Laboratory Evaluation of Hepatobiliary Disease

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Key Points

- Biochemical tests to evaluate liver function are mainly AST, ALT, GGT, bilirubin, ALP, and INR, but they are non-liver-specific, since, during pediatric age, they can be raised because of other organs' injury or the use of medications.
- Chronic liver disease can develop subclinically even in patients with normal aminotransferases. Overall, there is no correlation between the level of aminotransferases and the severity of liver disease.
- Finding raised aminotransferases is quite common in children with any acute illness; therefore, this test needs to be repeated serially to suspect a liver disease deserving further investigations.
- Noninvasive tests revealing the type and the severity of liver disease are missing, and the diagnosis of hepatopathies often requires a high index of suspicion as well as the inputs from clinical, biochemical, and radiological findings.
- Liver histology remains the gold standard to make the diagnosis of a chronic liver disease.

Research Needed in the Field

- Normal reference values based on large population-based cohorts of entirely healthy children of all different ages and of both sexes are still lacking for several of the commonly used liver function tests described herein.
- There is still a further need for more refined evaluation of the synthetic function of the liver, i.e., tests that are even more specific than, for example, INR.
- There is also a need to develop tests for daily clinical practice to measure the capacity to metabolize drugs.

4.1 Introduction

Most of the biochemical tests used to screen for and characterize possible liver disease are not actual tests of the global liver function but merely reflecting an isolated aspect of the liver. Thus, biochemical testing for liver disease should be done by combining different analyses and thereby looking at the possible aberrations from different perspectives, collectively adding up to a full picture. By liver function tests, we often mean serum markers for hepatocyte turnover (primarily AST and ALT), for cholestasis (total and conjugated bilirubin, bile acids, GGT, ALP), for portal hypertension (platelets, leukocytes, and hemoglobin), for possible malignancy (AFP, CA-19), and finally for liver synthetic function (albumin, PK/INR, single coagulation factors).

4.2 Markers of Hepatocyte Turnover: AST and ALT

The most commonly used biochemical markers for liver disease are aspartate aminotransferase (AST) and alanine aminotransferase (ALT). These two aminotransferases were first demonstrated in human blood from healthy subjects and

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from patients with varying disease entities, proposing that these enzymes could serve as markers of ischemic heart disease [1]. ALT and AST, formerly known as serum glutamic-pyruvic transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT), respectively, are nowadays most frequently used to detect liver damage. The terms transaminase and aminotransferase are interchangeable. Biochemically, they are both involved in the Krebs cycle, in transamination of amino groups from the amino acids alanine and aspartic acid, respectively. AST is found in the cytosol (cAST) and in the mitochondrion (mAST) in most cell types, whereas ALT is mainly cytosolic (cALT).

None of them are restricted solely to the hepatocyte and are found in large quantities in myocytes of the skeletal and heart muscle, in erythrocytes, and in renal tubular cells but also in pancreatic tissue, leukocytes, brain, and lung. Their half-life in blood differs depending on cellular localization and is approximately 48 h for cALT, 18 h for cAST, and >72 h for mAST [2, 3]. As with most serum markers, they are released into the blood stream from apoptotic cells and eliminated from the plasma at a relatively constant rate, thus keeping a stable plasma concentration. Enzyme activity of AST and ALT is often measured as units per mL (U/mL = μmol × min^{-1} × mL^{-1}), although the International System of Units (SI) recommends that catalytic activity of enzymes in serum should be expressed in the SI unit katal per liter (kat/L = mol × s^{-1} × L^{-1}) [4]. The conversion between the two units is as follows: 1 μkat/mL = 60 U/mL or 1 U/mL = 0.0167 μkat/mL.

When interpreting results of AST and ALT measurements, a few things must be remembered. First, think of extrahepatic causes; increased aminotransferase levels may be due to increased cellular turn-over in other tissues than the liver, most often myocyte degradation due to heavy exercise [5, 6] or underlying acute or chronic muscular disease. By testing for CK (creatinine kinase) or myoglobin, we can rule out damaged muscular tissue as the source. In addition to this, there is a plethora of other possible factors influencing the results, including day-to-day variations [7, 8], gender and age [9], specimen storage [10], and the rare presence of macroenzymemia including macro-AST [11, 12].

Second, the results must be put in a clinical context; normal aminotransferase levels do not rule out serious liver disease but merely reflect the actual number of damaged cells. Thus, in a liver with limited residual parenchyma, normal aminotransferase levels may be found. In fact, patients with end-stage liver disease with cirrhosis may have completely normal levels of aminotransferases. To this end, when end-stage disease could be suspected in a patient with completely or almost normal AST and ALT, the testing must also include markers of liver function, i.e., albumin and/or PK/INR.

Third, even slight elevations of AST and ALT should be noted and be reevaluated, as chronic liver disease in children may be very discrete and may reflect ongoing liver diseases, possibly progressing to end-stage liver disease, unless diagnosed and properly treated [13].

Looking closely at the dynamics of repeated aminotransferase analyses can often help us to understand the nature of the liver disease, including possible etiology, thereby guiding in differential diagnostics and predicting the natural course and prognosis of the liver disease, sometimes also aiding in evaluating effects of possible treatments. A steep rise of the aminotransferases followed by a quick decrease may hint toward a "one-hit etiology," such as temporary ischemia leading to liver damage, whereas a steep rise followed by a gradual decay of the liver enzymes instead would direct the clinician toward an ongoing process, such as a toxic or viral liver damage.

The ratio between ALT and AST in children may vary a lot. Increased AST/ALT ratios have been correlated with a less favorable outcome and may imply increased mitochondrial turnover or cirrhosis [14] or NAFLD [15]. Despite this, interpretations based on AST/ALT ratio should be done with caution.

Reference values in children have been published in several studies [16–23], and there are slight variations between age, gender, and race, but they should be interpreted with caution since the number of children especially in the youngest age groups often is too low for firm conclusions. It should be noted that the normal values in children are lower compared to adult normal values [24] and that AST activity in children and adolescents is somewhat higher than that of ALT [9].

4.2.1 Interpretation of Increased Levels of AST and ALT

Very high levels (>10 × ULN) are seen in acute liver disease—the highest levels in toxic and ischemic damage to the liver, sometimes also in acute viral hepatitis, less commonly in autoimmune hepatitis (AIH). These levels may also occur in Wilson's disease.

High levels (2–10 × ULN) are noted in all of the above and in metabolic liver disease including nonalcoholic fatty liver diseases (NAFLD) and autoimmune hepatobiliary diseases (including AIH and primary sclerosing cholangitis, PSC) but also in hereditary cholestatic liver diseases.

Slightly elevated liver enzymes (<2 × ULN) are commonly seen in all of the above but also in systemic and other GI diseases with liver involvement, alfa-1 antitrypsin disease, cystic fibrosis, celiac disease, and inflammatory bowel disease (Table 4.1).

Table 4.1 The aminotransferases AST and ALT markers of hepatocyte turnover

	AST activity	ALT activity	AST/ALT ratio	Weight (kg)	AST total	ALT total
Liver	7100	2850	2.5	1.5	10,650	4275
Kidney	4500	1200	3.8	0.25	1125	300
Heart	7800	450	17	0.3	2340	135
Muscle	5000	300	17	30	150,000	9000
Serum	1	1	1.0	3	3	3

Botros et al. [68]

4.3 Markers of Cholestasis

4.3.1 Alkaline Phosphatase (ALP)

The enzyme alkaline phosphatase (ALP), first described almost 100 years ago [25], with a half-life of approximately 7 days is abundant in many different human tissues including the sinusoidal membrane of the liver, intestine, kidney, bone, placenta, and white blood cells. It is represented by different isoenzymes, all catalyzing hydrolysis of phosphate esters in the different tissues, important in a number of basic physiologic mechanisms although still not exactly characterized [26, 27]. Despite its wide distribution, most ALP in humans emanates from bone tissue and from the hepatocyte, and increased levels are thus seen in bone disease and cholestatic liver disease. Although widely used as a reliable marker of obstructive cholangiopathy in adults, interpreting total ALP levels in growing children and adolescents with much higher osteoblast activity and turnover of bone tissue is therefore less straightforward. Thus, the value of total ALP activity as a marker of cholestatic liver disease in children and adolescents is limited as long as the exact origin of the isoenzyme is unknown. In other words, separating ALP into its isoenzymes and its isoforms would add value to the measurement of total enzyme activity. Increased levels of ALP activity are seen not only in bone and cholestatic liver disease but also in vitamin D deficiency, untreated celiac disease, and transient benign hyperphosphatasemia, where the serum levels of ALP may exceed 2000 U/L (or >30 μkat/L). The latter is not an uncommon entity in children less than 5 years of age, with no signs of bone or liver disease and ALP with isoforms of both bone and liver origin returning to normal levels within 4–6 months, and it is important to recognize to avoid misdiagnosis or unnecessary investigations [27–29]. Low serum levels of ALP in liver disease are found in patients with Wilson's disease, possibly due to the abundant copper ions competitively displacing zinc ions, a necessary cofactor of alkaline phosphatase, leading to low levels [30]. Low levels are seen in several non-hepatic diseases, including zinc deficiency, hypothyroidism, and congenital hypophosphatasia [26, 31].

4.3.2 Gamma-Glutamyltranspeptidase (GGT)

Gamma-glutamyltranspeptidase (GGT) belongs to a group of enzymes catalyzing the transfer of amino acids from one peptide to another amino acid or peptide. The main enzyme responsible for the transpeptidation of the gamma glutamyl group was initially studied in rat kidney tissue and named gamma-glutamyltranspeptidase [32].

GGT is abundant in renal, prostatic, pancreatic, and hepatobiliary tissue; smaller amounts are found in all other tissues except the muscle, and although liver tissue is the main source of serum GGT in humans, elevated GGT activity also occurs in patients with acute and chronic pancreatitis. Since the enzyme is found in as well the endoplasmic reticulum as in the canalicular membrane of the hepatocyte, increased activity indicates liver damage but does not perfectly discriminate between increased cell turnover and cholestasis [33].

Despite this, it is a valuable marker, especially in pediatric hepatology. Due to its tissue specificity, not found in bone or in muscle tissue, it adds valuable information when used in combination with less discriminate markers of cell turnover such as AST or ALT and those of cholestasis, such as ALP, with its low specificity in children and adolescents. Increased GGT activity is seen mainly in hepatobiliary disease, most often higher in cholestatic diseases than in non-cholestatic liver diseases, but elevated serum levels are also found in patients treated with certain enzyme-inducing drugs (antiepileptics) and in adult with alcohol overconsumption [34, 35]. In conclusion, GGT activity is a more useful marker of cholestasis than total ALP activity in children and adolescents due to their high bone tissue turnover. Despite this, there is a number of important cholestatic diseases including FIC1 deficiency, BSEP deficiency, and TJP2 deficiency, all characterized by decreased concentrations of biliary bile acids in the canaliculus, where GGT activity in serum remains low. Thus, in instances when infants or children present with clinical and biochemical signs of cholestasis, diseases of canalicular bile acid transport defects should be suspected [36].

4.3.3 Conjugated Serum Bilirubin

Bilirubin is a waste product from hemoglobin which undergoes conjugation in the liver. Conjugated bilirubin is secreted to the bile via the canalicular transporter multidrug resistance-associated protein 2 (MRP2). An elevated level of conjugated bilirubin in serum is considered one of the hallmarks of cholestasis in clinical practice. Using the SI unit system, a level of 30 μmol/L, corresponding to 1.75 mg/dL, or higher should definitely warrant further investigation, in particular if the conjugated fraction accounts for more than 20% of the total bilirubin level. In patients with isolated elevation of conjugated bilirubin but no other biochemical markers of cholestasis or hepatocellular injury, the alternative explanation of Dubin-Johnson syndrome may be considered. This benign state is caused by mutation in the gene encoding MRP2.

4.3.4 Serum Bile Acids

Bile acids are synthesized in the liver from cholesterol, excreted with the bile into the gut and very efficiently recirculated to the liver via the enterohepatic circulation. Under normal circumstances, the total bile acid levels in peripheral serum are therefore low, i.e., below 7 μmol/L. Cholestasis occurs if there is a blockage at any level of the enterohepatic circulation. This leads to a measurable increase of the bile acid levels in peripheral serum. While the occurrence of elevated levels, at least above 100 μmol/L, is a sensitive marker of cholestasis, it does not give any meaningful clue of the underlying cause. Thus, in a cholestatic infant, such levels can be seen both in biliary atresia and the different genetic cholestatic diseases, such as Alagille syndrome and the different types of progressive familial intrahepatic cholestasis. On the other hand, in cholestatic patients with inborn errors of bile acid synthesis, these levels, as analyzed by the hospital labs, are not elevated. The reason for this is that these routine methods measure only bile acids with a hydroxylation in the 3-alpha position, whereas the basic defect in these specific diseases causes accumulation of bile acids with hydroxylation in other positions of the molecule [37].

Mild elevations of bile acid levels in serum have been described in healthy infants below 6 months of age. This seems to be associated to an immature and therefore suboptimal transportation of bile acids at different points of the enterohepatic circulation and is denoted “physiologic cholestasis of the infant” [38].

Despite the limitations pointed out above, the bile acid level in peripheral serum is a useful biochemical marker of cholestasis, also in patients who are not obviously jaundiced, i.e., the serum levels of conjugated bilirubin may not be elevated.

4.4 Tumor Markers

4.4.1 α-Fetoprotein (AFP)

AFP is considered the most important binding protein in the fetus. During the first trimester, it is produced by the yolk sac; thereafter, the fetal liver is the main source of production. The steady fetal production results in high serum levels at birth, around 40,000 ng/mL. During the first year of life in a healthy infant, there is subsequently a logarithmic fall in the serum levels. However, the interindividual variation is large, and despite a half-life of 5–6 days, adult levels are not reached until 2 years of age. To assess single values obtained in infants, a nomogram should be used, and serial values are in fact strongly suggested [39, 40] (Fig. 4.1).

Elevated AFP is an important marker for germ cell tumors, hepatoblastoma (HB), and hepatocellular carcinoma. In HB, which is the most common hepatic malignancy in children, around 90% of the patients have elevated AFP. On the other hand, the minority with normal levels seem to have a poorer treatment outcome [41]. Repeated AFP testing is also useful, together with radiology, to estimate the effect of treatment with chemotherapy [42].

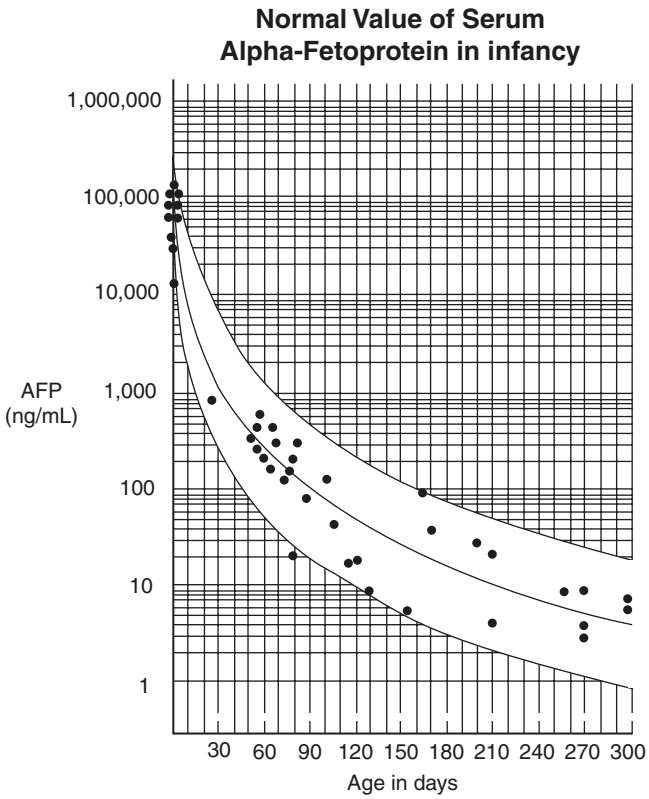

Fig. 4.1 Normal values of alpha-fetoprotein in infancy. Tsuchida et al. [69]

4.4.2 CA19-9

CA19-9 is a glycoprotein coated with sialylated blood group (carbohydrate) epitopes such as sialyl Lewis[a]. It is detected in low levels in healthy adult subjects, whereas pediatric data are scarce. Interestingly, Lewis-negative individuals, which are estimated to account for 7% of the population, have undetectable levels. Since the original descriptions in the late 1970s, CA19-9 has been suggested to be a marker for cholangiocarcinoma, for example, in adults with primary sclerosing cholangitis. However, the compiled results of several studies on this topic reveal a rather disappointing and diverging sensitivity of 38–89% and specificity of 50–98%. Other clinical situations than cholangiocarcinoma such as increasing cholestasis as well as bacterial cholangitis in patients with underlying hepatobiliary diseases will also cause an increase in the CA19-9 levels [43–45].

4.5 Markers of Synthetic Function

To make a relevant estimate of the remaining functional capacity in a patient with liver disease is complex. The different hepatic functions—for example, drug metabolism, excretion, and synthesis—may be more or less preserved in different types of diseases. To measure the synthesis or in fact the level of plasma proteins is therefore only one of several possible ways to assess this capacity. These analyses are extensively used, primarily because they are readily available to the clinician and the turnaround time at the laboratory is often relatively short.

4.5.1 Coagulation Factors

A majority of these proteins are produced in the liver, although it should be noted that coagulation factor 8 is also released from endothelial cells. If the functional liver cell mass is decreased, be it rapidly or over time, one would expect the production of coagulation factors to decrease.

The most widely used analysis is to measure prothrombin time. In recent years, the results obtained in this analysis have been standardized by comparison to reference tests, yielding the international normalized ratio (INR). However, the reference tests have for many years been based on samples from patients treated for thromboembolism with warfarin and not on patients with primary liver disease. Attempts have been made to standardize the results also in patients with liver disease [46, 47]. Furthermore, there are in fact two different laboratory methods available for prothrombin time, i.e., the Quick method which is dependent on the levels of factors 2, 7, and 10, which are vitamin K dependent, and on F5 and fibrinogen and on the other hand the Owren method which is only dependent on the levels of factors 2, 7, and 10 [47]. Finally, there are data both from adults and children with chronic liver disease that cholestasis may upregulate the synthesis of several coagulation factors. This can result in paradoxically high levels of certain coagulation factors despite progressive hepatic dysfunction, possibly making them less useful for evaluation of liver capacity in these patients [48, 49]. With the objections above in mind, it is still clear that INR is a widely used marker for synthetic function of the liver. For example, the definition of acute liver failure depends largely on the identification of an INR at or above 2.0 which does not improve on parenteral administration with vitamin K [50]. If the patient is encephalopathic, the corresponding INR level defining acute liver failure is set at 1.5. Since the half-life of the coagulation factors included in the analysis all varies between 6 and 48 h, repeated measurements of INR during the course of acute liver failure are often very relevant to predict the outcome [51].

Furthermore, INR is used as one of the parameters in several pediatric scoring systems, including PELD (pediatric end-stage liver disease) which several countries use for allocation of liver grafts for transplantation. PELD was primarily developed to assess short-term (i.e., 3 months) outcome in pediatric patients with severe chronic liver disease [52]. INR is also included in the King's College criteria for transplantation in acute liver failure [53], in the Wilson index for liver transplantation from the same institution [54], and also in the Liver Injury Unit scoring system suggested by the Pediatric Acute Liver Failure (PALF) Study Group [55].

Individual coagulation factors have for several decades also been used as prognostic markers in severe liver disease. The two main candidates have been factors 5 and 7, respectively.

For factor 5, there are several publications, mainly originating from French adult cohorts, suggesting it to be a useful predictor for outcome in patients with acute liver failure. In one of these, the authors assessed 115 patients above the age of 15 years with fulminant hepatitis B virus infection. In multivariate analysis, factor 5 levels, but not coma development, were found to predict outcome [56]. For comparison, in a British study of 110 patients above 13 years of age with fulminant liver failure, the positive predictive value (0.73) of low factor 5 levels was not as good as the previously mentioned King's College criteria (0.92) for the large subgroup of 88 patients with paracetamol intoxication as etiology. On the other hand, for the smaller subgroup with other

etiologies (i.e., mainly viral hepatitis and unknown etiology), the positive predictive values of these two methods were close to 1 both for low factor 5 levels and for the King's College criteria [57].

The usefulness of determining coagulation factor 7 levels to predict outcome was suggested already in the 1970s, i.e., in the largely "pretransplant era." For example, based on a detailed investigation of 12 mainly adult patients with fulminant liver failure, of whom 6 survived and 6 died, it was noted that all patients with a factor 7 level above 9% of the normal survived. This is one of the few studies where both factors 5 and 7 were studied. Interestingly, neither the coma grade nor factor 5 levels could predict outcome as precisely as factor 7 levels [58]. In another report from a tertiary hepatology unit for adults on 68 consecutive patients with acute hepatitis and INR greater than 1.7, factor 7 at admission was a better predictor of outcome than factor 5. However, after 3 days of hospitalization, factor 5 did in fact perform better when predicting outcome, possibly suggesting that repeated measurements could be of value [59].

Other coagulation factors than those previously mentioned have also been suggested as good prognostic markers, for example, antithrombin and fibrinogen [58, 60]. The advantage of using these parameters, in contrast to coagulation factors 5 or 7, would be that the analyses are readily available in most hospital labs and that results can be obtained rapidly. Furthermore, the levels are not dependent on vitamin K levels. On the other hand, data to compare the use of antithrombin and/or fibrinogen with INR or factors 5 and/or 7 as prognostic markers seem very scarce.

It should be noted that the majority of studies published so far deal with adult patients who often have a different disease spectrum than children with liver disease. Furthermore, the levels of basically all coagulation factors have been shown to be different in children. Thus, newborns and infants have significantly lower levels which do increase to near-adult levels by the age of 6 months but do not reach fully adult levels until after puberty [61, 62].

4.5.2 Other Plasma Proteins

Albumin is the most abundantly detected plasma protein produced in the liver, and the levels are easily analyzed. It has therefore been widely used to estimate the synthetic function of the liver. However, the levels are subject to interference for several reasons. They will drop due to losses in patients with concomitant gastrointestinal disease, such as inflammatory bowel disease or celiac disease. Similarly, renal losses, ongoing inflammation, or poor nutritional status may yield lower levels and thereby obscure the interpretation of the results. Additionally, with a half-life of approximately 3 weeks, it is less likely to be of use in the short-term situation of acute liver failure.

With all these limitations in mind, there are still studies showing the importance of low levels of albumin as a prognostic marker, in particular in chronic liver disease [63] but possibly also in more acute situations [64].

Cholinesterase is another plasma protein produced mainly in the liver, and the levels are followed routinely in liver patients in some parts of the world. Although these levels may also be influenced by the nutritional status of the patient, they seem to be useful when predicting outcome, at least for chronic conditions, as shown in a few studies both in adults and children [65, 66]. Interestingly, its half-life is 12 days, i.e., somewhat shorter than that of albumin (Figs. 4.2 and 4.3) [67].

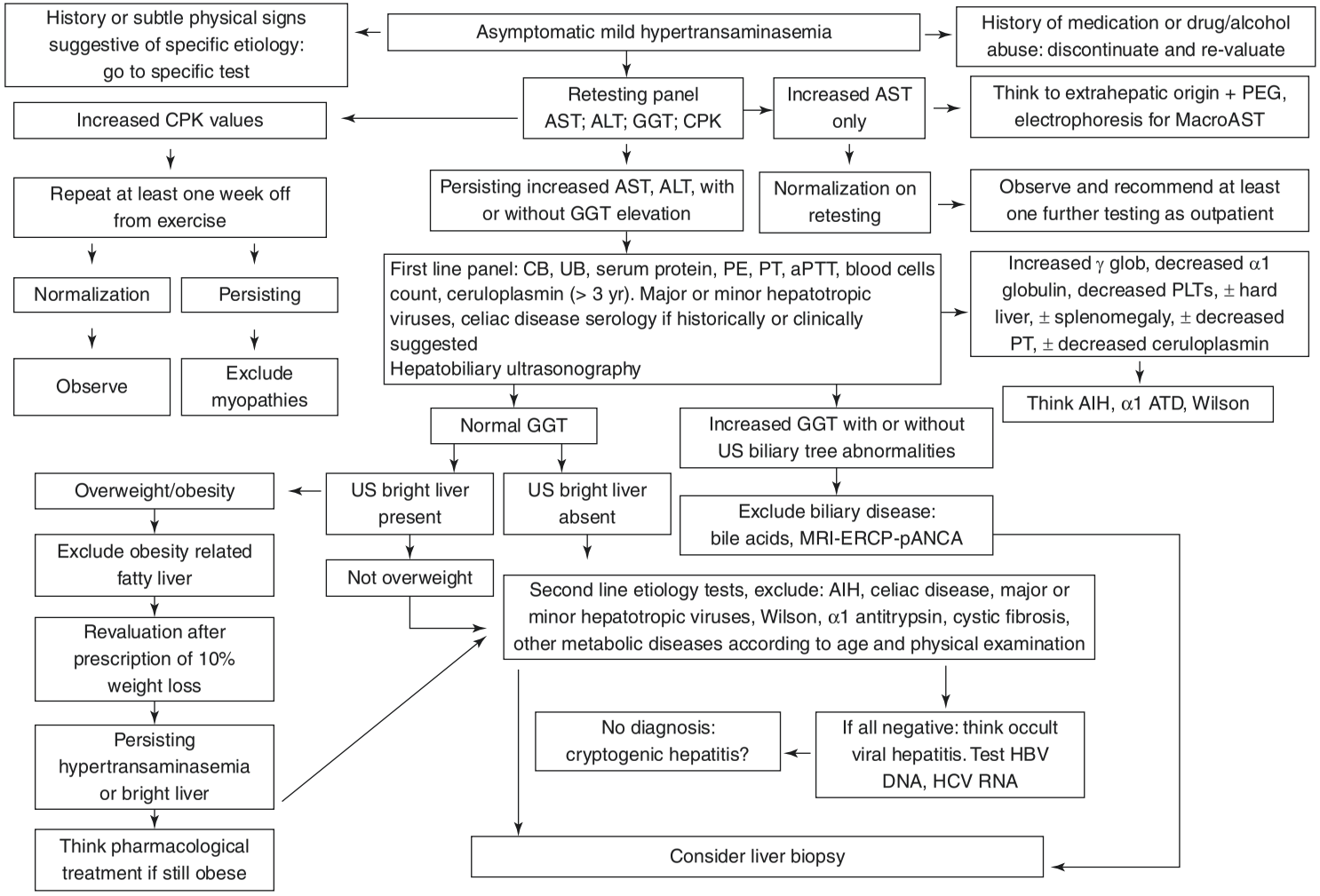

Fig. 4.2 Diagnostic algorithm for the diagnosis of pediatric mild chronic asymptomatic hypertransaminasemia. *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *CB* conjugated bilirubin, *UB* unconjugated bilirubin, *CPK* creatine kinase, *GGT* gamma-glutamyl transferase, *PE* pulmonary embolism, *PEG* polyethylene glycol, *PT* prothrombin time, *PTT* partial thromboplastin time, *US* ultrasound, *MRI* magnetic resonance imaging, *ERCP* endoscopic retrograde cholangiopancreatography, *pANCA* perinuclear anti-neutrophil cytoplasmic antibodies, *HBV* hepatitis B virus, *HCV* hepatitis V virus, *AIH* autoimmune hepatitis, *α1ATD* α1-antitrypsin deficiency. Suggested figure from Vajro P et al. [13]

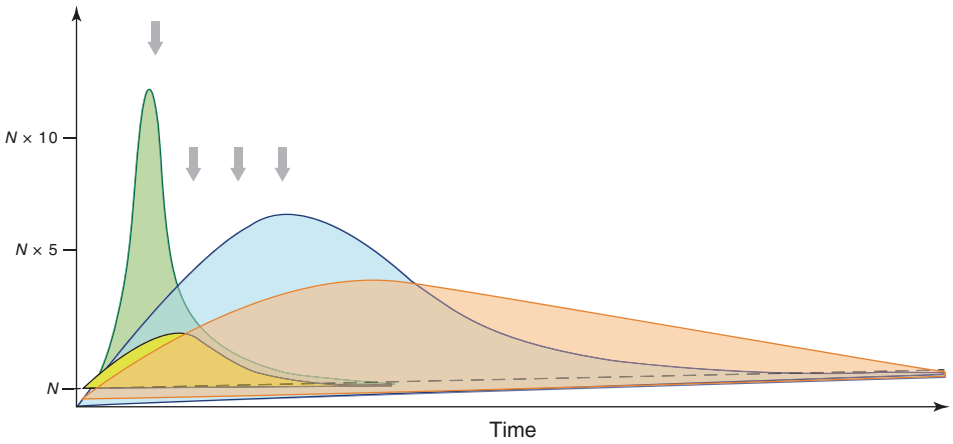

Fig. 4.3 Schematic representation of the rate of change of aminotransferase and bilirubin levels in a patient with acute ischemic hepatitis (green area, yellow area respectively) and acute viral hepatitis (blue area and orange area respectively). It is important to underscore that the pattern of enzyme alteration may vary and occasionally appear similar if a single observation point is taken into consideration (arrows) [70].

References

1. Karmen A, Wroblewski F, Ladue JS. Transaminase activity in human blood. J Clin Invest. 1955;34(1):126–31. PubMed PMID: 13221663; PubMed Central PMCID: PMC438594.
2. Panteghini M. Aspartate aminotransferase isoenzymes. Clin Biochem. 1990;23(4):311–9. Review. PubMed PMID: 2225456.
3. Price CP, Alberti KG. Biochemical assessment of liver function. In: Wright R, Alberti KGMM, Karran S, Millward-Sadler GH, editors. Liver and biliary disease—pathophysiology, diagnosis, management. London: WB Saunders; 1979. p. 381–416.
4. Dybkaer R. The tortuous road to the adoption of katal for the expression of catalytic activity by the General Conference on Weights and Measures. Clin Chem. 2002;48(3):586–90. PubMed PMID: 11861460.
5. Nuttall FQ, Jones B. Creatine kinase and glutamic oxalacetic transaminase activity in serum: kinetics of change with exercise and effect of physical conditioning. J Lab Clin Med. 1968;71(5):847–54. PubMed PMID: 5647686.
6. Pettersson J, Hindorf U, Persson P, Bengtsson T, Malmqvist U, Werkström V, Ekelund M. Muscular exercise can cause highly pathological liver function tests in healthy men. Br J Clin Pharmacol. 2008;65(2):253–9. PubMed PMID: 17764474.
7. Córdoba J, O'Riordan K, Dupuis J, Borensztajin J, Blei AT. Diurnal variation of serum alanine transaminase activity in chronic liver disease. Hepatology. 1998;28(6):1724–5. PubMed PMID: 9890798.
8. Rivera-Coll A, Fuentes-Arderiu X, Díez-Noguera A. Circadian rhythms of serum concentrations of 12 enzymes of clinical interest. Chronobiol Int. 1993;10(3):190–200. PubMed PMID: 8100488.
9. 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(5):361–7. PubMed PMID: 23581477.
10. Williams KM, Williams AE, Kline LM, Dodd RY. Stability of serum alanine aminotransferase activity. Transfusion. 1987;27(5):431–3. PubMed PMID: 3629675.
11. Caropreso M, Fortunato G, Lenta S, Palmieri D, Esposito M, Vitale DF, Iorio R, Vajro P. Prevalence and long-term course of macro-aspartate aminotransferase in children. J Pediatr. 2009;154(5):744–8. https://doi.org/10.1016/j.jpeds.2008.11.010. Epub 2008 Dec 25. PubMed PMID: 19111320.
12. Moriyama T, Tamura S, Nakano K, Otsuka K, Shigemura M, Honma N. Laboratory and clinical features of abnormal macroenzymes found in human sera. Biochim Biophys Acta. 2015;1854(6):658–67.
13. Vajro P, Maddaluno S, Veropalumbo C. Persistent hypertransaminasemia in asymptomatic children: a stepwise approach. World J Gastroenterol. 2013;19(18):2740–51. Review. PubMed PMID: 23687411.
14. Rosenthal P, Haight M. Aminotransferase as a prognostic index in infants with liver disease. Clin Chem. 1990;36(2):346–8.
15. 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.
16. Thierfelder N, Demuth I, Burghardt N, Schmelz K, Sperling K, Chrzanowska KH, Seemanova E, Digweed M. Extreme variation in apoptosis capacity amongst lymphoid cells of Nijmegen breakage syndrome patients. Eur J Cell Biol. 2008;87(2):111–21. Epub 2007 Oct 30. PubMed PMID: 17977616.
17. George J, Denney-Wilson E, Okely AD, Hardy LL, Aitken R. The population distributions, upper normal limits and correlations between liver tests among Australian adolescents. J Paediatr Child Health. 2008;44(10):579–85. PubMed PMID: 19012630.
18. 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(1):71–7. PubMed PMID: 19465871.
19. Lai DS, Chen SC, Chang YH, Chen CY, Lin JB, Lin YJ, Yang SF, Yang CC, Chen WK, Lin DB. Pediatric reference intervals for several biochemical analytes in school children in Central Taiwan. J Formos Med Assoc. 2009;108(12):957–63.
20. Southcott EK, Kerrigan JL, Potter JM, Telford RD, Waring P, Reynolds GJ, Lafferty AR, Hickman PE. Establishment of pediatric reference intervals on a large cohort of healthy children. Clin Chim Acta. 2010;411(19–20):1421–7. PubMed PMID: 20598674.
21. Colantonio DA, Kyriakopoulou L, Chan MK, Daly CH, Brinc D, Venner AA, Pasic MD, Armbruster D, Adeli K. Closing the gaps in pediatric laboratory reference intervals: a CALIPER database of 40 biochemical markers in a healthy and multiethnic population of children. Clin Chem. 2012;58(5):854–68. PubMed PMID: 22371482.

22. Hilsted L, Rustad P, Aksglæde L, Sørensen K, Juul A. Recommended Nordic paediatric reference intervals for 21 common biochemical properties. Scand J Clin Lab Invest. 2013;73(1):1–9. Epub 2012 Sep 26.
23. Bussler S, Vogel M, Pietzner D, Harms K, Buzek T, Penke M, Händel N, Körner A, Baumann U, Kiess W, Flemming G. New pediatric percentiles of liver enzyme serum levels (ALT, AST, GGT): Effects of age, sex, BMI and pubertal stage. Hepatology. 2018;68(4):1319–30. PubMed PMID: 28926121.
24. Schwimmer JB, Dunn W, Norman GJ, Pardee PE, Middleton MS, Kerkar N, Sirlin CB. SAFETY study: alanine aminotransferase cut-off values are set too high for reliable detection of pediatric chronic liver disease. Gastroenterology. 2010;138(4):1357–64, 1364. PubMed PMID: 20064512.
25. Robison R. The possible significance of hexosephosphoric esters in ossification. Biochem J. 1923;17(2):286–93. PubMed PMID: 16743183.
26. Van Hoof VO, De Broe ME. Interpretation and clinical significance of alkaline phosphatase isoenzyme patterns. Crit Rev Clin Lab Sci. 1994;31(3):197–293. Review. PubMed PMID: 7818774.
27. Sharma U, Pal D, Prasad R. Alkaline phosphatase: an overview. Indian J Clin Biochem. 2014;29(3):269–78. Review. PubMed PMID: 24966474.
28. Carroll AJ, Coakley JC. Transient hyperphosphatasaemia: an important condition to recognize. J Paediatr Child Health. 2001;37(4):359–62. PubMed PMID: 11532055.
29. Gualco G, Lava SA, Garzoni L, Simonetti GD, Bettinelli A, Milani GP, Provero MC, Bianchetti MG. Transient benign hyperphosphatasemia. J Pediatr Gastroenterol Nutr. 2013;57(2):167–71. PubMed PMID: 23539049.
30. Shaver WA, Bhatt H, Combes B. Low serum alkaline phosphatase activity in Wilson's disease. Hepatology. 1986;6(5):859–63.
31. Whyte MP. Hypophosphatasia and the role of alkaline phosphatase in skeletal mineralization. Endocr Rev. 1994;15(4):439–61. Review. PubMed PMID: 7988481.
32. Hanes CS, Hird FJ, Isherwood FA. Synthesis of peptides in enzymic reactions involving glutathione. Nature. 1950;166(4216):288–92. PubMed PMID: 15439292.
33. Goldberg DM. Structural, functional, and clinical aspects of gamma-glutamyltransferase. CRC Crit Rev Clin Lab Sci. 1980;12(1):1–58. Review. PubMed PMID: 6104563.
34. Vroon DH, Israili Z. Chapter 100. Alkaline phosphatase and gamma glutamyltransferase. In: Walker HK, Hall WD, Hurst JW, editors. Clinical methods: the history, physical, and laboratory examinations. 3rd ed. Boston: Butterworths; 1990. PubMed PMID: 21250047.
35. Cabrera-Abreu JC, Green A. Gamma-glutamyltransferase: value of its measurement in paediatrics. Ann Clin Biochem. 2002;39(Pt 1):22–5. Review. PubMed PMID: 11853185.
36. Wang NL, Li LT, Wu BB, Gong JY, Abuduxikuer K, Li G, Wang JS. The features of GGT in patients with ATP8B1 or ABCB11 deficiency improve the diagnostic efficiency. PLoS One. 2016;11(4):e0153114. PubMed PMID: 27050426.
37. Fischler B, Eggertsen G, Björkhem I. Genetic defects in synthesis and transport of bile acids. In: Pediatric endocrinology and metabolic diseases. 2017. p. 447–60.
38. Suchy FJ, Balistreri WF, Heubi JE, Searcy JE, Levin RS. Physiologic cholestasis: elevation of the primary serum bile acid concentrations in normal infants. Gastroenterology. 1981;80:1037–41.
39. Gitlin D, Perricelli A, Gitlin GM. Synthesis of α-fetoprotein by liver, yolk sac, and gastrointestinal tract of the human conceptus. Cancer Res. 1972;32:979–82.
40. Blohm ME, Vesterling-Hörner D, Calaminus G, et al. Alpha 1-fetoprotein (AFP) reference values in infants up to 2 years of age. Pediatr Hematol Oncol. 1998;15:135–42.
41. Murray MJ, Nicholson JC. α-Fetoprotein. Arch Dis Child Educ Pract Ed. 2011;96:141–7.
42. De Ioris M, Brugieres L, Zimmermann A, et al. Hepatoblastoma with a low serum alpha-fetoprotein level at diagnosis: the SIOPEL group experience. Eur J Cancer. 2008;44:545–50.
43. Koprowaski H, Steplewski Z, Mitchell K, et al. Colorectal carcinoma antigens detected by hybridoma antibodies. Somatic Cell Genet. 1979;5:957–72.
44. Nehls O, Gregor M, Klump B. Serum and bile markers for cholangiocarcinoma. Semin Liver Dis. 2004;24:139–54.
45. Lamerz R. Role of tumour markers, cytogenetics. Ann Oncol. 1999;10(Suppl. 4):S145–9.
46. Tripodi A, Chantarangkul V, Primignani M, Fabris F, Dell'Era A, Sei C, Mannucci PM. The international normalized ratio calibrated for cirrhosis (INR(liver)) normalizes prothrombin time results for model for end-stage liver disease calculation. Hepatology. 2007;46(2):520–7.
47. Magnusson M, Sten-Linder M, Bergquist A, et al. The international normalized ratio according to Owren in liver disease: interlaboratory assessment and determination of international sensitivity index. Thromb Res. 2013;132:346–51.
48. Cederblad G, Korsan-Bengtsen K, Olsson R. Observations of increased levels of blood coagulation factors and other plasma proteins in cholestatic liver disease. Scand J Gastroenterol. 1976;11:391–6.
49. Magnusson M, Fischler B, Svensson J, Petrini P, Schulman S, Németh A. Bile acids and coagulation factors: paradoxical association in children with chronic liver disease. Eur J Gastroenterol Hepatol. 2013;25:152–8.
50. Squires RH, Schneider BL, Bucuvalas J, et al. Acute liver failure in children: the first 348 patients in the Pediatric Acute Liver Failure Study Group. J Pediatr. 2006;148:652–8.
51. Franchini M, Lippi G. Prothrombin complex concentrates: an update. Blood Transfus. 2010;8(3):149–54.
52. McDiarmid SV, Anand R, Lindblad AS, Principal Investigators and Institutions of the Studies of Pediatric Liver Transplantation (SPLIT) Research Group. Development of a pediatric end-stage liver disease score to predict poor outcome in children awaiting liver transplantation. Transplantation. 2002;27:173–81.
53. O'Grady JG, Alexander GJ, Hayllar KM, Williams R. Early indicators of prognosis in fulminant hepatic failure. Gastroenterology. 1989;97:439–45.
54. Dhawan A, Taylor RM, Cheeseman P, et al. Wilson's disease in children: 37-year experience and revised King's score for liver transplantation. Liver Transpl. 2005;11:441–8.
55. Lu BR, Zhang S, Narkewicz MR, et al. Evaluation of the liver injury unit scoring system to predict survival in a multinational study of pediatric acute liver failure. J Pediatr. 2013;162:1010–6.
56. Bernuau J, Goudeau A, Poynard T, et al. Multivariate analysis of prognostic factors in fulminant hepatitis B. Hepatology. 1986;6:648–51.
57. Izumi S, Langley PG, Wendon J, et al. Coagulation factor V levels as a prognostic indicator in fulminant hepatic failure. Hepatology. 1996;23:1507–11.
58. Dymock IW, Tucker JS, Woolf IL, Poller L, Thomson JM. Coagulation studies as a prognostic index in acute liver failure. Br J Haematol. 1975;29:385–95.
59. Elinav E, Ben-Dov I, Hai-Am E, Ackerman Z, Ofran Y. The predictive value of admission and follow up factor V and VII levels in patients with acute hepatitis and coagulopathy. J Hepatol. 2005;42(1):82–6.
60. Rodzynek JJ, Preux C, Leautaud P, Abramovici J, Di Paolo A, Delcourt AA. Diagnostic value of antithrombin III and aminopyrine breath test in liver disease. Arch Intern Med. 1986;146:677–80.
61. Andrew M, Paes B, Milner R, et al. Development of the human coagulation system in the full-term infant. Blood. 1987;70:165–72.

62. Monagle P, Massicotte P. Developmental haemostasis: secondary haemostasis. Semin Fetal Neonatal Med. 2011;16:294–300.
63. Watanabe T. Short-term prognostic factors for primary sclerosing cholangitis. J Hepatobiliary Pancreat Sci. 2015;22:486–90.
64. Dabos KJ, Newsome PN, Parkinson JA, et al. Biochemical prognostic markers of outcome in non-paracetamol-induced fulminant hepatic failure. Transplantation. 2004;77:200–5.
65. Pferdmenges DC, Baumann U, Müller-Heine A, Framke T, Pfister ED. Prognostic marker for liver disease due to alpha1-antitrypsin deficiency. Klin Padiatr. 2013;225:257–62.
66. Abbas M, Abbas Z. Serum cholinesterase: a predictive biomarker of hepatic reserves in chronic hepatitis D. World J Hepatol. 2017;9:967–72.
67. Santarpia L, Grandone I, Contaldo F, Pasanisi F. Butyrylcholinesterase as a prognostic marker: a review of the literature. J Cachexia Sarcopenia Muscle. 2013;4(1):31–9.
68. Botros M, Sikaris KA. The de ritis ratio: the test of time. Clin Biochem Rev. 2013;34(3):117–30. Review. PubMed PMID: 24353357.
69. Tsuchida Y, Endo Y, Saito S, Kaneko M, Shiraki K, Ohmi K. Evaluation of alpha-fetoprotein in early infancy. J Pediatr Surg. 1978;13(2):155–62. PubMed PMID: 77324.
70. Giannini EG, et al. Liver enzyme alteration: a guide for clinicians. CMAJ. 2005;172(3):367–79.