REVIEW ARTICLE

REVIEWING THE USEFULNESS OF LABORATORY TESTS RESULTS IN HEPATITIS C

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ABSTRACT
Hepatitis C virus (HCV) infection is a common cause of chronic liver disease and is relatively frequent in certain populations. Liver disease progresses so slowly that a person can have hepatitis C for years without being feeling sick or having specific symptoms that would lead a clinician to suspect liver disease and diagnosis for hepatitis C often occurs only after they are found to have abnormal liver enzymes during routine blood workup or tested because of specific risk factors. Blood tests that assess liver function are frequently used to evaluate and treat patients with symptomatic and asymptomatic liver diseases. We scanned the literature in an effort to identify biochemical markers that may be helpful in the evaluation and management of chronic hepatitis C disease. This article discusses the utility and limitations of laboratory tests results in establishing the diagnosis and prognosis in HCV infected patients.

Key Words: Hepatitis C, Laboratory Tests results, Clinical usefulness, Predictive value.

INTRODUCTION TO BLOOD TESTS – BIOMARKERS OF LIVER FUNCTION

The liver is a vital organ and has a wide range of general functions. It is often called both the body's manufacturing unit and its filtration plant. Recognizing the different patterns of liver injury can be used as a guide to direct further evaluation of diseases that affect the liver. Evaluation of liver disease in hepatitis C is based only on the results of laboratory blood tests.[1-3]

Liver function tests (LFTs), are groups of clinical chemistry laboratory blood assays designed to give information about the state of a patient's liver. Some tests are associated with functionality (albumin and Prothrombin time); some with cellular integrity (transaminases – ALT and AST) and some with conditions linked to the biliary tract (Alkaline phosphatase and gamma – glutamyl transpeptidase).[4-7] These biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. These tests can be used to (1) detect the presence of liver disease, (2) distinguish among different types of liver disorders, (3) gauge the extent of known liver damage, and (4) follow the response to treatment.[4,7-9]

Some of the standard or routine blood tests that are used to check "liver function" are in reality only able to detect liver damage. These tests may not be sensitive enough to accurately reflect whether liver is functioning at its optimum level. These tests will usually be abnormal in significant liver disease or liver distress; however, they can still give normal readings in some cases of mild liver disease.[4,7-9]

The blood tests are objective, simple to perform, and easy to repeat, and therefore, may be useful to evaluate progression and prediction of patient outcome.[4,8,9] A number of biochemical tests are available that reflect the condition of the liver.[4,8,9] These tests may be normal in patients who have advanced liver disease. In addition, abnormal values can be caused by diseases unrelated to the liver.[7-12]

Regardless of the cause, all types of viral hepatitis affect liver cells. This accounts for the fact that many signs and symptoms for the various types are similar and not specific to the causative agent.[1,2,5] Diagnosing the specific agent responsible for viral hepatitis is not possible clinically and a definite diagnosis of viral hepatitis is only achievable by the use of viral – specific hepatitis markers.[3,13-17] The use of virologic markers has become essential in the management of HCV infection to diagnose infection, guide treatment decisions, and assess the virologic response to antiviral therapy.[3,13,18]

BLOOD TESTS USED TO DIAGNOSE HCV INFECTION

1. **Anti-HCV antibody detection** (Enzyme Immunoassays – Indirect tests) – markers of past or present HCV infection. In persons suspected to have hepatitis C should be tested for anti-HCV as an initial screening test. Anti-HCV test detects the presence of antibodies to the virus,

indicating exposure to HCV. This test cannot distinguish between someone with an active or a past HCV infection. Anti-HCV is detected by immunochromatographic test (ICT) device[19-22] or enzyme immunoassay (EIA) / enzyme linked-immunosorbent assays (ELISA).[23-27] The third – generation technique (EIA – 3) used today is more sensitive and specific than previous ones.[24] Usually, the test is reported as "positive" or "negative." A positive anti-HCV test may require additional or confirmatory testing especially when the anti-HCV test is "weakly positive."[13,16,17] According to the Centers for Disease Control and Prevention (CDC), a person can only be diagnosed with hepatitis C after a positive result for an anti-HCV screening test has been verified.[18]

2. **Confirmatory HCV assays** for the presence of hepatitis C infection are those tests that determine the presence of hepatitis C viral particles (HCV – RNA) in the blood.[13-17] A positive HCV – RNA in the serum confirms the diagnosis of active hepatitis C. This type of viral testing may be either qualitative or quantitative.[14,15]

Qualitative HCV RNA: The best approach to confirm the diagnosis of hepatitis C is to test for HCV – RNA using a sensitive assay such as polymerase chain reaction (PCR) or transcription – mediated amplification (TMA). [13-17,22-29] Results are reported as "positive" or "detected" if any HCV viral RNA is found; otherwise, the report will be "negative" or not detected. The presence of HCV – RNA in serum indicates an active infection and may be used to distinguish between a current or past infection. PCR and TMA amplification can detect low levels of HCV – RNA in serum.[22,24] In addition, testing for HCV – RNA is particularly useful when aminotransferase levels are normal or only slightly elevated, when anti-HCV is not present, or when several causes of liver disease are possible.[5-6,13,16]

Quantitative HCV – RNA test (HCV Viral Load) detects and measures the number of viral RNA particles in the blood. This powerful test allows clinicians to see, among other things, how an individual is responding to treatment.[36] Some newer viral load tests can detect very low amounts of viral RNA. Quantitative viral load testing should not be repeated frequently as it adds little to the care of the untreated patient other than increased expense and anxiety. These tests, however, should be followed serially in someone undergoing anti-viral therapy, as the goal of therapy is the loss of detectable serum HCV – RNA.[13,14,36,39]

HCV Recombinant Immunoblot Assays (RIBA) – also called "Western blots" are highly specific and valuable in verifying anti-HCV reactivity, although direct testing for HCV – RNA has largely replaced them as a means of confirming HCV infection.[15,17,37-39] It is of limited clinical utility because like other EIA antibody tests, it can not distinguish between an active or previous infection.[37,39] The NIH consensus conference on hepatitis C infection has recommended that it may be used as a confirmatory test in patients without known risk factors who test positive for the EIA anti-HCV to eliminate the possibility of a false positive EIA.[40]

Summary of Serological and Molecular Assays for HCV infection.[16-19]

Anti-HCV (EIA)	*HCV(RIBA)*	*HCV – RNA (Qualitative)*	*HCV Infection*
Negative	Not done	Not done	No infection
Positive	Not done	Not done	Indicates infection; Verify, more testing is required.
Positive	Negative	Not done	No infection; likely a false positive
Positive	Not done	Negative	No active infection; likely a false positive
Positive	Positive	Negative	Indicates past infection or low HCV viral load
Positive or Weak or Indeterminate	Not done or Positive	Positive	Indicates active HCV infection.

Key points

1. Liver tests are a group of laboratory blood assays designed to give information about the state of the individual's liver.
2. Abnormal liver tests may present in an asymptomatic individual.
3. Liver tests often become abnormal in non-hepatic disorders.
4. The commonly used liver tests primarily assess liver injury rather than liver function.
5. Liver tests can not be interpreted without clinical information (a good clinical history and physical examination are often rewarding).

Genotyping of HCV: Testing for HCV genotype is often clinically helpful and is used to determine the kind (genotype – specific antibodies) of the HCV virus present. There are 6 known genotypes and more than 50 subtypes of hepatitis C.[5,6,,32,33,39] Knowing the genotype or serotype of HCV is helpful in making recommendations and counselling regarding therapy and significantly reduces the duration of treatment. Patients with genotypes 2 and 3 are almost three times more likely to respond to antiviral therapy.[5,6,36,41] Once the genotype is identified, it need not be tested again; genotypes do not change during the course of infection.[5,6,39] HCV genotype is identified by using a laboratory test called RT – PCR (Reverse transcription – Polymerase Chain Reaction).[33,35]

BLOOD TESTS USED TO EVALUATE LIVER FUNCTION

Liver Function Tests generally refer to a group of blood tests that help to evaluate and monitor liver disease or damage in chronic hepatitis C and other liver diseases. The commonly used liver function tests (LFTs) primarily assess liver injury rather than hepatic function. The most common tests used in clinical practice include the serum aminotransferases, bilirubin, alkaline phosphatase, gamma-glutamyl transpeptidase, albumin, prothrombin time and INR.[4,7-9]

1. **Markers of Hepatocellular Injury:** As a biochemical marker of liver cell injury, aminotransferases are the most sensitive and widely used liver enzymes and include alanine aminotransferase (ALT) and aspartate aminotransferase (AST).[4,7,8] ALT is largely concentrated in the liver and consequently serves as a fairly specific indicator of current liver status and the most important indicator of HCV activity.[4,7] The degree of enzyme elevation may be important in acute phase of the disease but of limited importance in chronic liver disease.[4,7,9-12] The most common causes of elevated aminotransferases are viral hepatitis, drug induced hepatitis, autoimmune hepatitis, fatty liver and alcoholic liver disease.[4,7,9] These tests are a reflection of liver cell injury and death but are not liver function tests.[7] Therefore, an abnormality in these tests does not mean that the liver is not functioning. In fact, the vast majority of patients with elevated aminotransferases, regardless of degree, have normal liver function.[8-13]
2. **Markers of Cholestasis:** Alkaline phosphatase (ALP) and gamma – glutamyl transpeptidase (GGT) are more specific for biliary disease since they are made in the bile duct cells. Typically the blood levels of these two enzymes are often very high in people with cholestasis.[4,7-9] The main clinical utility of GGT is to exclude a bone source of ALP elevation.[4,9] Cholestatic liver conditions that commonly lead to the elevation of these enzymes include choelithiasis (gallstone disease), bile duct tumors, primary biliary cirrhosis, biliary atresia and primary sclerasing cholangitis. Liver diseases, such as cirrhosis, infections, and viral hepatitis are other causes of cholestasis.[4,7,9]
3. **Indicators of the Liver Functions:** Bilirubin is both a precursor and a product of liver metabolism. It is produced during the breakdown of hemoglobin and is formed from the breakdown of heme. A blood test that measures the level of bilirubin indicates the severity of the liver disease but not its cause.[7-9] Bilirubin may be elevated in conditions which lead to liver cell damage and cholestasis, such as hemolysis, hepatitis, drugs, chemotherapy, biliary stricture, neonatal hyperbilirubinaemia and Gilbert's syndrome. The level of serum bilirubin is not a sensitive indicator of liver dysfunction because it does not accurately reflect the extent of liver damage.[6-7]

Albumin and blood clotting factors are proteins synthesized in the liver. Blood tests such as the serum albumin level and prothrombin time (PT) are measures of these proteins. As these tests evaluate the functional integrity of the liver, they can be correctly called “liver function tests”. However, neither test is specific for liver disease. Albumin levels and prothrombin time are normal until late – stage disease. Abnormalities of these tests are of concern and are indicative of extensive liver damage.[6,36,41]

The most common laboratory abnormality seen in chronic hepatitis C infection is an isolated, elevated ALT activity. ALT is released into the blood stream as the result of liver cell injury. It therefore serves as a fairly specific indicator of liver status but serum ALT elevation does not correlate well with histological stage of the disease and may be normal in any stage of chronic hepatitis C.[6-12] Therefore, patients with minimal ALT elevations should be evaluated for the presence of chronic hepatitis.[4,7,10] In advanced disease, an elevation in serum alkaline phosphatase and total bilirubin level as well as thrombocytopenia may be seen.[7,9] ALT is often used to monitor the treatment of persons who have liver disease, to see if the treatment is working, and may be ordered either alone or along with other tests for this purpose.[4,7-9,13]

Several **other tests** are frequently obtained in patients with chronic hepatitis C.[4,6,36,41]

Lactate dehydrogenase (LDH) is an enzyme found in many body tissues, including the liver.

Elevated levels of LDH may indicate liver damage.[4,7]

Serum alpha – fetoprotein (AFP) is a biochemical marker of liver cancer but it may be mildly elevated in patients with chronic hepatitis C in the absence of liver cancer. If it is elevated, this test should be followed closely.[5,6,42-45]

Autoimmune markers may be present in as many as one quarter of patients with hepatitis C without the presence of autoimmune disease. These markers include an anti-nuclear antibody, smooth muscle antibody, anti-mitochondrial antibody or antithyroid antibodies. Patients in whom autoimmune disease is suspected should be adequately evaluated before the presence of auto-antibodies is attributed to HCV infection.[5,6,46,47]

Liver Biopsy: The liver biopsy has been widely regarded as the "gold standard" for defining the liver disease status. However, it is not necessary for diagnosis but is helpful for grading the severity of disease and staging the degree of fibrosis.[5,6,9,41-43,48]

Liver fibrosis can also be estimated by means of a number of commercial blood tests: 1- FIBROSpect II (Prometheus Laboratories), 2- HepaScore (Quest Diagnostics) and 3 – HCV FIBROSURE (LabCorp). In general, it is contended that these tests are accurate in determining the presence or absence of early (stage – 1) or advanced (stage – 4) fibrosis. However, these tests are less accurate when it comes to differentiating patients with moderate fibrosis.[5,36]

INTERPRETATION OF LIVER TESTS RESULTS

The approach to biochemical liver (function) tests may be challenging even to the experienced clinician. A number of pitfalls can be encountered in the interpretation of common blood liver function tests. These tests can be normal in patients with chronic hepatitis or cirrhosis. While these tests (LFTs) are commonly used to reflect how well the liver is working, this name can be misleading, as it is not possible for any blood test to accurately assess all of the liver's varied functions. Anyhow, laboratory test results can provide useful values to predict the progression of liver disease, but they generally cannot be interpreted without clinical information. LFTs when used in conjunction with other blood tests, give the clinician a better idea of what is wrong with the liver and how well the liver is working. All of these tests can be performed at the same time and should include a liver panel, complete blood count (CBC), prothrombin time PT), and international normalized ratio (INR).[4-6,13,36,41,43]

AST, ALT and alkaline phosphatase tests are most useful to make the distinction between hepatocellular and cholestatic liver disease. Testing for ALT and AST levels is an important component in orienting the diagnostic evaluation and assessing a patient with chronic hepatitis C. Although the serum aminotransferase level correlates poorly with liver histology but AST: ALT ratio of ≥ 1 (although specific for the presence of cirrhosis) had only a modest positive predictive value and should not be the sole determinant to identify cirrhosis.[36,39,41,43,48] Standard liver tests are of limited value in assessing the degree of fibrosis. In practice, a combination of factors is used to anticipate liver histology.[8-9,36,41-43] Increased INR and thrombocytopenia is also seen more frequently in cirrhosis.[5,6,36,41,43] However, liver biopsy remains the only definitive test for evaluation of fibrosis.[42,43,48] Commercially available blood markers used for predicting liver fibrosis have limited clinical utility and currently, evidence based data do not support their use.[5,36]

Additional laboratory studies that are useful include AFP level, HCV genotype and RNA level. The AFP is widely used in screening of hepatocellular carcinoma, while HCV genotype and RNA level can later guide the treatment process.[3,36,39,41-43] Three virologic markers are clinically useful: (1) anti-HCV antibodies, markers of past or present HCV infection, (2) HCV – RNA, a direct marker of HCV replication, and (3) HCV genotype, an intrinsic characteristic of HCV strains. Molecular or immunoassays are used in the diagnosis and management of hepatitis C but have no role in the assessment of disease severity or prognosis.[38,41,43] Thus, qualitative HCV – RNA testing is used for diagnosis while quantitative testing should be reserved for use during treatment.[13,14,35]

It is *concluded* that laboratory studies play a central role in assessing a patient and orienting the diagnostic evaluation but all tests of liver injury are neither highly sensitive nor specific. However, liver tests (LFTs) when used in conjunction with additional blood tests, give the "Clinician" a better idea of what is wrong with the liver and how well the liver is working. By keeping track of the results from the laboratory tests over a period of time ahead, the "Clinician" may have an idea whether the liver condition has stabilized, improved, resolved or worsened; whether a specific treatment is working, or if something different needs to be tried and whether it is time to refer the patient for further evaluation.

For monitoring HCV infection and the efficacy of therapy, a single normal value does not rule out active infection, progressive liver disease, or even cirrhosis. Therefore, frequent retesting is recommended to assess progression or regression.

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