Chapter 1
Assessment of Liver Function

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Case Study

A 50-year-old man has been referred to the Liver Clinic by his GP following the discovery of a mildly elevated ALT on a routine blood test. The letter states that the patient "does not drink too much alcohol" and has "no obvious reason" to have deranged LFTs.

The blood tests done by his GP reveal:
Normal Full Blood Count and renal function.
Random blood glucose (8.5 mmol/L).

LFT's

Bilirubin 10 μmol/L (3–17 μmol/L).
Alanine aminotransferase (ALT) 115 IU/L (10–45 IU/L).
Alkaline phosphatase (ALP) 95 IU/L (30–105 IU/L).
Gamma glutamyl transpeptidase (GGT) 75 IU/L (15–40 IU/L).
Albumin 38 g/L (35–50 IU/L).
Prothrombin time 11.7 s (9–12.7 s).

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Questions

1. Would you like any more information from the referral?
2. What is the differential diagnosis?
3. What additional tests should be done?
4. Which invasive and non-invasive investigations are you aware of to aid prognosis and guide subsequent management of liver disease?

An Overview of Anatomy and Functions of the Liver

The liver is the largest solid organ in the body weighing approximately 1600 grams in men and 1400 grams in women. It lies in the right upper quadrant of the abdomen with its upper border between the fifth and sixth ribs and its lower border along the right costal margin. 75% of hepatic blood flow is delivered by the portal vein while the hepatic artery provides 25% [1].

The liver is important in a wide variety of metabolic and immunological functions.

Metabolic

The liver is a central hub for carbohydrate, protein and lipid metabolism. It is responsible for glycogenolysis and gluconeogenesis and maintains plasma glucose levels via this pathway. Glucose homeostasis is reliant on the relative concentration of insulin and glucagon [1, 2].

The liver has diverse functions pertaining to protein metabolism including synthesis and degradation of amino acids and proteins including albumin, globulin and clotting factors. The production of ammonia from the deamination of amino acids and subsequent conversion to urea also happens almost exclusively in the liver, although there is some contribution from skeletal muscle [1, 2].

Cholesterol degradation and excretion is dependent on the liver. It performs lipogenesis; triglyceride production and the bulk of lipoproteins are synthesized in the liver. Bile production enables fat emulsification and absorption of fat soluble vitamins such as Vitamin A, D, E and K from the diet [2].

Immunologic

The liver is an important immunological site and acts as a sieve for vast amounts of pathogenic substances travelling via the portal vein from the intestine. Kupffer cells in the hepatic sinusoids have roles in phagocytosis, cytokine and chemokine release and activation of the hepatic stellate cells. Intrahepatic populations of lymphocytes, natural killer cells and dendritic cells also form part of the liver's defence mechanism [2].

Common Risk Factors for Deranged Liver Enzyme Tests

Alcohol

Assessment of alcohol intake is vital when considering causes for deranged liver function tests. This is done with open questions; but it is important to quantify the amount of alcohol consumed in units. Current UK guidelines set the maximum alcohol consumption at no more than 14 units per week for both men and women. A quick and easy guide to remember units:

1 unit: 1 single shot of spirits
1.5 units: 1 small glass of wine (125 mL)
2 units: 1 can of beer/lager/ale/cider or 1 medium glass of wine (175 mL)
3 units: 1 pint of beer/lager/ale/cider or 1 large glass of wine (250 mL)
9 units: 1 bottle of wine.

Metabolic Factors

Non-alcoholic fatty liver disease is on the rise and will soon be the most common cause for deranged liver enzymes. Metabolic risk factors including obesity, Type 2 Diabetes Mellitus, dyslipidaemia and hypertension should be taken into account when trying to identify the underlying cause for abnormal liver function test results.

Risk Factors for Viral Hepatitis

Always consider risk factors for viral hepatitis when investigating deranged liver function tests. These include:

Hepatitis A: Acquired through faecal-oral spread through contaminated food or water. Self-limiting illness and a vaccine is available.

Hepatitis B: Acquired through blood to blood contact, often through sexual contact, contaminated needles and perinatal transfer. Majority of infections will resolve spontaneously, but approximate 15% will acquire chronic infection. Vaccination is available.

Hepatitis C: Acquired through blood to blood contact, most often through injection drug use through contaminated needles, but can also be acquired less commonly through sexual contact and perinatal transfer. No vaccination available, but effective treatment results in cure.

Hepatitis D: Acquired as a superadded infection in the context of active Hepatitis B infection.

Hepatitis E: Acquired through faecal-oral spread through contaminated water. Can also be acquired via zoonotic spread especially undercooked pork products. Usually self-limiting, but can develop chronic disease in the immunosuppressed. No vaccine available.

Drug-Induced Liver Injury

Almost all available medications can cause a degree of liver enzymes derangement. New or recent medication along with

the full list of patient's medications should be included in the history taking, paying specific attention to any antibiotic use in the previous 2–3 months.

Interpretation of Liver Function Tests

As the liver is a multifunctional organ, with an extensive amount of reserve and ability to regenerate, clinical features may not manifest until the liver is near the end-stage of the spectrum of severity. The term liver function tests (LFTs) includes tests of:

- synthetic function (albumin, prothrombin time),
- excretory function (bilirubin),
- underlying necroinflammation (serum aminotransferases: ALT and aspartate aminotransferase (AST)) and,
- cholestasis (ALP, GGT).

The interpretation of abnormal LFTs requires a systematic approach with regards to:

- Taking a complete history including family history and eliciting the relevant physical signs and risk factors.
- Recognizing the pattern of LFT abnormality (i.e. impaired synthetic function, hepatocellular injury – "hepatitic picture", cholestasis or a mixed picture).
- An awareness of the duration and severity of elevation of LFT abnormalities.

Initial Approach to Potential Liver Disease

According to the recently published British Society of Gastroenterology (BSG) guidance, the initial investigation of potential liver disease should include bilirubin, albumin, ALT, ALP, gGT and FBC if not performed in the last 12 months.

A strategy of simply repeating abnormal tests can only be justified where there is a high degree of certainty that the abnormality will resolve in response to an identified acute insult. In other cases, detection of the first abnormality should trigger investigation of the aetiology or repeat testing

to assess progression or disease severity where there is a suspicion that the underlying cause may require urgent referral/admission.

Parenchymal Liver Screen

An **abdominal US** should be performed in all cases where the diagnosis is unclear in order to investigate the liver parenchyma in detail and exclude any obstructive causes.

"Full liver screen" is a loose term to describe a set of serum-based investigations to elucidate a cause of abnormal LFT's in the context of suspected chronic liver disease.

It consists of the following (Table 1.1):

Table 1.1 A typical "full liver screen"

Disease	Test
Chronic hepatitis B	Hepatitis B surface antigen
Chronic hepatitis C	Hepatitis C antibody Hepatitis C RNA (if antibody positive)
Autoimmune hepatitis	Anti-smooth muscle antibody Anti-nuclear antibody Anti-liver kidney microsomal antibody Immunoglobulin G
Primary biliary cholangitis	Antimitochondrial antibody Immunoglobulin M
Haemochromatosis	Ferritin Transferrin saturation (>45%) HFE genotype
Wilson's disease	Caeruloplasmin (normally low)
Alpha-1 antitrypsin deficiency	Alpha-1 antitrypsin level and phenotype (if level low)
Coeliac disease	Anti-TTG or Anti-endomysial antibody (if low IgA)

Tests of Hepatocellular Damage

Serum Aminotransferases (ALT and AST) [3, 4]

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are intracellular enzymes that are released into plasma during hepatocellular injury and are used as a marker of hepatic inflammation or necrosis.

ALT is a liver specific enzyme located in the cytosol of hepatocytes. Persisting abnormality in ALT should encourage the clinician to seek a cause.

AST is in the liver cytosol and mitochondria, but also in cardiac and skeletal muscle, kidney, brain and pancreas. It is, therefore, less specific than ALT as damage to any of these organs result in a rise.

An elevated AST to ALT ratio, especially if AST is more than twice that of ALT, may signify alcohol related liver disease (ALD) [5] (about 70% of patients with ALD have a ratio > 2). In Non-alcoholic fatty liver disease (NAFLD) an AST/ALT ≥0.8 is thought to signify significant liver fibrosis (Ishak stage 3/6).

Table 1.2 summarizes the wide differential diagnosis of elevated serum aminotransferases and Fig. 1.1 suggests a diagnostic algorithm.

Alkaline Phosphatase (ALP)

ALP is an enzyme that is present in the liver, bone, intestine and placenta. Levels can rise if there is damage to any of these tissues. It is usually elevated to at least four times the upper limit of normal in patients with cholestasis. A simultaneous rise in GGT is a very sensitive indicator of hepatobiliary disease. If still in doubt, serum electrophoresis can be used to determine the ALP isoenzyme, especially if more than one cause for ALP elevation is suspected. An elevation of ALP can indicate either intrahepatic or extrahepatic biliary disease, and patients would require an ultrasound to investigate the biliary tree.

Table 1.2 Causes of elevated serum transaminases.

Degree of elevation	Cause
Mild (<100 iu/L)	Non-alcoholic fatty liver disease (NAFLD) Alcohol consumption Chronic viral hepatitis B or C Haemochromatosis Coeliac disease Non-hepatic (thyroid disease, haemolysis, myopathy, strenuous exercise) (AST elevation)
Moderate (100–350 iu/L)	As above, plus Alcoholic hepatitis Autoimmune hepatitis Acute biliary obstruction Budd-Chiari syndrome Wilson's disease
Major (>1000 iu/L)	Paracetamol poisoning Acute viral hepatitis Autoimmune hepatitis Ischaemic hepatitis Budd-Chiari syndrome

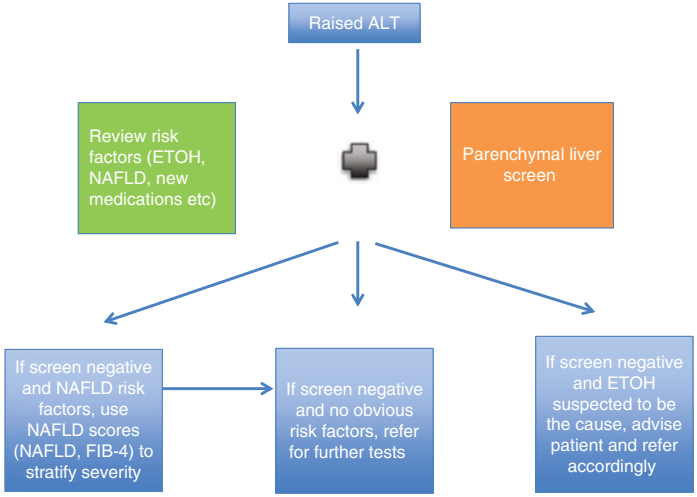

Figure 1.1 Diagnostic algorithm for elevated serum aminotransferases [6]

Table 1.3 Causes of elevated alkaline phosphatase in liver disease

Degree of elevation	Cause
Minor (<3× ULN)	All types of liver disease (hepatitis, cirrhosis, infiltrative diseases, sepsis, congestive cardiac failure)
Major (>3× ULN)	Biliary obstruction due to stones, pancreatic cancer, cholangiocarcinoma Liver malignancy/metastases Primary sclerosing cholangitis Primary biliary cholangitis Sepsis Cholestatic drug-induced liver injury (DILI) Sarcoidosis, amyloidosis

An isolated ALP elevation requires consideration of other conditions such as bone disorders, which include osteomalacia, Paget disease of the bone and bone metastases.

Low levels of ALP may occur in Wilson's disease and hypothyroidism. Table 1.3 shows causes of elevations in ALP and Fig. 1.2 proposes a diagnostic algorithm.

Isolated GGT Elevation

Apart from being a useful diagnostic marker for hepatobiliary diseases in association with ALP, GGT on its own can be elevated in many other conditions such as alcoholism, myocardial infarction, chronic obstructive pulmonary disease, renal failure, diabetes, obesity and pancreatic disease and is therefore often non-specific, thus investigation of an isolated GGT is not recommended.

Bilirubin

Understanding the physiology of bilirubin production and excretion within the digestive system is the key to recognizing the patterns of elevation.

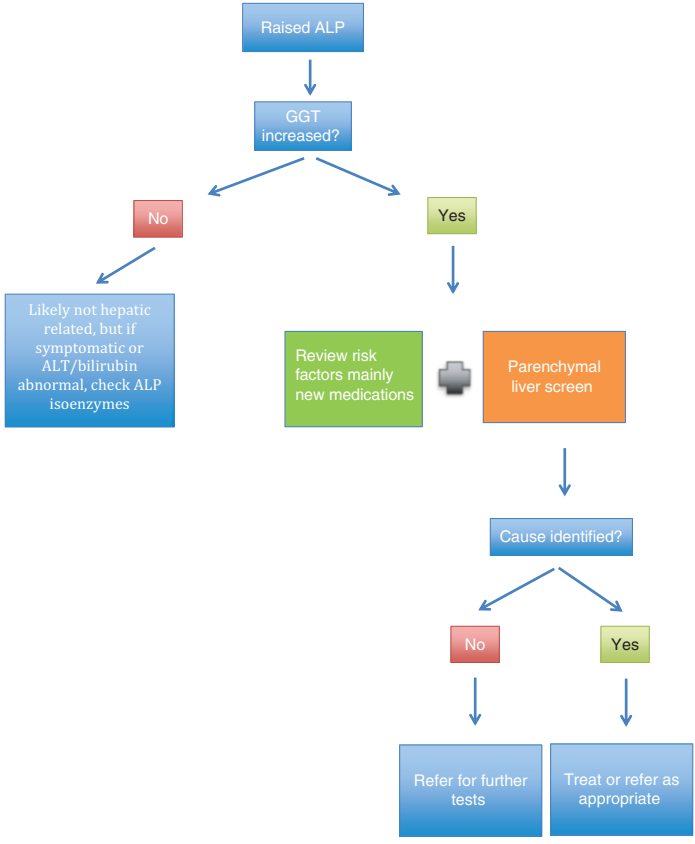

FIGURE 1.2 Diagnostic algorithm for elevated ALP [6]

Bilirubin is formed when haemoglobin is broken down within the reticulo-endothelial system in the liver, spleen and bone marrow. It is transported to the liver bound to albumin where it is conjugated into a water-soluble form. Conjugation facilitates its excretion into bile. Bile is then excreted via the common bile duct into the small bowel where it is reduced by gut bacteria into urobilinogen. Enterohepatic circulation enables reabsorption of the urobilinogen back into bile whilst a small proportion is excreted into urine and stool as stercobilinogen, giving stool its usual brown colour.

Hyperbilirubinaemia can either be predominantly unconjugated or conjugated. This measurement is most useful in determining at which level of bilirubin metabolism the pathology occurs. Unconjugated hyperbilirubinaemia is most commonly due to haemolysis and ineffective erythropoiesis whilst conjugated hyperbilirubinaemia usually reflects underlying parenchymal liver disease or biliary obstruction (Table 1.4, Fig. 1.3).

Table 1.4 Causes of hyperbilirubinaemia [7]

Unconjugated	**Conjugated**
Increased bilirubin production • Haemolysis • Ineffective erythropoiesis (G6PD, thalassaemia, sickle cell disease) • Blood transfusion • Haematoma	Hepatocellular diseases • Viral hepatitis • Chronic autoimmune hepatitis • DILI • Alcoholic hepatitis • PBC • PSC
Hereditary disorders (impaired hepatic uptake or conjugation of bilirubin) • Gilbert syndrome (most common) • Criggler-Najjar syndrome	Infiltrative diseases • Tumours (primary, metastatic) • Infections (tuberculosis, parasites)
Drugs • Rifampicin • Probenecid	Hereditary disorders • Dubin-Johnson syndrome • Rotor syndrome
	Extrahepatic biliary obstructive disease • Cholangiocarcinoma • Pancreatic disease (cysts, carcinoma, chronic pancreatitis) • Cholecystitis • Choledocholithiasis
	Drugs • Allopurinol • Phenytoin • Anabolic steroids • Statins • Chlorpromazine • Herbal medications
	Sepsis
	Parenteral nutrition

Abbreviations: *DILI* drug-induced liver injury, *PBC* primary biliary cholangitis, *PSC* primary sclerosing cholangitis, *G6PD* Glucose-6-phosphate isomerase deficiency

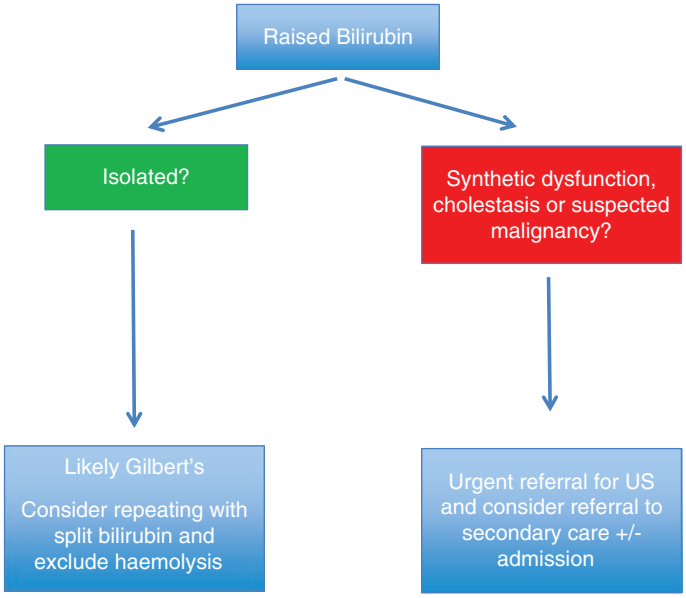

Figure 1.3 Algorithm for elevated bilirubin [6]

Test of Liver Synthetic Function

Prothrombin Time

The liver plays a significant role in haemostasis through production of coagulation factors (factor II, VII, IX, X), which are vitamin K dependent. Fat soluble vitamin K is dependent on bile salts for absorption. Conditions resulting in a reduction of bile salt production/excretion may therefore result in a prolongation of the prothrombin time. Administration of intravenous vitamin K will distinguish between true synthetic dysfunction and Vitamin K deficiency, resulting in correction of the PT in vitamin K deficiency and no effect in synthetic dysfunction.

The use of international normalized ratio (INR) minimizes variability in reporting of PT across different laboratories.

Albumin

Albumin is one of the major products of the liver. Hypoalbuminaemia in the context of liver disease suggest impaired synthetic function and provides information on prognosis. Due to its long half-life (17–30 days), low levels usually indicate chronic rather than acute liver dysfunction [8].

Causes of hypoalbuminaemia:

1. Decreased synthesis—severe liver disease, malnutrition, acute phase reaction, e.g., sepsis, surgery.
2. Plasma expansion—ascites, oedema.
3. Body losses—burns, protein losing enteropathy, nephrotic syndrome.
4. Increased catabolism—alcohol, malignancy, pregnancy.

Platelets

Thrombocytopaenia is quite common in chronic liver disease and is an indicator of advanced disease. The usual mechanisms are: decreased production, splenic sequestration and increased destruction. Decreased production is caused by bone marrow suppression (alcohol, iron overload, drugs) and by a reduction in thrombopoietin levels. Splenomegaly (as caused by portal hypertension in advanced liver injury) causes splenic sequestration. Platelet destruction is increased non-specifically in liver cirrhosis because of shear stress and fibrinolysis whereas in specific causes of autoimmune liver disease, immunologically mediated destruction of platelets occurs owing to antiplatelet immunoglobulin.

Liver Fibrosis

Chronic liver disease results in the development of fibrosis which subsequently progresses to cirrhosis. Cirrhosis may be complicated by portal hypertension, liver parenchymal failure and hepatocellular carcinoma. Although liver fibrosis was

originally thought to be irreversible, there is now evidence that in certain disease processes it may be a dynamic process with the potential of significant regression [9].

Liver biopsy has historically been the gold standard for grading and assessing progression of liver fibrosis. Biopsy remains a useful way of assessing fibrosis, inflammation and also in identifying a second pathology where uncertainty exists with regards to the primary diagnosis. There are, however, significant limitations with liver biopsy being an invasive procedure with complications, sampling error given heterogenicity of the organ and inter-observer variability [10, 11].

Non-invasive methods of assessing liver fibrosis have now become popular including serological and radiological methods of assessment.

Serological testing has the advantage of high applicability and inter-laboratory reproducibility but is limited due to lack of liver specificity and so results can be influenced by other factors such patient co-morbidities. However, the use of FIB-4 or NAFLD fibrosis score, or ELF testing when NAFLD is suspected should be first line investigation in primary care. Depending on the results, a referral for elastography should be considered.

Radiological methods, such as transient elastography are becoming the modality of choice to ascertain the degree of fibrosis. It is quick, cost effective and reproducible and examines a significantly larger mass of liver tissue compared to liver biopsy. Interpretation of results should always take into account patient demographics, disease aetiology and laboratory parameters. The applicability however is limited in obese patients and has been shown to produce unreliable results. It can also not be performed in patients with ascites. Cholestasis, acute hepatitis, congestive cardiac failure, food and alcohol intake can increase liver stiffness and results need to be interpreted with caution in these circumstances [10–12].

Special Circumstances: Pregnancy [6]

Pregnancy results in a hyperdynamic circulation state, which has some similarities to the hyperdynamic state present in patients with decompensated chronic liver disease. On physical examination of a pregnant woman, it is not uncommon to see palmar erythema or multiple spider naevi.

There are also some physiological changes in LFTs which are given in Table 1.5. ALP is raised in third trimester due to placental production and foetal bone development. Alpha-fetoprotein (AFP) can also be raised as it is produced by the foetal liver.

Table 1.5 Physiological changes in blood markers during pregnancy

Blood marker	First trimester	Second trimester	Third trimester
ALT/AST	No change	No change	No change
Bilirubin	No change	No change	No change
ALP	No change	No change or increased	Increased up to fourfold
gGT	No change	No change	No change
Albumin	Decreased	Decreased	Decreased
Prothrombin time	No change	No change	No change
Platelets	No change	No change	No change

Answer to Question 1: History Clarification

The past medical history of the patient includes type 2 diabetes and hypertension. He is taking Metformin 500 mg BD and Amlodipine 5 mg OD. He has not had any over the counter medication, herbal remedies or new medications prescribed by the GP. He lives with his long-term partner, has not travelled abroad and does not have any tattoos. He has never had a blood transfusion in the past or injected drugs intravenously. He drinks a bottle of wine per week and denies drinking more than that in the past. His BMI is 31 kg/m^2.

Examination reveals an obese but otherwise well patient with no signs of chronic liver disease, no palpable hepatosplenomegaly and no evidence of decompensation, e.g. Jaundice, ascites, encephalopathy or bleeding.

Answer to Question 2

The differential diagnosis in this patient would include:

- Non-alcoholic fatty liver disease (NAFLD).
- Autoimmune hepatitis.
- Haemochromatosis.
- Chronic viral hepatitis (B or C).

He is at risk of NAFLD because he has diabetes, hypertension and an elevated BMI. The ALT and GGT are usually raised in NAFLD, although an elevation in ALP can also be seen. Ultrasound of the liver would reveal a fatty liver and features of portal hypertension (e.g. large spleen, ascites and reduced portal vein flow <20 cm/sec) may be present in the presence of cirrhosis.

Answer to Question 3

It is important to exclude other liver disorders before a diagnosis of NAFLD is made. The parenchymal liver screen is a fundamental component in the diagnostic approach. In this patient, his liver screen was unremarkable. Given the metabolic risk factors and negative parenchymal liver screen, the likely diagnosis is NAFLD. Assessment of any liver fibrosis would be important, ideally using non-invasive tests.

Answer to Question 4

Assessment of the presence of liver fibrosis has significant prognostic implications. Liver biopsy is an invasive option which provides valuable information, however, does have limitations.

Non-invasive options include:

Serological markers - This can be divided into direct and indirect biomarkers. Examples of indirect markers include the AST to platelet ratio index (APRI), FIB-4 score and NAFLD fibrosis score. Indirect markers available include procollagen type III amino-terminal peptide (PIIINP) and serum hyaluronic acid [10, 11].

Radiological markers - Transient elastography is an established tool to assess liver fibrosis non-invasively. It is applicable in a wide range of aetiologies and guides further management and informs the need to proceed to liver biopsy where appropriate.

References

1. Tortora GJ, Derrickson BH. Principles of anatomy and physiology. 12th ed. Hoboken: Wiley; 2008.
2. Nash K, Guha IN. Basic science. In: Hepatology: clinical cases uncovered. 1st ed. New York, NY: Wiley-Blackwell; 2011.
3. Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. Clin Chem. 2000;46(12):2050–68.
4. Limdi JK, Hyde GM. Evaluation of abnormal liver function tests. Postgrad Med J. 2003;79(932):307–12.
5. Cohen JA, Kaplan MM. The SGOT/SGPT ratio--an indicator of alcoholic liver disease. Dig Dis Sci. 1979;24(11):835–8.
6. Newsome PN, et al. Figure 1–3 adapted from Guidelines on the management of abnormal liver blood tests. Gut Jan. 2018;67(1):6–19.
7. Bloom S, et al. Table 4 adapted from Oxford Handbook of Gastroenterology and Hepatology. 2nd edition, December 2011.

8. Friedman LS, Keefe EB. Handbook of liver disease. 3rd ed. Philadelphia, PA: Elsevier Health Sciences; 2011.
9. Pellicoro A, Ramachandran P, Iredale JP. Reversibility of liver fibrosis. Fibrogenesis Tissue Repair. 2012;5(Suppl 1):S26.
10. Cui XW, Friedrich-Rust M, De Molo C, Ignee A, Schreiber-Dietrich D, Dietrich CF. Liver elastography, comments on EFSUMB elastography guidelines 2013. World J Gastroenterol. 2013;19(38):6329–47.
11. Ferraioli G, Filice C, Castera L, et al. WFUMB guidelines and recommendations for clinical use of ultrasound elastography: part 3: liver. Ultrasound Med Biol. 2015;41(5):1161–79.
12. Westbrook RH, Dusheiko G, Williamson C. Pregnancy and liver disease. J Hepatol. 2016;64(4):933–45. https://doi.org/10.1016/j.jhep.2015.11.030.