Laboratory evaluation of the patient with liver disease

M. ASTEGIANO, N. SAPONE, B. DEMARCHI, S. ROSSETTI, R. BONARDI, M. RIZZETTO

Molinette Hospital, SC Gastrohepatology DU - Turin (Italy)

Abstract. – Because the liver performs multiple functions, no single laboratory test or battery of tests is sufficient to provide a complete estimate of the function of the liver in every clinical situation. A broad array of biochemical tests are used to assess the many functions of the liver and to evaluate patients with suspected or established liver disease. These tests are referred to collectively as "liver function tests" (LFTs).

LFTs are used to screen people for the presence of liver disease, suggest the underlying cause, estimate the severity, assess prognosis, and monitor the efficacy of therapy. Abnormal LFTs may be the first indication of sub clinical liver disease and may thereby guide further diagnostic evaluation. After the existence of hepatic dysfunction is recognized, the specific pattern of liver test abnormalities may suggest the category of the underlying liver disease, such hepatitis, biliary obstructions, or infiltrative liver disease.

The value of screening healthy, asymptomatic persons for liver disease with the use of LFTs is controversial and may not be cost-effective. If screening is performed a panel of tests (e.g., AST, alkaline phosphatase, bilirubin, albumin) is preferable to using a single test because of superior sensitivity and specificity for liver disease and lower cost than the sum of individually performed tests.

Key Words:

Liver, Function test, AST.

Introduction

Because the liver performs multiple functions, no single laboratory test or battery of tests is sufficient to provide a complete estimate of the function of the liver in every clinical situation. A broad array of biochemical tests are used to assess the many functions of the liver and to evaluate patients with suspected or established liver disease. These tests are referred to collectively as "liver function tests" (LFTs).

LFTs are used to screen people for the presence of liver disease, suggest the underlying cause, estimate the severity, assess prognosis, and monitor the efficacy of therapy. Abnormal LFTs may be the first indication of sub clinical liver disease and may thereby guide further diagnostic evaluation[1] (Figure 1). After the existence of hepatic dysfunction is recognized, the specific pattern of liver test abnormalities may suggest the category of the underlying liver disease, such hepatitis, biliary obstructions, or infiltrative liver disease. Additional testing may permit an assessment of the severity of liver dysfunction, and certain tests, especially when performed repeatedly, may provide an estimate of prognosis. Finally, sequential measurements of LFTs may be useful in assessing response to therapy, as in the case of declining serum aminotransferase levels after therapy for chronic viral hepatitis.

There are limitations to the use of LFTs in the diagnosis and management of liver disease. There are limitation in sensitivity and specificity. LFT abnormalities rarely provide a specific diagnosis but rather suggest a general category of the liver disorder.

In approaching patients with liver disease, it is helpful to classify laboratory tests into several broad categories. These include tests (1) that reflect injury to hepatocytes or bile ducts, such as serum aminotransferase levels (hepatocellular damage) and alkaline phosphatase levels (cholestasis); (2) that measure the capacity of the liver to transport organic anions and clear endogenous or exogenous substances from the circulation, such as serum bilirubin and bile acid levels; (3) that measure
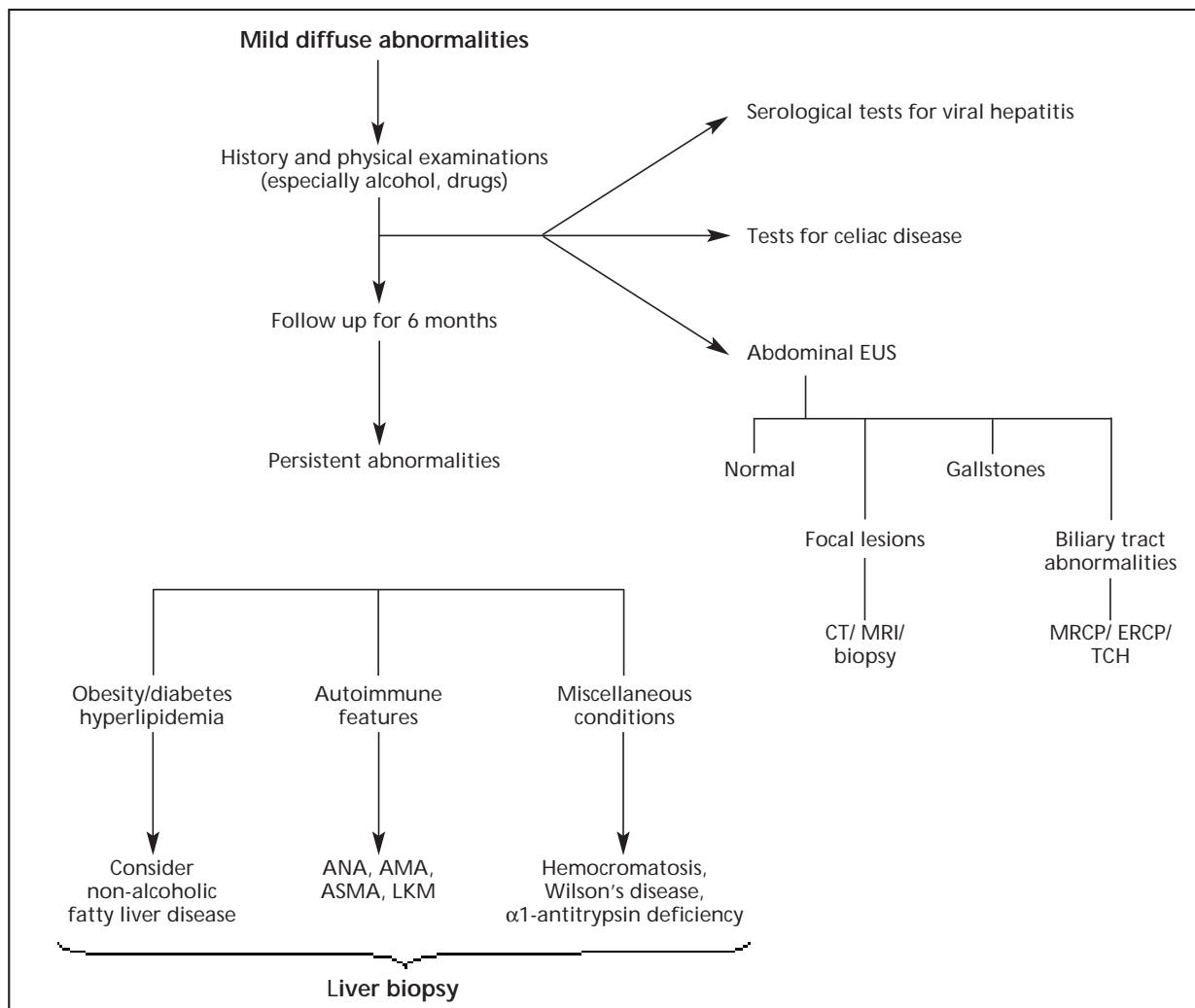
Figure 1.

the capacity of the liver to metabolise drugs; (4) that measure hepatic synthetic function, such as levels of serum albumin and coagulation factors; and (5) that contribute to accurate diagnosis in liver disease but do not necessarily assess liver function, including levels of immunoglobulin, specify autoantibodies, and serologic tests for viral hepatitis.

General screening of the population for chronic hepatic injury is not cost-effective and should be limited to high risk individuals. These include those with a family of genetic disease known to affect the liver or risk factors for chronic viral infection (Table I).

- Screening for chronic hepatitis is recommended in asymptomatic high-risk individuals

- ALT is the most cost-effective screening test for metabolic or drug induced liver injury; AST should also be measured with history of alcohol abuse
- Specific viral serologies (HBsAg and anti-HCV), as well as ALT, should be used in individuals at high risk for viral hepatitis
- Confirmation of chronic HCV infection in an anti-HCV-positive individuals should be made by HCV-RNA tests; if negative and ALT is increased, HCV-RNA should be repeated

In patients with increased ALT, negative viral markers, and a negative history for drug or alcohol ingestion, the workup should include less common causes of chronic hepatic injury

Table I. Risk factors for chronic viral hepatitis.

Establishing risk factors
• Injection drug use
• Chronic hemodialysis
• Blood transfusion or transplantation prior to 1992 (HCV)
• Receipt of blood (including needlestick) from a donor subsequently testing positive for HCV
• Receipt of clotting factor concentrates produced before 1987
• Asian ancestry (HBV)
• Unvaccinated healthcare workers (HBV)
• Birth to mother with chronic HBV or HCV
Possible risk factors
• Body piercing or tattooing
• Multiple sexual partners or sexually transmitted diseases
• Healthcare workers (HCV)
• Contacts of HCV positive persons

• Initial evaluation should include a detailed drug history along with measurement of HBsAg and anti-HCV. If anti-HCV is positive, chronic infection should be confirmed by qualitative HCV-RNA measurement

• With persistently increased ALT and negative viral markers, the workup should include anti-nuclear antibodies (ANAs) and iron and iron-binding capacity (or unsaturated iron-binding capacity)

• In patients under age 40, ceruloplasmin should also be measured

• In patients negative for these markers, α_1-antitrypsin phenotype may be usefuls

• NASH is the most common cause of chronic hepatic injury other than viruses and alcohol and the most common cause of cryptogenic cirrhosis

• Although NASH occurs most commonly in middle-aged women with obesity and/or diabetes, it also occurs in men and in patients without these risk factors

• If these tests are negative or inconclusive, diagnostic liver biopsy should be performed

Tests that Reflect Hepatobiliary Injury

Aminotransferase

The aminotransferase – aspartate aminotransferase (AST) and alanine aminotransferase (ALT) – are the most frequently used indicators of hepatic injury and represent markers of hepatocellular necrosis.

It is important to note that ALT is relatively liver-specific, whereas AST is found in skeletal and cardiac muscle, kidney, brain, pancreas, and blood cells, in addition to hepatocytes.

Serum levels of AST and ALT are elevated to some extent in almost all liver disease. The highest elevations occur in severe viral hepatitis, drug- or toxin-induced hepatic necrosis, and circulatory shock (ischemic hepatitis)[2].

Although enzyme levels may reflect the extent of hepatocellular necrosis, they do not correlate with eventual outcome. Moderately elevated levels of serum aminotransferase (threefold to twenty fold) are typical of acute or chronic hepatitis including viral hepatitis and autoimmune, drug-induced, and alcoholic hepatitis, whereas mild elevation (less than threefold) are seen in fatty liver, non-alcoholic steatohepatitis, drug toxicity, and chronic hepatitis C[3].

Acute biliary tract obstruction may result in elevation of AST and ALT of greater than 300 U/l; these levels peak early and decline rapidly over 24 to 72 hours despite unresolved obstruction. Determinations of serum aminotransferase have proved useful as screening tests for sub clinical liver disease in asymptomatic person[4] and an abnormal result may lead to a diagnosis of hemocromatosis, Wilson disease, or α_1-antitrypsin deficiency and non-hepatic disease, such as coeliac disease, Addison's disease, and anorexia nervosa. Elevated serum aminotransferase levels have been found in as many as 40 percent of adult patients[5] and 54 percent of children[6] with coeliac disease.

The ratio of AST to ALT in serum may be helpful diagnostically. An AST to ALT ratio of more than 2.0 is characteristically observed in alcoholic liver disease. In viral hepatitis the ratio of AST to ALT is typically less than 1.0.

If autoimmune hepatitis is a possibility, testing for anti-nuclear and anti-smooth muscle antibodies and serum globulins should be performed. In a recent study from Spain[7] a serologic evaluation failed to reveal a cause in 10 percent of patients with elevated serum aminotransferase levels. Liver biopsy in these 10 percent disclosed chronic hepatitis or cir-

rhosis (often attributable to occult viral infection) in approximately 50 percent of patients and NAFLD or non-specific changes in the remainder.

Alkaline Phosphatase

In the human body alkaline phosphatase has been identified in liver, bone, intestine, placenta, kidney, and leukocytes. In patients with an elevated level of serum alkaline phosphatase, the source is the liver in a majority of cases; but in up to one third of such individuals, no evidence of liver disease can be found.

The highest elevations of serum alkaline phosphatase in patients with liver disease occur in cholestatic disorders. Elevations occur as a result of both intrahepatic and extra hepatic obstruction to bile flow, and the degree of elevation does not help to distinguish the two.

Elevated serum levels of alkaline phosphatase of hepatic origin also may result from other infiltrative liver disease, such as abscesses, granulomatous liver disease, and amyloidosis.

Low levels of serum alkaline phosphatase may occur in hypothyroidism, pernicious anemia, zinc deficiency, and congenital hypophosphatasia.

γ-Glutamyl Transpeptidase

In liver disease γ-glutamyl transpeptidase activity in serum correlates well with serum alkaline phosphatase levels. Serum γ-glutamyl transpeptidase is a sensitive test for hepatobiliary disease and is the most sensitive indicator of biliary tract disease. Rarely the serum γ-glutamyl transpeptidase level is normal in intrahepatic cholestasis.

An elevated γ-glutamyl transpeptidase level may be caused by systemic inflammatory disorders, chronic alcoholism, liver disease, renal disease, obesity, use of enzyme inducers (such as alcohol, phenytoin, phenobarbital, or rifampin), diabetes mellitus, chronic obstructive pulmonary disease, myocardial infarction, and pancreatic disease. The rationale for the execution of liver biopsy is that serum γ-glutamyl transpeptidase is considered one of the most sensitive tests for detecting hepatobiliary disease, and thus persistent and significant elevation of γ-glutamyl transpeptidase may be the only reflection of NAFLD, alcoholic liver disease, chronic hepatitis, or cirrhosis and may have important implications

for patient management. Additionally, a liver biopsy may simply confirm fatty liver or disclose otherwise unsuspected disorders such as amyloidosis, sarcoidosis, oral-antitrypsin deficiency.

If the liver biopsy shows granulomas or only non-specific changes (and in some cases even when it is normal), serologic tests for granulomatous disorders should be obtained, including antibodies to coccidioidomycosis, histoplasmosis, brucellosis, and cryptococcosis; a PPD should be placed. Nevertheless, many patients with granulomas in the liver may lack a specific diagnosis, even after intensive diagnostic investigation.

Other Enzymes

Lactate dehydrogenase (LDH) is often included in liver biochemistry panels but has poor diagnostic specificity for liver disease. Markedly increased levels of LDH in serum may be seen in hepatocellular necrosis, shock liver, cancer, or haemolysis associated with liver disease. The ratio of ALT to LDH has been reported to distinguish acute viral hepatitis (> 1.5) from shock liver and acetaminophen toxicity (< 1.5) with a sensitivity of 94 percent and specificity of 84 percent[8].

Tests that Measure the Capacity of the Liver to Transport Organic Anions

Bilirubin

Measurement of bilirubin levels in serum is important in the assessment of hepatic function.

Hyperbilirubinemia is classified as either predominantly unconjugated or predominantly conjugated. Levels between 1.2 and 5 mg/dl, representing unconjugated hyperbilirubinemia, may result from increased production of bilirubin, impaired transport of bilirubin into hepatocytes, or defective bilirubin conjugation in hepatocytes.

Serum bilirubin levels higher than 5 mg/dl or bilirubin levels between 1.2 and 5 mg/dl in association with other LFT abnormalities usually signify the presence of liver disease. In these situations at least 50 percent of the serum bilirubin is conjugated. Conjugated hyperbilirubinemia results from impaired intrahepatic excretion of bilirubin or extra hepatic

obstruction. Extreme hyperbilirubinemia with levels above 35 mg/dl usually signifies severe parenchymal liver disease in association with haemolysis, as in sickle cell anemia, or renal failure. The serum bilirubin level has prognostic value in chronic liver disease particularly primary biliary cirrhosis and other cholestatic liver disease[9], and in hepatic failure[10], in which deep jaundice is associated with increased mortality.

If the bilirubin is predominantly unconjugated, the concomitant presence of anaemia should lead to a thorough investigation for haemolysis, generally performed by examining the peripheral blood smear, obtaining a reticulocyte count, determining the levels of lactate dehydrogenase isoenzymes and aptoglobin, and performing an anti-globulin (Coombs') test.

If the initial fractionation of the serum bilirubin reveals predominantly conjugated hyperbilirubinemia, further evaluation for liver disease, including a liver biopsy, may eventually have to be considered to clarify the diagnosis. The diagnosis of the Dubin-Johnson or Rotor syndromes, which are characterized by an excretory defect of bilirubin, may be considered in this situation. Usually, Dubin-Johnson syndrome is associated with the presence of an abnormal pigment in hepatocytes on the liver biopsy. In contrast, Rotor's syndrome is characterized by a normal liver biopsy, without pigmentation.

Serum Bile Acids

Although several studies suggest that measurement of the serum bile acid concentration is a sensitive marker of liver disease, there is little evidence that its determination offers any advantage over conventional biochemical tests for early detection of liver disease. Moreover, the utility of serum bile acid measurements in assessing prognosis in acute or chronic liver disease is uncertain and such measurements are not widely used.

Tests that Measure the Capacity of the Liver to Metabolize Drugs

Hepatic function may be assessed with substances that are metabolised selectively by the liver. The most widely performed tests as-

sess hepatic drug metabolism, such as the determination of plasma clearance of antipyrine and the ^{14}C-aminopyrine breath test. Additional tests include determination of caffeine clearance, galactose elimination, the maximum rate of synthesis of urea, and the formation of metabolites of lidocaine[11].

Tests that Measure Hepatic Synthetic Function

The liver synthesizes and releases a variety of proteins, including albumin, coagulation factors, and lipoproteins. These proteins have variable rates of synthesis, release, and degradation. Abnormal serum levels of other proteins such as immunoglobulins, which are not produced by the liver, reflect impairment of the ability of the liver to filter portal venous blood or anatomic shunting of blood around the liver and also provide information about liver function.

Albumin

Albumin is quantitatively the most important protein in plasma synthesized by the liver and is a useful indicator of hepatic function.

Moreover, the serum albumin level at a single point in time may not reflect synthesis because the turnover of albumin can be affected by disturbances in distribution, catabolism, and synthesis. Albumin synthesis is affected not only by liver disease but also by nutritional status, hormonal balance, and osmotic pressure.

Serum albumin levels are typically depressed in patients with cirrhosis and ascites. In patients with or without ascites the serum albumin level correlates with prognosis. Other non hepatic causes of hypoalbuminemia are nephritic syndrome, protein losing enteropathy, and burns.

Prothrombin Time and Serum Coagulation Factor Levels

The liver synthesizes coagulation factors I (fibrinogen), II (prothrombin), V, VII, IX and X.

In acute or chronic hepatocellular disease the prothrombin time may serve as useful prognostic indicator. In acute hepatocellular

disease a markedly prolonged and worsening prothrombin time suggests an increased likelihood of acute hepatic failure.

The differential diagnosis of an elevated INR includes vitamin K deficiency (caused by malnutrition, malabsorption, or antibiotic use) warfarin administration (which interferes with the vitamin K-dependent gamma-carboxilation), consumptive coagulopathy (e.g., disseminated intravascular coagulation [DIC], rare congenital deficiencies and liver disease.

Because of the short half-life of some of the coagulation factors measured by the INR, changes in the INR and in factor VII levels in particular are useful in monitoring hepatic synthetic function in patients with acute liver disease.

Abnormally high levels of des-γ-carboxy prothrombin have been found in plasma in up to 90 percent of patients with biopsy-proven hepatocellular carcinoma[12].

As a marker for hepatocellular carcinoma, the plasma level of des-γ-carboxy prothrombin does not correlate with levels of alpha fetoprotein. But the two tests are complementary with a combined sensitivity of up to 85 percent in patients with documented hepatocellular carcinoma and a greater specificity for des-γ-carboxy prothrombin than for alpha fetoprotein[12].

Miscellaneous Tests

A large number of diagnostic tests are available for the specific diagnosis of liver disease, these include specific serologic tests for hepatitis viruses; an array of autoantibodies useful in the diagnosis of primary biliary cirrhosis and the classification of autoimmune hepatitis; a variety of protein such as ceruloplasmin, ferritin, α_1 antitrypsin, and α fetoprotein, abnormal levels of which are associated with specific disease such as Wilson's disease, hemochromatosis, α_1 antitrypsin deficiency, and hepatocellular carcinoma, respectively; and serum ammonia, elevation of which is associated with hepatic encephalopathy and urea cycle enzyme deficiencies.

Liver Biopsy

Liver biopsy continues to have a central role in the evaluation of patients with suspected liver disease[13]. Definitive diagnosis often depends on liver biopsy, and much

knowledge regarding characteristic features and the natural history of liver diseases is based on information obtained by serial liver biopsies. Determination of the precise nature of hepatic damage (e.g., necrosis, inflammation, fibrosis) can only be made by liver biopsy, which has assumed increasing importance with regard to staging and prognosis since specific drug therapies for liver disease have been introduced. Unfortunately, this procedure is invasive, prone to complications and has a high risk of sampling error. Biochemical markers for liver fibrosis (FibroTest) and necroinflammatory features (ActiTest) are an alternative to liver biopsy in patients with chronic hepatitis C. The test combine five components (α_2-macroglobulin, haptoglobin, apolipoprotein A1, γ-glutamil transpeptidase, and total bilirubin) for FibroTest and same plus alanine aminotransferase (ALT) for ActiTest[14].

Because of their predictive values and their reproducibility in different populations, biochemical markers could be used as surrogate markers for liver biopsy both for the initial decision of liver biopsy and for the follow-up of chronic hepatitis C.

It is therefore possible that biochemical markers may provide a more accurate (quantitative and reproducible) picture of fibrogenic events occurring within the liver. Furthermore, and because treatment is now so effective in patients with genotype 2 or 3 infection, the utility of biopsy in this setting could be challenged.

Assessing Prognosis

The biochemical tests of liver disease, especially the markers of hepatic synthetic function and bilirubin are useful for predicting prognosis in certain settings. For examples prolongation of the INR and elevation of the serum bilirubin level are associated with poor prognosis in nonacetaminophen-related acute hepatic failure. Both tests are also predictive of early mortality from severe alcoholic hepatitis and are thus useful in deciding whether glucocorticoid therapy is likely to be beneficial. Prognosis in PBC correlates directly with the serum bilirubin level, and a composite score derived from the serum bilirubin, INR, serum albumin, patient's age, and severity of fluid retention is useful in assessing prognosis.

Conclusions

Because the liver performs multiple functions, no single laboratory test or battery of tests is sufficient to provide a complete estimate of the function of the liver in every clinical situation. A broad array of biochemical tests are used to assess the many functions of the liver and to evaluate patients with suspected or established liver disease. These tests are referred to collectively as "liver function tests" (LFTs).

LFTs are used to screen people for the presence of liver disease, suggest the underlying cause, estimate the severity, assess prognosis, and monitor the efficacy of therapy. Abnormal LFTs may be the first indication of sub clinical liver disease and may thereby guide further diagnostic evaluation. After the existence of hepatic dysfunction is recognized, the specific pattern of liver test abnormalities may suggest the category of the underlying liver disease, such hepatitis, biliary obstructions, or infiltrative liver disease. Determination of the precise nature of hepatic damage (e.g., necrosis, inflammation, fibrosis) can only be made by liver biopsy, which has assumed increasing importance with regard to staging and prognosis since specific drug therapies for liver disease have been introduced.

References

1) Pratt DS, Kaplan MM. Evaluation of abnormal liver function: principles and practice. J Gastroenterol Hepatol 1990; 5: 468.

2) Johnson RD, O'Connor ML, Kerr RM. Extreme serum elevations of aspartate aminotransferase. Am J Gastroenterol 1995; 90: 1244.

3) Hay JE, Czaja AJ, Rakela J, et al. The nature of unexplained chronic aminotransferase elevations of a mild to moderate degree in asymptomatic patients. Hepatology 1989; 9: 193.

4) Goddard CJ, Warnes TW. Raised liver enzymes in asymptomatic patients: investigation and outcome. Dig Dis 1992; 10: 218.

5) Bardella MT, Vecchi M, Conte D, et al. Chronic unexplained hypertransaminasemia may be caused by occult celiac disease. Hepatology 1999; 29: 654.

6) Bonamico M, Pitzalis G, Culasso F. Hepatic damage during celiac disease in childhood. Minerva Pediatr 1986; 38: 959.

7) Berasain C, Betés M, Panizo A, et al. Pathological and virological findings in patients with persistent hypertransaminasemia of unknown etiology. Gut 2000; 47: 429.

8) Cassidy WM, Reynolds TB. Serum lactic dehydrogenase in the differential diagnosis of acute hepatocellular injury. J Clin Gastroenterol 1994; 19: 118.

9) Dickson ER, Grambsch PM, Fleming TR, et al. Prognosis in primary biliary cirrhosis: model for decision making. Hepatology 1989; 10: 1.

10) O'Grady JG, Alexander GJM, Hayllar KM, et al. Early indicators of prognosis in fulminant hepatic failure. Gastroenterology 1989; 97: 439.

11) Jalan R, Hayes PC. Review article: quantitative tests of liver function. Aliment Pharmacol Ther 1995; 9: 263.

12) Weitz IC, Liebman HA. Des-γ-carboxy (abnormal) prothrombin and hepatocellular carcinoma: a critical review. Hepatology 1993; 18: 990.

13) Grant A, Neuberger J. Giudelines on the use of liver biopsy in clinical practice. Gut 1999; 45 (Suppl IV): IV 1.

14) Halfon P, Imbert-Bismut F, Messous D, et al. A prospective assessment of the inter-laboratory variability of biochemical markers of fibrosis (FibroTest) and activity (ActiTest) in patients with chronic liver disease. Comp Hepatol 2002; 1 (1): 3.