Clinical Practice Guidelines

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EASL Clinical Practice Guidelines on genetic cholestatic liver diseases☆

European Association for the Study of the Liver*

Summary

Genetic cholestatic liver diseases are caused by (often rare) mutations in a multitude of different genes. While these diseases differ in pathobiology, clinical presentation and prognosis, they do have several commonalities due to their cholestatic nature. These Clinical Practice Guidelines (CPGs) offer a general approach to genetic testing and management of cholestatic pruritus, while exploring diagnostic and treatment approaches for a subset of genetic cholestatic liver diseases in depth. An expert panel appointed by the European Association for the Study of the Liver has created recommendations regarding diagnosis and treatment, based on the best evidence currently available in the fields of paediatric and adult hepatology, as well as genetics. The management of these diseases generally takes place in a tertiary referral centre, in order to provide up-to-date approaches and expertise. These CPGs are intended to support hepatologists (for paediatric and adult patients), residents and other healthcare professionals involved in the management of these patients with concrete recommendations based on currently available evidence or, if not available, on expert opinion.

Introduction

These Clinical Practice Guidelines (CPGs) focus on several monogenic disorders causing defects in bile formation and/or flow. They each lead to an accumulation of bile acids and frequently conjugated (direct) bilirubin in the liver and systemic circulation. While these disorders most commonly manifest at a young age, advances in genetics have led to an increased detection of variants that may become apparent at a later age. Heterozygous carriers may display less overt symptoms and milder biochemical abnormalities, but often carry a predisposition for elevated serum liver tests, increased gallstone formation, episodic or chronic cholestasis, intrahepatic cholestasis of pregnancy (ICP), drug-induced liver injury (DILI), increased risk of hepatobiliary malignancies and progressive forms of cholestatic disorders upon aging. Finally, the widespread availability of genetic testing as well as family screening has led to the discovery of genetic variants of uncertain significance (VUS) that pose a great challenge in patient counselling. Among the covered disorders, alpha-1 antitrypsin deficiency (AATD) is somewhat unique as it may present as cholestasis, progressing to hepatitis-like liver disease, portal hypertension and cirrhosis in young children, while primarily causing pulmonary symptoms, including chronic obstructive pulmonary disease, in adults.[1,2]

Methods

The European Association for the Study of Liver Disease (EASL) Governing Board initiated these CPGs in July 2021 by selecting a panel of experts and describing the remit of the assignment. The development of these CPGs followed a standard operating procedure set out by EASL and meets the international standards for CPGs set out by the Guidelines International Network. The process involves identification of key questions pertinent to the subject matter. The CPG panel drafted questions according to the PICO format. P – patient, problem or population, I – intervention, C – comparison, control or comparator, O – outcome. PICO questions were vetted through a simplified Delphi process in the broader community including 32 physicians, scientists, patient representatives, and other stakeholders competent in the field of genetic cholestatic liver diseases beyond the CPG panel and the EASL Governing Board. This was followed by a systematic literature review process. A literature search was performed using PubMed, and expanded to Embase, Google Scholar and Scopus when needed. Additional articles were obtained through citation snowballing to locate primary sources. Each expert took responsibility and made proposals for statements for a specific section of the guideline and shared tables of evidence and text with the full panel. The panel met virtually on 12 occasions, and all recommendations were discussed and approved by all

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participants. The level of evidence was graded according to the Oxford Centre for Evidence-Based Medicine system (Table 1) and the strength of the recommendations was categorized as either 'weak' or 'strong' (Table 2). The higher the quality of the evidence, the more likely a strong recommendation was made. If no clear evidence was available, recommendations were based on the expert opinion of the panel members. All recommendations were subsequently approved through a second Delphi round and were ultimately brought to the attention of the EASL Governing Board for final approval.

General recommendations

Genetic testing

Genetic testing for cholestatic liver disease without a clear cause is recommended early in the diagnostic work-up in infants and children, and after exclusion of more frequent causes of cholestatic liver disease in adults (Fig. 1). In adults, testing is particularly recommended if there are unusual features or if they are unresponsive to initial treatment. In early onset disease, genetic variants may be the major determinant of the phenotype. In adult presentations, the association may be less direct. Next-generation sequencing (NGS) techniques such as panel sequencing and whole-exome sequencing (WES) are recommended. Since genetic variants in a number of different genes can present with a similar phenotype, Sanger sequencing of individual genes is now discouraged, perhaps with the exception of *ABCB4* gene testing in (young) patients with small duct primary sclerosing cholangitis.[3] In the case that WES analysis does not identify mutations likely responsible for the phenotype of the patient, one could consider whole-genome sequencing (WGS). Both WES and WGS may allow for reanalysis of the data once novel cholestasis-associated genes are identified. We recommend to re-analyse the data at least every 3 years to check for newly discovered variants in patients who did not receive a diagnosis after the first round of testing. Genetic counselling must precede genetic testing and handling of incidental findings should be discussed in advance.

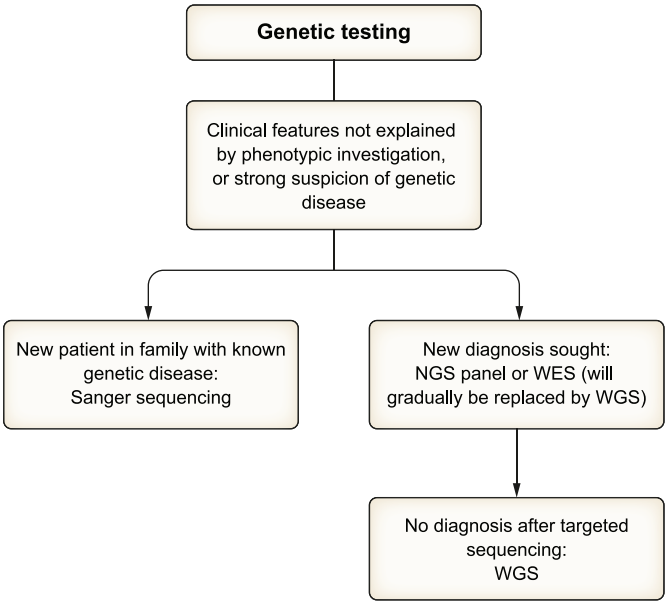

Fig. 1. Genetic testing for cholestatic liver disease. NGS, next-generation sequencing; WES, whole-exome sequencing; WGS, whole-genome sequencing.

Table 1. Level of evidence based on the Oxford Centre for Evidence-based Medicine.

Level	Criteria	Simple model for high, intermediate and low evidence
1	Systematic reviews (SR) (with homogeneity) of randomised-controlled trials (RCT)	Further research is unlikely to change our confidence in the estimate of benefit and risk
2	RCT or observational studies with dramatic effects; SR of lower quality studies (*i.e.* non-randomised, retrospective)	
3	Non-randomised-controlled cohort/follow-up study/control arm of randomised trial (systematic review is generally better than an individual study)	Further research (if performed) is likely to have an impact on our confidence in the estimate of benefit and risk and may change the estimate
4	Case-series, case-control, or historically controlled studies (systematic review is generally better than an individual study)	
5	Expert opinion (mechanism-based reasoning)	Any estimate of effect is uncertain

Table 2. Grades of recommendation.

Grade	Wording	Criteria
Strong	Shall, should, is recommended. Shall not, should not, is not recommended.	Evidence, consistency of studies, risk-benefit ratio, patient preferences, ethical obligations, feasibility
Weak or open	Can, may, is suggested. May not, is not suggested.	

According to the American College of Medical Genetics and Genomics guideline, mutations are classified based on their likelihood of impacting on disease phenotype.[4] While classes 1 and 2 comprise likely benign variants, class 3 denotes VUS. A large number of variants detected in patients with cholestatic liver disease fall into this category, which indicates that it is unclear whether the variant may contribute to the phenotype of the patient. *In silico* prediction tools, testing of affected and unaffected family members, *in vitro* experimental analysis as well as emergence of further data from other families may help to determine whether the novel variant contributed to the phenotype and should be upgraded to 'likely pathogenic' (class 4) or 'pathogenic' (class 5).

The severe forms of each disease have been most intensely studied and, in the cases of familial intrahepatic cholestasis type 1 (FIC1) and bile salt export pump (BSEP) deficiency, are largely referred to in these guidelines. Relapsing disease has been described in patients with FIC1, BSEP, multidrug resistant resistance protein 3 (MDR3), TJP2 (tight junction protein 2) and USP53 (ubiquitin specific peptidase 53) deficiencies. Genetic testing by means of WES (or WGS) now allows for the identification of variants in individuals who may develop cholestatic disease later in life.

In late onset disease, in particular, the phenotype results from the interaction of one or more variants with environmental factors, such as drugs, pregnancy, infections, or other acquired causes of liver diseases.[5–7]

For the severe forms of each disease, a wide variety of treatments have been described. These include pharmacological treatments, most recently the medical interruption of the enterohepatic circulation (EHC), as well as temporary or permanent depletion of the bile acid pool via naso-biliary drainage or surgical interruption of the EHC by partial internal or external biliary diversion. Patients with milder or recurrent forms of these diseases have been excluded from clinical trials and most retrospective studies. However, it seems reasonable that strategies for their management are based on those applied for the more severe forms of each disease, but comprehensive evidence is lacking.

Pruritus management

The management of pruritus is challenging. Pruritus is also one of, if not the, most debilitating symptoms of many of the diseases featured herein. Hence, we offer a comprehensive approach to pruritus treatment that can be applied across all cholestatic liver diseases, separately for children and for adults (Fig. 2, Table 3). Where possible these recommendations are based on scientific evidence, however, due to the lack of high-quality studies, many suggestions are based on expert opinion and consensus of the CPG committee.

The present CPG has concentrated on the following diseases: AATD, Alagille syndrome (ALGS), FIC1 deficiency (PFIC1), BSEP deficiency (PFIC2), MDR3 deficiency (PFIC3 and other associated phenotypes), and bile acid synthesis defects (BASD). These CPGs do not include a detailed description of the clinical and biochemical characteristics and the pathophysiology of the different diseases. Rather, at the beginning of each topic, the reader is respectfully referred to excellent recent reviews on the different diseases.

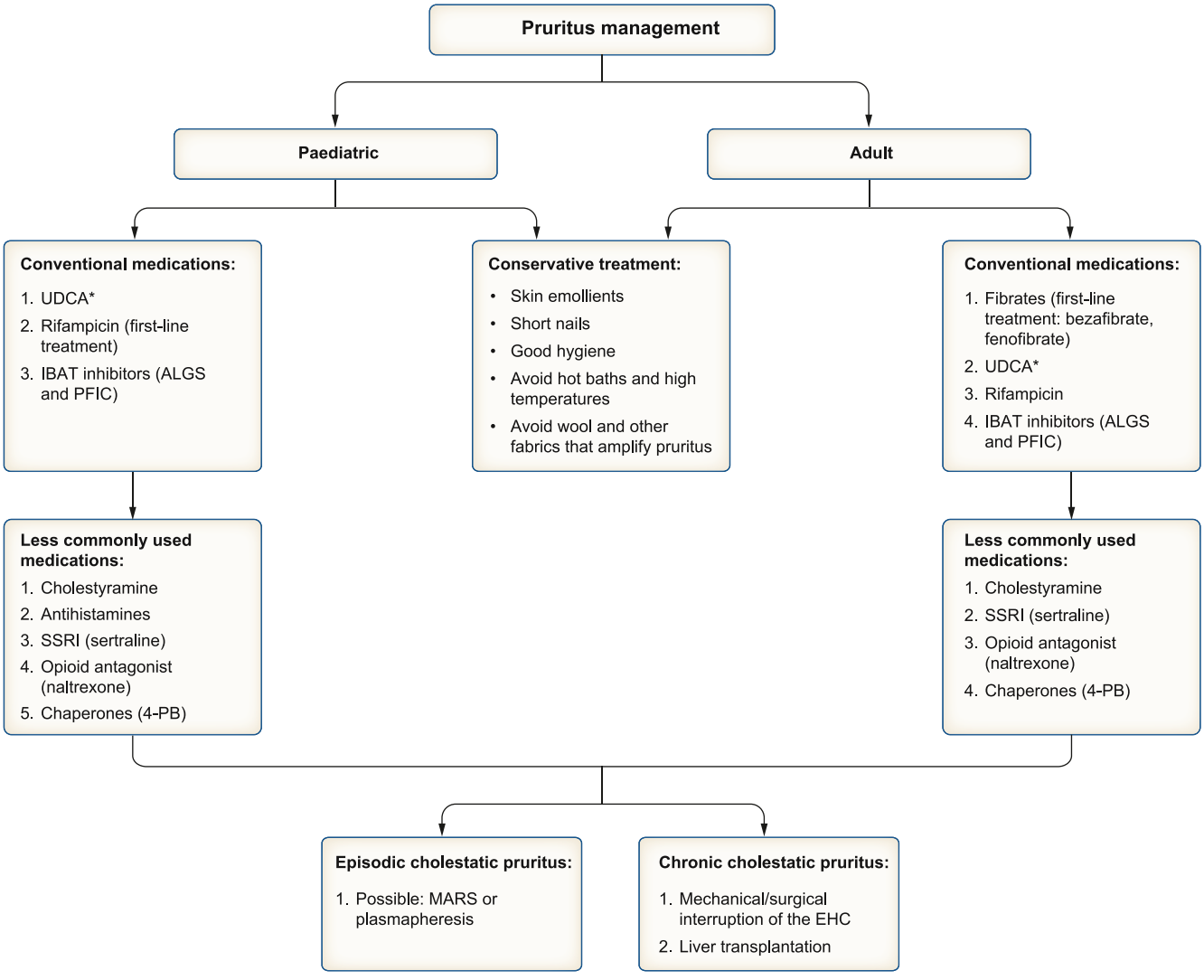

Fig. 2. Pruritus management flowchart. *UDCA is not generally considered a first-line treatment due to lack of evidence, however, because of its low risk profile, it is often tried as one of the first options in the management of cholestatic pruritus. ALGS, Alagille syndrome; EHC, enterohepatic circulation; IBAT, ileal bile acid transporter; MARS, molecular adsorbent recirculating system; PFIC, progressive familial intrahepatic cholestasis; SSRI, selective serotonin reuptake inhibitor; UDCA, ursodeoxycholic acid; 4-PB, 4-phenylbutyric acid.

Table 3. Drugs and their targets in genetic cholestatic liver diseases.

Drug class	Drug name	Target	Mechanism of action	Effect	Efficacy	Approved use	Experimental/off label use
Antibiotics[8–10]	Rifampicin	Bactericidal and acts on both intracellular and extracellular organisms	Unknown how it suppresses pruritus, possibly through PXR activation which also results in detoxification of bile acids and other potential pruritogens	Decrease in cholestatic pruritus	Moderate	—	ALGS, FIC1 deficiency, BSEP deficiency, MDR3 deficiency (PFIC in general)
Antihistamines[8–10]	Chlorpheniramine; alimemazine	H1 receptor	Binds to the histamine H1 receptor; this blocks the action of endogenous histamine	Decrease in cholestatic pruritus	Marginal (sedative)	—	ALGS, FIC1 deficiency, BSEP deficiency, MDR3 deficiency (PFIC in general)
Bile acid sequestrants[8,9]	Cholestyramine	Bile acids	Bile acid-binding resins form an insoluble complex with bile acids in the intestine, thereby interrupting the EHC; the excretion of this complex with the faeces stimulates the conversion of cholesterol into bile acids in the liver	Decrease in cholestatic pruritus	Marginal	—	ALGS, FIC1 deficiency, BSEP deficiency, MDR3 deficiency (PFIC in general)
Bile acids (primary)[11–13]	Cholic acid	Bile acid composition	Replacement therapy to prevent accumulation of hepatotoxic atypical bile acids and restore bile acid homeostasis	Restoring normal liver function	High	BASD	
Bile acids (secondary)[8,10,14–19]	Ursodeoxycholic acid	Bile acid composition	Changing the bile acid composition from hydrophobic to more hydrophilic; it reduces the amount of toxic (hydrophobic) bile acids and increases bile production; it also has an immunomodulatory effect on liver cell membranes	Decrease in cholestatic pruritus; decreasing cholestasis, reducing bile acid toxicity, reducing and preventing cholelithiasis	Low in FIC1 and BSEP deficiency; variable range from none to high in MDR3 deficiency, related to genotype	—	AATD, ALGS, FIC1 deficiency, BSEP deficiency, MDR3 deficiency (PFIC in general)
Chaperones/ potentiators/ correctors[8,9,20–24]	4-phenylbutyrate; Glycerol phenylbutyrate; Ivacaftor	Hydrophobic segments of unfolded protein	Enhancing the cell surface expression and transport capacity of mutated plasma membrane proteins by interacting with the exposed hydrophobic segments of the unfolded protein; this interaction protects the protein from aggregation, promotes the folding of proteins, and reduces ER stress	Decreasing cholestatic pruritus; decreasing cholestasis	Limited number of case reports and fundamental studies, low efficacy in specific missense mutations	—	FIC1 deficiency, BSEP deficiency
Fibrates[10,25]	Bezafibrate	Lipoprotein and hepatic lipase	Lowers VLDL and LDL levels and increases HDL levels; regulates bile-transporter expression and inflammation; the mechanism of action is not yet fully understood	Decrease in cholestatic pruritus, mechanism not fully understood	Unknown in paediatrics; intermediate in adults	—	ALGS, FIC1 deficiency, BSEP deficiency, MDR3 deficiency (PFIC in general)
IBAT inhibitors[8,10,26–28]	Maralixibat; Odevixibat	IBAT	Interruption of the EHC of bile acids by inhibition of bile acid reabsorption in the terminal ileum by the IBAT	Decrease in cholestatic pruritus and/or circulating bile acids	Variable range from none to high; in PFIC, depending on type of PFIC and partially on genotype	ALGS, FIC1 deficiency, BSEP deficiency, MDR3 deficiency (PFIC in general)	

(*continued on next page*)

Table 3. (*continued*)

Drug class	Drug name	Target	Mechanism of action	Effect	Efficacy	Approved use	Experimental/off label use
Opioid antagonists[9,10]	Naltrexone	Opioid receptors	Naltrexone competitively binds to opioid receptors in the CNS, blocking the effect of endogenous and exogenous opioids	Decrease in cholestatic pruritus, blocking opioid receptors in the CNS	Marginal	–	ALGS, FIC1 deficiency, BSEP deficiency, MDR3 deficiency (PFIC in general)
Selective serotonin reuptake inhibitors[9]	Sertraline	Serotonin channel at the terminal neuron	Inhibiting the reuptake of serotonin at the terminal neuron, thereby increasing serotonin activity in the brain	Decrease in cholestatic pruritus through modulation of the CNS pruritoception	Marginal	–	ALGS, FIC1 deficiency, BSEP deficiency, MDR3 deficiency (PFIC in general)

ALGS, Alagille syndrome; BASD, bile acid synthesis defects; BSEP, bile salt export pump; CNS, central nervous system; EHC, enterophepatic circulation; ER, endoplasmic reticulum; FIC1, familial intrahepatic cholestasis type 1; HDL, high-density lipoprotein; IBAT, ileal bile acid transporter; LDL, low-density lipoprotein; MDR3, multidrug resistance protein 3; PFIC, progressive familial intrahepatic cholestasis; PXR, pregnane X receptor; VLDL, very low-density lipoprotein.

Alpha-1 antitrypsin deficiency

AATD is caused by mutations in the *SERPINA1* gene and the clinical and biochemical characteristics and pathophysiology have recently been reviewed (Fig. 3).[29,30]

Should testing for AATD be performed by first measuring AAT levels in serum and, in case of decreased values, by genotyping/phenotyping?

Recommendations

- Measuring serum AAT levels as a triage tool for AATD testing is recommended **(LoE 3, strong recommendation, consensus).**
- Serum AAT levels should be measured in situations without inflammation **(LoE 3, strong recommendation, consensus).**
- A cut-off of 50 mg/dL (9.2 μmol/L) can be used as a triage tool that raises suspicion of severe AATD **(LoE 3, weak recommendation, consensus).**
- Phenotyping can be used when a quick decision is needed, whereas genotyping should be used for definitive diagnosis when available **(LoE 3, weak recommendation, consensus).**

AATD is a relatively common genetic disease affecting the production of AAT in the liver, which is responsible for protecting lung tissue against the enzyme neutrophil elastase. AAT (*SERPINA1*) is highly expressed by hepatocytes and, at lower concentrations, by intestinal epithelial cells, neutrophils, lung epithelial cells and macrophages. Upon secretion, it is an important acute phase protein and the major serine protease inhibitor in blood. Amongst other functions, AAT inhibits the activity of neutrophil elastase (ELANE2). The main site of AAT activity is in the lung, where it protects the fragile connective tissue of the lower respiratory tract from the uncontrolled proteolysis triggered by neutrophils during inflammation.[1] Mutations in the *SERPINA1* gene can not only lead to pulmonary but also to hepatic disease manifestations, as a result of the stress induced by defective protein accumulation in hepatocytes (Fig. 3).[1] While more than 100 genetic variants in the *SERPINA1* gene coding for AAT have been found, 95% of severe AATD cases result from the homozygous Pi*ZZ genotype (Fig. 4).[1] Accordingly, Pi*ZZ was detected in >90% of children with severe AATD-associated liver disease.[31,32] In adults, the Pi*ZZ genotype greatly increases the risk of cirrhosis while other assessed genotypes carry only mild or moderate risk (Fig. 5).[2] Individuals with Pi*ZZ have an ~85% decrease in plasma AAT levels and can thereby be distinguished from those without AAT mutations (normal range 90-175 mg/dL [16.6-32.2 μmol/L]).[1] Accordingly, the majority of existing guidelines recommend the measurement of AAT levels, which is typically performed by nephelometry (a light scatter technology using the formation of AAT-antibody immunocomplexes to quantify the AAT level[33]) as the first step of testing.[34,35] While individuals with Pi*ZZ typically display

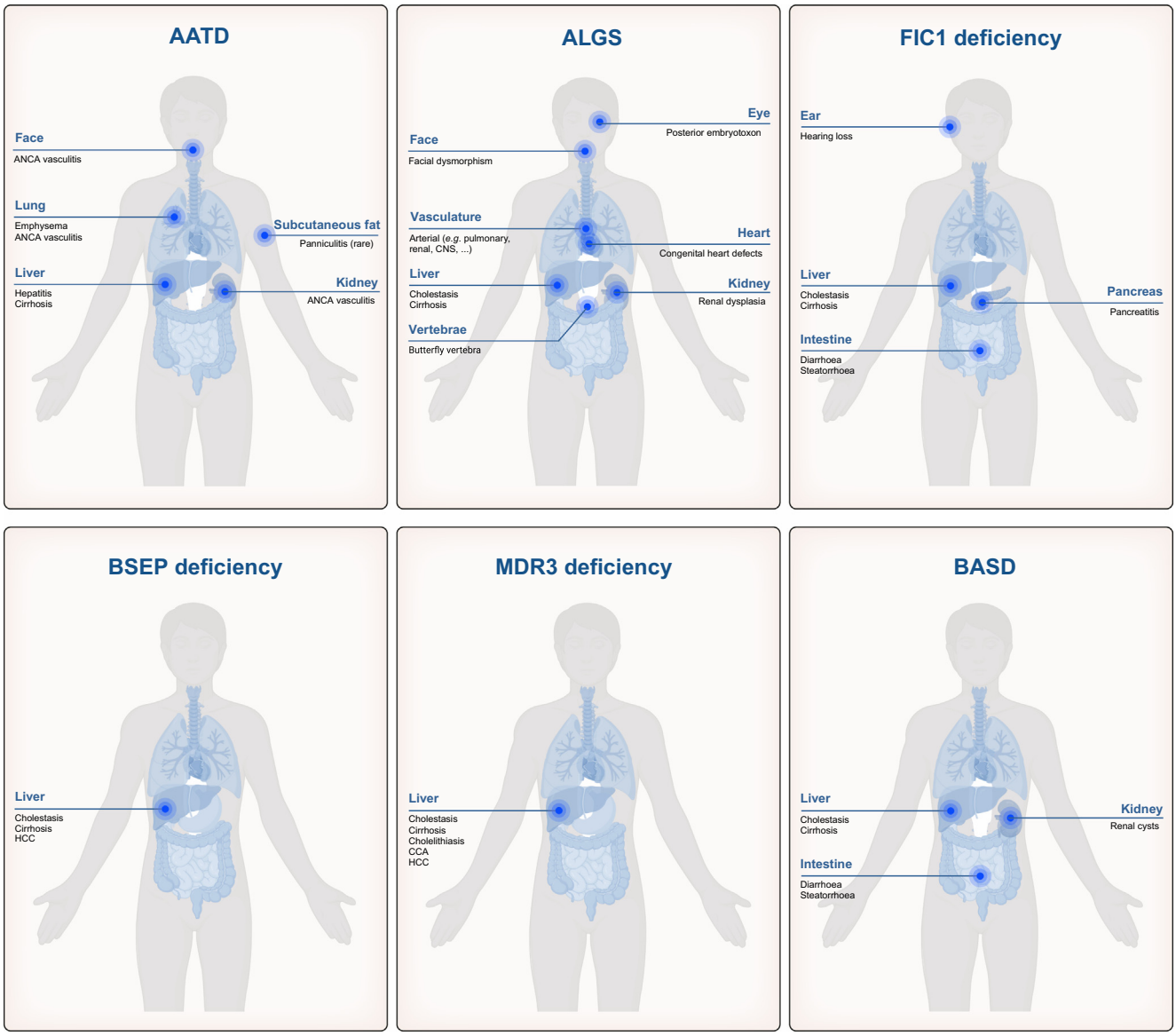

Fig. 3. Disease manifestations in genetic cholestatic liver disease. AATD, alpha-1 antitrypsin deficiency; ALGS, Alagille syndrome; ANCA, anti-neutrophil cytoplasmic antibody; BASD, bile acid synthesis defects; BSEP, bile salt export pump; CCA, cholangiocarcinoma; CNS, central nervous system; FIC1, familial intrahepatic cholestasis protein 1; HCC, hepatocellular carcinoma; MDR3, multidrug resistance protein 3. *Created with BioRender.com*.

levels <50 mg/dL (9.2 μmol/L), plasma measurements cannot reliably distinguish between individuals without AATD and those with mild AATD. Notwithstanding this limitation, a cut-off of 110 mg/dL (20.2 μmol/L) has often been used as a level yielding an acceptable sensitivity of 73.4% and a fair specificity of 88.5%.[34,36] Due to this, further AATD testing is warranted in cases with high suspicion, for example in case of family testing, even if levels exceed 110 mg/dL. Since AAT levels rise during the acute phase response, re-testing in inconclusive cases with a parallel assessment of C-reactive protein is sometimes recommended.[34,36] The second step typically includes AAT genotyping (*i.e.*, detection of specific mutations) or phenotyping (*i.e.*, analysis of AAT variants present in the blood via isoelectric focusing). While genotyping provides an unambiguous assignment of a genetic variant, phenotyping has the potential benefit of increased availability, faster turnaround times, and the ability to detect variants that are not included in the local genotyping panel. In inconclusive cases, sequencing of the entire *SERPINA1* gene provides definitive results.[34]

In children with AATD-associated liver disease, does treatment with UDCA postpone/prevent abnormal liver enzymes, liver decompensation and liver transplantation?

Recommendation

- There are insufficient data to advise for/against UDCA treatment **(LoE 3, strong recommendation, consensus).**

The available data on drug treatment in AATD-related liver disease are insufficient for definitive recommendations. In some children with Pi*ZZ, treatment with ursodeoxycholic acid (UDCA) was associated with improved clinical status and serum liver tests, while no beneficial effect was seen in children with the most severe liver involvement.[37] Notably, norUDCA, a derivative of UDCA, improved liver injury in a mouse model of AATD.[38] Despite this, no general recommendation can be given and treatment should be accompanied by regular measurement of serum liver tests. In adults with Pi*ZZ, inhibition of AAT production by the small-interfering RNA fazirsiran efficiently decreased both serum and hepatic AAT levels, which was associated with improved serum liver tests.[39] While the only publicly available data stems from a small, open-label phase II study, the inclusion of eligible patients into the corresponding clinical trials should be openly discussed.

Compared to no evaluation, does non-invasive monitoring of fibrosis and portal hypertension facilitate clinical management and counselling in patients with Pi*ZZ?

Recommendations

- Impaired liver synthetic function or decompensation of cirrhosis should be used to identify severe disease associated with poor outcomes **(LoE 3, strong recommendation, strong consensus).**
- Liver stiffness measurements can be used in adults with Pi*ZZ to estimate the level of histological fibrosis **(LoE 3, weak recommendation, strong consensus).**

The recommendation of an appropriate method of non-invasive liver disease monitoring in AATD is hampered by the lack of data. In children with Pi*ZZ, some studies suggested using the presence of neonatal cholestasis to predict an adverse disease course, while others reported no or only a poor predictive value.[31,32,40] Similarly conflicting results are available on the usefulness of the serum liver tests aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT).[40] While the formal evidence is limited, there is a general agreement that signs of impaired liver synthetic function such as prolonged international normalised ratio (INR), recurrence of jaundice or decreased albumin values constitute markers of severe disease associated with poor outcomes.[40] The same is true for signs of portal hypertension or cirrhosis decompensation such as ascites, varices, gastrointestinal bleeding, or

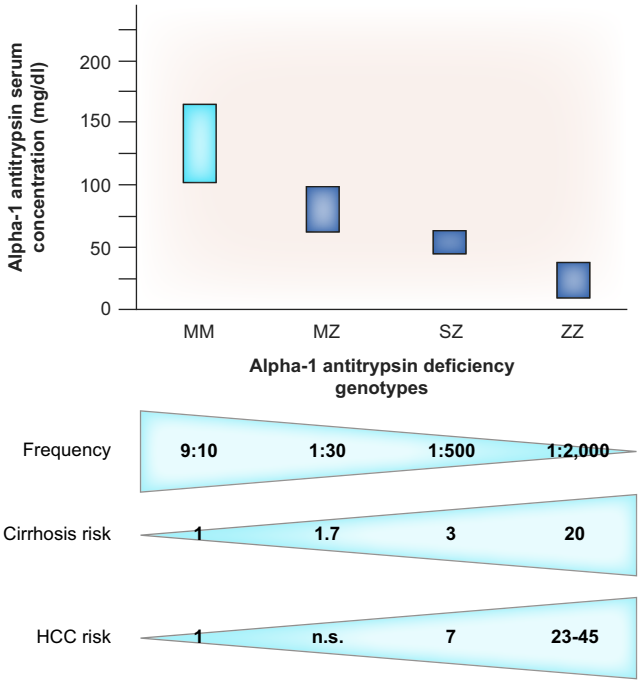

Fig. 4. AATD genotypes. AATD, alpha-1 antitrypsin deficiency; HCC, hepatocellular carcinoma.

hepatic encephalopathy that should trigger consideration for liver transplantation.[40,41] The exact timing should be based on established parameters for (cholestatic) end-stage liver disease. There is limited data on liver stiffness measurements in children with Pi*ZZ, but the measurements are feasible and correlate with serum liver tests and the presence of portal hypertension.[42] In adults with Pi*ZZ included in the Swedish national AATD register, repeatedly elevated serum liver tests were associated with the development of liver disease,[43] while the usefulness of alanine aminotransferase (ALT) measurement for liver disease was questioned in a large study from the USA,

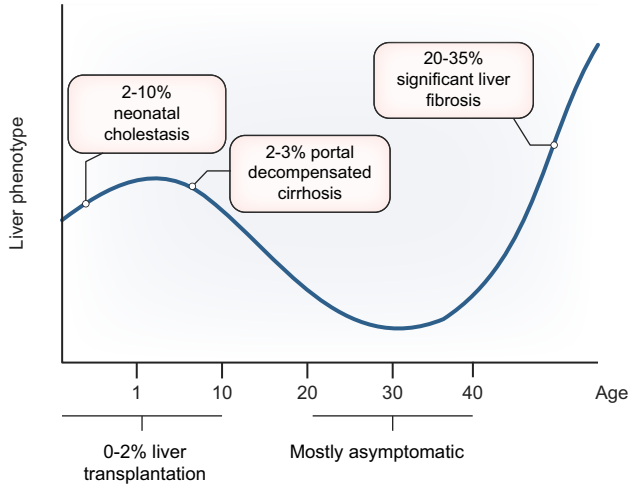

Fig. 5. Timeline of the liver phenotype in AATD. AATD, alpha-1 antitrypsin deficiency.

due to its poor sensitivity.[44] In biopsy-controlled studies, liver stiffness measurement (LSM) by vibration-controlled transient elastography (VCTE) was well-suited to detect advanced liver fibrosis, while it was less useful for prediction of significant fibrosis.[44,45] For the latter, VCTE-LSM seemed comparable to widely available fibrosis scores such as AST-to-platelet ratio index (APRI) and Fibrosis-4 index (FIB-4) and a large non-invasive study reported a moderate correlation between APRI and VCTE-LSM.[44,46] In conclusion, prospective, longitudinal studies are needed to clearly determine the usefulness of non-invasive liver fibrosis assessments in clinical routine.

Can lifestyle counselling improve the pulmonary and hepatic prognosis of patients with AATD?

Recommendations

- Lifestyle counselling is recommended as smoking, obesity and alcohol consumption have negative effects on the health of patients with AATD **(LoE 3, strong recommendation, strong consensus).**
- Despite the lack of evidence of the impact of lifestyle counselling on actual lifestyle change, the authors suggest lifestyle counselling given the important influence of lifestyle on disease outcome **(LoE 5, weak recommendation, strong consensus).**

A large amount of evidence demonstrates that smoking accelerates the development of lung disease in individuals with Pi*ZZ.[47–49] While the diagnosis of severe AATD by genetic testing has been shown to trigger attempts to quit smoking, there is a lack of convincing data on the impact of lifestyle counselling.[50] Even though the data on obesity and alcohol consumption are limited in individuals with Pi*ZZ, both factors clearly promote the development of advanced liver fibrosis in individuals with Pi*MZ.[2,51,52] Based on inference and expert opinion, appropriate lifestyle counselling seems justified, despite the fact that evidence of its efficacy is lacking.

In adults with AATD with otherwise unexplained, recurrently elevated liver enzymes, does liver biopsy aid in diagnosing the underlying problem compared to no biopsy?

Recommendation

- Liver biopsy should be considered when careful non-invasive evaluation remains inconclusive **(LoE 3, strong recommendation, strong consensus).**

No studies are available that systematically evaluated the diagnostic usefulness of liver biopsy in adults with AATD. Several reports indicate that elevated liver transaminases are only found in 10-15% of adults with Pi*ZZ and these numbers are even lower for milder AATD genotypes.[44,46,53,54] At the same time, elevated serum liver tests are associated with higher fibrosis grades.[46,53] Therefore, liver biopsy should be discussed when careful clinical evaluation remains inconclusive.

Can family members with heterozygous AATD mutations (Pi*MZ genotype) be used as donors for liver transplantation when no suitable organs are available?

Recommendations

- In the setting of living donor transplantation, Pi*MZ organs should only be considered for liver transplantation when other suitable organs are lacking and no signs of liver injury are present **(LoE 5, weak recommendation, strong consensus).**
- Livers from individuals with known homozygous Pi*Z mutations (Pi*ZZ genotype) should not be used as donor organs **(LoE 3, strong recommendation, strong consensus).**

Heterozygous Pi*Z mutations (Pi*MZ genotype) are seen in 2-4% of Caucasians and are associated with ca. 1.7-fold increased risk of liver-related mortality at the population level.[2] Accordingly, the presence of additional risk factors is typically required for development of a clinically significant liver disease.[52] Since the vast majority of serum AAT is produced in the liver, individuals receiving an organ from a Pi*MZ donor display mildly decreased AAT serum levels and a Pi*MZ phenotype (*i.e.* presence of a “Z” band in isoelectric focusing).[55,56] There are barely any studies systematically analysing the post-transplant outcomes of individuals receiving Pi*MZ grafts. One study reported transiently elevated alkaline phosphatase levels in recipients of Pi*MZ grafts, but no other abnormalities were noted.[56] A retrospective study identified six individuals who received livers carrying AATD mutations and did not report any adverse consequences.[57] Similar findings were reported in an additional, smaller case series,[58,59] while several case reports described either post-transplant complications or a rapid development of large numbers of AAT globules within hepatocytes.[60,61] In conclusion, Pi*MZ donor organs without significant liver injury can be considered for liver transplantation, especially in older recipients or when other suitable organs are lacking. Restrictive use seems justified in obese recipients, since obesity increases the risk of liver-related outcomes in individuals with Pi*MZ, and in paediatric patients, who are at higher risk of developing advanced liver disease due to their young age.[54] Since individuals with homozygous Pi*Z mutations (Pi*ZZ genotype) display a markedly increased risk of developing end-stage liver disease, these grafts should not be routinely used for liver transplantation.[54]

Alagille syndrome

ALGS is a multi-system autosomal dominant disease with predominantly hepatic manifestations (Fig. 3). The majority of patients carry damaging mutations in the *JAG1* gene and a small minority in the *NOTCH2* gene.[62] *JAG1* is expressed in portal mesenchymal cells, endothelial cells and biliary epithelial cells and functions as a ligand for *NOTCH2*, which is expressed in hepatoblasts during liver development. The disruption in

JAG1-NOTCH2-mediated cell-cell communication between biliary epithelial cells and hepatoblasts caused by mutations in either of these genes disturbs proper development of the biliary tree. This results in bile duct paucity in human patients with ALGS. Kamath and colleagues recently reviewed the clinical and biochemical features of the syndrome.[63]

In patients with a genetic diagnosis of ALGS, does a liver biopsy (histopathology) aid in prognosis and/or management?

Recommendation

- The routine use of liver biopsy for determining prognosis or management of liver disease in ALGS is not recommended **(LoE 3, strong recommendation, strong consensus).**

There are very few data to support the use of liver biopsy in determining the prognosis or management of ALGS-related liver disease. Multiple papers describe the use of histopathology in differentiating ALGS from other causes of neonatal cholestasis as a diagnostic tool, however, the relative insensitivity of the presence of bile duct paucity in young infants with ALGS is also well documented.[62] A liver biopsy can be valuable for diagnostic purposes in limited situations, such as in the presence of a genetic VUS.

Only one publication specifically addressed the use of histopathology as a prognostic marker. Mouzaki *et al.* performed a retrospective multicentre study in 144 children with ALGS to identify predictive markers of liver disease outcome in early life.[64] Histopathology reports were retrospectively reviewed in children under the age of 5 and the presence of fibrosis (reported as a binary variable) was associated with a severe hepatic outcome later in childhood (defined as death from liver-related complications; cholestasis with complications such as pruritus requiring biliary diversion; portal hypertension; or (listing for) liver transplantation) in univariate analysis. The risk of a severe long-term hepatic phenotype was more than three-fold higher in patients with fibrosis present on a liver biopsy performed during the first 5 years of life. In mixed model analysis, the presence of fibrosis combined with xanthoma and a serum bilirubin threshold of 65 μmol/L reliably predicted a severe hepatic outcome. Recent data from Shiau *et al.* reported findings from a study of 40 children with ALGS in which a single hepatopathologist reviewed all the liver biopsies and staged them for fibrosis.[65] Biochemical parameters were compared between children staged F3-4 and F0-2. No differences were observed in standard laboratory parameters between the two groups, however, children with ALGS staged F3–F4 on liver biopsy had higher APRI than those with F0–F2 (3 *vs.* 1.7; p = 0.029). In addition, APRI and FIB-4 were associated with increased risk of liver transplantation over time, with a 50% increase in APRI being associated with an almost 2-fold higher odds ratio for liver transplantation. These data may support the use of non-invasive tools such as APRI to predict prognosis in ALGS. Thus, there is insufficient data to justify the use of liver biopsy to aid in the prognosis or management of a cholestatic child with genetically proven ALGS.

Are IBAT inhibitors a useful addition to standard of care in reducing cholestasis-associated pruritus in patients with ALGS?

Recommendation

- When available, IBAT inhibitor treatment should be offered to patients with ALGS and cholestatic pruritus **(LoE 2, strong recommendation, strong consensus).**

Two randomised-controlled trials have investigated the role of IBAT inhibition in the treatment of cholestatic pruritus in ALGS. Shneider *et al.* reported the results of ITCH, a double-blind, randomised, placebo-controlled phase IIb trial of maralixibat in children with ALGS.[66] The ITCH study was run in 13 clinical sites in the USA and Canada, using maralixibat as the intervention. The primary endpoint was ItchRO, a caregiver observed pruritus score. The primary endpoint was not met for the total cohort. Based on a follow-up study (ICONIC, see below) in which patients received higher doses of maralixibat, it has been speculated that a relatively low dosage of maralixibat in the ITCH study contributed to the insignificant findings.

ICONIC was an international, multicentre, phase IIb, double-blind, placebo-controlled drug-withdrawal study with open-label extension of maralixibat in children with ALGS. The primary endpoint was the mean change in fasting serum bile acid (sBA) levels from week 18 to week 22 in patients who had achieved a reduction in sBA of ≥50% from baseline to week 12 or 18. The trial started with all patients receiving the drug from 0-18 weeks, followed by a randomised withdrawal period from weeks 19-22, a stable dosing period from weeks 23-48 and an open-label extension portion from weeks 49-204. Gonzales *et al.* reported statistically significant reductions in sBA from baseline to week 18, 48 and 204 (88 μmol/L [p = 0.0005], 96 μmol/L [p = 0.0058], 181 μmol/L [p = 0.0020], respectively) at doses of 380 μg/kg once per day, which are all statistically significant reductions. Thus, the primary efficacy endpoint of at least a 50% reduction in sBA from baseline to week 12 or 18 was met through treatment with maralixibat. Gonzales *et al.* also reported a statistically significant mean decrease in clinical severity scale score of 1.8 and significant reductions in ItchRO from baseline to weeks 18, 48, and 204, following treatment with maralixibat in the ICONIC trial.[26]

Shneider *et al.* went on to report results of the extension phase of ITCH and another UK-based intervention study of maralixibat in ALGS.[66] In this combined analysis of 57 children with ALGS, statistically and clinically significant improvements in pruritus and quality of life were observed. These changes were durable at week 72.

Taken together, these data support the use of maralixibat to treat cholestatic pruritus in children with ALGS. In addition, a phase III randomised-controlled trial has recently been completed for the treatment of pruritus in ALGS using odevixibat, another IBAT inhibitor (ASSERT study).[27] The data presented to date are positive, as the primary endpoint of a clinically meaningful reduction in pruritus with odevixibat has been met.

It should be noted that liver transplantation currently remains the standard of care for progressive cholestasis in ALGS

and decompensated portal hypertension; however, initial data have shown that reductions in sBAs are linked to improved outcomes such as transplant-free survival in ALGS.[67] Referral to a liver transplant centre is strongly encouraged for patients with ALGS and substantial liver involvement.

In patients with ALGS, is the use of statins indicated for the management of hypercholesterolemia and/or xanthomas?

Recommendation

- Statins are not recommended for hyperlipidaemia or xanthomas in ALGS **(LoE 4, strong recommendation, strong consensus).**

It is well established that children with ALGS have elevated total serum cholesterol, triglycerides, and phospholipids. Additionally, it has been shown that they have higher very-low-density lipoprotein-cholesterol and low-density lipoprotein-cholesterol levels and lower high-density lipoprotein-cholesterol levels than controls.[68] Lipoprotein-X has been shown to account for a major portion of the hyperlipidaemia in ALGS and is not thought to be atherogenic.[69] There is only a single case report on the use of statins to treat xanthomas and therefore, at this time, they cannot be recommended for hypercholesterolemia or xanthomas in ALGS.

FIC1 deficiency

FIC1 deficiency is caused by mutations in the *ATP8B1* gene, encoding FIC1. It is one of the members of the group of progressive familial intrahepatic cholestasis (PFIC) diseases, which were recently reviewed.[8,70] FIC1 is most highly expressed in hepatocytes and intestinal epithelial cells and functions as a aminophospholipid translocase that helps cellular membranes maintain a detergent-resistant state, particularly at the bile canalicular and intestinal apical epithelium, with its deficiency leading to hepatic and extrahepatic disease manifestations (Fig. 3).[71,72]

Different adult phenotypes, such as benign recurrent intrahepatic cholestasis (BRIC) and chronic liver diseases are caused by heterozygous variants in the *ATP8B1* gene.[6,73]

A few cases of ICP have been described in patients with *ATP8B1* mutations, while no link with hepatobiliary cancers has been reported.[74,75]

Does medical/surgical interruption of the EHC in patients with FIC1/ATP8B1 deficiency prolong native liver survival compared to other conservative (non-EHC interruption) treatments?

Recommendation

- Surgical interruption of the EHC is not recommended as a routine treatment to prolong native liver survival for patients with FIC1 deficiency **(LoE 3, strong recommendation, consensus).**

There have been no randomised studies of surgical interruption of the EHC in FIC1 deficiency (Fig. 6). There have been a few small retrospective cohort studies and one large multinational retrospective study.[76] These studies did not demonstrate a clear improvement in native liver survival for patients undergoing surgical interruption of the EHC. Almost nothing is known about local, historical decision-making regarding the use of surgery. Thus, it is very difficult to know how comparable the groups were. There was a non-significant trend towards benefit. In the patients that did undergo surgical interruption of the EHC there was a benefit, and, if nothing else, a delay in the requirement for transplantation in those who responded to the surgery, as measured by a reduction in sBAs to <65 μmol/L.[76]

In a randomised study on the use of odevixibat (an IBAT inhibitor) in PFIC, the endpoints were pruritus and reduction in sBAs.[28] As the placebo phase was only 6 months, this is most likely too short to obtain data regarding the need for transplantation. Preliminary data comparing the PEDFIC (odevixibat) trial to data from the NAPPED (NAtural course and Prognosis of PFIC and Effect of biliary Diversion) consortium indicate a benefit of odevixibat on native liver survival.[77] Full long-term data from these studies are eagerly awaited and may change the level of evidence for this particular PICO question. (https://clinicaltrials.gov/study/NCT04185363?cond=pfic1&rank=8~).

In patients with FIC1/ATP8B1 deficiency, are there medical/symptomatic interventions to prevent or improve diarrhoea after liver transplantation compared to current standard of treatment?

Recommendation

- Medical or surgical interruption of the EHC at transplantation or post-transplant could be used to treat diarrhoea and steatohepatitis in patients with FIC1 deficiency **(LoE 5, weak recommendation, strong consensus).**

There are a handful of reports of improvement in both diarrhoea and fatty liver following surgical interruption of the EHC, at or following liver transplantation in FIC1 deficiency.[78–81] An improvement has been seen following the administration of an IBAT inhibitor after liver transplantation in a small number of patients.[82,83] These data could be compared to previously published cases of patients with liver transplantation and subsequent surgical interruption of EHC by total biliary diversion.[79,81]

BSEP deficiency

BSEP deficiency is caused by a multitude of different mutations in the *ABCB11* gene. It is part of the PFIC disease spectrum, which was recently reviewed (Fig.3)[8,70] and is also described as PFIC type 2. BSEP, encoded by *ABCB11*, is the transporter that pumps conjugated bile acids from the hepatocyte into the bile canaliculus.

Heterozygous *ABCB11* mutations have been associated with several phenotypes in adults, such as BRIC, chronic cholestasis, ICP or DILI, which are often triggered by environmental factors.[6,84–88]

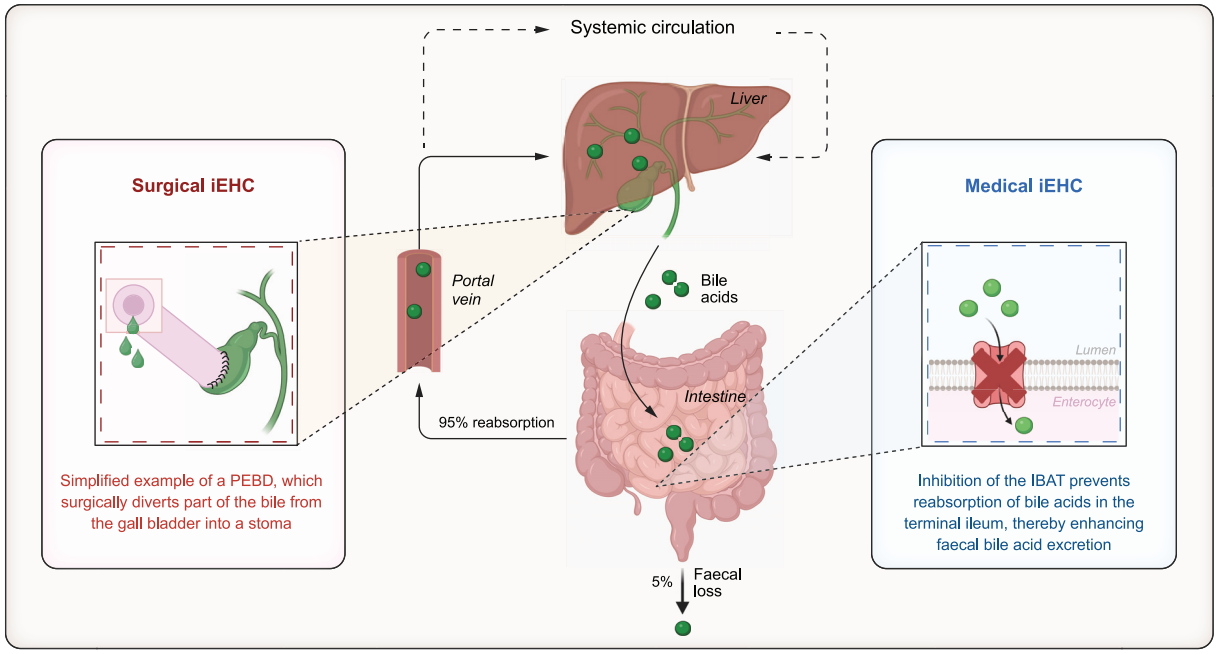

Fig. 6. Medical *vs.* surgical interruption of the EHC. IBAT, ileal bile acid transporter; iEHC, interruption of the enterohepatic circulation; PEBD, partial external biliary diversion. *Created with BioRender.com*.

Does the predicted effect of mutations on BSEP/ABCB11 protein function have consequences for follow-up, screening and treatment decisions in BSEP/ABCB11 deficiency?

Recommendations

- Management including follow-up/screening and treatment, including interruption of the EHC, should be based on the existing evidence for specific genotypes **(LoE 3, strong recommendation, strong consensus).**
- The screening intervals for hepatocellular carcinoma should be based on genotype (specific recommendations provided in a subsequent section) **(LoE 3, strong recommendation, consensus).**

Many studies show that the natural course of BSEP deficiency greatly differs between different genotypes.[89–98] For different mutations, the level of evidence regarding the natural course, prognosis and response to treatment varies, and the overall quality of evidence is low to moderate. Most studies are based on retrospective data. Several studies have shown that two specific missense mutations in *ABCB11* (p.D482G and p.E297G) are associated with a less severe BSEP deficiency phenotype, which is likely due to residual bile acid transport function.[89,91,94,98] Patients with homozygous p.D482G and/or p.E297G have a median survival with native liver of more than 18 years and a sustained response to surgical interruption of the EHC, which delays or even prevents the need for liver transplantation.[89,94] It would therefore be warranted to interrupt the EHC before considering liver transplantation in these patients, provided that there are no other risk factors for hepatocellular carcinoma (HCC). On the other hand, mutations leading to a predicted protein-truncating mutation (PPTM) are associated with poor native liver survival and response to surgical interruption of the EHC, and a high incidence of HCC at a young age.[89,90,95–97] These patients require a different treatment approach to patients with other (missense) mutations, including closer monitoring for HCC, no surgical interruption of the EHC and consideration of liver transplantation at an early age.[89,97] Recently, a new study by Felzen *et al.* showed that a combination of either the p.E297G or p.D482G mutation together with a PPTM was associated with a poor response to surgical interruption of the EHC and short native liver survival.[90] Patients with these specific compound heterozygous genotypes may require a treatment approach similar to patients with two PPTMs. We recommend that management, including follow-up/screening and treatment, be based on the existing evidence for specific genotypes, particularly for patients with homozygous p.D487G, p.E297G, compound heterozygous p.D487G or p.E297G with one PPTM, or those with two PPTMs. This is a field of active investigation and may be subject to modifications in upcoming years due to the recently approved IBAT inhibitors whose efficacy in the different BSEP genotypes remains to be determined.

Can medical/surgical interruption of the EHC in patients with BSEP/ABCB11 deficiency prolong native liver survival compared to other (non-EHC interruption) treatments?

Recommendations

- To prolong native liver survival, surgical interruption of the EHC should be considered in patients with a responsive genotype **(LoE 3, strong recommendation, consensus).**
- Due to the current lack of data, it is not yet possible to recommend medical rather than surgical interruption of the EHC to prolong native liver survival **(LoE 5, weak recommendation, strong consensus).**

There are no randomised-controlled trials available addressing the superiority of medical/surgical interruption of the EHC (Fig. 6) over other (non-EHC interruption) treatments with respect to delaying or preventing the need for liver transplantation. In retrospective studies, surgical interruption of the EHC was associated with prolonged native liver survival in patients with BSEP deficiency, provided they did not have two PPTMs in *ABCB11*.[89] An sBA level below 102 μmol/L postoperatively has been associated with long-term native liver survival.[89]

Odevixibat was approved for the medical interruption of EHC by the EMA and FDA in 2021 after the first randomised-controlled trial in a PFIC population.[28] The outcomes of this study demonstrated positive effects of odevixibat, such as reduction in pruritus and in sBA concentrations, during 24 weeks of treatment. Preliminary data comparing the PEDFIC (odevixibat) trial to NAPPED data indicate a benefit of odevixibat on native liver survival.[77] No published long-term results on the ability of odevixibat to delay or prevent liver transplantation are presently available but they are expected in due course. (https://clinicaltrials.gov/ct2/show/NCT03659916?term=pedfic&draw=2&rank=2).

Is medical/surgical interruption of the EHC in patients with BSEP/ABCB11 deficiency superior to other (non-EHC interruption) treatments in preventing or decreasing pruritus?

Recommendations

- Surgical interruption of the EHC can be used to reduce pruritus in patients with a responsive genotype prior to considering liver transplantation **(LoE 3, weak recommendation, consensus).**
- Medical interruption of the enterohepatic circulation by IBAT inhibition should be considered to reduce pruritus prior to considering liver transplantation in patients with missense mutations **(LoE 2, strong recommendation, strong consensus).**

BSEP deficiency is known for causing debilitating pruritus and interruption of the EHC is one available treatment option. The mechanism behind this technique (be it surgical or medical) is to decrease the reabsorption of bile acids at the level of the intestinal ileum and thereby to interrupt the EHC of bile acids (Fig. 6). This in turn is hypothesised to decrease intrahepatic bile acid accumulation, which is thought to be one of the main triggers of cholestatic pruritus.[99] Due to the subjective nature of pruritus, reliable reporting is challenging, especially in children. In accordance with this, it has been difficult to accurately determine the effect of interventions on pruritus as an outcome. Nonetheless, due to its clinical relevance, several studies have been performed to show the usefulness of interruption of the EHC in decreasing pruritus.

Surgical interruption of the EHC preceded the recently licensed medical interruption of the EHC and has yielded variable results on pruritus improvement in patients with BSEP deficiency. One retrospective study by Van Wessel *et al.* has shown an overall sustained partial or complete pruritus relief rate of 54% (22/41 patients) after surgical interruption of the EHC.[89] They also demonstrated that the response rate is dependent on the genotype. Patients with at least one p.E297G or p.D482G missense mutation had a response rate of 66% while patients with at least one other missense mutation had a response rate of 36%. No improvement in long-term pruritus was observed upon surgical interruption of the EHC in any of the three patients with two PPTMs for whom data was available.[89] Several other studies have also shown that surgical interruption of the EHC can (at least transiently) improve pruritus.[100–103]

As for medical interruption of the EHC, the first randomised, placebo-controlled trial in patients with PFIC demonstrated that the IBAT inhibitor odevixibat reduced pruritus and sBA concentrations in patients with BSEP and FIC1 deficiency.[28] Patients in both arms of the study were allowed to continue UDCA, rifampicin and/or antihistamine treatment during the trial. Thus, this study tested the value of odevixibat as an add-on treatment and not in direct comparison to other medications/strategies. Patients with two PPTMs were excluded from the trials on theoretical grounds and due to their unresponsiveness to surgical interruption of the EHC in phase II studies. To date, no results on sustained anti-pruritic effects have been published, however, trials are ongoing and results are expected in due course (https://clinicaltrials.gov/ct2/show/NCT03659916?term=pedfic&draw=2&rank=2).

Does screening for HCC improve prognosis and long-term survival in patients with BSEP/ABCB11 deficiency and what is the optimal screening age, interval and modality?

Recommendations

- For patients with missense mutations, an HCC screening interval of 3 to 6 months is recommended based on expert opinion **(LoE 3, strong recommendation, strong consensus).**
- For patients with biallelic PPTMs, an HCC screening interval of 3 months is recommended **(LoE 3, strong recommendation, strong consensus).**

BSEP deficiency is a risk factor for the development of HCC early in life, even in the absence of end-stage liver disease. Depending on the genotype, the incidence of HCC in children <16 years of age varies between 4-34%. Patients with biallelic PPTMs (the most severe genotype) are at the highest risk of developing HCC, with an incidence of around 15% before the age of 5.[89,96,97] There are no clear prospective studies on the impact of screening for HCC on the prognosis and long-term survival of patients with BSEP deficiency. However, several retrospective studies have provided grounds to indicate that screening should be offered to these patients.[89,95–97] Davit-Spraul *et al.* suggested that monitoring for HCC should be offered from the moment of diagnosis onwards, which is supported by the observations of Knisely *et al.* that 7/10 patients with HCC were below 2 years of age at the time of HCC diagnosis.[95,97] Alpha-fetoprotein and ultrasound are recommended as standard screening modalities for HCC in paediatric patients.[97] Screening should be performed even when there is a good response to surgical interruption of the EHC, as this does not necessarily seem to protect from HCC development.[89,97] A screening interval of 3 to 6 months is recommended by expert opinion, but epidemiological or prospective data to support this recommendation are lacking.[95,104] In patients with biallelic PPTMs, who carry the highest risk of HCC development, closer monitoring, *e.g.* 3-month screening intervals, seems necessary.

What is the optimal way to diagnose and treat BSEP alloimmunisation in transplanted patients with BSEP/ABCB11 deficiency?

Recommendations

- BSEP alloimmunisation should be diagnosed through the detection of anti-BSEP antibodies in plasma or anti-canalicular antibodies upon immunofluorescence staining of liver tissue **(LoE 3, strong recommendation, strong consensus).**
- Treatment should focus on adapting the immunosuppressive regimen and when necessary, a combination of rituximab and plasmapheresis should be considered **(LoE 3, strong recommendation, strong consensus).**
- Re-transplantation(s) may become necessary if treatment fails **(LoE 3, weak recommendation, strong consensus).**

If patients carry mutations resulting in no canalicular BSEP expression, alloimmunisation can develop after transplantation, this leads to an immune response against the donor BSEP and the production of anti-BSEP IgG alloantibodies.[105–107] The resulting clinical picture mimics the BSEP deficiency phenotype with cholestasis, pruritus and low GGT. BSEP alloimmunisation can present itself similarly to the original disease in the absence of other common causes of cholestasis in liver-transplanted patients, such as rejection or biliary complications.[106] It can be diagnosed through the detection of anti-BSEP antibodies in plasma or anti-canalicular antibodies via immunofluorescence staining of liver tissue.[105–108] Upon liver biopsy, cholestasis with giant cell transformation of hepatocytes and sometimes fibrosis in the absence of signs of rejection or biliary obstruction may be observed.[105–107] There are insufficient data to support an evidence-based stepwise treatment approach, however, often the first step in the treatment of BSEP alloimmunisation is to intensify the immunosuppressive regimen.[105–107] In case symptoms do not resolve, more aggressive treatment could be considered using a combination of rituximab and plasmapheresis.[105,107] If the disease proves refractory to treatment, re-transplantation has been performed although not always with long-term success.[105–107]

MDR3 deficiency

MDR3 deficiency is caused by mutations in the *ABCB4* gene. MDR3 is responsible for flopping phosphatidylcholine molecules at the canalicular membrane in the liver.[109] MDR3 deficiency is one of the members of the group of PFIC diseases recently reviewed (Fig. 3) [51,52] and is also described as PFIC type 3.

Patients with MDR3 deficiency show high GGT levels, unlike those with FIC1 and BSEP deficiencies, where GGT is low.

Heterozygous mutations in the *ABCB4* gene can cause chronic cholestasis, ICP, DILI, low phospholipid-associated cholelithiasis (LPAC) syndrome and HCC.

Does the ABCB4 genotype affect management?

Recommendations

- In patients with biallelic *ABCB4* variants, genetics should be used to predict phenotype and response to UDCA therapy **(LoE 2, strong recommendation, consensus).**
- Patients with known pathogenic variants in *ABCB4* should be offered follow-up for liver-related complications, including malignancy **(LoE 2, strong recommendation, strong consensus).**

No prospective cohort or registry study has addressed this question. In severe MDR3-deficient patients, who carry biallelic *ABCB4* variants, the genotype helps to partially predict response to UDCA. Patients with missense variants are more likely to respond to UDCA therapy, while patients carrying two PPTMs usually do not, or only partially respond to UDCA therapy (Tables 3 and 4). It also helps to partially predict native liver survival. Patients carrying two severe variants usually require liver transplantation before the age of 10 years. Other patients treated with and responding to UDCA treatment (especially those carrying at least one missense variant) may reach adulthood with their native liver and remain healthy for decades.[14,109–112]

In a population analysis in Iceland, individuals heterozygous for a rare missense variant (p.G622E) were at a higher risk of fibrosis/cirrhosis development (odds ratio [OR] 5.52) while those heterozygous for either another rare missense (p.N510S, OR 4.74) or a rare frameshift (p.L445GfsX22, OR 3,07) variant were at a higher risk of HCC or cholangiocarcinoma (CCA) development.[113] A risk of liver disease progressing to biliary

Table 4. Follow-up and treatment of *ABCB4*-associated diseases.

	Monoallelic variants					Biallelic variants (homozygous or compound heterozygous)
Manifestation type	**Asymptomatic heterozygous carriers (family screening)**	**ICP**	**Gallstone disease**	**LPAC**	**Biliary fibrosis**	**MDR3 deficiency**
Follow-up	Individualised	At least twice after delivery	With known pathogenic *ABCB4* variant; depending on lab values	Yes	Yes	Yes
Follow-up interval	Normal lab values, no signs of advanced liver fibrosis: 3-year intervals; abnormal serum liver tests: yearly follow-up	6-8 weeks post-partum, 2nd follow-up depending on lab values, clinical presentation: either in 2-3 years or yearly	Depending on values, once per year or every 3-5 years	Yearly	Every 6-12 months depending on signs of portal hypertension	Every 3-6 months to 6-12 months according to response to UDCA therapy and signs of portal hypertension
HCC/CCA screening (US)	Patients with abnormal liver biochemistry, yearly	Yearly for those with abnormal liver function tests after pregnancy	Ultrasound every 3-5 years	Once per year	Yes, at least once a year, in advanced fibrosis every 6 months	Yes, at least once a year, every 6 months in those with portal hypertension
UDCA therapy	Recommended for those with signs of cholestatic liver disease such as ICP, gallstone disease, LPAC; treatment should be considered in patients with abnormal serum liver tests	From 2nd trimester to delivery, lifelong dependent on lab values and genetic variant	Recommended	Life long	Life long, but may not be beneficial in those with 1 PPTM	Lifelong until LTx when required, but may not be beneficial in those with two PPTMs

CCA, cholangiocarcinoma; HCC, hepatocellular carcinoma; ICP, intrahepatic cholestasis of pregnancy; LPAC, low phospholipid-associated cholelithiasis; LTx, liver transplantation; MDR3, multidrug resistance protein 3; PPTM, predicted protein-truncating mutation.

fibrosis has been reported in case series, however, the prevalence of fibrosis development in heterozygous carriers is still unclear.[111] In these patients, reduced secretion of phospholipids into bile increases the concentration of free bile acids and leads to bile duct injury. However, reduced phospholipids also decrease the solubility of cholesterol, promoting its crystallization and thereby gallstone formation. The LPAC syndrome criteria include age at onset of biliary symptoms under 40 years, recurrence of biliary symptoms after cholecystectomy and hyperechoic intrahepatic foci or comet tail images within intrahepatic bile ducts on ultrasound.[114]

In two large case series of patients with LPAC syndrome (n = 122) and a mixed group of liver disease phenotypes associated with heterozygous damaging *ABCB4* variants (n = 67), the authors observed that the majority of patients did not suffer from progressive liver disease, although most patients were on UDCA therapy.[115,116] Despite the lack of liver fibrosis/cirrhosis, both studies described an increased risk of hepatobiliary malignancy in patients heterozygous for damaging *ABCB4* variants.[115,116] One study analysed a cohort of 308 patients with LPAC syndrome of whom 122 were carriers of a potentially pathogenic *ABCB4* gene variant. Hepatobiliary malignancy was significantly more frequent in the *ABCB4* heterozygotes compared to the patients with non-*ABCB4* LPAC (10.1% *vs.* 2.2%, OR on multivariate analysis 5.0, 95% CI 1.2-25.5, $p = 0.026$).[116] An analysis of 67 adult heterozygous carriers of damaging *ABCB4* variants also found an increased risk of hepatobiliary malignancy.[115] CCA development in carriers of *ABCB4* pathogenic variants has also been reported sporadically.[117]

In patients with severe MDR3 deficiency and biallelic variants, genetics can partially predict the phenotype and response to UDCA, however, heterozygous adult *ABCB4* carriers exhibit a broad range of clinical presentations. This is despite identical *ABCB4* variant carrier status, suggesting that other factors, including environmental factors, contribute to the phenotype.[93,118–120]

Based on the two larger studies and the Icelandic population study, follow-up surveillance should be offered to patients with known damaging *ABCB4* variants (expert consensus, 92%).[6,113,121] Since no data on interval and type of work-up are available to date, a first follow-up after 6 months and further follow-up at yearly intervals is suggested by the expert panel. During these visits, medical history, laboratory analysis, a non-invasive test for screening of higher degree fibrosis (*e.g.* FIB-4, elastography) and liver ultrasound are recommended.[122]

In another retrospective single-centre study, 60 adult carriers of pathogenic, likely pathogenic and VUS heterozygous *ABCB4* variants with LPAC syndrome experienced features of complications on MRI when compared with a cohort of 65 patients with LPAC without *ABCB4* mutations (35 *vs.* 15%; OR 2.9, 95% CI 1.1–7.8, $p < 0.05$): cholangitis-like multiple stenosis, abscess formation, portal hypertension and CCA were described.[123]

However, prospective registries including a predefined genetic work-up as well as clinical risk factors for liver diseases and hepatobiliary malignancy are needed in order to determine the prevalence of HCC/CCA and progressive liver disease in this cohort.

Should first degree relatives of patients with MDR3/ABCB4 deficiency gene variants (Class III-V) be offered genetic testing in the context of proper genetic counselling?

Recommendation

- Genetic testing should be offered to first degree relatives of patients with MDR3 deficiency **(LoE 2, strong recommendation, strong consensus).**

Based on the population analysis in Iceland by Gudbjarsson *et al.*, genetic testing should be offered to first degree relatives of patients with MDR3 deficiency. This study showed that carriers of a rare missense variant (p.G622E) had a higher risk of fibrosis/cirrhosis development (OR 5.52) while carriers of either a rare missense (p.N510S, OR 4.74) or a rare frameshift (p.L445GfsX22, OR 3,07) variant were at a higher risk of HCC/CCA development.[113]

How should follow-up be performed in patients with pathogenic MDR3/ABCB4 deficiency gene variant(s)?

Recommendations

- Follow-up should be adjusted to clinical presentation and detected genetic variant (expert opinion) **(LoE 5, strong recommendation, strong consensus).**
- Medical history, laboratory analysis and non-invasive screening for higher degree fibrosis should be carried out during follow-up visits (at least) every 6 months **(LoE 3, strong recommendation, strong consensus).**
- Liver ultrasound and/or MRI with MRCP are recommended at least on a yearly basis **(LoE 4, strong recommendation, strong consensus).**

Based on the two larger cohort studies and the Icelandic population study, follow-up surveillance can be offered to patients with potentially pathogenic *ABCB4* variants (Table 4).[111,113,115] Based on the current data, it seems logical to adopt a management strategy based on the clinical presentation of patients with MDR3 deficiency, however, data supporting these recommendations are weak.

During follow-up visits, a medical history, laboratory analysis (including AST, ALT, alkaline phosphatase, GGT, bilirubin, albumin, prothrombin time, INR, platelets) and a non-invasive test for screening of higher degree fibrosis (*e.g.* serum fibrosis tests such as FIB-4 or elastography) should be performed.[122] Liver ultrasound and/or MRI with MRCP are recommended on a regular basis, ranging from 3-month to <2-year intervals, depending on the manifestations and response to UDCA.

ICP: It is assumed that genetic risk contributes about 20% of the risk of ICP. Patients with severe ICP (sBA >100 μmol/L), recurrent ICP and/or early onset ICP should be offered genetic testing. UDCA is recommended for ICP. A follow-up clinical visit, including laboratory analysis about 6-8 weeks following delivery, is encouraged. In women with complete normalisation of liver function tests, a second follow-up after 2-3 years is recommended since patients also have increased risk for hepatobiliary disease as well as immune-mediated disease (*e.g.* diabetes mellitus, thyroid disease).[124–126] If serum liver tests do not normalise and the patient has a class 4/class 5 *ABCB4* variant, lifelong UDCA can be considered based on retrospective data, although no prospective data are available. A recent study from Finland confirmed the good long-term outcomes of mothers who experienced at least one pregnancy with ICP, who had similar long-term survival as controls.[127]

LPAC syndrome: A heterozygous missense variant can be found in 40-50% of patients with LPAC. Although randomised prospective trials are not available for UDCA in LPAC, retrospective analysis shows transplant-free survival of >90% in these patients on UDCA.[115,116] Thus, lifelong use of UDCA is recommended in patients with LPAC. Since two studies reported an increased risk of hepatobiliary malignancy in LPAC as well as in carriers of heterozygous *ABCB4* variants, a yearly follow-up including serum liver tests and liver imaging (*e.g.* ultrasound) is recommended (expert opinion).[115,116]

Heterozygous parents and first degree relatives of patients with severe MDR3 deficiency: If these family members are asymptomatic and have normal laboratory values and no signs of advanced liver fibrosis (*e.g.* FIB-4, elastography) or gallstone disease, an individualised follow-up strategy is recommended based on the individual's preference. A follow-up every 3 years seems adequate. In family members with symptoms of hereditary cholestasis such as ICP, gallstone disease, LPAC and/or cholestasis or DILI, UDCA therapy can be considered and yearly follow-up (as described above) is recommended by the expert panel.

The natural history of children with severe MDR3 deficiency is typically marked by a moderate chronic cholestasis responsible for growth failure, pruritus, fat soluble vitamin deficiencies and the development of biliary cirrhosis, resulting in significant portal hypertension and liver failure. These patients need to be clinically and biologically evaluated at least every 6 months and to undergo a liver ultrasound every year. This minimal follow-up should be sufficient in patients fully responding to UDCA therapy (normal serum liver tests) without cirrhosis and significant portal hypertension. On the other hand, patients with MDR3 deficiency diagnosed at the stage of decompensated cirrhosis or those evolving toward end-stage liver disease (despite UDCA therapy) require close monitoring to prevent and treat complications of cirrhosis (ascites, oesophageal/gastric variceal bleeding, hepatic encephalopathy). In addition, these patients should receive nutritional (oral, enteral and sometimes parenteral) support and be prepared for liver transplantation. In patients with a mild form of MDR3 deficiency, which fully responds to UDCA (normalisation of serum liver tests), compliance to UDCA treatment is critical. Treatment holidays and non-compliance to treatment have been shown to result in abnormal serum liver tests and are likely to decrease native liver survival.[15] In addition, it has been suggested that long-term treatment with UDCA could result in a decrease of liver fibrosis.[16,17]

Should patients with known potentially damaging MDR3/ABCB4 deficiency gene variants be treated with UDCA to prevent or decrease liver-related complications?

Recommendations

- UDCA treatment is recommended for those with at least one *ABCB4* missense variant and a clinical phenotype **(LoE 3, strong recommendation, strong consensus).**
- Partial response to UDCA has been reported in patients carrying biallelic protein-truncating MDR3/*ABCB4* gene variants and UDCA may therefore be offered **(LoE 4, weak recommendation, strong consensus).**

Whether UDCA improves liver-related outcomes in patients with known pathogenic MDR3 deficiency gene variants has not been addressed in any randomised-controlled trial. The only data available are retrospective cohort analyses and case reports.

A first report that UDCA may be beneficial, especially in patients carrying *ABCB4* missense variants, came from a prospective study of 39 patients with PFIC, including 13 with elevated serum GGT levels.[15] These data were later confirmed in larger cohorts of patients with MDR3 deficiency and a genetically proven disease.[14,17,128] No response to UDCA therapy (no change in serum liver tests) was found in patients carrying a premature stop codon (no response of 8 patients), while 21 of 23 patients carrying at least one missense variant responded with normalisation of serum liver tests. The latter was accompanied by a stabilisation of liver disease.[17,128] The ability of UDCA to improve or normalise liver biology in the subset of MDR3-deficient patients carrying at least one missense variant has been shown in other retrospective cohorts of patients.[109,110–112,16,17] A longer native liver survival in these patients carrying at least one missense variant has also been confirmed when compared to patients carrying two severe variants. However, the association between response to UDCA and longer native liver survival has not been demonstrated in a randomised prospective trial.

In patients with **LPAC syndrome**, UDCA (8-10 mg/kg body weight up to 13-15 mg/kg body weight) is widely used.[116,129] This is based on the initial description of six patients who responded to UDCA therapy with complete resolution of symptoms as well as normalisation of serum liver tests.[130] Therefore, lifelong UDCA is recommended for LPAC.

In a retrospective cohort study of 67 adult carriers of **heterozygous potentially pathogenic *ABCB4*** variants, 62 carriers (93%) with different MDR3 deficiency phenotypes received UDCA at some point during their treatment. Transplant-free survival was 91% with a median follow-up of 14 years. Only three patients in the study cohort died, two from CCA and one patient from decompensated biliary cirrhosis. Liver stiffness was normal (<6.3 kPa) in 75% of the cohort, suggesting stabilisation of disease progression.[115]

As described above in ICP, UDCA is recommended starting from the second trimester until delivery. Whether to continue UDCA following delivery should be decided based on individual family history, clinical presentation, presence of gallstones, laboratory values and presence of liver disease. A follow-up is recommended as described above.

Besides these observational case series and cohort studies, a number of case reports describe beneficial responses of patients with *ABCB4* variants to UDCA treatment. While no or only a partial beneficial effect of UDCA has been reported in patients with biallelic protein-truncating *ABCB4* gene variants, it may still be offered. UDCA treatment is recommended by the expert panel for those with at least one missense variant and a clinical phenotype (see above).

Should a recipient of an MDR3 monoallelic deficient liver graft (i.e., from a donor carrying one pathogenic MDR3 variant) receive long-term UDCA after liver transplantation?

Recommendation

- Long-term UDCA therapy is recommended for recipients of an MDR3 monoallelic deficient graft **(LoE 4, strong recommendation, strong consensus).**

There are plenty of data which address the potential liver diseases related to a heterozygous status for one damaging *ABCB4* variant in patients who still possess their native liver (Table 4). There is also sufficient evidence for the positive safety profile of UDCA therapy,[18,131] including in pregnant women.[19,132] It therefore seems highly logical to recommend long-term UDCA therapy for the recipients of a MDR3 monoallelic deficient liver graft. However, data are lacking to fully support this recommendation. Heterozygosity for one damaging MDR3 variant in the graft may be known or easy to assess before living donor liver transplantation is performed in the setting of MDR3 deficiency. However, whether such a study would be clinically relevant and cost-effective as part of the systematic evaluation of all liver grafts remains to be studied.

Other types of PFIC

Thanks to the development of NGS, WES or WGS over recent decades, the spectrum of PFIC diseases has expanded vastly from the more commonly known types 1-3, mentioned above, to even more rare subtypes including diseases caused by TJP2 deficiency, FXR (farnesoid X receptor) deficiency, MYO5B (myosin 5B) deficiency, USP53 deficiency and deficiencies in several more associated genes like *SLC51A, KIF12, ZFYVE19, SEMA7A* and *VPS33B* (Table 5). (https://www.omim.org/entry/211600) While many of the diagnostic and treatment recommendations given above for the more common PFIC diseases will apply to these conditions as well, due to the current lack of data, they fall outside the scope of these CPGs.

Of patients with cirrhosis on the UK liver transplant list, 5–30% do not have a diagnosis, and when an NGS cholestasis gene panel was performed (10/165 patients, 6.1%), a variant was identified in 6/10 (60.0%) cases (five *ABCB4* and one *DCDC2*, a gene responsible for neonatal sclerosing cholangitis via a loss of function of normally constituted primary cilia in cholangiocytes).[133]

In another study, WES yielded a positive diagnosis in 5/19 (26%) adults with idiopathic liver disease, underlining the clinical utility of genome analysis.[134]

Therefore, such patients should only be cared for by tertiary care physicians.

Bile acid synthesis defects

Bile acids are synthesised from cholesterol by several enzymatic conversions. Accordingly, the synthesis can become compromised by genetic mutations in different genes of the synthesis pathway, resulting in different phenotypes (Fig. 3). Inborn errors of primary bile acid synthesis are ultra-rare disorders with autosomal recessive inheritance. The most frequent defects responsible for liver diseases are the 3β-Δ^5hydroxy-C_{27}-steroid oxidoreductase (3β-HSD, also known as 3β-hydroxysteroid-dehydrogenase) deficiency (OMIM 607764) due to variants in *HSD3B7*, and the Δ^4-3-oxosteroid-5β-reductase (Δ^4-3-oxoR) deficiency (OMIM 604741) due to variants in *AKR1D1*.[135–140]

Should patients with a bile acid synthesis defect (namely HSD3B7 and AKR1D1 deficiencies) be treated with an oral primary bile acid to improve survival and other liver-related outcomes?

Recommendation

- Patients with a primary bile acid synthesis defect (namely HSD3B7 and AKR1D1 deficiencies) should be treated with an oral primary bile acid **(LoE 3, strong recommendation, strong consensus).**

In Europe, the prevalence of these diseases could be estimated as 1.13 cases per 10 million (0.99 and 0.14 for 3β-HSD and Δ4-3-oxoR deficiencies, respectively).[141] Defects in these enzymes catalysing key reactions in the formation of primary bile acids, namely cholic acid (CA) and chenodeoxycholic acid in humans, lead to an inadequate synthesis of primary bile acids that are critical for bile formation and to the accumulation of atypical and hepatotoxic bile acid intermediates.[142–145]

HSD3B7 and AKR1D1 deficiency most commonly manifest as cholestasis in neonates or infants which may progress to early cirrhosis and liver failure unless treated.[135,136,143,144] Absence of pruritus, normal serum GGT and normal or low total serum primary bile acid concentrations are diagnostic features of these conditions.[143,144,146] Specific diagnosis is based on mass spectrometry analysis of urinary bile acids showing typical bile acid profiles and on the identification of disease-causing mutations in the *HSD3B7* or the *AKR1D1* gene.[135–144] Oral primary bile acid replacement by CA or chenodeoxycholic acid is required for these defects to restore bile flow and to downregulate endogenous bile acid synthesis.[143,144,11,12,147,148] CA is the major primary bile acid in humans and is now recognised as the bile acid of choice for the treatment of 3β-HSD and Δ4-3-oxo-R deficiencies, since it is neither hepatotoxic nor embryotoxic/teratogenic.[11,13,143,144,148–150] CA is the only primary bile acid with a marketing authorisation in both the USA and Europe for

Table 5. Overview of the different types of PFIC.

Disease	Affected gene	Affected protein
FIC1 deficiency (PFIC1)	*ATP8B1*	FIC1
BSEP deficiency (PFIC2)	*ABCB11*	BSEP
MDR3 deficiency (PFIC3)	*ABCB4*	MDR3
TJP2 deficiency	*TJP2*	TJP2
FXR deficiency	*NR1H4*	FXR
SLC51A deficiency	*SLC51A*	SLC51A
USP53 deficiency	*USP53*	USP53
KIF12 deficiency	*KIF12*	KIF12
ZFYE19 deficiency	*ZFYVE19*	ZFYE19
MYO5B deficiency	*MYO5B*	Myosin 5B
SEMA7A deficiency	*SEMA7A*	SEMA7A
VPS33B deficiency	*VPS33B*	Vps33

FIC1, familial intrahepatic cholestasis protein 1; BSEP, bile salt export pump; MDR3, multidrug resistance protein 3; TJP2, tight junction protein 2; FXR, farnesoid X receptor; SLC51A, solute carrier family 51, subunit alpha; USP53, ubiquitin specific peptidase 53; KIF12, kinesin family member 12; ZFYVE19, zinc finger fyve domain-containing protein 19; MYO5B, myosin 5B; SEMA7A, semaphorin 7A; VPS33B, vacuolar protein sorting 33, yeast, homolog of, B; ATP8B1, ATPase phospholipid transporting 8B1; ABCB11, ATP binding cassette subfamily B member 11; ABCB4, ATP binding cassette subfamily B member 4; NR1H4, nuclear receptor subfamily 1 group H member 4. Based on most recent entries on https://www.omim.org/entry/211600 at the time of CPG writing.

these two indications.[151,152] In case reports, case series and cohort studies, prolonged oral CA therapy has been shown to be safe and lifesaving during childhood as it leads to normalisation of clinical features, serum liver tests and liver imaging, as well as a substantial improvement of bile acid profiles (mass spectrometry) and liver histology.[11,13,148–150] Only a few studies have reported on the long-term safety and efficacy of CA therapy during adulthood. These studies indicate that the effects of CA are maintained long term and that CA is safe during adulthood, including in pregnant women.[153] Thus, with a successful transition of care and follow-up by a specialised hepatologist, CA therapy will likely guarantee patients, including those diagnosed as adults, a normal quality of life throughout their life.[154–157]

Future perspectives

Recent years have witnessed great advances in our understanding of the pathobiology of genetic cholestatic liver diseases together with major improvements in treatment.

The general understanding of the natural course of disease and prognosis has only in recent years been (partly) illuminated in large numbers by the international consortia of the GALA study for ALGS and the NAPPED study for FIC1 deficiency (PFIC1) and BSEP deficiency (PFIC2). While they have added greatly to the understanding of these diseases, much is still unknown about the role of genetics and other factors (environmental influences, modifier genes) that could alter the course of disease and change the responsiveness to treatment. In addition, more (very rare) genetic types of intrahepatic cholestasis have only recently been identified and (partly) characterised. Much is still unknown regarding the natural disease course of these new subtypes, since patient numbers remain extremely low. This underlines the importance of global collaboration to characterise these rare genetic diseases.

The introduction of IBAT inhibitors as the first FDA- and EMA-approved treatments for ALGS and PFIC have opened up a new horizon for medical therapeutic strategies, next to the previously common surgical options (*e.g.* surgical interruption of the EHC and liver transplantation). Long-term follow-up data will determine their roles in altering the natural course of disease. Initial data suggest that in responsive patients, IBAT inhibitor treatment delays the need for liver transplantation due to pruritus. This needs to be studied in more patients and with longer follow-up. It also remains to be established if IBAT inhibitor treatments affect the (rate of) progression of the indicated types of cholestasis towards end-stage liver disease and/or the risk of HCC development. It has become evident that not all FIC1- and BSEP-deficient patients respond to IBAT inhibition. Therefore, there is a clear unmet need to understand the mechanism(s) by which patients do or do not respond to IBAT inhibition and find alternative treatments for patients who are non-responsive to the currently available drugs. Finally, several fundamental studies have also evaluated the effects of other drugs (*e.g.* potentiators and correctors, combination treatments, small-interfering RNAs) in the treatment of these diseases, and while clinical trials are eagerly awaited, their implementation will be difficult given the low number of (often paediatric) patients.

Appendix. Delphi round agreement on the recommendations of the present clinical practice guidelines.

Recommendation	Consensus
Measuring serum AAT levels as a triage tool for AATD testing is recommended **(LoE 3, strong recommendation).**	78%
Serum AAT levels should be measured in situations without inflammation **(LoE 3, strong recommendation).**	86%
A cut-off of 50 mg/dL (9.2 μmol/L) can be used as a triage tool that raises suspicion of severe AATD **(LoE 3, weak recommendation).**	80%
Phenotyping can be used when a quick decision is needed, whereas genotyping should be used for definitive diagnosis when available **(LoE 3, weak recommendation).**	73%
There are insufficient data to advise for/against UDCA treatment **(LoE 3, strong recommendation)**.	82%
Impaired liver synthetic function or decompensation of cirrhosis should be used to identify severe disease associated with poor outcomes **(LoE 3, strong recommendation)**.	100%
Liver stiffness measurements can be used in adults with Pi*ZZ to estimate the level of histological fibrosis **(LoE 3, weak recommendation).**	95%
Lifestyle counselling is recommended as smoking, obesity and alcohol consumption have negative effects on the health of patients with AATD **(LoE 3, strong recommendation).**	100%
Despite the lack of evidence of the impact of lifestyle counselling on actual lifestyle change, the authors recommend lifestyle counselling given the important influence of lifestyle on disease outcome **(LoE 5, weak recommendation).**	96%

(*continued on next page*)

(*continued*)

Recommendation	Consensus
Liver biopsy should be considered when careful non-invasive evaluation remains inconclusive **(LoE 3, strong recommendation).**	100%
In the setting of living donor transplantation, Pi*MZ organs should only be considered for liver transplantation when other suitable organs are lacking and no signs of liver injury are present **(LoE 5, weak recommendation).**	96%
Livers from individuals with known homozygous Pi*Z mutations (Pi*ZZ genotype) should not be used as donor organs **(LoE 3, strong recommendation).**	96%
The routine use of liver biopsy for determining prognosis or management of liver disease in ALGS is not recommended **(LoE 3, strong recommendation).**	100%
When available, IBAT inhibitor treatment should be offered to patients with ALGS and cholestatic pruritus **(LoE 2, strong recommendation).**	100%
Statins are not recommended for hyperlipidaemia or xanthomas in ALGS **(LoE 4, strong recommendation).**	100%
Surgical interruption of the EHC is not recommended as a routine treatment to prolong native liver survival for patients with FIC1 deficiency **(LoE 3, strong recommendation).**	90%
Medical or surgical interruption of the EHC at transplantation or post-transplant could be used to treat diarrhoea and steatohepatitis in patients with FIC1 deficiency **(LoE 5, weak recommendation).**	95%
Management including follow-up/screening and treatment, including interruption of the EHC, should be based on the existing evidence for specific genotypes **(LoE 3, strong recommendation).**	96%
The screening intervals for hepatocellular carcinoma should be based on genotype (specific recommendations provided in a subsequent section) **(LoE 3, strong recommendation).**	91%
To prolong native liver survival, surgical interruption of the EHC should be considered in patients with a responsive genotype **(LoE 3, strong recommendation).**	91%
Due to the current lack of data, it is not yet possible to recommend medical rather than surgical interruption of the EHC to prolong native liver survival **(LoE 5, weak recommendation).**	100%
Surgical interruption of the EHC can be used to reduce pruritus in patients with a responsive genotype prior to considering liver transplantation **(LoE 3, weak recommendation).**	82%
Medical interruption of the enterohepatic circulation by IBAT inhibition should be considered to reduce pruritus prior to considering liver transplantation in patients with missense mutations **(LoE 2, strong recommendation).**	100%
For patients with missense mutations, an HCC screening interval of 3 to 6 months is recommended based on expert opinion **(LoE 3, strong recommendation).**	100%
For patients with biallelic PPTMs, an HCC screening interval of 3 months is recommended **(LoE 3, strong recommendation).**	100%
BSEP alloimmunisation should be diagnosed through the detection of anti-BSEP antibodies in plasma or anti-canalicular antibodies upon immunofluorescence staining of liver tissue **(LoE 3, strong recommendation**	96%
Treatment should focus on adapting the immunosuppressive regimen and when necessary, a combination of rituximab and plasmapheresis should be considered **(LoE 3, strong recommendation).**	100%
Re-transplantation(s) may become necessary if treatment fails **(LoE 3, weak recommendation).**	96%
In patients with biallelic *ABCB4* variants, genetics should be used to predict phenotype and response to UDCA therapy **(LoE 2, strong recommendation).**	92%
Patients with known pathogenic variants in *ABCB4* should be offered follow-up for liver-related complications, including malignancy **(LoE 2, strong recommendation).**	100%
Genetic testing should be offered to first degree relatives of patients with MDR3 deficiency **(LoE 2, strong recommendation).**	100%
Follow-up should be adjusted to clinical presentation and detected genetic variant (expert opinion) **(LoE 5, strong recommendation**	96%
Medical history, laboratory analysis and non-invasive screening for higher degree fibrosis should be carried out during follow-up visits (at least) every 6 months **(LoE 3, strong recommendation).**	96%
Liver ultrasound and/or MRI with MRCP are recommended at least on a yearly basis **(LoE 4, strong recommendation).**	100%
UDCA treatment is recommended for those with at least one *ABCB4* missense variant and a clinical phenotype **(LoE 3, strong recommendation).**	100%
Partial response to UDCA has been reported in patients carrying biallelic protein-truncating MDR3/*ABCB4* gene variants and UDCA may therefore be offered **(LoE 4, weak recommendation).**	100%
Long-term UDCA therapy is recommended for recipients of an MDR3 monoallelic deficient graft **(LoE 4, strong recommendation).**	96%
Patients with a primary bile acid synthesis defect (namely HSD3B7 and AKR1D1 deficiencies) should be treated with an oral primary bile acid **(LoE 3, strong recommendation).**	100%

Abbreviations

AAT, alpha-1 antitrypsin; AATD, alpha-1 antitrypsin deficiency; ALGS, Alagille syndrome; ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; BASD, bile acid synthesis defects; BRIC, benign recurrent intrahepatic cholestasis; BSEP, bile salt export pump; CA, cholic acid; CPGs, Clinical Practice Guidelines; DILI, drug-induced liver injury; EASL, European Association for the Study of the Liver; EHC, enterohepatic circulation; ICP, intrahepatic cholestasis of pregnancy; INR, international normalised ratio; LPAC, low phospholipid-associated cholelithiasis; LSM, liver stiffness measurement; NAPPED, NAtural course and Prognosis of PFIC and Effect of biliary Diversion; NGS, next-generation sequencing; PFIC, progressive familial intrahepatic cholestasis; PPTM, predicted protein-truncating mutation; UDCA, ursodeoxycholic acid; VCTE, vibration-controlled transient elastography; VUS, variants of uncertain significance; WES, whole-exome sequencing; WGS, whole-genome sequencing.

Conflict of interest

Please refer to the accompanying ICMJE disclosure forms for further details.

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Supplementary data

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