



SPANISH ASSOCIATION OF PAEDIATRICS

Diagnostic action against hypertransaminasemia in paediatrics: Consensus document of Sociedad Española de Gastroenterología, Hepatología y Nutrición Pediátrica (SEGHNP), Asociación Española de Pediatría de Atención Primaria (AEPap) and Sociedad Española de Pediatría de Atención Primaria (SEPEAP)[☆]



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Abstract Hypertransaminasemia is a frequent finding in paediatrics, which could reflect potentially treatable serious disease. The aim of this document is to establish, by reviewing the available evidence, a consensus for an adequate management of hypertransaminasemia, from

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Pediatrics;
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its detection until the study is complete. To this end, a working group was formed with the participation of members of the Society of Pediatric Gastroenterology, Hepatology and Nutrition (SEGHNP), the Spanish Association of Primary Care Pediatrics (AEPap) and the Spanish Society of Primary Care Pediatrics (SEPEAP). Twenty-one recommendations are established with a marked practical component that will be useful in hospital clinical practice and primary care.

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PALABRAS CLAVE

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Diagnóstico

Actuación diagnóstica ante hipertransaminasemia en pediatría: documento de consenso de Sociedad Española de Gastroenterología, Hepatología y Nutrición Pediátrica (SEGHNP), Asociación Española de Pediatría de Atención Primaria (AEPap) y Sociedad Española de Pediatría de Atención Primaria (SEPEAP)

Resumen La hipertransaminasemia es un hallazgo frecuente en pediatría, puede ser banal o reflejar enfermedad grave potencialmente tratable. El objetivo de este documento es establecer, mediante la revisión de la evidencia disponible, un consenso para un adecuado enfoque práctico desde la detección de la hipertransaminasemia hasta completar su estudio en la edad pediátrica. Para ello, se constituyó un grupo de trabajo con participación de miembros de la Sociedad de Gastroenterología, Hepatología y Nutrición Pediátrica (SEGHNP), Asociación Española de Pediatría de Atención Primaria (AEPap) y Sociedad Española de Pediatría de Atención Primaria (SEPEAP). Se establecieron 21 recomendaciones con el objetivo de que sirvan de utilidad en la práctica clínica habitual tanto en atención primaria como hospitalaria.

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Introduction

Transaminases are intracellular enzymes found in hepatocytes and other cells. The most important ones are aspartate aminotransferase (AST), also known as glutamic oxaloacetic transaminase (GOT), which is less specific due to its ubiquity, and alanine aminotransferase (ALT), also known as glutamic-pyruvic transaminase (GPT), which is more specific to the liver.¹⁻⁵

Hypertransaminasaemia can be detected in the evaluation of liver disease or can be an incidental finding in blood tests performed for other reasons.⁶ Mild elevations of transaminase levels frequently lead to performance of incomplete evaluations.⁷ Persistent hypertransaminasaemia calls for performance of a comprehensive evaluation to determine the specific aetiology.⁶⁻⁸

In developing this document, the objective was to establish the steps to take from the initial detection of hypertransaminasaemia in a blood test to the completion of the diagnostic process through a consensus process involving paediatricians specialised in primary care and in gastroenterology, hepatology and nutrition. It includes the sequence of tests to include in the initial diagnostic evaluation (Fig. 1). This document does not address the evaluation of patients with isolated cholestasis or cholestasis associated with hypertransaminasaemia, the diagnosis process for each specific disease once it is suspected, the treatment of the different causes of hypertransaminasaemia or the management of abnormal liver function or acute liver failure.

Methods

A working group (WG) was established that comprised 8 members that represented the Sociedad Española de Gastroenterología, Hepatología y Nutrición (Spanish Society of Gastroenterology, Hepatology and Nutrition, SEGHNP), Asociación Española de Pediatría de Atención Primaria (Spanish Association of Primary Care Paediatrics, AEPap) and Sociedad Española de Pediatría Extrahospitalaria y Atención Primaria (Spanish Society of Outpatient and Primary Care Paediatrics, SEPEAP). The group broached 15 clinical questions regarding what, when and how to do from the moment of the initial detection of hypertransaminasaemia to the completion of its evaluation in both the primary care and hospital settings.

The WG conducted a systematic literature search in PubMed for articles published between January 2010 and January 2020 with the search terms "liver test" "liver disease" in patients aged 0–18 years, articles published at any time with the search terms "aminotransferase" "hypertransaminasemia" in ages 0–18 years, and articles published at any time with the terms "genetic liver disease" "histology liver disease". We conducted a second search for articles published between January 2020 and September 2020 with the same approach. We used the Zotero 5.0 open-source citation management system. The search yielded 248 articles in English or Spanish, for which the group obtained the full text. Of this total, 113 were considered relevant to the consensus documents and selected because they were

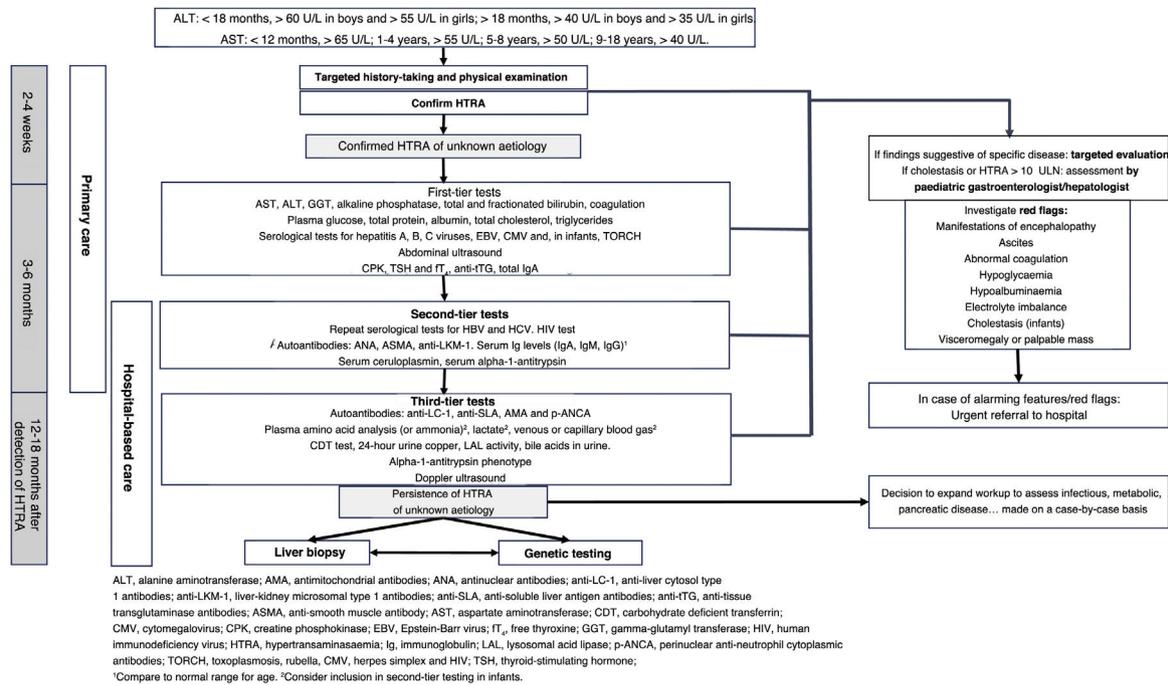


Figure 1 ALT, alanine aminotransferase; AMA, antimitochondrial antibodies; ANA, antinuclear antibodies; anti-LC-1, anti-liver cytosol type 1 antibodies; anti-LKM-1, liver-kidney microsomal type 1 antibodies; anti-SLA, anti-soluble liver antigen antibodies; anti-tTG, anti-tissue transglutaminase antibodies; ASMA, anti-smooth muscle antibody; AST, aspartate aminotransferase; CDT, carbohydrate deficient transferrin; CMV, cytomegalovirus; CPK, creatine phosphokinase; EBV, Epstein-Barr virus; fT₄, free thyroxine; GGT, gamma-glutamyl transferase; HIV, human immunodeficiency virus; HTRA, hypertransaminasaemia; Ig, immunoglobulin; LAL, lysosomal acid lipase; p-ANCA, perinuclear anti-neutrophil cytoplasmic antibodies; TORCH, toxoplasmosis, rubella, CMV, herpes simplex and HIV; TSH, thyroid-stimulating hormone.

¹Compare to normal range for age. ²Consider inclusion in second-tier testing in infants.

helpful in addressing the questions posed for development of the guideline.

Development of the document

The WG answered each of the clinical questions (each of which was addressed by 2 members) based on the available evidence and with the aim of establishing a structured approach to the diagnostic evaluation of hypertransaminasaemia based on the consensus of the group. The group did not perform a systematic review or meta-analysis of the contemplated diagnostic tests. The full document was revised 5 times by every member of the group, with a virtual meeting conducted after each of the revisions through Gotomeeting (access granted by the SEGHP and Zoom). All the recommendations presented in this document were reached by consensus and approved by the members of the group (informal consensus). The full consensus document can be consulted in the webpage of any of the participating professional associations.

Definition of hypertransaminasaemia and its main causes

Given the absence of a widely accepted abbreviation for hypertransaminasaemia, it was agreed that HTRA would be used for this document.

Hypertransaminasaemia (HTRA) is defined as elevation of serum levels of transaminases past the upper limit of normal (ULN)⁹ established in a healthy and representative sample of the population of interest. These levels exhibit substantial physiological variability within and between individuals. Their elevation reflects the lysis or increased permeability of the membrane of cells that contain them.^{2,5,6} In the paediatric population, through extrapolation of the threshold traditionally used in adults, HTRA is usually defined as levels greater than 40 U/L, but different authors have published thresholds that may differ to some extent and with some variations¹⁰⁻¹⁹ (Table 1). There is considerable overlap between healthy and unhealthy populations, which hinders the establishment of cut-off points with an adequate sensitivity and specificity.⁹ In addition, some authors note that in adolescence normal levels are too high and therefore this threshold loses sensitivity when used for initial screening of fatty liver or chronic hepatitis caused by hepatitis C virus (HCV), so that a lower ULN should be applied in these circumstances.¹⁰

There is no reliable correlation between the degree of transaminase elevation and its severity or prognosis. Nevertheless, very high levels are usually associated with extensive injury, which has led to the arbitrary categorization of HTRA into 3 degrees: mild (< 5 × ULN), moderate (=5–10 × ULN) and very high (>10 × ULN). On the other hand, based on experience and long-lasting consensus, HTRA is considered chronic if it lasts longer than 6 months.⁶

Table 1 Paediatric transaminase level ranges published by different authors.

Author (year)	Values in U/L, by age range and/or sex			
Fraser (2007)	Male and female adolescents ALT LSN = 30			
England (2009)	<18 months, male	<18 months, female	>18 months, male	female
Lai (2009)	ALT ULN = 60 ALT 2.5–97.5 percentile = 8–38		ALT ULN = 40	ALT ULN = 35
Schwimmer (2010)	Male adolescents ALT ULN = 26		Female adolescents ALT ULN = 22	
Rodoo (2013)	6 months–8 years, male ALT ULN = 23.4	6 months–8 years, female ALT ULN = 17.4	9–18 years, male ALT ULN = 30.6	9–18 years, female ALT ULN = 30.6
	6–12 months, male and female AST ULN = 66	1–4 years, male and female AST ULN = 55.8	5–8 years, male and female AST ULN = 48	9–18 years, male and female AST ULN = 43.2
Zierk (2015)	ALT and AST expressed as a continuous reference spectrum in the form percentile curves (P2.5–P97.5) for age			
Klietherme (2017)	Adolescents, male ALT ULN = 28		Adolescents, female ALT ULN = 21–24	
Bussler (2018)	11 months–16 years, male Several age ranges ALT ULN male = 29.9–38 AST ULN male = 41.5–68.7		11 months–16 years, female Several age ranges ALT ULN female = 24.2–31.7 AST ULN female = 35.2–62.9	
Kim (2018)	Adolescents, male ALT ULN = 33		Adolescents, female ALT ULN = 25	
Liu (2019)	9 age ranges, differences based on sex only in adolescents ALT ULN = 39–56 AST ULN = 32–67			

ALT, alanine aminotransferase; AST, aspartate aminotransferase; P, percentile; ULN, upper limit of normal.

The detection of HTRA, transient as it may be in some cases, should be considered a potential marker of disease and be interpreted in the clinical context of the patient. Therefore, it should always be confirmed and monitored until the levels normalize or the cause is identified.^{1,20}

In the paediatric population, there are multiple possible causes of HTRA, hepatic and extrahepatic, so its differential diagnosis is broad (Table 2).^{8,20} Isolated or disproportionate elevation of AST usually has an extrahepatic aetiology: difficult blood draw, haemolysis, rhabdomyolysis, etc. It may also be due to the formation of macro-AST.³

The most frequent cause of acute mild-to-moderate elevation that normalises within 6 months is viral respiratory or gastrointestinal infection in infants, and infection by cytomegalovirus or Epstein-Barr virus in older children.⁸ A pharmacological or toxic aetiology should also be considered. The drugs most frequently involved in HTRA are antibiotic and antiepileptic agents, but there is a broad range of pharmaceuticals that can cause it. Other possible causative substances are illicit drugs (such as alcohol or cocaine), solvents, pesticides or natural remedies (such as St John's wort, ephedra or gentian).

The main cause of persistent HTRA of hepatic aetiology in older children and adolescents is non-alcoholic fatty liver disease (NAFLD) associated with overweight or obesity.⁸

Recommendations:

- 1 We recommend the following ALT cut-off points for the healthy European paediatric population, stratified by age and sex: age less than 18 months, >60 U/L in boys and >55 U/L in girls, and age greater than 18 months, >40 U/L in boys and >35 U/L in girls. We propose the following ALT thresholds for initial screening of hepatic steatosis or chronic hepatitis caused by HCV in adolescents: 26 U/L in male adolescents and 22 U/L in female adolescents.
- 2 We recommend the following AST cut-off points, stratified by age: >65 U/L up to age 12 months, >55 U/L for ages 1–4 years, >50 U/L for ages 5–8 years and >40 U/L for ages 9–18 years.
- 3 The aetiology of HTRA may be hepatic and/or extrahepatic. We recommend a stepwise diagnostic evaluation based on age and the most frequent hepatic and extrahepatic causes.

Initial assessment following detection of HTRA

The history-taking and physical examination are the first tools to use in the diagnostic evaluation of HTRA.^{5,21,22} Their findings sometimes provide information on the aetiology or severity of the disease (Table 3).^{5,20–22} The history

Table 2 Causes of hypertransaminasaemia in the paediatric population.

Hepatic		Hepatic	
Infectious	Acute hepatitis (A, B, C, D, E) and chronic hepatitis (B, C) Systemic with hepatic tropism: EBV, CMV, HSV, VZV, HIV, toxoplasmosis, SARS-CoV-2. Sepsis and bacteraemia. Brucellosis, leptospirosis, typhoid fever. Other (more frequent in young children): respiratory and gastrointestinal viruses (adenovirus, RSV, parvovirus, echovirus, rotavirus), gastroenteritis caused by <i>Salmonella</i> , UTI	Neoplastic	Primary: liver tumour (hepatoblastoma, hepatocarcinoma) Secondary neuroblastoma, lymphoma, leukaemia, metastasis.
Immune	Autoimmune hepatitis, autoimmune cholangitis. Liver disease associated with immune disorder: collagenosis and other	Hepatobiliary	Cholelithiasis, choledochal cyst Cholestatic pattern of disease, require separate detailing and evaluation.
Obesity	Non-alcoholic fatty liver disorder (hepatic steatosis, steatohepatitis).	Traumatic	Obstetric trauma (subcapsular haematoma), abdominal trauma
Metabolic	Alpha-1 antitrypsin deficiency, Wilson disease, galactosaemia, fructosaemia, tyrosinaemia, glycogenosis (I, III, IV, VI, IX), lipidosis (Gaucher, Niemann-Pick), fatty acid β -oxidation disorders, lysosomal acid lipase deficiency, congenital disorders of glycosylation, Inborn errors of bile acid synthesis, urea cycle disorders, lipoprotein metabolism disorders (HBL), porphyria, other.	Miscellaneous	Chromosomal disorder (Turner syndrome), amyloidosis, sarcoidosis, Reye syndrome, CMPA (in infants), vascular diseases
Ischaemic-vascular	Low cardiac output, hepatic artery or portal vein obstruction, congestive heart failure, Budd-Chiari syndrome.	Hepatotoxicity	Prescription drugs, illicit drugs, toxins, herbal and alternative medicine remedies.
		Gastrointestinal or systemic disease	Coeliac disease, thyroid dysfunction, cystic fibrosis, chronic inflammatory bowel disease, Shwachman-Diamond syndrome.
		Extrahepatic	
		Elevation of AST and ALT	Muscular and neuromuscular diseases (AST > ALT): Duchenne and Becker muscular dystrophy, caveolinopathies, limb-girdle muscular dystrophy, polymyositis, dermatomyositis, metabolic myopathies (glycogen storage disease type V or McArdle disease). Other disorders involving muscle: major trauma, high-degree burns, surgery, vigorous exercise.
		Isolated AST elevation	Thyroid disorders: hypothyroidism and hyperthyroidism. Renal diseases. Adrenal insufficiency. Anorexia nervosa.
			Haemolysis: difficult blood draw, haemolytic disease. Heart disease (pericarditis, myocarditis, acute myocardial infarction). Isolated macroaspartasaemia.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CMPA, cow's milk protein allergy; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HSV, herpes simplex virus; HBL, hypobetalipoproteinaemia; HIV, human immunodeficiency virus; RSV, respiratory syncytial virus; UTI, urinary tract infection; VZV, varicella-zoster virus.

*The most frequent aetiologies are presented in boldface.

Table 3 Signs and symptoms in the history-taking and physical examination.

Nonspecific manifestations	Asthenia, anorexia, nausea, vomiting, abdominal distension, abdominal pain
Suggestive of liver disease	Hepatomegaly, jaundice, acholia, choloria
Suggestive of chronic liver disease	Telangiectasias, palmar erythema, malnutrition, ascites, splenomegaly, collateral vessels, gastrointestinal bleeding
Dysmorphic features	Characteristic phenotype (Alagille syndrome)
Neurologic manifestations	Irritability, changes in behaviour, changes in muscle tone, psychomotor retardation.
Manifestations of muscular involvement	Abnormal gait, myalgia, muscle hypertrophy or weakness.

Table 4 Comprehensive liver panel.

<p>Markers of cytolysis or hepatocellular injury</p> <ul style="list-style-type: none"> • Transaminase levels: ALT and AST • Other tests with lower diagnostic yield: LDH, GGT and aldolase
<p>Markers of cholestasis</p> <ul style="list-style-type: none"> • Alkaline phosphatase (liver isoenzyme) • GGT • Direct bilirubin • Serum bile acids
<p>Markers of hepatic synthesis</p> <ul style="list-style-type: none"> • Blood glucose • Cholinesterase • Albumin • Clotting factors

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; LDH: lactic dehydrogenase.

taking should include the personal and family medical history, use of pharmaceuticals or drugs, performance of vigorous activity, results of previous blood tests and systemic symptoms.^{20,22} The physical examination will include a thorough review of systems, as there is a broad range of extrahepatic conditions that may manifest with HTRA⁶ (Table 2).

When it comes to diagnostic tests, the first step is to confirm the presence of HTRA.⁴ The comprehensive liver panel includes markers of hepatocellular liver injury, cholestasis and hepatic synthesis (Table 4).^{5,20} An abdominal ultrasound examination may be useful to assess liver and bile duct morphology, rule out tumours and, in adolescents with excess weight, indicate the possibility of fatty liver. Serological tests for viral detection will be performed based on the history of the patient.⁶

Many episodes of HTRA are self-limited, but there is variability in the time elapsed to resolution (weeks or months).^{6,22} There is no evidence to establish how much time should elapse before retesting. Traditionally, an interval of 2–4 weeks is recommended in the case of HTRA in the absence of any other warning signs.^{5,20–22} This interval should be individualised in the context of transient HTRA (associated with consumption of hepatotoxic pharmaceuticals or herbal remedies, infection, vigorous physical activity) likely to take longer to resolve.^{6,20}

If additional warning signs are present (Table 5), the patient should be referred to a hospital on an urgent basis. It is particularly important to rule out acute liver failure (inability of the liver to perform its biosynthesis, regulation and detoxification functions).²³ If there are signs of encephalopathy, impaired renal function requiring dialysis, haemodynamic instability or respiratory failure, the patient should be treated in an intensive care unit, and, in the

presence of encephalopathy, hypoglycaemia, renal failure or an international normalised ratio (INR) greater than 2.5,²⁴ transfer to a hospital that can perform liver transplantation should be contemplated.

Recommendations:

- 4 Performance of a thorough anamnesis and physical examination, which in some cases may reveal information that can guide the diagnosis and the assessment of severity.
- 5 We recommend considering detection of HTRA, even if transient, as a potential marker of disease to be evaluated taking into account the clinical context of the patient and with performance of a comprehensive liver panel for initial screening (Table 4).
- 6 Hypertransaminasaemia should always be confirmed. In the absence of other warning signs, we suggest performance of a confirmatory test 2–4 weeks after the initial detection. If a specific temporary cause is suspected, the follow-up test can be delayed to up to 8 weeks if resolution of the transient process is expected within that timeframe. If there are clinical or laboratory features suggestive of severity, the follow-up test will be performed on an urgent basis.
- 7 We recommend screening for the most frequent or relevant causes of HTRA by means of blood tests and an ultrasound examination (first-tier tests) following confirmation of HTRA or at the time the confirmatory test is performed (Table 6).
- 8 We recommend assessment of every warning sign that would warrant urgent referral to a hospital (severe liver disease, infantile cholestasis or suspected tumour) in ever follow-up visit. Children with cholestasis (excluding infants) should be referred for assessment on a priority basis.

Table 5 Red flags in patients with hypertransaminasaemia.

Severe liver disease
<ul style="list-style-type: none"> • <u>Clinical manifestations:</u> <ul style="list-style-type: none"> • General malaise • Encephalopathy (changes in behaviour and level of consciousness) • Impaired synthesis (haematoma formation and bleeding at puncture sites) • Ascites • <u>Blood chemistry findings:</u> <ul style="list-style-type: none"> • Abnormal coagulation (INR < 1.5 and/or prothrombin activity decrease to < 60%) • Hypoglycaemia (< 50 mg/dL) • Hypoalbuminaemia (albumin < 3 g/dL) • Electrolyte and acid-base imbalance
Infantile cholestasis
<ul style="list-style-type: none"> • <u>Clinical manifestations:</u> <ul style="list-style-type: none"> • Prolonged jaundice > 2 weeks (except if secondary to breastfeeding with evidence of progressive improvement) • Acholia or hypocholia, choluria • <u>Blood chemistry findings:</u> <ul style="list-style-type: none"> • <u>Elevation of direct bilirubin</u> >1 mg/dL
Tumour
<ul style="list-style-type: none"> • Palpable abdominal mass • <u>Visceromegaly</u>

Table 6 First-, second- and third-tier tests.

First tier	Comprehensive liver panel	AST, ALT, GGT, alkaline phosphatase, total and fractionated bilirubin, coagulation study
	Blood chemistry	Glucose, total protein, albumin, total cholesterol, triglycerides, CPK
	Acute infectious disease	Viral serology tests for hepatitis A, B and C, Epstein-Barr virus, cytomegalovirus and, in infants, the TORCH test
	Imaging	Abdominal ultrasound
Second tier	Other	Thyroid function (TSH and fT4), coeliac disease (tissue transglutaminase IgA), total IgA
	Infectious disease	Repeat serologic tests for hepatitis B and C and HIV.
	Autoantibodies	ANA, ASMA, anti-LKM-1
Third tier	Other	Serum ceruloplasmin, serum alpha-1 antitrypsin, serum Ig levels (IgA, IgG and IgM) ^a
	Autoantibodies	Anti-LC-1, anti-SLA, AMA, p-ANCA
	Imaging	Abdominal Doppler ultrasound
	Other	Plasma amino acid analysis (or ammonia) ^b , venous or capillary blood gas analysis, ^b lactate, ^b CDT test, alpha-1 antitrypsin phenotype, urine copper (24-h urine), LAL activity, bile acids (plasma and/or urine),

ALT, alanine aminotransferase; AMA, antimitochondrial antibodies; ANA, antinuclear antibodies; anti-LC-1, anti-liver cytosol type 1 antibodies; anti-LKM-1, liver-kidney microsomal type 1 antibodies; anti-SLA, anti-soluble liver antigen antibodies; ASMA, anti-smooth muscle antibody; AST, aspartate aminotransferase; CDT, carbohydrate deficient transferrin; CPK, creatine phosphokinase; fT4, free thyroxine; GGT, gamma-glutamyl transferase; HIV, human immunodeficiency virus; Ig, immunoglobulin; LAL, lysosomal acid lipase; p-ANCA, perinuclear anti-neutrophil cytoplasmic antibodies; TORCH, toxoplasmosis, rubella cytomegalovirus, herpes simplex, and HIV; TSH, thyroid-stimulating hormone.

^a Compare to normal range for age.

^b Consider inclusion in second-tier testing in infants.

Table 7 Assessment of specific diseases.

Diseases	Characteristics	Tests
Inborn errors of metabolism Diagnosis guided by specialist in metabolic diseases	Many are included in newborn screening protocols. They are rarely asymptomatic, and their main manifestations (faltering growth, short stature, vomiting, psychomotor retardation, hepatomegaly, hypoglycaemia cholestasis, etc.) usually appear in infancy.	Review newborn screen results Plasma amino acids, ammonia, lactate, accumulation of metabolites in blood and/or urine, specific enzyme assays and genetic testing.
Infections Diagnosis guided by specialist in infectious disease	Patients in risk group for HBV or HCV with initial negative screen or immunocompromised patient. History of travel	PCR for detection of HBV DNA and HCV RNA Opportunistic infections Parasites and bacteria that with low prevalence Spain
Pancreatic involvement	Cystic fibrosis Shwachman-Diamond syndrome	Review newborn screen results Sweat chloride test/CFTR gene Cytopenias Genetic testing
Macro-AST	Isolated, sustained and exclusive elevation of AST due to the presence of macroenzymes	Percent polyethylene glycol precipitable activity (%PPA) Electrophoresis

AST, aspartate aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus.

- 9 If acute liver failure is suspected, we recommend considering admission to the intensive care unit and transfer to a hospital with an active paediatric liver transplantation programme.

Evaluation of confirmed HTRA of unknown aetiology

In the absence of signs or symptoms that could guide the diagnostic evaluation, it is important to conduct an organised and systematic evaluation, with inclusion of specific diseases in the differential diagnosis.^{25–28}

It must be taken into account that NAFLD is currently the most frequent cause of HTRA in children aged more than 10 years. Obesity and/or liver hyperechogenicity in the initial ultrasound scan suggest, but do not confirm, the presence of NAFLD and require evaluation according to the established protocol.²⁹

Doppler ultrasonography allows diagnosis of diseases that could manifest with abnormal hepatic vascularization³⁰ and is useful for screening of portal hypertension.³¹

The assessment of other diseases that are less frequent or that do not chiefly manifest with isolated HTRA should be personalised based on the age, clinical context, existing symptoms and associated clinical features (Table 7). Table 6 details the first, second and third-tier diagnostic tests.

Recommendations:

- 10 After performance of first-tier tests, if HTRA persists and its aetiology is unknown, we propose performance of laboratory tests for assessment of autoimmune hepatitis, Wilson disease, alpha-1-antitrypsin deficiency, lysosomal acid lipase deficiency, bile acid synthesis

disorders, congenital disorders of glycosylation and urea cycle defects and repetition of serology tests for hepatitis B virus (HBV) and HCV.

- 11 In children with sonographic features compatible with fatty liver and/or with excess weight, we recommend following the specific protocol for these diseases to complete the evaluation of HTRA.
- 12 In the case of persistent HTRA, we propose completing the imaging assessment with a liver Doppler ultrasound scan to evaluate hepatic venous and arterial vascularization.
- 13 In the presence of findings compatible with metabolic disease, and depending on the type of newborn screening performed, the clinical presentation, the age of the patient and the laboratory findings, we recommend performance of a targeted metabolomic analysis with participation of a specialist in metabolic disorders. In the cases of suspected cystic fibrosis, we recommend the sweat chloride test and, depending on the results, genetic testing to screen for variants in the *CFTR* gene.
- 14 In the case of children with risk factors for HBV or HCV (immunocompromised, from high-prevalence areas, of parents who are carriers of HBV or HCV), even if the hepatitis B surface antigen or HCV antibody test turn out negative, we recommend performance of PCR tests for detection of HBV DNA and HCV RNA.
- 15 In certain epidemiological situations, especially in children of immigrant origin, patients with suspected or known immunosuppression or undergoing evaluation of a systemic disease, the search for infectious aetiologies should be expanded in collaboration with a specialist in infectious disease.
- 16 In the case of isolated and sustained elevation of AST, we recommend testing for detection of macro-AST.

Diagnostic tests for follow-up of confirmed isolated HTRA

Specific studies have not been performed to validate the appropriate timing of follow-up tests in the management of HTRA. The specific circumstances of the patient are very important in determining when to perform follow-up tests. Normal transaminase levels at a single time point do not rule out liver disease, as some of these diseases have a fluctuating course.⁶ In addition, some diseases may manifest with mild transaminase elevation and still cause acute liver failure.^{6–32}

In the absence of alarming findings (Table 5), patients should be referred to the hospital to complete the diagnostic evaluation or treatment when investigation or therapeutic options are exhausted in primary care or if there are findings indicative of severe disease (such as HTRA > 10 × ULN, cholestasis).²⁰

Recommendations:

- 17 We recommend follow-up testing in 3–6 months in patients with unexplained HTRA that resolved after its detection, avoiding collection of the blood sample in the context of situations that may cause secondary hypertransaminasaemia (infection, medication, physical activity. . .).
- 18 In asymptomatic patients with HTRA, in the absence of red flags, we recommend performance of first- and second-tier tests in the first 3–6 months. If HTRA persists and its aetiology remains unknown, we suggest a comprehensive evaluation (first-, second- and third-tier tests) within 12 months of the initial detection of HTRA.
- 19 Patients with associated cholestasis or very high transaminase elevation (>10 × ULN) should be referred for assessment by a paediatric gastroenterologist/hepatologist on a priority basis.
- 20 In the absence of alarming signs, we recommend referral to a paediatric gastroenterologist/hepatologist of patients with HTRA and a suspected diagnosis requiring specific diagnostic procedures or treatment or with HTRA of unknown aetiology requiring performance of third-tier tests. The care setting where the second-tier tests are performed will depend on the organization, experience and resources of health care facilities in the region.

Other investigations in the evaluation of sustained and asymptomatic HTRA

Genetic testing

The results of previous tests, the phenotype of the patient and the study of the case with a geneticist will guide the selection of the genetic tests to be performed. At present, genetic testing is performed by means of next-generation sequencing (NGS) techniques. Next-generation sequencing allows detection of point mutations, microdeletions and intragenic insertions in several genes at once. The NGS tests used in clinical practice are targeted gene panels,³³ clinical exome sequencing (CES), which may be targeted based on the key phenotypic features of the patient using the Human Phenotype Ontology (HPO) nomenclature³⁴ and whole-exome sequencing (WES).³⁵ Due to advances in molecular genetics and the increasing availability of these

techniques, NGS will be used increasingly frequently and at earlier stages in clinical practice.

Liver biopsy (LB)

Since this is an invasive procedure, it is only performed after an exhaustive initial evaluation with non-invasive methods. The ultrasound-guided needle biopsy of the liver is the criterion standard procedure for sampling liver tissue, using cutting or suction needles of varying gauge based on age.³⁶ Persistent HTRA is the main indication for performance of LB in children.³⁷ The right time to perform it is not as clearly established. Some authors have reported a low diagnostic yield of LB in the case of sustained HTRA of unknown aetiology, and performance of this procedure could be delayed to 14–16 months after initial detection of HTRA.⁸ The parents or legal guardians must be informed of the potential benefits and risks of the procedure based on the nature of the liver disorder experienced by the child.

The LB specimen can provide information on specific pathological and histological features of different liver diseases.^{38,39} This technique is also useful in the prognostic assessment of chronic liver disease through the determination of histological activity indices (inflammation, fibrosis, necrosis, steatosis etc). Different techniques may be used in the histological examination: basic staining with haematoxylin and eosin, special histochemical stains and immunostains or electron microscopy.³⁷ Adequate communication between the pathology and clinical teams is crucial to ensure the correct processing and assessment of histological samples.

In recent years, non-invasive techniques have been developed to assess liver fibrosis by ultrasound elastography. Although this approach provides relevant information in the followup, due to the absence of established normal ranges validated in the paediatric population or specific cut-off values for the different diseases that may be present, ultrasound elastography cannot currently replace performance of a LB.⁴⁰

Recommendations:

- 21 In the case of persistent HTRA without an aetiological diagnosis, genetic testing should be performed in collaboration with specialists in clinical genetics. In these patients in whom HTRA is the only warning sign, we recommend clinical exome sequencing.
- 22 We recommend performance of an additional LB (preferably guided by ultrasound) at 12–18 months from initial detection of asymptomatic and persistent HTRA, in the absence of other warning signs, in patients in whom the previous diagnostic evaluation did not establish the aetiology of HTRA.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Liu Z, Que S, Xu J, Peng T. Alanine aminotransferase-old biomarker and new concept: a review. *Int J Med Sci*. 2014;11:925–35.

2. Senior JR. Alanine aminotransferase: a clinical and regulatory tool for detecting liver injury-past, present, and future. *Clin Pharmacol Ther.* 2012;92:332–9.
3. Lledín Barbancho D, Vecino López R. Exploraciones clínicas, bioquímicas y técnicas de imagen en la valoración de la patología digestiva y hepatobiliar. *Pediatr Integral.* 2015;XIX, 66.e1–66.e18.
4. Lamireau T, McLin V, Nobili V, Vajro P. A practical approach to the child with abnormal liver tests. *Clin Res Hepatol Gastroenterol.* 2014;38:259–62.
5. Marugán de Miguelsanz JM, Torres Hinojal MC, Alonso Vicente C. Aproximación diagnóstica al paciente con enfermedad hepática. *Pediatr Integral.* 2020;XXIV:6–14.
6. Vajro P, Maddaluno S, Veropalumbo C. Persistent hypertransaminasemia in asymptomatic children: a stepwise approach. *World J Gastroenterol.* 2013;19:2740–51.
7. Fernández Ventureira V, Ros Arnal I, Rodríguez Martínez G, García Rodríguez B, García Romero R, Ubalde Sainz E. Evaluación del seguimiento de niños con hallazgo de hipertransaminasemia. *An Pediatría.* 3 de septiembre de 2020. Available from: <https://www.sciencedirect.com/science/article/pii/S1695403320302411>. [Accessed 16 March 2021].
8. Iorio R, Sepe A, Giannattasio A, Cirillo F, Vegente A. Hypertransaminasemia in childhood as a marker of genetic liver disorders. *J Gastroenterol.* 2005;40:820–6.
9. Pacifico L, Ferraro F, Bonci E, Anania C, Romaggioli S, Chiesa C. Upper limit of normal for alanine aminotransferase: quo vadis? *Clin Chim Acta.* 2013;422:29–39.
10. England K, Thorne C, Pembrey L, Tovo PA, Newell ML. Age- and sex-related reference ranges of alanine aminotransferase levels in children: European paediatric HCV network. *J Pediatr Gastroenterol Nutr.* 2009;49:71–7.
11. Kliethermes S, Ma M, Purtell C, Balasubramanian N, Gonzalez B, Layden TJ, et al. An assessment of racial differences in the upper limits of normal ALT levels in children and the effect of obesity on elevated values. *Pediatr Obes.* 2017;12:363–72.
12. Bussler S, Vogel M, Pietzner D, Harms K, Buzek T, Penke M, et al. New pediatric percentiles of liver enzyme serum levels (alanine aminotransferase, aspartate aminotransferase, γ -glutamyltransferase): effects of age, sex, body mass index, and pubertal stage. *Hepatology.* 2018;68:1319–30.
13. Fraser A, Longnecker MP, Lawlor DA. Prevalence of elevated alanine aminotransferase among US adolescents and associated factors: NHANES 1999–2004. *Gastroenterology.* 2007;133:1814–20.
14. Lai DS, Chen SC, Chang YH, Chen CY, Lin JB, Lin YJ, et al. Pediatric reference intervals for several biochemical analytes in school children in Central Taiwan. *J Formos Med Assoc.* 2009;108:957–63.
15. Schwimmer JB, Dunn W, Norman GJ, Pardee PE, Middleton MS, Kerkar N, et al. SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. *Gastroenterology.* 2010;138:1357–64, 1364.e1–2.
16. Rödöö P, Ridefelt P, Aldrimer M, Niklasson F, Gustafsson J, Hellberg D. Population-based pediatric reference intervals for HbA1c, bilirubin, albumin, CRP, myoglobin and serum enzymes. *Scand J Clin Lab Invest.* 2013;73:361–7.
17. Zierk J, Arzideh F, Rechenauer T, Haeckel R, Rascher W, Metzler M, et al. Age- and sex-specific dynamics in 22 hematologic and biochemical analytes from birth to adolescence. *Clin Chem.* 2015;61:964–73.
18. Kim JW, Lee KJ, Yang HR, Chang JY, Moon JS, Khang YH, et al. Prevalence and risk factors of elevated alanine aminotransferase among Korean adolescents: 2001–2014. *BMC Public Health.* 2018;18:617.
19. Liu J, Dai Y, Lee Y, Yuan E, Wang Q, Wang L, et al. Pediatric reference intervals of liver and renal function tests from birth to adolescence in Chinese children as performed on the Olympus AU5400. *Clin Chim Acta.* 2019;490:142–6.
20. Blesa Baviera L, Vegas Alvarez A, Albañil Ballesteros M. Guía de Algoritmos en Pediatría de Atención Primaria. Hipertransaminasemia. *AEPap.* 2016 (en línea). Available from: algoritmos.aepap.org.
21. Lesmes Moltó L, Albañil Ballesteros MR. Aumento aislado de transaminasas: aproximación diagnóstica. *Form Act Pediatr Aten Prim.* 2013;6:35–42.
22. González Jiménez D, Santos Rodríguez PM. Hipertransaminasemia en Pediatría. *Bol Pediatr.* 2013;53:137–45.
23. Alonso EM, Horslen SP, Behrens EM, Doo E. Pediatric acute liver failure of undetermined cause: a research workshop. *Hepatology.* 2017;65:1026–37.
24. Zeeshan M, Hamidi M, O’Keefe T, Hanna K, Kulvatunyou N, Tang A, et al. Pediatric liver injury: physical examination, fast and serum transaminases can serve as a guide. *J Surg Res.* 2019;242:151–6.
25. Socha P, Janczyk W, Dhawan A, Baumann U, D’Antiga L, Tanner S, et al. Wilson’s disease in children: a position paper by the Hepatology Committee of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr.* 2018;66:334–44.
26. Cakir M, Sag E, Islek A, Baran M, Tumgor G, Aydogdu S. Liver involvement in children with Alpha-1 antitrypsin deficiency: a multicenter study. *Pediatr Gastroenterol Hepatol Nutr.* 2020;23:146–53.
27. Marques-da-Silva D, Dos Reis Ferreira V, Monticelli M, Janeiro P, Videira PA, Witters P, et al. Liver involvement in congenital disorders of glycosylation (CDG). A systematic review of the literature. *J Inherit Metab Dis.* 2017;40:195–207.
28. Mieli-Vergani G, Vergani D, Baumann U, Czubkowski P, Debray D, Dezsöfi A, et al. Diagnosis and management of pediatric autoimmune liver disease: ESPGHAN Hepatology Committee Position Statement. *J Pediatr Gastroenterol Nutr.* 2018;66:345–60.
29. Vos MB, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli R, et al. NASPGHAN clinical practice guideline for the diagnosis and treatment of nonalcoholic fatty liver disease in children: recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). *J Pediatr Gastroenterol Nutr.* 2017;64:319–34.
30. Baiges A, Turon F, Simón-Talero M, Tasayco S, Bueno J, Zekrini K, et al. Congenital extrahepatic portosystemic shunts (abernethy malformation): an international observational study. *Hepatology.* 2020;71:658–69.
31. Hegarty R, Deheragoda M, Fitzpatrick E, Dhawan A. Paediatric fatty liver disease (PeFLD): all is not NAFLD - Pathophysiological insights and approach to management. *J Hepatol.* 2018;68:1286–99.
32. Newsome PN, Cramb R, Davison SM, Dillon JF, Foulerton M, Godfrey EM, et al. Guidelines on the management of abnormal liver blood tests. *Gut.* 2018;67:6–19.
33. Nicastro E, D’Antiga L. Next generation sequencing in pediatric hepatology and liver transplantation. *Liver Transpl.* 2018;24:282–93.
34. Groza T, Köhler S, Moldenhauer D, Vasilevsky N, Baynam G, Zemojtel T, et al. The human phenotype ontology: semantic unification of common and rare disease. *Am J Hum Genet.* 2015;97:111–24.
35. Vilarinho S, Mistry PK. Exome sequencing in clinical hepatology. *Hepatology.* 2019;70:2185–92.
36. Dezsöfi A, Baumann U, Dhawan A, Durmaz O, Fischler B, Hadzic N, et al. Liver biopsy in children: position paper of the ESPGHAN Hepatology Committee. *J Pediatr Gastroenterol Nutr.* 2015;60:408–20.

37. Schady DA, Finegold MJ. Contemporary evaluation of the pediatric liver biopsy. *Gastroenterol Clin North Am.* 2017;46:233–52.
38. Rockey D, Caldwell S, Goodman Z, Nelson R, Smith A. American Association for the Study of Liver Diseases. *Liver Biopsy Hepatol.* 2009;49:1017–44.
39. Ovchinsky N, Moreira RK, Lefkowitz JH, Lavine JE. Liver biopsy in modern clinical practice: a pediatric point-of-view. *Adv Anat Pathol.* 2012;19:250–62.
40. Andersen S, Ewertsen C, Carlsen J, Henriksen B, Nielsen M. Ultrasound elastography is useful for evaluation of liver fibrosis in children—a systematic review. *J Pediatr Gastroenterol Nutr.* 2016;63:389–99.