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Liver Function Laboratory Tests: Conventional Markers and Hepatocellular Carcinoma Biomarkers

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Abstract

Liver function tests represent a cornerstone in the evaluation of hepatobiliary disease, providing a non-invasive assessment of hepatic injury, synthetic capacity, and excretory function, offering valuable insight into hepatocellular integrity, cholestasis, and hepatic synthetic capacity. Despite their widespread use, many of these parameters reflect liver injury rather than true hepatic function, requiring careful clinical interpretation. This narrative review summarizes the physiological basis, diagnostic significance, and limitations of conventional liver biochemical markers, including aminotransferases, alkaline phosphatase, bilirubin, gamma-glutamyl transferase, albumin, total protein, platelet count, and prothrombin time. Particular emphasis is placed on the interpretation of laboratory patterns that distinguish hepatocellular from cholestatic injury. In addition, the review highlights emerging biomarkers for hepatocellular carcinoma (HCC), a leading cause of cancer-related mortality worldwide. Current evidence regarding alpha-fetoprotein, microRNA-122, and inflammatory mediators such as tumor necrosis factor-alpha is discussed, with attention to their diagnostic performance, clinical applications, and limitations in HCC surveillance. Understanding the strengths and shortcomings of both conventional and emerging biomarkers is essential for improving early detection of liver disease and optimizing surveillance strategies for hepatocellular carcinoma. Future approaches integrating multi-marker panels with imaging and clinical risk stratification may enhance diagnostic accuracy and patient outcomes.

Keywords: AST, ALT, Bilirubin, Alkaline Phosphatase (ALP), AFP, microRNA-122.

1. Introduction

The liver is the largest internal organ and performs a wide range of essential physiological functions that are critical for maintaining metabolic homeostasis. Located in the right upper quadrant of the abdomen beneath the diaphragm, it serves as the central organ for metabolism, detoxification, and synthesis of numerous biologically important molecules [1,2]. Hepatic functions include the metabolism of carbohydrates, lipids, and proteins; detoxification of endogenous metabolites and xenobiotics; synthesis of plasma proteins such as albumin and coagulation factors; and production of bile necessary for digestion and excretion of waste products [1–3]. The liver also plays a pivotal role in bilirubin metabolism, transforming the breakdown products of hemoglobin from senescent erythrocytes into

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conjugated bilirubin that can be excreted through the biliary system [2,3].

Given the liver's diverse physiological roles, laboratory assessment of hepatic health relies on a panel of biochemical tests commonly referred to as liver function tests (LFTs) [4]. However, this terminology can be misleading, as many of these laboratory markers do not directly measure hepatic functional capacity but rather indicate hepatocellular injury, inflammation, or biliary obstruction [4,5]. Interpretation, therefore, requires integration of multiple laboratory parameters together with clinical findings and imaging studies [4]. Patterns of abnormalities such as hepatocellular, cholestatic, or mixed injury often provide important diagnostic clues that guide further evaluation [5].

In addition to their role in diagnosing liver injury, laboratory markers are increasingly being investigated for their utility in the surveillance and early detection of hepatocellular carcinoma (HCC), the most common primary malignancy of the liver and one of the leading causes of cancer-related death globally [6]. Early detection is crucial for improving prognosis, yet current surveillance strategies remain suboptimal [7]. While alpha-fetoprotein (AFP) remains the most widely used serum biomarker, its limited sensitivity for early-stage disease has stimulated research into novel molecular markers, including circulating microRNAs and inflammatory mediators [7,8].

This review aims to provide a comprehensive overview of conventional liver biochemical tests and their clinical interpretation, while also examining emerging biomarkers with potential utility in hepatocellular carcinoma detection and surveillance. By integrating traditional laboratory parameters with evolving molecular markers, this review highlights current challenges and future directions in laboratory assessment of liver disease.

2. Conventional Liver Function Tests

The term "liver function tests" is acknowledged as somewhat misleading, as most measured parameters do not directly assess hepatic function but rather indicate hepatocyte injury or cholestasis [9,10]. Patients with advanced liver disease may demonstrate normal results on these tests, necessitating careful clinical correlation [10].

2.1 Aspartate Aminotransferase (AST/SGOT)

Aspartate aminotransferase is an enzyme present in both cytosolic and mitochondrial isoenzymes, catalyzing the transfer of amino groups from aspartic acid to ketoglutaric acid [11]. While AST is found in the highest concentrations within the liver, it is also distributed in cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leukocytes, and erythrocytes [11,12]. This broad tissue distribution renders AST less specific for hepatic injury compared to alanine aminotransferase [12]. The half-life of AST is approximately 17 ± 5 hours [11]. In clinical practice, AST elevation may reflect hepatocellular injury, but extrahepatic sources, including myocardial infarction, rhabdomyolysis, and hemolysis, must be considered [12]. The ratio of AST to ALT provides diagnostic utility, with a ratio exceeding 2:1 characteristic of alcohol-related liver disease, attributable to pyridoxal-5'-phosphate deficiency in patients with alcohol use disorder [12].

2.2 Alanine Aminotransferase (ALT/SGPT)

Alanine aminotransferase is a cytosolic enzyme concentrated predominantly in the liver, catalyzing the transfer of amino groups from alanine to ketoglutaric acid [11]. With a half-life of approximately 47 ± 10 hours, ALT demonstrates greater hepatic specificity than AST, as it is not present in significant quantities in extrahepatic tissues [11,12]. Hepatocellular injury, rather than cell death itself, triggers the release of enzymes into the circulation [11]. Normal reference ranges demonstrate sex-based variation, with true normal

values ranging from 29 to 33 IU/L in men and 19 to 25 IU/L in women—values lower than those reported by many commercial laboratories [12]. ALT levels correlate positively with body mass index, with elevated reference ranges observed in obese individuals [11]. Markedly elevated ALT (exceeding 500 IU/L) typically indicates acute hepatocellular necrosis from viral hepatitis, toxin-induced injury, ischemic hepatitis, or autoimmune hepatitis [12].

2.3 Alkaline Phosphatase (ALP)

Alkaline phosphatase comprises a family of zinc metalloenzymes concentrated in the microvilli of bile canaliculi, with additional distribution in bone, intestine, and placenta [11,13]. Four isoenzymes have been characterized: placental ALP, germ cell ALP, intestinal ALP, and tissue-nonspecific ALP [11]. In healthy non-smoking adults, placental and germ cell isoenzymes constitute less than 1% of total serum ALP activity [11]. Elevated ALP levels primarily indicate cholestasis, whether intrahepatic or extrahepatic in origin [12,13]. Following biliary obstruction, ALP levels increase to at least four times normal within one to two days, persisting for several days after obstruction resolution due to the enzyme's seven-day half-life [12]. Mild-to-moderate ALP elevation (up to three times normal) occurs in various hepatic disorders, including viral hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, drug-induced liver injury, and space-occupying lesions [12]. Physiologic ALP elevation occurs during childhood and adolescence due to bone growth, in pregnancy from placental isoenzyme production, and following fatty meals due to intestinal isoenzyme release [12,13].

2.4 Total and Direct Bilirubin

Bilirubin represents the end product of heme catabolism, with approximately 80% derived from hemoglobin breakdown from senescent erythrocytes [12,14]. Unconjugated (indirect) bilirubin is water-insoluble and transported bound to albumin, precluding urinary excretion [12]. Hepatic conjugation with glucuronic acid produces water-soluble bilirubin diglucuronide (conjugated or direct bilirubin), which is excreted into bile and ultimately metabolized to urobilins in the duodenum [12,14]. Normal total bilirubin measures below 1.2 mg/dL (<20 μmol/L), predominantly unconjugated [12]. Fractionation into conjugated and unconjugated fractions proves most valuable when other liver tests remain normal, suggesting non-hepatic hyperbilirubinemia [12]. Unconjugated hyperbilirubinemia (direct fraction <15%) reflects increased bilirubin production from hemolysis or impaired hepatic uptake/conjugation as in Gilbert syndrome [12,14]. Conjugated hyperbilirubinemia (direct fraction >50%) indicates decreased bile formation or excretion from hepatocellular dysfunction or biliary obstruction [12]. Severe hyperbilirubinemia in primary biliary cholangitis, alcohol-related hepatitis, or acute liver failure portends a poor prognosis [12].

2.5 Gamma-Glutamyl Transferase (GGT)

Gamma-glutamyl transferase is a glycoprotein located on cell membranes with high secretory or absorptive activities, catalyzing the transfer of gamma-glutamyl groups from peptides to amino acids [11]. While GGT is abundant in the kidney, pancreas, intestine, prostate, and other tissues, its absence from bone renders it more specific for hepatobiliary disease compared to ALP [11,13]. In obstructive liver disease, GGT increases approximately 12-fold compared to ALP's 3-fold elevation, demonstrating superior sensitivity [11]. GGT levels correlate with body mass index, with values 50% higher in individuals with BMI exceeding 30 kg/m² due to hepatic steatosis [11]. Clinical applications include confirming the hepatic origin of elevated ALP and detecting alcohol-related liver injury [13]. However, isolated GGT elevations occur frequently and often prove clinically unhelpful, leading some institutions to exclude this test from routine liver panels [10].

2.6 Total Protein

Serum total protein measurement encompasses albumin and globulins, providing insight into the liver's synthetic function and immune status [15]. While not specific for hepatic dysfunction, total protein levels may be decreased in chronic liver disease due to reduced albumin synthesis, or increased in autoimmune hepatitis due to hypergammaglobulinemia [15]. The protein electrophoretic pattern aids in distinguishing various liver disease etiologies.

2.7 Albumin

Albumin is synthesized exclusively by hepatocytes, serving as the primary determinant of plasma oncotic pressure and a transport protein for numerous endogenous and exogenous compounds [11,12]. With a half-life of approximately three weeks, serum albumin reflects hepatic synthetic capacity over a prolonged timeframe [15]. Hypoalbuminemia indicates chronic liver disease with impaired synthetic function, though non-hepatic causes, including protein-losing nephropathy or enteropathy, malnutrition, and chronic inflammation must be considered [12,15]. Normal albumin ranges from 3.5 to 4.5 g/dL (35-45 g/L) [14]. Serial albumin measurements aid in monitoring disease progression and treatment response in chronic liver disease [15].

2.8 Platelets

Thrombocytopenia represents a common hematologic abnormality in chronic liver disease, primarily resulting from portal hypertension-induced hypersplenism and reduced thrombopoietin production [16,17]. Platelet counts below 150,000/μL warrant consideration of chronic liver disease with clinically significant portal hypertension [14,17]. While thrombocytopenia raises concerns regarding bleeding risk, recent evidence demonstrates that platelet count poorly predicts procedural bleeding in patients with cirrhosis [16,17]. In the PROLIVER study of 280 patients with chronic liver disease, 91% of major bleeds were attributable to portal hypertension rather than thrombocytopenia [17]. Current guidelines recommend against routine platelet transfusion for low-risk procedures based solely on platelet count [17].

2.9 Prothrombin Time (PT)

Prothrombin time measures the integrity of the extrinsic coagulation pathway, assessing hepatic synthesis of vitamin K-dependent clotting factors (II, VII, IX, X) [11,15]. Unlike albumin's three-week half-life, factor VII has a half-life of only six hours, rendering PT a sensitive indicator of acute hepatic synthetic dysfunction [15]. Prolonged PT indicates significant hepatocellular injury and correlates with prognosis in acute liver failure [12]. However, in chronic liver disease, PT poorly predicts bleeding risk due to concomitant alterations in procoagulant and anticoagulant factors that may preserve hemostatic balance [17]. The international normalized ratio standardizes PT reporting across laboratories [9]. Progressive PT prolongation in acute or chronic liver disease typically indicates deterioration toward liver failure [9].

3. Hepatocellular Carcinoma Biomarkers

Hepatocellular carcinoma represents the fourth leading cause of cancer mortality worldwide, typically arising in the context of cirrhosis from viral hepatitis, alcohol-related liver disease, or metabolic dysfunction-associated steatotic liver disease [18,19]. Late diagnosis with limited treatment options contributes to poor survival, underscoring the critical need for effective surveillance biomarkers [19,20].

3.1 Alpha-Fetoprotein (AFP)

Alpha-fetoprotein remains the most widely used serum biomarker for HCC surveillance, though its clinical utility remains controversial [19,20]. Current guidelines either do not recommend AFP or consider it optional in conjunction with ultrasound [19]. The principal limitations include suboptimal sensitivity for early-stage HCC and false-positive elevations in patients with viral hepatitis,

cholangiocarcinoma, and other malignancies [19]. A meta-analysis demonstrated that ultrasound alone yields 45% sensitivity for early-stage HCC, increasing to 63% when combined with AFP [19]. Serial AFP monitoring may identify patients at high risk for HCC development up to 15 years before clinical diagnosis, with area under the receiver operating characteristic curve values ranging from 0.73 to 0.83 [19,21]. The GALAD score, incorporating gender, age, AFP-L3 (a lectin-binding glycoform of AFP), AFP, and des-gamma-carboxyprothrombin, achieves AUROC values exceeding 0.88 for HCC detection irrespective of disease stage [19]. In North American validation cohorts, the GALAD score demonstrated an AUROC of 0.95 compared to 0.82 for ultrasound alone [19].

3.2 MicroRNA-122 (miR-122)

MicroRNAs are small non-coding RNA molecules approximately 22-24 nucleotides in length that regulate gene expression through post-transcriptional mechanisms [19,20]. miR-122 is highly specific to liver tissue and constitutes approximately 70% of total hepatic miRNA [20,22]. Serum miR-122 levels correlate strongly with conventional liver enzyme elevations and necroinflammatory activity, reflecting hepatocellular injury [20]. Beyond its role as a liver injury marker, miR-122 demonstrates critical importance in hepatitis C virus replication, serving as an essential host factor for viral infection [20,22]. In HCC, miRNA expression profiles differ according to underlying etiology, with distinct patterns observed in hepatitis B virus-related versus hepatitis C virus-related tumors [20,23]. Monitoring changes in circulating miRNA profiles may provide an earlier warning of neoplastic transformation preceding clinically apparent HCC [20]. While promising, miRNA biomarkers require standardization of detection methods and validation in large prospective cohorts before clinical implementation [19].

3.3 Tumor Necrosis Factor Alpha (TNF-α)

Tumor necrosis factor alpha represents a pro-inflammatory cytokine implicated in hepatocarcinogenesis through activation of nuclear factor-kappa B and downstream pathways promoting cell proliferation and survival [19]. Chronic inflammation characteristic of viral hepatitis and metabolic liver disease creates a microenvironment rich in TNF-α and other inflammatory mediators that may accelerate malignant transformation [19,23]. As an immune mediator biomarker, TNF-α forms part of a broader class of circulating cytokines and chemokines under investigation for early HCC detection [19,24]. The complexity of inflammatory processes in chronic liver disease complicates the interpretation of immune markers, as ongoing hepatitis activity may elevate these mediators independent of tumor presence [19]. Combination panels incorporating multiple inflammatory markers with protein biomarkers and miRNAs may enhance diagnostic accuracy for early-stage HCC [19].

4. Conclusion

Liver function tests provide essential information for screening, diagnosis, prognostication, and monitoring of hepatobiliary disease. Pattern recognition, distinguishing hepatocellular, cholestatic, and mixed injury patterns, guides differential diagnosis and further evaluation. While conventional markers, including aminotransferases, alkaline phosphatase, bilirubin, and synthetic function tests, remain clinical mainstays, understanding their limitations is paramount. Platelet count and prothrombin time, though frequently abnormal in liver disease, poorly predict bleeding risk and should not trigger routine prophylactic transfusions. For hepatocellular carcinoma surveillance, alpha-fetoprotein demonstrates modest performance that improves when combined with ultrasound or incorporated into multi-marker panels. Emerging biomarkers, including microRNA-122 and inflammatory

mediators, show promise for early HCC detection but require rigorous validation before integration into clinical practice. Future directions include refined biomarker panels and algorithmic approaches that integrate laboratory parameters with clinical and imaging data to optimize patient outcomes.

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