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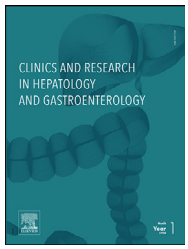

PRACTICE GUIDELINES

Non-invasive diagnosis and follow-up of rare genetic liver diseases

KEYWORDS
Wilson' disease, copper balance;
Exchangeable copper and relative exchangeable copper;
molecular analysis gene;
ATP7B gene;
alpha-1 antitrypsin deficiency serum testing;
isoelectric gel electrophoresis phenotyping;
SERPINA-1 gene;
Cystic fibrosis neonatal screening;
CFTR gene;
non-invasive fibrosis markers

Abstract
Rare genetic liver diseases can result in multi-systemic damage, which may compromise the patient's prognosis. Wilson's disease and alpha-1 antitrypsin deficiency must be investigated in any patient with unexplained liver disease. Cystic fibrosis screening of new-borns is now implemented in most high-prevalence countries. The diagnosis of these diseases can be strongly suggested with specific non-invasive tests. Molecular analysis gene for these diseases is long and tedious but is recommended to confirm the diagnosis and help for the family screening. Liver biopsy is not systematic and is discussed when it helps diagnosis. Currently, for these three diseases, non-invasive fibrosis markers could identify patients with risk of cirrhosis and complications.

Screening for alpha-1 antitrypsin deficiency is recommended in patients with chronic obstructive pulmonary disease or chronic liver disease. The screening is based on the demonstration of a decrease in serum alpha-1 antitrypsin concentration and phenotyping can be performed by isoelectric focusing gel electrophoresis. In the event of pulmonary involvement, it is recommended to investigate signs of chronic liver disease.

Cystic fibrosis screening of new-borns is now implemented in most high-prevalence countries. Neonatal screening programmes, with blood sample taken shortly after birth by heel prick-testing for immunoreactive trypsin, reduce the severity of the disease, prevent delayed diagnosis and improve prognosis. After diagnostic or during patient follow-up with cystic fibrosis, a liver assessment is recommended to look in particular for portal hypertension.

In its various diseases, the liver biopsy is not systematic and is discussed when it helps diagnosis. Currently, for these three diseases, non-invasive fibrosis markers could identify patients with risk of cirrhosis and complications. Molecular analysis gene for these diseases is long and tedious but is recommended to confirm the diagnosis and help for the family screening.

Wilson's disease

Wilson's disease is an autosomal recessive genetic disorder that causes the accumulation of copper in the tissues of

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several organs, including the liver, brain and cornea[1,2]. It is estimated that between 1000 and 1500 subjects have Wilson's disease in France. The Wilson gene, located on chromosome 13, encodes an intracellular copper transporter protein, ATP7B. This protein allows the cellular excretion of excess copper. The functional deficiency of ATP7B in Wilson's disease leads to a defect in the elimination of copper in the bile, causing its accumulation in the liver and its subsequent release in free form into the bloodstream. Thus, Wilson's disease is initially a liver disorder. Left undiagnosed, it progresses to a systemic condition with copper accumulation in other organs, including the brain and eye.

Question 1: under what circumstances is it recommended to investigate for Wilson's disease?

1. **Wilson's disease must be investigated in any patient with unexplained liver disease and/or unexplained neurological or neuropsychiatric disorders (B1)**
2. **The diagnosis must not be ruled out on age alone (B1)**

Strong agreement

The signs of the disease are highly polymorphic and manifest in the majority of cases in children or young adults. Patients with an initial presentation of neurological or psychiatric symptoms are most often slightly older than patients with a hepatic presentation of the disease. Neurological damage is commonly already associated with liver damage, the symptoms of which usually go unnoticed.

The diagnosis is based on a range of clinical, biological, radiological (brain MRI) and even histological features. Molecular biology makes it possible to confirm the diagnosis in more than 90% of cases. The treatment combines patient diet, copper chelators or zinc salts. Liver transplantation is the treatment for fulminant hepatitis-related forms and decompensated cirrhosis, and it is also considered for the treatment of very severe neurological forms. This rare genetic disease has a good prognosis if treatment is initiated early and continued throughout life.

Question 2: what non-invasive methods are recommended for the diagnosis of wilson's disease?

Diagnosis is based on a combination of clinical and biological features:

- **Decreased ceruloplasmin and increased urinary excretion of copper over 24 hours (B1)**
- **The REC (Relative Exchangeable Copper = exchangeable copper/total copper) is currently the most sensitive and specific biochemical test for the diagnosis of Wilson's disease. A threshold above or equal to 18% is strongly in favour of Wilson's disease (B1)**
- **Investigation for a Kayser-Fleischer ring by slit lamp examination must be performed by an experienced operator. Its absence does not exclude diagnosis (B1)**
- **MRI imaging of the brain with expert neurologist advice is recommended (B1)**
- **Molecular analysis by complete sequencing of the ATP7B gene is recommended to confirm the diagnosis following advice from an expert reference centre (B1)**

Strong agreement

The clinical diagnosis of Wilson's disease is not based on a single element but on a combination of features with a set of symptoms, most often non-specific and affecting several organs; hence the potential confusion with other diseases. Classically in Wilson's disease, routine monitoring shows low blood copper levels, ceruloplasmin deficiency and high urine copper levels. The diagnosis can easily be made in young people with neurological disorders or cirrhosis, associated with a Kayser-Fleischer ring and low ceruloplasmin levels. The absence of a Kayser-Fleischer ring does not rule out the diagnosis, but it is almost always present in neurological forms.

The disease is caused by abnormalities in the gene encoding ATP7B. Currently, more than 500 mutations have been identified as well as some deletions in the gene. Analysis of the mutations by complete gene sequencing is currently possible.

Blood and urine copper assessment (diagnosis and follow-up)

The first-line examinations for copper level assessment are non-invasive and can be routinely performed. Ceruloplasmin levels are usually low or even deficient. The quantification of this serum protein is based on nephelometric techniques and a concentration of less than 0.2 g/l is generally observed during the course of the disease (normal level 0.2—0.5 g/l). In adult patients, ceruloplasmin is lowered in about 95% of homozygous patients and in 20% of heterozygous patients [3]. Ceruloplasmin is within its normal range among 15—36% of paediatric cases [4]. According to some studies, up to 50% of people with decompensated liver disease have normal ceruloplasmin concentrations [5].

Urinary copper is an important diagnostic parameter that is almost always higher than 100 μg/24 h (1.6 μmol/24 h). This assay is also of great interest for monitoring treatment efficacy and compliance. However, interpretation of the urine copper assay can be difficult in other liver diseases, especially in severe disease. Urinary copper excretion can be moderately increased in heterozygous patients.

Urinary excretion of copper after administration of D-penicillamine was put forward as a diagnostic test [6,7]. This test is still offered to children. After an initial oral administration of 500 mg D-penicillamine, 12 hours later a 24-hour urine sample is collected. Evidence of urinary copper excretion above 25 μmol/24 h (1600 μg/24 h) is considered in favour of Wilson's disease. D-penicillamine administration methods and cut-off values vary among studies. This test does not exclude diagnosis in asymptomatic siblings. In adults with the disease, the predictive values

and utility of this test are still poorly defined [8]. Therefore, this test is not currently recommended for use in adults.

Exchangeable copper

Relative exchangeable copper for diagnostic purposes

In view of the invasive nature of percutaneous liver biopsy and the often long delays for genetic analyses, being able to perform rapid diagnosis by non-invasive methods remains attractive. It is in this context that the exchangeable copper assay was developed. Exchangeable copper is a good indication of circulating and toxic copper. After incubation with a chelating agent (EDTA), the serum is membrane ultra-filtrated (for the elimination of the main copper vector proteins), then centrifuged, releasing the exchangeable copper (eliminating ceruloplasmin-bound copper) [9]. Exchangeable copper is stable for 24 hours at room temperature and up to 14 days when frozen. A new marker has been identified; REC (relative exchangeable copper) = exchangeable copper/ total copper. In a study of 16 patients with the disease, a REC above 18.5% was demonstrated to be a good marker for the diagnosis of Wilson's disease, with a sensitivity and specificity close to 100% [10]. Thus, the REC assay is a non-invasive test with results that can be obtained within just a few days and it can be very useful for the diagnosis of index patients [10,11]. A study evaluating REC in 127 asymptomatic relatives with Wilson's disease compared the results with other standard tests (serum copper, ceruloplasmin, urinary copper excretion and molecular analysis). Using a threshold of 15%, REC determination made it possible to significantly differentiate between non-diseased subjects and patients with the disease [11].

Exchangeable copper in disease severity assessment

In a study of 48 patients with recent diagnosis of Wilson's disease, exchangeable copper values were found to be significantly higher in patients with ocular and/or brain damage. In addition, exchangeable copper at diagnosis was positively correlated with a score assessing neurological symptoms and disability, the severity of corneal copper deposition and MRI brain lesions [10,12]. Thus, exchangeable copper can indicate the severity of extra-hepatic damage. However, in fundamental liver disease, exchangeable copper does not illustrate the ability to be used as a marker for liver damage severity.

Brain MRI

Brain MRI (T1, T2 and FLAIR sequences) is an important tool in the diagnosis of the disease, even if the abnormalities detected are not specific. Patients with neurological symptoms in particular, but also some patients who are asymptomatic or have a hepatic form of the disease, may have signal abnormalities within the brain parenchyma. The damage mainly involves the grey matter. The damage affects the grey matter nuclei, dentate nuclei and the dark matter, and is usually symmetrical. The "face of giant panda" appearance has been described for mesencephalic involvement. Lesions most often demonstrate varying degrees of T1 hyposignal and a generally clear T2 hypersignal. Diffuse cerebral atrophy is common [17,18].

Question 3: what are the recommended non-invasive methods for the family screening for Wilson's disease?

1. **When a case of Wilson's disease is found in a family, screening is recommended for siblings of the index case from the age of 3 years (B1)**
2. **It is recommended to extend screening to the parents of the index case (D2)**
3. **Family screening for Wilson's disease must involve:**
   - **Copper assessment: ceruloplasmin, 24-hour blood and urine copper levels (B1)**
   - **Relative Exchangeable Copper (REC) testing (B1)**
   - **Molecular analysis of the ATP7B gene (B1)**

Strong agreement

The REC (Relative Exchangeable Copper) assay is a test that can be very useful for screening related subjects. Specific screening for known mutations or haplotype analysis must be the main mode of screening for first-degree relatives of patients with the disease. For example, molecular analysis of the ATP7B gene takes a long time and is tedious, taking possibly several weeks to several months. However, thanks to advanced molecular biology techniques, only 6% of cases remain unconfirmed in the most recent diagnosed families.

Question 4: what are the recommended non-invasive methods for follow-up of patients with Wilson's disease?

1. **For specific routine monitoring of treatment with copper chelators, liver function tests, exchangeable copper and copper urine tests must be regularly performed, twice a year in a well-balanced patient (B2)**
2. **In cirrhosis, screening for portal hypertension and hepatocellular carcinoma is required (A1)**
3. **Remote patient monitoring is recommended in patients with Kayser-Fleischer rings and/or brain MRI lesions (B2)**
4. **Measurement of liver stiffness could help identify patients with cirrhosis and monitor the progression of the disease (C2)**

Strong agreement

Urinary copper is of great interest for monitoring efficacy and compliance with treatment with copper chelators.

Ultrasound, as for other chronic liver diseases, is useful in the diagnosis of cirrhosis. The existence of hepatic morphological abnormalities and signs of portal hypertension are grounds for suspecting cirrhosis. Semi-annual abdominal ultrasound is also useful for detecting hepatocellular carcinoma in cirrhotic patients. CT and MRI scans then allow the characterisation of certain abnormalities observed on ultrasound and/or suspicious nodules. Given copper does not harbour magnetic properties like iron, quantification of copper by MRI cannot be performed.

Given the opportunity to diagnose Wilson's disease by non-invasive methods (numerous features, relative exchangeable copper, molecular genetics), liver biopsy has become less critical in the diagnosis of the disease. Moreover, there is often reluctance to perform this biopsy in a context of often very young patients or abnormal liver movements. However, it is still useful to estimate fibrosis level at the time of diagnosis and during treatment follow-up to improve follow-up adaptation (screening for nodules in cirrhotic patients, features favouring portal hypertension).

Therefore, similarly to other liver diseases and in particular viral-related liver diseases, non-invasive diagnostic markers for liver fibrosis are increasingly being used in routine practice in patients with Wilson's disease. Although today there are some preliminary results, there is little data correlating liver stiffness values and fibrosis scores to Wilson's disease in large cohorts. One study demonstrated that 35 patients under treatment had a liver stiffness measurement that correlated with liver biopsy results. The liver stiffness measurement limit for the diagnosis of significant fibrosis was 6.6 kPa, with a sensitivity of 66.7%, specificity of 81.2%, positive predictive value of 67%, negative predictive value of 82% and diagnostic performance of 79.9% (AUROC 0.799). The best liver stiffness threshold for the diagnosis of severe fibrosis was 8.4 kPa, with a sensitivity of 89.5%, a specificity of 88.9%, a positive predictive value of 94%, a negative predictive value of 80% and a diagnostic performance of 90.6% (AUROC 0.906) [13]. In addition, preliminary data among children has shown that liver stiffness is high at diagnosis and decreases during specific treatment [14-16].

The brain lesions observed on MRI may be reversible under copper chelation therapy [19]. Thus, to monitor treatment effectiveness, it is advisable to repeat brain MRI scan at one year from treatment initiation and then later depending on the clinical course.

Question 5: in what situations is liver biopsy required for the diagnosis or follow-up of Wilson's disease?

1. **Liver biopsy must be performed if there is diagnostic doubt. Liver copper quantification must be carried out at the time of the liver biopsy (B1)**
2. **Liver biopsy with hepatic copper quantification must be considered by an expert reference centre for asymptomatic patients with isolated mutations in favour of the disease (D2)**
3. **Liver biopsy can also be performed in patients with comorbidities or other related liver diseases (C2)**

Strong agreement

In patients with an early "liver" presentation of the disease, diagnosis can sometimes be difficult. In such cases or in case of diagnostic doubt, a liver biopsy with a high intra-hepatic copper level can confirm the diagnosis. An aspect of chronic hepatitis is often observed, involving cirrhosis in approximately 50% of cases.

Liver biopsy may provide additional information. Early steatosis, nuclear glycogen inclusions and focal hepatocellular necrosis can be seen. Rhodamine labelling can be increased but its expression remains heterogeneous and highly variable (especially in the early stages of the disease). Positive rhodamine staining can be observed in severe chronic cholestasis.

X-ray fluorescence allows the quantitative analysis of several tissue elements. Copper level determination, as well as the levels of other metals including iron, has been shown possible within just a few minutes from percutaneous needle biopsies that have been fixed with formalin and embedded in paraffin. It has thus been shown possible to significantly differentiate patients with Wilson's disease from other chronic liver diseases with a specificity of 97.6% and a sensitivity of 100% [20]. This rapid and versatile method carried out on paraffin-fixed biopsies could easily be implemented in a clinical setting.

Alpha-1 antitrypsin deficiency

Alpha-1 antitrypsin deficiency is a genetic disease that mainly presents lung impairment with emphysema and fibrotic liver disease. Alpha-1 antitrypsin deficiency is characterised by the deficiency of an important protease inhibitor, alpha-1 antitrypsin [21]. The disease is often underdiagnosed or misdiagnosed as asthma, chronic obstructive pulmonary disease or "cryptogenic" liver disease.

Prevalence in the general Western European population is approximately 1/2500 and it is highly dependent on the number of Scandinavian descendants in the population [22]. Alpha-1 antitrypsin deficiency is due to mutations in the SERPINA1 gene (14q32.1) coding for alpha-1 antitrypsin. Its transmission is autosomal recessive.

Question 6: under what circumstances is it recommended to investigate alpha-1 antitrypsin deficiency?

1. **Screening for alpha-1 antitrypsin deficiency is recommended in patients with chronic obstructive pulmonary disease or chronic liver disease (B1)**

Strong agreement

Clinical manifestations can widely vary among patients; some patients may be asymptomatic and others may have severe liver or lung disease.

The ZZ and SZ phenotypes are risk factors for the development of respiratory symptoms (dyspnea, coughing), the early onset of emphysema and the development of

obstructive lung disease in early adulthood. Environmental factors, such as smoking and exposure to dust, are additional risk factors and have been linked to accelerated disease progression. There are several alleles that may be deficient. In Northern Europe, the most common deficiency alleles are PI*Z and PI*S. The majority of patients with severe alpha-1 antitrypsin deficiency are homozygous for the Z allele (PI*ZZ). The ZZ phenotype in patients with alpha-1 antitrypsin deficiency can also lead to the development of subacute liver failure or chronic liver disease in childhood or adulthood: characteristic signs are persistent jaundice associated with increased conjugated bilirubin after birth and liver enzyme abnormalities. Cirrhosis can arise around the age of 50 years after a long asymptomatic period. In very rare cases, necrotising panniculitis and secondary vasculitis can occur.

Question 7: what non-invasive methods are recommended for the diagnosis of alpha-1 antitrypsin deficiency?

1. **Screening for alpha-1 antitrypsin deficiency is based on the demonstration of a decrease in serum alpha-1 antitrypsin concentration (A1)**
2. **In alpha-1 antitrypsin deficiency, phenotyping can be performed by isoelectric focusing gel electrophoresis in order to identify normal alleles (M), the most frequent variants (S or Z) as well as rarer variants (Mmalton) (B1)**
3. **When in doubt, targeted genotyping for the most frequent mutations (S and Z) can confirm the diagnosis but must be interpreted in relation to the serum alpha-1 antitrypsin concentration (B2)**
4. **Complete sequencing of the SERPINA-1 gene may be necessary to identify the cause of an unexplained deficiency (B2)**

Strong agreement

Diagnosis can be made by the detection of decreased serum alpha-1 antitrypsin levels [23]. In alpha-1 antitrypsin deficiency, phenotyping by isoelectric focusing gel electrophoresis is performed. Differential diagnosis must rule out other causes of chronic liver disease. The majority of patients with severe alpha-1 antitrypsin deficiency are homozygous for the Z allele (PI*ZZ). This technique, which is commercially available, makes it possible to identify the most common variants [24]. The normal PiM allele is characterised by medium-speed electrophoretic mobility, the PiS-deficient allele is slower and the PiZ allele is very slow. Certain medical conditions, such as inflammation, may cause the plasma baseline alpha-1 antitrypsin concentration to vary and it increases in response to inflammatory stimuli. This could distort the alpha-1 antitrypsin assay results obtained and particularly conceal the detection of subjects with intermediate deficiency (subjects with a heterozygous mutated allele).

Despite the establishment of this technique as a reference method for the exploration of alpha-1 antitrypsin, complementary means of analysis provided by molecular biology techniques are being increasingly used in the diagnosis of alpha-1 antitrypsin deficiency. The first molecular approach is investigation of the two deficient alleles, PiS and PiZ, which are most commonly reported [23]. However, deficient cases lacking mutations in the exons coding for alpha-1 antitrypsin have been reported. In these exceptional and unexplained cases, analysis of polymorphisms in the alpha-1 antitrypsin gene (non-coding exons, introns, 3′ and 5′ regions) and/or other serpin genes in close proximity could elucidate the cause of the deficiency.

Question 8: what non-invasive methods are recommended for the assessment of the severity of alpha-1 antitrypsin deficiency?

1. **It is recommended to investigate signs of chronic liver disease in order to assess liver disease severity: liver function tests, morphological evaluation by abdominal ultrasound and/or cross-sectional imaging (B2)**
2. **Measurement of liver stiffness can identify patients with cirrhosis (C2)**
3. **Expert advice is recommended for the assessment of extra-hepatic disease severity (A1)**

Strong agreement

Diagnostic tests are non-invasive and currently include determination of blood alpha-1 antitrypsin concentration, phenotyping by isoelectric focusing gel electrophoresis and genotyping. However, given the silent nature of liver disease, particularly in the early stages, the use of non-invasive methods for the diagnosis of liver fibrosis is increasingly developing in routine practice as it is for diagnosis of other chronic liver diseases.

Abdominal ultrasound, as for other chronic liver diseases, is useful in the diagnosis of cirrhosis. The existence of hepatic morphological abnormalities and signs of portal hypertension indicate the suspicion of cirrhosis. Semi-annual abdominal ultrasound is also useful for the detection of hepatocellular carcinoma in cirrhotic patients. CT and MRI scans can then be used to characterise certain abnormalities observed on ultrasound and/or suspicious nodules.

Percutaneous liver biopsy is the reference method for the quantification of liver fibrosis.

However, given its invasive nature (pain, risk of complications) and diagnostic limitations, percutaneous liver biopsy is not adequate for the screening or follow-up of asymptomatic patients.

There is limited data on non-invasive methods for the assessment of liver fibrosis in patients with alpha-1 antitrypsin deficiency. A pilot study involving 33 patients has suggested that measurement of liver stiffness by MRI could be an accurate means of estimating liver fibrosis in patients with alpha-1 antitrypsin deficiency [26]. The MRI measurement of liver stiffness had an AUROC of 0.90 ($p < 0.0001$). The threshold value of 3.0 kPa had an accuracy of 89%, with a sensitivity of 80% and specificity of 100% for detecting stage 1 fibrosis according to the Ishak classification. In another study of 15 patients with alpha-1 antitrypsin deficiency with no clinical hepatic manifestations, different non-invasive methods for diagnosing liver fibrosis were evaluated: measurement of liver stiffness by

MRI, by pSWE and by 2D-SWE [27]. It was shown that these different liver stiffness measurement methods enabled the early and consistent identification of patients with liver fibrosis compared to healthy subjects [27].

Question 9: what non-invasive methods are recommended for the follow-up of patients with alpha-1 antitrypsin deficiency?

1. **Liver damage monitoring is recommended at least once a year (B2)**
2. **Screening for hepatocellular carcinoma must be performed semi-annually in cirrhotic patients (A1)**
3. **The methods used and monitoring regularity remain uncharacterised for patients lacking evidence of liver damage during severe alpha-1 antitrypsin (PiZZ) deficiency. However, they must take into account the presence or absence of liver disease co-factors (metabolic syndrome, excessive alcohol consumption...) (C2)**

Strong agreement

Question 10: what are the recommended non-invasive methods for family screening for alpha-1 antitrypsin deficiency?

1. **Family screening must include serum testing and targeted phenotyping or genotyping (B1)**
2. **It is recommended to screen first-degree parents of the index case (B1)**

Strong agreement

Question 11: in what situations is liver biopsy necessary for the diagnosis or follow-up of alpha-1 antitrypsin deficiency?

1. **Liver biopsy must be considered:**
   - **During the pre-lung transplant assessment when there is any suspected advanced liver disease (C2)**
   - **In patients with comorbidities or other related chronic liver disease (C2)**

Strong agreement

Liver biopsy reveals PAS-positive cytoplasmic inclusions, particularly in the periportal region, corresponding to the accumulation of alpha-1 antitrypsin. Percutaneous liver biopsy is used to assess the extent of fibrosis and to determine the presence or not of cirrhosis [25]. Other damage can be associated with varying degrees; this is particularly the case for biliary obstructions.

Cystic fibrosis

Cystic fibrosis is the most common genetic disorder among children in populations of European origin. Its transmission is autosomal recessive and there are 5,000−6,000 patients in France, half of whom are adults. There are approximately 200 new patients born with this disorder each year [28].

The genetic abnormality is located on chromosome 7, in the region that codes for the synthesis of the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) protein. CFTR is composed of 1480 amino acids and it regulates the transport of sodium and chlorine ions across the epithelial cell apical membrane. Nearly 2000 mutations have been described since the discovery of the gene, the most frequent of which concerns the elimination of an amino acid (phenylalanine) located at position 508 on the CFTR protein (delta F508 mutation). This mutation is found in 70−75% of patients.

Dysfunction of the CFTR gene results in defective mucus secretion, particularly at the bronchopulmonary and digestive levels. The clinical expression of the disorder is very heterogeneous from one patient to another. This heterogeneity is not only due to the high number of mutations; genes other than the CFTR gene, known as modifiers, can also influence the clinical expression of the disorder.

Pulmonary damage manifests from the first months of life due to inflammation and obstruction of the bronchial tubes, favouring bronchopulmonary superinfections, in particular by Staphylococcus aureus and Pseudomonas aeruginosa. Colonisation by pyocyanin affects approximately 50% of patients, more than 80% of these being adults. This represents a turning point in the prognosis of cystic fibrosis, with the risk of the development of chronic respiratory failure.

Exocrine pancreatic insufficiency is present from birth in 70−80% of patients. CFTR protein dysfunction results in hyperviscose pancreatic secretions, which obstruct the pancreatic ducts and contribute to the secondary destruction of acini. Insufficient external lipase secretion leads to maldigestion and malabsorption syndrome which has a deleterious effect on nutritional status and is responsible for vitamin deficiencies.

Abnormal CFTR protein expression in bile epithelial cells can cause obstruction of the bile ducts. These lesions, initially focal (focal biliary cirrhosis), progress to multilobular biliary cirrhosis. Liver damage incidence varies according to the extent of the abnormalities. Although histologic abnormalities are present in the autopsies of patients who have died from other complications, biliary cirrhosis with or without portal hypertension is present in only about 10% of patients [29].

The prognosis of cystic fibrosis, which depends above all on respiratory impairment, has considerably improved thanks to its earlier diagnosis been made possible by neonatal screening (widespread in France) and the setting up of multidisciplinary teams in specialised care centres. Cystic fibrosis has long been a disease considered exclusive to children. The median survival rate did not exceed 5 years 60 years ago, but now almost all children currently followed in specialised paediatric centres will become adults, with a life expectancy of around 45 years.

Cystic fibrosis screening of new-borns is now implemented in most high-prevalence countries [28]. Neonatal screening programmes reduce the severity of the disease, prevent delayed diagnosis and improve prognosis. A blood sample is taken shortly after birth by heel prick-testing for immunoreactive trypsin (IRT) quantification, corresponding to a group of molecules related to a pancreatic enzyme

demonstrating increased blood levels in subjects with cystic fibrosis. This assay carried out on dried blood provides information on the risk of cystic fibrosis and thus consequent diagnostic investigations. The increase in IRT is not specific for cystic fibrosis because temporary duct obstruction can also lead to an increase in IRT in the blood.

A sweat test must be performed afterwards as a final diagnostic test. After stimulation of sweating, sweat is collected and the chloride concentration is measured. The test is considered normal when this level is below 40 mmol/l (30 mmol/l in infants). The diagnosis is positive if two measures are above or equal to 60 mmol/l. When the level is between 40 (30 in infants) and 60 mmol/l, interpretation is debatable and the diagnosis of cystic fibrosis cannot be excluded. It is then necessary to repeat the tests and use other clinical and/or paraclinical features, such as nasal potential difference. Ion transport through the respiratory epithelium generates a transepithelial potential difference which can be measured in vivo, especially in the nasal mucosa [30,31].

Screening can also be improved by additionally performing the pancreatitis-associated protein (PAP) assay to the IRT assay. PAP is a protein synthesised in large quantities by the pancreas under stress. This can be carried as soon as from the in utero life of children with cystic fibrosis [32,33].

Investigation into the most frequent mutations in the CFTR gene using molecular biology techniques can be used to confirm or exclude the diagnosis of cystic fibrosis. Most CFTR gene mutations are missense alterations, but frameshifts, splice mutations, nonsense, deletions and insertions have also been described. Approximately 15% of the identified genetic variants are not associated with the disease. CFTR mutations can be divided into different classes according to their effects on protein function [28].

Question 12: what non-invasive methods are recommended for the assessment of liver damage in patients with cystic fibrosis?

1. **Liver damage assessment is recommended by carrying out liver function tests and abdominal ultrasound (B2)**
2. **The absence of liver damage is confirmed by the normality of the tests repeated annually: clinical examination, abdominal ultrasound (with the exception of steatosis), ALT and GGT (composite set of criteria) (B2)**
3. **Different diagnostic markers for liver fibrosis (APRI, FIB-4, Fibrotest®, liver stiffness) could be used for the identification of patients with cirrhosis (C2)**

Strong agreement

Diagnosis of liver damage is usually made on a composite set of criteria combining repeated liver function test abnormalities and abdominal ultrasound abnormalities, apart from steatosis (and in the absence of other causes in patients with cystic fibrosis: sepsis, undernutrition, right heart failure). Liver damage is a diffuse impairment of the liver parenchyma, with non-dilated intra-hepatic bile ducts, that can progress to significant macronodular cirrhosis (described as focal biliary cirrhosis and then multilobular biliary cirrhosis).

The value of non-invasive markers for the diagnosis of liver damage has not yet been demonstrated. However, non-invasive markers could be useful in detecting or assessing the severity of liver damage.

Measurement of liver stiffness by FibroScan®, APRI and Fibrotest® was compared in the diagnosis of hepatic impairment in a Canadian cohort of 127 patients with cystic fibrosis [15]. The prevalence of liver fibrosis associated with the disease was 14%. The AUROC for the diagnosis of liver disease associated with cystic fibrosis using FibroScan®, APRI and Fibrotest® was 0.78 (95% CI 0.65—0.92), 0.72 (95% CI 0.56—0.87) and 0.76 (95% CI 0.62—0.90), respectively, with no significant difference between the different methods used. At a threshold of 5.2 kPa, the sensitivity, specificity, positive and negative predictive values of liver stiffness by FibroScan® for the detection of chronic liver disease were 67%, 83%, 40% and 94%, respectively.

Question 13: what non-invasive methods are recommended for the assessment of significant portal hypertension in patients with cystic fibrosis?

1. **Liver stiffness can be useful in assessing significant portal hypertension (C2)**

Strong agreement

Abdominal ultrasound is useful in assessing liver damage and particularly in the investigation of signs of portal hypertension. Liver morphological abnormalities and evidence of portal hypertension are suggestive of cirrhosis. Semi-annual abdominal ultrasound can detect hepatocellular carcinoma in cirrhotic patients. CT and MRI scans can then be used to characterise certain abnormalities observed on ultrasound and/or suspicious nodules.

Question 14: what non-invasive methods are recommended for the follow-up of patients with cystic fibrosis?

1. **It is recommended during patient follow-up to perform liver assessment and abdominal ultrasound for the investigation of hepatic echotexture abnormalities and/or evidence of portal hypertension (B2)**
2. **Various non-invasive markers for the diagnosis of liver fibrosis (APRI, FIB-4, Fibrotest®, liver stiffness) could identify patients with liver disease with an associated risk of portal hypertension (C2)**

Strong agreement

In a German study, 36 patients were prospectively followed for 4—5 years with FibroScan®, APRI and FIB-4 [34]. A

subgroup of 9 of these 36 patients had an increase in stiffness >6.3 kPa during follow-up with a delta score of >0.38 kPa. The APRI and FIB-4 scores confirmed the rationale for grouping these 9 patients with a significant increase in fibrosis.

Question 15: in what situations is liver biopsy necessary for the evaluation of liver damage in cystic fibrosis?

1. **No recommendation can be made (B2)**

Strong agreement

Liver biopsy is rarely necessary. It may reveal the most typical feature: macronodular cirrhosis with an initial focal biliary cirrhosis or multilobular biliary cirrhosis.

Declaration of Competing Interest

BOURLIÈRE Marc: Abbott, BMS, Boehringer Ingelheim, Gilead, GSK, Idenix, Intercept, Janssen, Merck, Novartis, Roche, Vertex

BOUZBIB Charlotte: AbbVie, Gilead, Novartis

BUREAU Christophe AbbVie, Gilead, Gore

DE LÉDINGHEN Victor: AbbVie, Alfasigma, BMS, Diafir, Echosens, Gilead, Indivior, Intercept, Medac, Myr Pharma, Pfizer, Promethera, Spimaco, Supersonic Imagine

DUCLOS-VALLÉE Jean-Charles: No conflict of interest

GANNE-CARRIÉ Nathalie: Bayer, Gilead, Ipsen, Roche, Shionogi

GUILLAUD Olivier: Declared no conflicts of interest

POUJOIS Aurélia: Declared no conflicts of interest

SOBESKY Rodolphe: Declared no conflicts of interest

SOGNI Philippe: AbbVie, BMS, Galmed, Genfit, Gilead, Intercept, Janssen, MSD, Viking

WOIMANT France: Declared no conflicts of interest

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