Practical Approaches to Assessing Liver Function

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Advances in medical technology have greatly facilitated the evaluation of "hepatic function," but most endeavors to evaluate such function in clinical medicine are a qualitative effort at best. Rather, biochemical tests employed to determine "hepatic function" measure degrees of dysfunction or the lack thereof, as indicated by a biochemical profile. However, with such a profile, supplemented by immunologic and virologic tests, and by ultrasonography and radionuclear imaging, the clinician has readily available means to identify hepatobiliary disease, dysfunction and changes in tissue density.

The biochemical tests most often employed provide information on the following: hepatocellular necrosis; cholestasis; capacity to synthesize proteins; uptake, conjugation, and excretion of organic anions; function of endoplasmic reticulum-associated enzymes; hepatic regeneration and tumors; immune responses to viral infections. In addition, ultrasonography and computerized tomography yield information on metabolic and hepatobiliary excretory function, and on morphologic changes within the liver and biliary tract.

HEPATOCELLULAR NECROSIS
Aminotransferases
Aspartate aminotransferase (AST; SGOT), and alanine aminotransferase (ALT; SGPT) are sensitive indicators of hepatocellular injury. Although elevations of both precede other evidence of viral, toxic, or drug related hepatitis, and are elevated in chronic hepatic injury, a quantitation of the extent of such injury is not possible; aminotransferases may be more than 1,000 times normal in acute metabolic disease, hereditary fructose intolerance, whereas they may be minimally elevated in acute fulminating hepatitis (viral, Wilson's disease). Very high values may be observed at the onset of an acute extrahepatic biliary obstruction.1

Whereas aminotransferases are quite sensitive in indicating hepatocellular necrosis, their specificity is limited; activity of AST is found in myocardium, skeletal muscle, liver, brain, pancreas and kidney, and injury to any of those tissues results in a rise of enzyme activity. An isolated elevation of AST without obvious liver disease is suspect of a myopathy, or in small infants, may follow repeated intramuscular injections. In chronic inflammatory liver disease, AST alone is a reasonably good indicator of disease activity. ALT activity is also found in several organs, but its greatest activity is in the liver; that makes ALT a more specific indicator of hepatocellular injury. In the course of acute viral hepatitis, ALT activity often follows a biphasic curve, whereas multiphasic elevations with intercurrent periods of normalcy are common in chronic non-A, non-B hepatitis.2

AST/ALT ratios have been employed to differentiate acute (ratio < 1.0) from chronic (ratio > 1.0) liver disease, but the absolute value of these ratios is limited.

Other enzymes which indicate liver cell necrosis, such as sorbitol dehydrogenase, isocitric dehydrogenase, alcohol dehydrogenase, guanase or ornithin carbamoyl transferase have been used in clinical situations1 but in terms of availability and economics, none have a particular advantage over determinations of AST and of ALT.

Lactic Acid Dehydrogenase (LDH)
Despite significant activity in hepatocytes, LDH is neither a sensitive, nor specific indicator of hepatocellular necrosis, and is of no value in the detection of liver disease. However, in patients with liver disease, elevations of LDH greater than 3 times normal, suggest either malignant disease, megaloblastic anemia, or centrilobular necrosis of chronic passive

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In acute liver disease . . . a decrease in albumin means severe hepatocellular injury and follow-up of albumin levels are helpful for prognosis.

Congestion. Determination of isoenzyme LDH-V provides greater sensitivity and specificity in regard to liver cell injury, but only in the absence of uremia and muscle cell injury. However, measurements of this isoenzyme are not superior to determinations of AST and of ALT.

CHOLESTASIS
Conjugated Serum Bilirubin (CB)
Determination of CB provides quick and reliable information on the presence or absence of cholestasis. Biochemically, cholestasis may be defined by elevations of CB greater than 2.0 mg/dl or when it exceeds 30% of the total bilirubin.

Serum Alkaline Phosphatase (AP)
Elevations of AP occur in both intra- and extrahepatic cholestasis, but levels of AP are not helpful in distinguishing between these two conditions. Higher values are more commonly associated with obstructive biliary tract disease. Increased AP (in absence of bone disease), may also indicate granulomatous (tuberculosis, sarcoidosis), or tumorous involvement of the liver; elevated AP may be the sole indicator of liver involvement in Epstein-Barr virus infection, or in chronic inflammatory bowel disease.

Several factors render AP non-specific: activity in serum is age-dependent, levels are elevated during catch-up growth and during pubertal growth spurt; AP may be elevated in hyperthyroidism and, rarely, it may be elevated without evidence of disease.

Total AP is a composite of liver/biliary tract/bone/intestinal isoenzymes, but the greatest contribution comes from liver and bone; determination of isoenzymes may be helpful in distinguishing the origin of a major AP fraction.

Gamma-glutamyltransferase (GGT)
GGT catalyses the transfer of y-glutamyl compounds to a variety of amino acid and dipeptide acceptors. The distribution of GGT is widespread throughout the body, notably in the biliary tract, but also in pancreas, seminal vesicles, and brain. GGT is a membrane-bound enzyme, and 80% to 90% of the enzyme activity is found in the biliary tract, and therefore helpful as part of a hepatic biochemical profile.

High GGT serum levels are associated with obstructive biliary tract disease ie, biliary atresia, with the Pi-Z type of alpha-1-antitrypsin deficiency during cholestasis, and in cirrhosis. GGT concentrations are higher in cholestatic forms of liver disease, as compared with viral hepatitis, where GGT levels may either be normal or only slightly elevated. GGT is also a sensitive indicator of intrahepatic biliary tract disease appearing during prolonged total parenteral nutrition. GGT/bilirubin ratios tend to be higher in intrahepatic cholestasis. GGT activity is induced by phenobarbital, dilantin and ethanol.

In cholestasis, a large but variable amount of GGT is associated with lipoprotein-X (LP-X), forming a high molecular mass complex which could explain, in part, low levels of LP-X in cholestatic liver disease, or in biliary atresia. Furthermore, GGT complexes with high density lipoproteins and with AP.

The specificity of GGT is rather low; levels may be increased in pancreatitis, in hyperthyroidism and in rheumatoid arthritis.

Biliary Glycoprotein I
Serum concentrations of this compound in patients with liver disease correlate positively with GGT, but it is not known whether sensitivity or specificity of biliary glycoprotein I are superior to those of GGT.

Lipoprotein-X (LP-X)
LP-X is the abnormal low-density lipoprotein of cholestasis, and has been used for the differentiation of biliary atresia from various types of intrahepatic cholestasis. Correctly employed, determinations of LP-X have been in the experience of the author, more useful, sensitive and specific, than any other test for the identification of biliary atresia. False negative results may be obtained by freezing plasma and by collecting plasma in the non-fasting state; the presence of fatty acids inhibits the characteristic cathodal migration of LP-X during electrophoresis in agar gel.

Recently, an automated test for LP-X has been developed and free fatty acids do not inhibit migration of LP-X to the cathode.

5'-Nucleotidase (5'N)
Elevations of this enzyme are largely the results of bile ductal (ductular) pathology, with or without obstructive jaundice. In the presence of cholestasis, 5'N is more specific than alkaline phosphatase, since 5'N is not significantly elevated in bone disease. However, determinations of GGT are often favored over measurements of 5'N as part of a biochemical profile in liver disease.

PROTEIN SYNTHESIS
Albumin
This protein, as most other circulating proteins with the exception of immunoglobulins, is synthesized by hepatocytes. The liver's reserve capacity for the synthesis of protein is considerable, and decreased serum levels of albumin, in the presence of liver disease, are usually associated with severe acute, or chronic advanced and decompensated liver disease. In acute liver disease, such as in acute viral hepatitis, a decrease in albumin means severe hepatocellular injury and follow-up of albumin levels are helpful for prognosis. Hypoalbuminemia, in association with

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increased levels of gammaglobulins, is common in chronic active hepatitis.

Serum protein electrophoresis provides a good quantitative measurement of major serum protein fractions, and is suitable for follow-up of chronic liver disease. The absence of the α1-globulin peak is highly suspect of α1-antitrypsin deficiency.

Clotting Factors
A depression of vitamin K-dependent factors (II, VII, X) occurs in patients with acute and chronic liver disease, including infants with congenital or perinatal viral hepatitis (cytomegalovirus, etc.), and with α1-antitrypsin deficiency. Non vitamin-K-dependent factors (V, VIII, IX) may also be depressed in either chronic or acute liver disease. Hence, measurements of prothrombin and partial thromboplastin times should be part of a biochemical profile in acute severe, in chronic, and in cholestatic liver disease.

Other Serum Proteins
Fibrinogen is low in severe acute, or in chronic decompensated liver disease; levels of α1-antitrypsin and ceruloplasmin are often increased in chronic liver disease; C3 may be low in acute viral hepatitis, and elevated in obstructive liver disease, but it is not suitable as a liver-specific index because of its ability to form complexes. Ceruloplasmin is useful as a first screening test for Wilson's disease, and levels below 20 mg/dl are suspect, and should be followed up by a 24 hour urine copper determination and by measurements of copper in biopsied liver tissue.

UPTAKE, CONJUGATION AND EXCRETION OF ORGANIC ANIONS
The appropriate handling by the liver of endogenous organic anions eg, bilirubin, bile acids and exogenous ones such as indocyanine green, is dependent on appropriate hepatocyte function, as it involves active transport of anion into the hepatocyte, its conjugation (indocyanine green excepted), and excretion from the liver cell into bile.

Serum Bilirubin
The normal liver is able to handle a three-fold increase of bilirubin production, before elevations of serum levels are detected. Total serum bilirubin is not a sensitive index of hepatic dysfunction, but is useful to monitor trends, particularly in acute, as well as in chronic decompensated disease; unlike changes in aminotransferase activity, continued rises of serum bilirubin raise concern. Conjugated bilirubin is a sensitive index to hepatic dysfunction, cholestasis in particular. Elevations of conjugated bilirubin are found in 30% of patients with liver disease, in whom total serum bilirubin levels are normal. In health, normal levels of conjugated bilirubin are usually less than 0.3 mg/dl.

Along with OGT, serial determinations of conjugated bilirubin are most helpful to identify and to monitor hepatic dysfunction which may develop during prolonged total parenteral nutrition.

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Serum Bile Acids
Levels of serum bile acids, conveniently measured by radioimmunoassay, or by the 3-OH steroid dehydrogenase method, are the result of a "spill-over" of bile acids from the portal into the systemic circulation during re-cycling of bile acids from the intestine to the liver. Measurements of postprandial, not fasting, serum bile acids are considered equal, but not superior to determinations of aminotransferases for the assessment of acute or mild hepatocellular injury. The specificity of the 2-hour postprandial serum bile acid determination is good, the sensitivity fair, and measurements qualify as a screening test for hepatic dysfunction. The sensitivity of the postprandial serum bile acid test is perhaps higher in chronic liver disease. Experience in children with liver disease is limited.

Determination of plasma disappearance and conjugation of cholic acid seems to have good prognostic sensitivity for testing residual hepatic function in acute liver failure, but more information is required.

Excretion of Bromosulfophthalein (BSP)
Measurements of serum disappearance of injected BSP is quite sensitive for the detection of hepatic dysfunction, particularly when transport maxima are measured. The BSP test is very sensitive in cirrhosis. Because of several and rather serious side effects following BSP injection, the clinical use of this test has declined.

Excretion of Indocyanine Green (ICG)
ICG is a triarboxycyanine dye, which is handled by the liver in much the same manner as BSP, but is not conjugated with glutathione. ICG also has characteristics suitable to evaluate hepatic transport function. Toxic reactions have not occurred. Experience with ICG is still limited, particularly in regard to its use in children. Optimal doses as well as sampling times following injection of ICG still have to be determined.

TESTS OF MICROSOMAL FUNCTION
Function and integrity of microsomal enzyme systems in hepatocytes is measured by analysis of expired 14CO2 after injection of a labeled pharmaceutical. The aminopyrine breath test is considered useful, reproducible and sensitive for the screening of cirrhosis. Aminopyrine labeled with 14C, a stable isotope, may be employed in children, because no radiation-emitting isotope is employed; however, this distinct advantage is offset by the need of specialized equipment.

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ment for mass-analysis, and no large data base is available at present. Breath analysis of labeled phenacetin is the latest addition for the assessment of the drug-oxidizing microsomal function. In addition to specific technical requirements, some problems remain, such as possible ambiguity in interpretation, and the availability of liver biopsy, which may still be more accurate.

VIRAL MARKERS

Viral Hepatitis A and B

Infection with and evolution of the host response to infection with hepatitis A and hepatitis B virus can be monitored with appropriate serological tests, most commonly by solid phase radioimmunoassays. Schematic presentation of host response to infection with viral hepatitis A and B, as well as test patterns in hepatitis B are shown in the Table. No test systems are currently available for routine use to diagnose non-A, non-B hepatitis, or delta-hepatitis.

Other Viruses

The acronym “TORCH” is used to aid in serologic testing for infectious agents responsible for neonatal hepatitis. With variable license, the initials are used as follows: T (toxoplasmosis); O (others: syphilis, etc.); R (rubella); C (cytomegalovirus, coxsackie); H (herpetoviridae—cytomegalovirus, Epstein-Barr virus, herpes simplex virus; hepatitis B). However, criticism has been raised in regard to the routine use of the “TORCH-battery,” rather, it is recommended to use and to follow specific tests. Serologic tests for recrur type 3, implicated in the pathogenesis of biliary atresia, are only available in a few research laboratories.

REGENERATION, TUMOR MARKERS

Alpha-fetoprotein (AFP)

AFP is synthesized by embryonal liver cells and is present in high concentrations in the developing fetus. AFP is elevated in instances of enhanced cell production, such as in regenerating liver or in neoplastic tissue. Theoretically, determinations of AFP could be used as a measure of hepatic regeneration following acute hepatocellular injury. However, in acute fulminating hepatitis, the onset of hepatocyte regeneration, as measured by increases in serum AFP, may be delayed until the second week of illness; furthermore, a rise in AFP is not always consonant with recovery from acute fulminating hepatitis, since damage to other organs, such as the pancreas, the central nervous system, or kidneys may be more important for mortality, despite evidence of liver cell regeneration.
TABLE

HEPATITIS B VIRUS INFECTION: ASSOCIATION BETWEEN SEROLOGIC TESTS AND STATUS OF INFECTION

<table>
<thead>
<tr>
<th>HBs-Ag</th>
<th>HBe-Ag</th>
<th>Anti-HBc</th>
<th>IgM</th>
<th>Anti-HBe</th>
<th>Anti-HBs</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Incubation period; chronic asymptomatic carrier (infective)*</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Late incubation period; chronic asymptomatic carrier, probably also with DNA-polymerase (highly infective)*</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Acute hepatitis; chronic asymptomatic carrier, probably also with DNA-polymerase (highly infective)*</td>
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<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Late stage of acute hepatitis; chronic asymptomatic carrier (moderately infective)*</td>
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<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Convalescent stage following acute infection</td>
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<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Late convalescent stage following acute infection</td>
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<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Recovering from acute hepatitis</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Recovered from infection; immunized against HBV; presence of infection possible</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Chronic infection, active viral replication</td>
</tr>
</tbody>
</table>

*Blood, secretions
†High-titer antibody compatible with immunity; low-titer antibody does not indicate unequivocal immunity

AFP may also be employed as a tumor marker, as very high values in a patient with chronic liver disease, suggests the presence of a hepatoma. AFP is also elevated in hereditary tyrosinemia, type 1.

5'Nucleotide Phosphodiesterase-Isoenzyme V

This enzyme is present in a significant proportion of patients with hepatic metastases. It may be an important diagnostic aid for cancer patients, an early predictor of hepatic metastases, and marker for hepatoma.29

RADIONUCLIDE IMAGING, HEPATOBILIARY EXCRETION

These tests monitor hepatobiliary excretory function and, in pediatrics, are primarily employed to aid the diagnosis of biliary atresia. Biliary excretion of Rose Bengal labeled with 1311, or 125I is quite sensitive and specific for this purpose, particularly when used in modified form.30 Nowadays, there is a tendency to replace the labeled Rose Bengal test by tests utilizing newer radiopharmaceuticals; however, the ability to obtain scintiphotos as late as 72 hours after the injection, is a distinct advantage.31

 Organic derivatives of imino-diatomic acid (IDA) labeled with 99mTc are also readily taken up by the hepatocyte and excreted into the biliary tract, and have found widespread use as "second-generation" hepatobiliary imaging agents. Primarily used are 99mTc-PI-IDA, HIDA, 99mTc DIS-IDA, and 99mTc-B-IDA. HIDA, DIS-IDA and B-IDA have gained good acceptance in hepatobiliary imaging of infants,11-13 particularly when used after a "priming" dose of phenobarbital, 5 mg/kg/day given 5 days before the test to stimulate bile flow. The advantage of these radiopharmaceuticals are readily available results, but no imaging beyond 18 to 24 hours is possible because of the short half life of the isotope. Sensitivity is very good, specificity approaches 90%.

ULTRASONOGRAPHY AND COMPUTERIZED TOMOGRAPHY

Compared with other techniques, such as the Tc-sulfur colloid scan, ultrasonography (US) and computerized tomography (CT) have a much greater specificity and, because of their cross-sectional imaging, play a very important role in the evaluation of the liver.

Ultrasonography (US)

US relies on the distribution of echogenic surfaces. In addition to detailed clinical information, US of the liver and biliary tract is usually the first choice of investigation if a child presents with cholestatic jaundice, right upper quadrant pain or mass. US is most valuable to complement biochemical investigations in infections of the neonate and in infancy, in congenital or acquired diseases, or in anomalies of the hepatobiliary system, in metabolic diseases, tumors, and in hepatobiliary complications of total parenteral nutrition.14

Computerized Tomography (CT)

Supplemented by US, CT detects and provides information on the nature of an abnormality, it helps determine the extent of a disease process, which may be important for surgery and is useful in monitoring progress or failure of specific treatments. CT relies on differentiation of tissue densities, as is reflected in the differential alterations of an x-ray beam. New contrast agents, such as EOE 13 (ethiodized

*HIDA: 99mTc-N-[1,6-diethyl-acetanilide]-aminodiacetic acid, PIIDA: 99mTc-N-[p-isopropanilacetanilide]-aminodiacetic acid, DISIDA: 99mTc-N-(di-isopropylacetanilide)-aminodiacetic acid, BIDA: 99mTc-p-Butyl-imino-diatomic acid.
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oil emulsion (13) should be helpful to detect small (1 cm) lesions. However, no distinction can be made between malignant and benign disease.

NEW IMAGING TECHNIQUES

In the not too distant future, the physician interested in liver disease should have at his disposal new and more powerful imaging techniques not only for the demonstration of anatomic abnormalities, but also for a more quantitative study of certain metabolic functions of the liver. However, at present, practical applications are likely to remain limited because of cost and the need for specialized equipment.

Positron Emission Tomography

This technique measures perfusion, metabolic distribution and myocardial metabolism of positron-emitting isotopes. This technique may, for instance, permit localization of metabolically active tissue.

Nuclear Magnetic Resonance (NMR)

This type of imaging may play a major role in hepatic diagnosis, but current experience is limited, particularly with regard to childhood liver disease.

NMR allows the formation of two-dimensional images through the use of very weak interactions with endogenous stable magnetic atomic nuclei, which have the property of nuclear spin. Their spin can be oriented in an external magnetic field, impulses of which create a resonance phenomenon, which is followed by a measurable energy absorption. The latter provides the basis for a computer-generated image. There are no known hazards, but NMR requires careful application of appropriate methods and interdisciplinary cooperation to achieve its full potential. Recent practical applications are a demonstration of focal fatty changes, measurements of metals such as iron and copper, as well as excellent discrimination between normal and neoplastic tissue.

REFERENCES