartate aminotransferase ; UDCA, ursodeoxycholic acid; HSCs, hepatic stellate cells; PPARγ, peroxisome proliferator-activated receptor gamma; PMFs, portal myofibroblasts; HSC-MFs, HSC-derived myofibroblasts; FXR, farnesoid X receptor; GGT, gamma-glutamyl transferase; FGF, fibroblast growth factor ; TE, transient elastography ; LT, liver transplantation

Conflicts of Interest

Michael Trauner serves as a consultant for Albireo, Falk, Genfit, Gilead, Intercept, MSD, Novartis and Phenex and is a member of the speakers' bureau of Falk, Gilead, MSD and Roche ; he further received travel grants from Falk, Roche and Gilead and unrestricted research grants from Albireo, Falk, Intercept and MSD ; he is also co-inventor of a patent on the medical use of *nor*UDCA. Michael Narkewicz is consultant for Vertex and AbbVie, and received research grants from Vertex, Merck and AbbVie. Peter Witters is supported by the Clinical Research Foundation of UZ Leuven, Belgium. For the remaining authors, none are declared.

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ABSTRACT

Objectives. Hepatobiliary complications are a leading cause of morbidity and mortality in cystic fibrosis (CF) patients. However, knowledge of the underlying pathological aspects and optimal clinical management is sorely lacking.

Methods. We provide a summary of the lectures given by international speakers at the ESPGHAN monothematic conference on CF-related liver disease (CFLD) held in Paris in January 2016, to discuss the status of our current knowledge of liver disease in CF patients, to define the critical areas that need to be addressed and to resolve actions to elucidate relevant mechanisms of disease to optimise future therapeutic options.

Conclusions. The need for a universal consensus on the definition of CFLD to clarify disease stage and to identify relevant biomarkers to assess disease severity was highlighted. A deeper understanding of the pathophysiology and prognostic factors for the long-term evolution of CFLD is fundamental to move forward, and has a strong bearing on identifying potential treatments. Novel experimental models and new treatment options under investigation are discussed and offer hope for the near future of CFLD.

Keywords: cirrhosis; portal hypertension; biomarkers; bile acids; therapy

What is known?

- CFLD is characterized histologically by biliary obstruction, inflammation and focal biliary cirrhosis, with progression to multilobular cirrhosis in a minority of patients.
- Hepatobiliary complications are a leading cause of morbidity and mortality.
- CFLD is frequently asymptomatic and has a wide spectrum of manifestations, leading to challenges in early diagnosis.

What is new ?

- The monothematic meeting on CFLD was a catalyst to obtain a transatlantic agreement from major professional associations (ESPGHAN, NASPGHAN) for a consensus definition and classification (likely in 2017-2018) which will be critical for addressing epidemiology, and natural disease course.
- This agreement will be useful, if not essential, to assess effects on CFLD of novel therapeutic strategies, including bile salt analogues, CFTR correctors and potentiators, and antifibrotics.
- Key research directions were identified including addressing the mechanisms behind inflammation and fibrosis, the anti-inflammatory/anti-fibrotic properties of drugs targeting nuclear receptors, the role of the microbiome, and longitudinal studies to optimize the management of oesophageal varices and the timing of liver transplantation.

INTRODUCTION

With improvements in treatment of the pulmonary complications of cystic fibrosis (CF), gastrointestinal disorders have acquired an increasingly prominent place in terms of CF-related morbidities. In 2014, CF-related liver disease (CFLD) was the third most common cause of mortality after cardiorespiratory and transplant-related causes, responsible for approximately 2.8% of deaths (1). European guidelines recommend annual screening for liver involvement with abdominal examination by a gastroenterologist, biochemical evaluation and ultrasonography (2). The only medical therapy currently widely used for CFLD, despite the lack of long-term randomized controlled trials and proven long-term efficacy, is the natural bile acid, ursodeoxycholic acid (UDCA) (3).

Three areas of development are expected to have major influence on the prevention and treatment of CFLD: 1) experimental models allowing for pathophysiologic and mechanistic studies to identify possible targets for intervention, 2) biomarkers and non-invasive tools to measure liver fibrosis, and, 3) novel therapies including CFTR correctors or potentiators.

The first ESPGHAN CFLD conference held in Paris, France in January 2016 (see Supplemental Digital Content, Program, http://links.lww.com/MPG/B41) brought together international experts to review gaps in current knowledge, and define critical areas to address including changes in registry data collection, non-invasive monitoring of progression of liver disease, and relevant disease mechanisms to optimise future therapeutic options.

CLINICAL ASPECTS

Spectrum of hepatobiliary disease in CF

The phenotypic expression of CFLD is extremely heterogeneous in terms of the type and severity of alterations. Focal biliary cirrhosis is reported in up to 70% of CF patients in autopsy studies,

although it progresses to multilobular cirrhosis in 5 to 10% of patients, generally by the end of the first decade of life (4). Mild steatosis is reported with a wide range of frequencies (10 to 70%) but without a proven relationship to developing liver fibrosis. The frequency of biliary manifestations of CF, such as sclerosing cholangitis, microgallbadder, gallbladder dyskinesia, and symptomatic cholelithiasis remains unclear. Obliterative portal venopathy was recently recognized in a subset of CF patients with non-cirrhotic portal hypertension, however its cause remains obscure (5).

Towards a consensus definition of CFLD

With its frequently asymptomatic presentation and wide spectrum of manifestations, CFLD can be very difficult to diagnose in the early phases of development and might be largely underdiagnosed. This increases the risk that important aspects of this disease are ignored and that minor transaminase elevations are over interpreted. A key issue is that the diagnosis of CFLD should be made after other causes of liver disease are excluded. There are currently two widely adopted definitions of CFLD (2, 6), often leading to epidemiological confusion. In North America, CFLD is defined on the basis of the presence of liver cirrhosis and portal hypertension, or liver involvement denoted by persisting or intermittent liver enzyme elevations, steatosis, fibrosis, cholangiopathy and/or ultrasound abnormalities (6). According to the European definition, at least two of the following conditions must be present after exclusion of steatosis: hepatomegaly, liver enzyme abnormalities in at least at three consecutive determinations over 12 months, or evidence of liver disease or portal hypertension by ultrasonography (2).

It is thus urgent to agree on a common definition of CFLD .along with standardized evaluations of pathology and ultrasound data to ensure harmonized international data. Given the current diagnostic tools at our disposal, we believe that a phenotypic characterization of the liver involvement outlined in Table 1 is the most pertinent for clarity in reporting. As the average life expectancy of CF patients increases, the paediatric focus of epidemiologic investigations must extend to encompass the adult population using a similar classification.

A NASPGHAN/ESPGHAN consensus meeting will be held in 2017 to define a common definition of CFLD including different phenotypes for epidemiological purposes, long-term outcomes, and selection of patients for inclusion in therapeutic studies. This will also serve as the basis for the critical assessment of new diagnostic tools such as elastography and help define the essential screening tests to perform, their frequency and timing of implementation.

Optimizing biomarker use

Relevant biomarkers are important to identify patients at risk of bile duct injury, cirrhosis or portal hypertension, and to assess the effects of novel therapies. Such biomarkers are however largely lacking,.Existing biomarkers are often limited by a lack of sensitivity or specificity, or are only applicable in late disease phases. Whilst abnormal liver enzymes are common in CF, data are conflicting as to whether this correlates with the presence or severity of CFLD (7). Persisting elevated gamma-glutamyl transferase activity (GGT) (8) and heterogenous increased liver echogenicity at ultrasound may identify patients at risk of progressive liver disease (9). But a normal ultrasound does not preclude significant liver fbrosis (10). The AST to Platelet Ratio Index (APRI) is reliable at predicting severe fibrosis, but not for differentiating fibrosis at earlier stages (11). Serum biomarkers under investigation include circulating microRNAs (12), and biomarkers of intestinal bile salt malabsorption such as plasma fibroblast growth factor 19 (FGF19) and 4-cholesten-3-one (C4), an intermediate of bile acid synthesis and a marker of Cyp7a activity (13).

Non-invasive methods to quantify liver fibrosis such as transient elastography (TE) measuring liver stiffness may be useful in assessing early diagnosis and progression of liver fibrosis, but better quality studies and further validation are still needed particularly for the diagnosis of mild to moderate fibrosis (14, 15). Liver stiffness evaluated with ultrasound may be more accurate for assessing early stages of fibrosis and warrants further investigations (16). Hepatic steatosis and necroinflammatory activity may be confounding factors for liver stiffness assessments. This

highlights the need for concurrent biomarkers of necro-inflammatory histological activity and steatosis, or a controlled attenuation parameter.

TREATMENT ASPECTS

Multilobular cirrhosis is the most clinically-relevant CFLD lesion, given the possibility of progression to portal hypertension and related complications (17, 18). Long -term outcomes lack clarity and large prospective multicentre studies are needed to determine the optimal therapeutic management for portal hypertension and the indications for liver, lung or lung-liver transplantation when CFLD is present.

Prevention of oesophageal variceal bleeding

In CF children with portal hypertension, variceal bleeding is relatively rare in terms of episodes per patient years, and is associated with low mortality, but with significant morbidity (including ascites and infections) (19-21). The risk of bleeding may continue to arise in adulthood (17). Non-invasive means to identify children at risk of variceal bleeding include predictive scores based on simple biomarkers such as platelet count and spleen size (22). TE of the liver or spleen may improve bleeding prediction (23).

Although primary variceal prophylaxis has proven benefit for adults with non-CF cirrhosis, evidence of its safety and efficacy in CF patients is still lacking. Nonetheless, many centres offer endoscopic screening and treatment of large varices (2). Endoscopic variceal ligation requires intensive physiotherapy and intravenous antibiotics before anaesthesia, but is generally preferred over non-selective beta-blockers due to concerns over poor tolerance and bronchoconstriction. Recurrent variceal bleeding is managed on a case-by-case basis. Options include repeated endoscopic variceal ligation, a transjugular intrahepatic portosystemic shunt, a surgical portosystemic shunt, or liver transplantation (LT).

An important consideration when proposing a program for endoscopic screening and primary prophylaxis of variceal bleeding in patients with CF is the safety of repeated general anaesthesia. Prospectives studies to assess screening methods for early identification of oesophageal variceal, and the indications, optimal timing, and benefits of different therapeutic interventions are needed.

The role of liver or combined liver and pancreas transplantation

LT is an effective therapeutic option for CF patients with end-stage liver failure, treatment resistant and complicated portal hypertension (2). Survival after LT (median 10-year survival of 80%) is similar to that in other groups of children but the long-term outcome is dependent on other CF manifestations (24, 25). Timing is clinically relevant as poor growth and nutritional status are associated with deteriorating lung function and increased post-transplant mortality. Pre-emptive LT before significant and irreversible pulmonary and nutritional deterioration have developed in CF children with cirrhosis but no liver failure remains controversial. Studies in children and adults are required to better define when LT is indicated depending on the presence of portal hypertension.

A consensus on the indication of combined pancreas and LT in CF is yet to be clearly established although it may potentially restore endocrine and exocrine function improving diabetes control and nutritional status. An analysis of United Network Organ Sharing data from 1987 to 2014 showed low rates (<1%) of pancreas transplants (with or without liver, lung and kidney transplant) in the CF population, despite generally encouraging outcomes (26). In a recent poll of 50 paediatric transplantation centres, 94% reported that they would consider combined liver/pancreas transplantation for CFLD with diabetes, 50% for CFLD with glucose intolerance, and 24% for CFLD with pancreatic insufficiency (27).

CFLD PHYSIOPATHOLOGY

A thorough understanding of the complete pathologic process is fundamental to driving therapeutic development.

Questioning current theories

The primary hypothesis for CFLD aetiology revolves around decreased or absent CFTR function in the bile duct, causing decreased chloride, bicarbonate and osmotically-coupled water transport into the bile, leading to increased viscosity, reduced bile flow and increased bile salt accumulation. Abnormalities in mucin secretion may also contribute to increased bile viscosity, and retention of endogenous hydrophobic bile acids may be responsible for cell membrane injury. This in turn causes inflammation and collagen deposition around the bile ducts and portal tracts, leading to focal biliary and peri-portal fibrosis which in a minority of patients progresses to multilobular cirrhosis with portal hypertension.

However, these hypotheses are not solidly supported by histological findings. Bile inspissation is surprisingly infrequent (<7% of CFLD patients) and does not correlate with the degree of fibrosis (28), while electron microscopy does not show evidence of cholestasis (29). The presumption that this leads to bile duct inflammation has also been questioned as often only sparse inflammatory cells are seen around bile ducts (28, 29), although periductal fibrosis is encountered in most patients.

The gut-liver axis theory

Data in animals with CFTR defects as well as in CF patients supports differences in the microbiome between CF and non-CF subjects (30, 31). Several factors predispose CF patients to dysbiosis such as prolonged small bowel transit, frequent antibiotic exposure, and small intestinal bacterial overgrowth. The role of the microbiome and whether it plays a truly causative part or is a secondary effect remains to be clarified. Elucidating potential mechanisms of action of adsorbed bacterial products and lipotoxins in the development of CFLD is critical. From a clinical

perspective, multi-centre longitudinal microbiome studies in CF patients at higher risk for developing liver disease are needed and should include collection and centralized analysis of the faecal microbiome. Large international serum and plasma biobanks are needed for measuring markers of bacterial translocation (e.g., lipopolysaccharides).

Valuable lessons from animal models

Although the majority of ongoing studies are in CF mouse models, the advent of new genome editing techniques means that CFTR null or mutant animals can now be created for any species.

- The role of bile acids in CFLD pathogenesis

Faecal bile acid loss is increased in in CF mouse models, indicating decreased intestinal reabsorption (32). Faecal loss is compensated by increased hepatic synthesis of the primary bile acids, cholic and chenodeoxycholic acid and, indeed bile acid composition in CF mice has a higher cholate contribution than controls leading to higher hydrophobicity (33). However bile cytotoxicity in CF mice with hepatobiliary pathology is unaltered suggesting that this does not impact development of CFLD (32). These findings, together with the above-mentioned CFLD histological features, bring the research focus towards other potentially contributing disease mechanisms, including the effects of CFTR deficiency on cellular processes within cholangiocytes and the activation of hepatic stellate cells (HSCs), the drivers of hepatic fibrogenesis.

- Cellular and molecular mechanisms behind fibrosis

Fibrosis is thought to develop in response to cholangiocyte cytotoxicity and resulting inflammation. HSCs are the major source of myofibroblasts in the injured liver, although mesenchymal cells, distinct from HSCs and located in the portal tract, can also give rise to myofibroblasts referred to as portal myofibroblasts (PMFs) (34). PMFs outnumber HSC-derived myofibroblasts (HSC-MFs) at early stages of biliary-type liver fibrosis (35) and express several genes at higher levels than HSC-MFs including collagen type XV alpha 1. *In vivo* and *in vitro*

findings in a rat model of biliary cirrhosis indicate that PMFs signal endothelial cells via vascular endothelial growth factor A - containing microparticles, and act as mural cells for newly formed vessels driving scar progression from portal tracts into the parenchyma (35). This pattern of fibrosis progression from portal tracts with PMFs playing a key role is particularly relevant in CF-related liver fibrosis and requires further investigation.

- Defective peroxisome proliferator-activated receptor gamma signaling

Several members of the nuclear receptor superfamily can suppress inflammation by transrepression, interfering with pro-inflammatory transcription factors via protein-protein interactions (36). Nuclear receptors also appear to have a potential role in the reversible wound-healing process of fibrosis. CFTR-deficient mice have defective nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) signaling and administration of the synthetic PPARγ ligand rosiglitazone partially normalizes the altered gene expression pattern and reduces disease severity (37). Stimulation of PPARγ with rosiglitazone significantly attenuates biliary damage and inflammation in CFTR-knockout mice with induced portal endotoxemia (38). Thus, impaired PPARγ activation has a contributing role in the chronic inflammatory state of CFTR-defective cholangiocytes, representing a potential target for controlling biliary epithelium inflammation.

- Lessons from the CF pig model

Animal-specific differences may provide insights into the underlying mechanisms of CFLD. More than 50% of all CFTR null and F508del pigs are born with clear CFLD symptoms and all new-born CF pigs have a micro-gallbladder (39). Possible explanations for early onset and severe CFLD in newborn CF pigs versus mild or absent CFLD in CF mice are the abundance of compensatory calcium-activated chloride channels in CF mice gallbladders relative to pig gallbladders or species differences in the expression of other bicarbonate transporters or apical proton channels or pumps (40). Verification of a causal relationship is urgently needed, e.g. by

creating an inducible, biliary-specific TMEM16A (encoding the Ca2+ activated Cl- channel)/CFTR double knockout mouse model to study the predicted development of CFLD and approaches to reverse this phenotype.

In contrast to CF pigs, the low prevalence and late onset of CFLD in humans suggests that factors other than bile duct defective CFTR function contribute to liver disease. Pathophysiologic mechanisms must explain why only about one-third of CF patients develop liver disease and less than 10% severe liver disease. CF patients who carry the SERPINA 1 Z allele appear at greater risk of developing severe liver disease with portal hypertension (41).The identification of genetic modifiers for liver disease is a research priority, as it may allow early identification of patients at risk who might benefit from prophylactic strategies.

FUTURE MEDICAL TREATMENT APPROACHES

Investigational CFLD drug candidates

It is critical that potential therapeutic agents are evaluated with well-designed randomized clinical studies, large sample sizes and long-term follow-up (≥10 years), ensuring that the populations most likely to benefit from treatments are identified to minimize unnecessary exposure in patients unlikely to benefit.

A number of investigational avenues targeting different aspects of liver disease are being explored.

- Bile acid analogues: NorUDCA (a side chain-shortened homologue of UDCA with one less methylene group) undergoes cholehepatic shunting leading to a bicarbonate-rich hyperchloresis, has direct anti-inflammatory, anti-fibrotic and anti-proliferative properties, and stimulates alternative bile acid detoxification and elimination routes. It has shown encouraging effects in the clinic improving serum liver tests in primary sclerosing cholangitis, an immune-mediated liver disease with biliary morphological similarities to CFLD, although antibiotics, but not norUDCA, reduced biliary injury in a CF mouse model (42, 43).

- FXR agonists : FXR (a nuclear receptor) suppresses bile acid synthesis via FGF19, stimulates bile acid and bicarbonate secretion, modulates fibrosis and regulates lipid and glucose metabolism (44). An alternative approach is to modify bile acid enterohepatic transport by inhibiting ileal ASBT, although the enterohepatic bile acid circulation may be altered in CF.

- FGF1 was recently identified as a PPARγ target in visceral adipose tissue and is critical to adipose remodelling (45). FGF1 improved hepatic inflammation, steatosis and damage in leptin-deficient ob/ob and choline-deficient mice, two etiologically different NAFLD models (46).

- Vitamin D receptors (VDRs) also offer potential for treatment intervention with their activation implicated in preventing hepatic fibrosis involving TGFβ1 signalling via pro-fibrotic genes (47).

Targeting the causative CFTR defect

- The CFTR potentiator ivacaftor that enhances chloride transport of CFTR on the cell surface was developed by high-throughput screening, giving impressive clinical improvement in pulmonary function and in exocrine pancreatic function in individuals with gating mutations. Few, if any, CFLD-specific analyses have been published, probably due to liver disease being an exclusion criterion in all CFTR modulator trials because of abnormalities in liver enzymes occurring in a few patients during treatment (48). Only one case report documented an improvement in hepatic steatosis (49).

- Combination therapy with lumacaftor, a CFTR corrector that increases trafficking of phe508del CFTR to the cell surface, and ivacaftor is currently used for deltaF508 homozygous patients but once again the effect on CFLD is unknown (50). A Phase 3 study is underway with ataluren, a drug allowing read-through of CFTR defects with a premature termination codon (51).

Prospective therapies focusing on high-risk groups and a better understanding of the genetic profiles of CFLD patients are needed.

In conclusion, agreement on definitions and classification of CFLD is critical for investigating epidemiology, natural history, and response to interventions. Characterizing biomarkers for

specific categories of liver involvement and age groups is an important research axis that needs to be urgently addressed to determine the effects of novel therapeutic strategies on CFLD, including bile salt analogues, antifibrotics, and CFTR correctors and potentiators. Underappreciated aspects of disease management to take into consideration in research and clinical management were highlighted. Studies to improve management of oesophageal variceal bleeding and to define the optimal timing of liver transplantation or combined liver-pancreas transplantation are needed. Classic views of pathophysiological mechanisms underlying CLFD are under question, prompting interest in novel areas including the mechanisms behind inflammation and fibrosis, the anti-inflammatory/anti-fibrotic properties of drugs targeting nuclear receptors, and the role of the microbiome.

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Table 1: Proposed "phenotypic" reporting of CFLD, as modified from Flass & Narkewicz (6).

Phenotypic characterization of liver involvement

1. **Multilobular cirrhosis** by imaging [ultrasound, CT, MRI], biopsy, direct visualization, or elastography [at the extremes of staging]
    a. Without portal hypertension
    b. With portal hypertension (to describe by one or more of the underlying features)
        i. Hypersplenism (platelets <150,000 10^9/L and WBC <3,000 10^9/L) with splenomegaly
        ii. Oesophageal or gastric varices (imaging, endoscopy)
        iii. Ascites
        iv. Encephalopathy
    c. With liver failure (INR > 1.5 X normal; vitamin K resistant)
2. **Liver involvement without cirrhosis** (to describe by one or more of the underlying features)
    a. Abnormal ALT (1.5 X upper limit of normal)
        i. Persistent (2 or 3 consecutive measurements over >6 months above threshold)
        ii. Intermittent
    b. Abnormal GGT
        i. Persistent (2 or 3 consecutive measurements over >6 months above threshold)
        ii. Intermittent
    c. Imaging abnormalities
        i. Ultrasound heterogeneous increased echogenicity pattern
        ii. Ultrasound homogeneous increased echogenicity pattern
    d. Hepatic steatosis (biopsy proven)
    e. Hepatic fibrosis (biopsy proven)
    f. Hepatomegaly
    g. Portal hypertension
        i. Hypersplenism (platelets <150,000 10^9/L and WBC <3,000 10^9/L) with splenomegaly
        ii. Oesophageal or gastric varices (imaging, endoscopy)
3. **No evidence of liver involvement** (Normal exam, imaging, ALT and GGT)