Chapter 6.3. Studies on Hepatic Metabolic Disorders Driven by ESPGHAN Members: The Case of Alpha1-Antitrypsin Deficiency, Cystic Fibrosis, and Urea Cycle Defects

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Cystic fibrosis (CF), alpha1-antitrypsin deficiency (A1ATD), and urea cycle defects (UCD) are well known inborn metabolic disorders characterized by specific abnormalities that may severely affect one or more organ systems, including the liver. Hepatic involvement may be and/or became the overriding problem burdened by difficult management. As there are not completely effective medical therapies, it may require sooner or later liver transplantation. In the last 3 decades many efforts have been made by ESPGHAN members in getting further insights and improved clinical management of these rare hepatic metabolic disorders.

CYSTIC FIBROSIS

CF (OMIM #219700) is a genetic multiorgan disorder caused by mutations in the CF conductance regulator gene (CFTR), located on chromosome 7. The identification of CF gene was published for the first time in 1989. CF manifestations are related to the disruption of exocrine function of the pancreas, intestinal glands, biliary tree, bronchial glands, and sweat glands; infertility occurs in males and females [1]. Biliary obstruction and progressive periportal fibrosis resulting from lack/dysfunction of the CFTR at the apical membrane of bile duct cells determine a focal biliary cirrhosis. Extension of the initially focal fibrogenic process may lead to multilobular biliary cirrhosis, portal hypertension and eventually liver failure. Why some patients develop liver disease (LD), sometimes with progressive outcome is currently still uncertain [2,3].

CF-related Liver Disease

In a cohort of 241 CF children, Thierry Lamireau and colleagues showed that cystic fibrosis-related liver disease (CF-LD) occurred mainly in the first decade of life (prevalence 41% at age 12 years) [4]. Although LD does not influence the clinical course of CF, in some patients it may progress rapidly and require LT. In an Italian series of 177 CF patients without LD, Carla Colombo described during a 14-year follow-up, 48 patients (mainly with history of meconium ileus, male sex, or severe genotype) that developed subsequent LD, 5 with cirrhosis [5]. In 1990, an Italian study coauthored by Pietro Vajro, demonstrated that the gallbladder hypokinesia with impaired/slower emptying predisposes CF patients to gallstones even in early childhood [6]. Based also on these clinical premises, 3 recent studies, conducted on CFTR knockout Cftr−/− mice and wild-type controls, shed light on the pathogenetic mechanisms of impaired biliary function in CF. Dominique Debray demonstrated that Cftr−/− and CftrΔF508 mice have defects in gallbladder emptying that disrupt enterohepatic circulation of biliary acids (BA) and create a cholecystohepatic shunt that restricts the amount of toxic secondary BA entering the liver [7]; in 2 studies coauthored by Henkjan Verkade it has been shown that LD in Cftr−/− mice is not related to increased bile hydrophobicity but probably to alterations in intestinal bacterial biotransformation of bile salts; smaller quantity of faecal Bacteroides bacteria found in Cftr−/− suggest Cftr dependent alterations in intestinal bacterial biotransformation of bile salts [8]. Furthermore, prolonged cholate exposure did not induce CF-LD in Cftr−/− mice [9]. Further studies in Cftr−/− mice authored by Dominique Debray have suggested that a high fat diet has a critical impact, mainly via gut dysbiosis, on the emergence of CF-related bile duct injury [10].

Cirrhosis in CF patients has been shown to be significantly associated with either homozygous or compound heterozygous mutations in the *MBL2* gene encoding mannose-binding lectin [11,12]. In a 2-stage case-control multicentric study coauthored by Carla Colombo, Dominique Debray and Florence Lacaille, it has been shown that SERPINA1 Z allele is a risk factor (odds ratio = approximately 5) for LD in CF with portal hypertension [13], adding therefore an important piece of jigsaw by clarifying that a rare variant with large penetrance (such as the Z allele) may be more useful than a common variant with low penetrance in screening for genetic polymorphism.

Diagnosis and Management of Advanced CF-LD

In the last 25 years the paediatric hepatology research made important progress in the evaluation and management of CF-LD. An interesting collaborative study coauthored by several ESPGHAN members [14] led to recommend an updated clinically useful diagnostic workup (including the more recent fibroscan assessment) and the therapeutic and surgical management of oesophageal varices, integrating previous guidelines [15]. Surgical portosystemic shunting may be considered to relieve portal hypertension in CF-LD patients without progressive liver failure and severe lung disease as an alternative to LT [15].

Pietro Vajro proved that correct diagnosis of cirrhosis in CF patients may be difficult unless liver biopsy is made under laparoscopy control [16]. Among serological markers of advanced CF-LD persistently high GGT levels [17], and serum hyaluronic acid concentrations [18], have shown quite specific features of increased risk of cirrhosis and/or hepatic fibrogenesis. These studies were

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coauthored by Henkjan Verkade [17], Anil Dhawan and Giorgina Mieli-Vergani [18], ESPGHAN members.

In a multicenter study coauthored by Carla Colombo, severe LD was shown to significantly increase the risk of developing CF-related diabetes thus representing a red flag for earlier diabetes screening to identify and possibly treat glucose intolerance [19].

UDCA exerts in mice a choleretic effect and influences the bile salt fractional turnover rate and profile with a more hydrophilic bile salt pool independent of the presence of functional CFTR [20]. Early UDCA treatment revealed beneficial in patients at risk of developing CF-LD, eg, those with meconium ileus [21]. Based on results of a large double-blind multicenter trial conducted by Carla Colombo, UDCA appeared to improve clinical and biochemical parameters. Brigitta Strandvick group in Sweden demonstrated the efficacy of 2 years UDCA treatment with liver biopsies and liver function tests in 10 CF-LD patients aged from 8 to 28 years. This study showed that UDCA modulates inflammation in CF-LD and improves liver morphology [22]. Three retrospective studies conducted or coauthored by a number of ESPGHAN members [23–25] revealed that the common indications for LT in CF patients are hepatic failure and/or severe portal hypertension with well-preserved pulmonary function. In these conditions, LT is also associated with long-term beneficial effects on the nutritional status and seems to favour bone mineralisation. [26]. Deirdre Kelly and Carla Colombo coauthored the first European large survey on LT in patients with advanced CF-LD. This study highlighted that liver failure, hypersplenism, gastrointestinal bleeding and malnutrition represented the major indications for LT and that most European liver centres perform LT before the development of end-stage liver disease or overt pulmonary or other clinical decompensation [27]. Furthermore, en bloc liver-pancreas transplantation could be an appealing option since it concurrently restores exocrine function and prevents insuline-dependent diabetes [25]. In January 2016 during the ESPGHAN CF monothematic conference in Paris it has been discussed the need for a universal consensus on the definition of CF-LD to clarify disease stage and to identify relevant biomarkers (eg, biomarkers of intestinal bile salt malabsorption) to assess disease severity. Future medical treatment approaches have been also evaluated. CF-LD drug candidates other than drug targeting the causative CFTR (ie, CFTR potentiator ivacaftor and combination therapy with lumacaftor, a CFTR corrector) are: bile acid analogues, Farnesoid X receptor (FXR) agonists, fibroblast growth factor 1, and vitamin D receptors [28].

ALPHA1-ANTITRYPSIN DEFICIENCY

A1ATD (OMIM #613490) is not a rare disease but a disease that is rarely diagnosed [29]. It is an autosomal recessive disorder characterised by both liver and lung injury. Pulmonary manifestations become evident in adults, whereas LD occurs already in paediatric age. Mutation incidence is around 1:2500 live birth in Europe [1]. In Sweden, the prevalence of A1ATD (PiZZ) is around 1:1600. This prevalence was established by a screening program of all 200,000 newborns during the period 1972–1974 [30]. The pathogenesis of cholestatic LD remains to be largely clarified, although liver injury in ZZ genotype is due to thoroughly investigated toxic effects of the abnormal A1AT molecule accumulating within the endoplasmic reticulum of liver cells.

In a Swedish series of neonatal cholestasis, Björn Fischler showed that A1ATD together with progressive familial intrahepatic cholestasis was the second most common diagnosis after extrahepatic biliary atresia, contrary to other countries where A1ATD is reported to be a rarer cause [31]. Only 12% to 15% of individuals with ZZ genotype develop LD due to the influence of other genetic and epigenetic/behavioural factors which determine this susceptibility [32]. The role of rare allelic variants in chronic liver disease is even less clear; heterozygous rare allelic variants may result in transient early onset hepatopathy as recently reported by Pietro Vajro [33]. Deficiency state of another serine proteinase inhibitor, namely alpha 1-antichymotrypsin, may predispose to obstructive lung disease and influence the course of LD [34]. As an example, in a case control study coauthored by Nedim Hadzic an identified single nucleotide polymorphism conferred a significant risk for LD. This was the first description of a genetic modifier of LD in homozygous ZZ children [35].

Miranda et al (Nedim Hadzic, coauthored this study) have developed a conformation-specific monoclonal antibody (2C1) that recognizes pathological polymers formed by A1AT and demonstrated that Z and shutter domain mutants of A1AT form polymers with a shared epitope likely having a similar structure [36].

In a retrospective study on 42 children with ZZ A1ATD coauthored by Alain Dabadie and Emmanuel Jacquemin, UDCA therapy improved clinical status and liver tests in some children with LD but not in those with most severe liver involvement. Elevated initial levels of GGT and total bilirubin have been shown of prognostic value for therapy effectiveness [37]. More recently, in a study coauthored by Ulrich Baumann it has been shown a positive correlation between simple laboratory parameters (number of platelets and common liver function tests) and LT and/or death [38]. The presence of arteriovenous anastomoses in the lungs (ie, hepatopulmonary syndrome) can be assessed by scintiscanning as in other chronic liver diseases [39]. In a retrospective study (n = 21 children; 1 with A1ATD) co-authored by Piotr Socha no correlation was found between the severity of hepato-pulmonary syndrome and the aetiology and stage of the underlying disease [39]. These combined series of useful information may considerably help the paediatrician to take decisions during patients' difficult follow-up.

Liver Transplantation in Alpha1-Antitrypsin Deficiency Children

LT in A1ATD patients with decompensated disease offers good long-term outcome [33,40], also in small babies [41], with excellent results achievable provided the reduction of waiting time list as reported by Anil Dhawan, Giorgina Mieli-Vergani and Nedim Hadzic [42]. In a tertiary care centre LT in A1ATD was the second common indication to LT (8.1%), preceded by biliary atresia (41.9%) [40]. This study was coauthored by Loreto Hierro and Paloma Jara. Based on results of a large retrospective study at King's College coauthored by Giorgina Mieli-Vergani and Nedim Hadzic, LT resulted to have significantly improved the prognosis of LD associated with PiZZ A1ATD. Duration of jaundice, severity of histological features and biochemical abnormalities predict outcome in early stage of the disease [43]. In PiZZ patients with portal hypertension, oesophageal varices, or deterioration of hepatic function, LT should not be delayed, as reported in a single centre retrospective study (n = 59 children homozygous for A1ATD) coauthored by Piotr Socha, ESPGHAN member [44].

UREA CYCLE DISORDERS

UCDs result from several defects in the metabolism of waste nitrogen derived from the breakdown of protein and other nitrogen-containing molecules. Severe deficiency or total absence of activity of any of the first 4 enzymes in urea cycle or the cofactor producer results in the accumulation of ammonia and other precursor metabolites. The incidence of UCD is estimated to be at least 1:35,000 births; partial defects may make the figure much higher [45,46].

Hepatic involvement, for example, liver steatosis initially, is a frequent finding [47] with more frequent severe damage in

argininosuccinate lyase (ASL) deficiency [48,49] and Ornithine transcarbamylase deficiency (OTCD). Episodes of hepatocellular injury, liver dysfunction, and acute liver failure have been identified in a high proportion of children with symptomatic OTCD. Two recent studies both coauthored by Etienne Sokal, described the initial presentation [48] and the evolving clinical phenotype [49] of 343 UCD compared to 452 patients with Organic acidurias (OA). It was found that neurological impairment is common in OAD and UCD, whereas the involvement of other organs follows a disease-specific pattern. Hepatic involvement was more frequent in UCD patients, in particular in ASL.

Methyl malonic aminoacidemia and propionic acidemia are the 2 main Organic Acidurias which present also hyperammonemia. Their clinical neonatal presentation is very similar, with neonatal period onset, when feeds containing protein have been started. If not recognized and treated promptly, OA patients progress to severe brain damage and death. Those presenting in the neonatal period have a worse prognosis. Immediate treatment is based on removal of toxic metabolites. Long-term treatment is dietary protein restriction and essential amino acid supplementation. Hyperornithinaemia-hyperammonaemia-homocitrullinuria syndrome (Mitochondrial Ornithine Transporter Deficiency) (OMIM #238970) is an autosomal recessive disorder caused by a defect in ornithine translocase. The clinical presentation is variable, including episodic hyperammonaemia, chronic neurological manifestations and hepatic derangement which may appear as a fulminant hepatitis-like condition. Its early recognition in the scenario of hepatic failure is important due to possible recovery without resorting to LT as reported by Pietro Vajro, in a case series of 3 children with fulminant hepatitis [50].

Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) also known as Neonatal-onset type II citrullinemia (OMIM #605814), is caused by homozygous or compound heterozygous mutation in the *SLC25A13* gene [1]. Mutations have a carrier rate of 1:65 in Japan and China, whereas they are much less frequent in the Western world. The same gene is involved also in the adult-onset phenotype (citrullinemia 2, OMIM #603471) which is mainly characterized by fatty liver and late recurrent hyperammonemic neurological disturbance due to the decreased associated liver-specific argininosuccinate synthetase (ASS) enzyme activity.

NICCD typically presents as a self-limiting neonatal intrahepatic cholestasis. Fatty liver, and increased serum citrulline may make it resembling an UCD. However, NICCD patients show a unique carbohydrate aversion in contrast to protein dislike seen in other UCD or in lysinuric protein intolerance. Fondness for proteins and lipids should therefore not be discouraged [51,52]. ESPGHAN members, Ulrich Baumann and Patrick McKiernan, reviewed this subject and reported 2 cases of NICCD children from UK, one Caucasian and one of Pakistan origin. Both had typical clinical and biochemical changes with a diagnosis confirmed by previously unreported mutations in the SLC25A13 gene. Citrin deficiency needs to be considered in the diagnosis of any neonate with an unexplained cholestasis independent from ethnic origin. MRI and spectroscopy will probably become a powerful tool to measure the effects of pharmacological neuroprotective approaches [53].

Transplantation in Urea Cycle Defect

In patients with severe types of UCD, LT also in case of normal liver function remains the most effective means of preventing further hyperammonemic crises; it is associated with excellent survival rate if performed before irreversible brain damage. Auxiliary partial orthotopic LT has shown encouraging results in UCD. Apart from neonatal onset of OTC deficiency which is a clear indication for LT, in all other UCD conditions the indication is based on the failure to maintain metabolic compensation with medical treatment as discussed in the article coauthored by Lorenzo D'Antiga [54,55]. Hepatocytes transplantation represents a more and more promising therapeutic approach for patients with liver-based metabolic disorders. Anil Dhawan and Etienne Sokal have pioneered in ESPGHAN the concept of hepatocyte transplantation as an alternative to LT in patients with liver-based metabolic disorders, focusing on protocols (isolation of human hepatocytes with collagenase perfusion, preparation in clean GMP conditions with cells meeting criteria of function and lack of microbial contamination, infusion of cells intraportally into the patient's liver) and on aetiologies of liver disease in which this technique has been used (including UCDs) [56]. The Brussel's cell transplant program has further demonstrated the long term engraftment of hepatocytes with de novo enzyme activity following hepatocyte transplantation [57], and has pioneered the second generation of cell therapy, using in vitro expanded liver derived stem cells as a new source of cells to treat patients with inborn errors of the urea cycle [58,59].

Even if LT has an excellent survival rate this is not exempt from peri- and post-operative complications. Hepatocyte cell transplantation instead avoids the risks of major surgery and can help bridge a patient to whole-organ transplantation or leave the option of gene therapy, if and when it becomes available in future.

The ability to reproducibly generate a well-characterized source of engraftable and functional liver cells remains a challenge. In this regard, the use of metabolic profiling (NMR spectroscopy of urine and plasma) could be a promising method for evaluating the efficacy of cell infusions and the capability for the early detection of response to therapy in real time. In 2009 it has been published the first successful hepatocyte transplantation as a bridge to auxiliary partial orthotopic LT in a child antenatally diagnosed with severe ornithine transcarbamylase deficiency. In this single patient study coauthored by Anil Dhawan, NMR spectroscopic profiles of urine and plasma has been used for the first time for evaluating the efficacy of cell infusions. NMR profiles indicated that the disrupted urea cycle could be normalized by hepatocyte cell infusion and this was confirmed using orthogonal partial least-squares-based chemometrics [60].

The limited availability of donor tissue implies the search for new resources of liver tissue for isolation of high-quality hepatocytes. The group of King's College with Anil Dhawan and Giorgina Mieli-Vergani suggested the use of segment IV with or without the caudate lobe obtained from split-liver procedures. Tissue-derived stem/progenitor cells and pluripotent stem cell-derived cells represent now a new opportunity to remove/reduce challenges of hepatocyte cells transplantation. [61].

Finally, cryopreserved cells have proved to allow short- to medium-term metabolic control and urea synthesis while waiting for OLT [62].

CONCLUSIONS

Several ESPGHAN members' studies in this group of metabolic based liver diseases have substantially contributed to shed light on several disease-mechanisms, understanding why some affected patients develop a progressive outcome and other do not, and evaluating old and new medical therapies.

For CF, A1ATD, and some UCD, LT has become an accepted treatment capable of correcting totally or in part, the metabolic defect while replacing the diseased organ, and sometimes may even improve pulmonary function (eg, in CF-LD). Correct timing for organ replacement has been an issue largely explored. In UCD, and in some OA it may be more difficult in phenotypes with a

(still) little or even absent liver injury, and potential involvement of other organ system. Ideally, LT should be considered as soon as possible when dietary and medical treatments cannot reliably prevent metabolic decompensation and damage of severe extrahepatic organs (eg, CNS) [63–65]. Auxiliary partial orthotopic LT, and hepatocyte cells transplantation appear feasible alternatives.

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