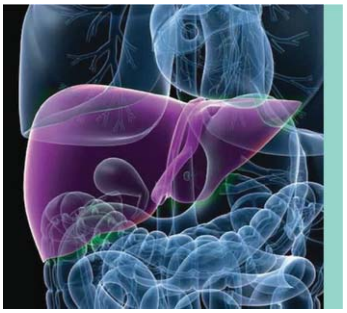

# The Holy Grail of a Biomarker for "Liver Function"

*Avik Majumdar, M.B.B.S.(Hons), M.P.H.T.M., F.R.A.C.P., and Massimo Pinzani, M.D., Ph.D., F.R.C.P.*

The past decade has given rise to many innovations in hepatology, particularly in the fields of noninvasive fibrosis assessment and viral hepatitis. Comparatively, advances in the functional assessment of the liver have progressed at a much slower rate. It is an ambitious proposal to develop a single unifying biomarker to assess global liver function, but one that highlights the inadequacies of current methods of assessment. A major impediment to the pursuit of this ''holy grail'' is the broad range of functions performed by the liver. Moreover, liver function per se is only one determinant of prognosis in chronic liver diseases, with portal hypertension and hepatocarcinogenesis being two other major factors. For this reason, any progress in the pure functional assessment of the liver has been made predominantly in the settings of acute liver failure and the preoperative assessment for liver resection. This brief review aims to discuss the current and future status of the hepatic functional assessment.

## FUNCTIONS OF THE LIVER

The primary functions of the liver and corresponding tests are summarized in Table 1. The breadth and heterogeneity of sites of hepatocyte function demonstrate the difficulty in developing a single functional biomarker.

## ROUTINE LABORATORY ASSESSMENT OF LIVER FUNCTION

Among the routinely available laboratory tests, only serum albumin, bilirubin, and prothrombin time, or its derivative international normalized ratio, have the capability to assess liver function. Despite widespread and frequent use, these basic tests have drawbacks (Table 2). To improve practical utility, these tests have been combined with other biochemical or clinical parameters to devise clinical scores to stratify prognosis in chronic liver disease, the most common being Child-Turcotte-Pugh and the Model for End-Stage Liver Disease. Although originally derived for other purposes, both of these scores are currently used with the endpoints of mortality or transplantation in mind, rather than liver function in absolute terms.[1,2] Additional biochemical measures of liver function have been investigated in acute liver failure, such as arterial ammonia and lactate, of which the latter has been incorporated into prognostic scores such as the

Abbreviations: ICG, indocyanine green; PDR, plasma disappearance rate; R15, percentage retained 15 minutes after intravenous bolus injection.

From the UCL Institute for Liver and Digestive Health and Sheila Sherlock Liver Unit Royal Free Hospital, Rowland Hill Street, NW3 2PF, London, United Kingdom.

Potential conflict of interest: Nothing to report.

Received 3 March 2016; accepted 30 March 2016

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**TABLE 1. SELECTED FUNCTIONS OF THE LIVER AND CORRESPONDING TESTS**

| Function | Test |
|---|---|
| Bile synthesis | |
| Bile salt dependent | Serum and urine bilirubin |
| Bile salt independent | Bile acids |
| Metabolic function | |
| Glucose homeostasis | Capillary blood glucose |
| Amino acids | Lactate |
| Fat-soluble and water-soluble vitamins | |
| Lipid metabolism | |
| Detoxification and inactivation | |
| Drugs/Toxins (cytochrome P450 function) | Aminopyrine clearance |
| | Bromsulphthalein |
| Endogenous steroids | Caffeine clearance |
| | Galactose elimination capacity |
| | Indocyanine-green clearance |
| | Lignocaine metabolites |
| | Molecular imaging techniques |
| | Serum ammonia |
| Plasma protein synthesis | |
| Albumin | Coagulation tests |
| Clotting factors | Serum albumin |
| Acute-phase proteins | Serum globulin |
| Hormone-binding proteins | |
| Protein catabolism | Urea |
| Immune function | |

King's College Hospital criteria.[3,4] However, these are of limited use in the chronic disease setting.

## QUANTITATIVE ASSESSMENT OF LIVER FUNCTION

Quantitative methods of assessing liver function have traditionally focused on the excretory capacity of the liver. Bromsulphthalein clearance was first described for this purpose in 1924; however, its use was discontinued in the 1970s because of a high perceived risk for anaphylactic reactions.[5] Indocyanine green (ICG) clearance and galactose elimination capacity have since superseded bromsulphthalein and are described later in this article. Other excretory tests that have been described include caffeine clearance and lidocaine metabolite formation.[6] Quantitative methods have not yet been adopted to routine clinical practice because of their lack of specificity and dependence on specialized equipment, and thus are mainly used in research or referral centers.

**TABLE 2. NEGATIVE CHARACTERISTICS OF ROUTINE LIVER LABORATORY TESTS FOR ASSESSING FUNCTION**

| Test | Characteristics |
|---|---|
| Bilirubin | Affected by inflammatory states, biliary obstruction, and hemolysis |
| Albumin | Not specific for impaired hepatic protein synthesis (e.g., negative acute-phase reactant, nutrition dependent, nephrotic syndrome) |
| Coagulation factors | May be affected by vitamin K deficiency, inherited or acquired coagulopathies |
| Ammonia | Generally not useful in chronic liver disease but is of prognostic utility in acute liver failure |
| Lactate | Affected by many confounders including dehydration, sepsis, inflammatory states, and ischemia; mainly useful in acute liver failure and critically ill cirrhosis |
| Serum bile acids | Little or no utility with regard to liver function |
| Glucose | Hypoglycemia only seen in severe acute liver injury or end-stage chronic liver disease |
| Urea | Low serum urea may be seen in end-stage chronic liver disease or severe acute liver failure; however, it is confounded by nutrition, muscle mass, overhydration, and urea cycle disorders. |

### Indocyanine Green Clearance

The clearance of ICG has been used to assess functional hepatocyte mass, although originally designed to estimate hepatic blood flow using the Fick equation. ICG binds to albumin, alpha1-lipoproteins, and beta-lipoproteins and is entirely excreted by the liver into the bile.[5] ICG test results are commonly expressed as the plasma disappearance rate (ICG-PDR) or the percentage retained 15 minutes after intravenous bolus injection (ICG-R15) of ICG at doses of 0.5 mg/kg. These parameters can be quantified with serial arterial or venous blood sampling or using noninvasive pulse densitometry. Several cutoff values have been reported in the literature for safe hepatic resection, with ICG-PDR greater than 15% per minute or ICG-R15 less than 15% generally considered as cutoffs for normal values. ICG testing may be affected by hepatic blood flow variations caused by thrombosis or intrahepatic shunting, and competitive inhibition by excessive bilirubin. Furthermore, ICG uptake

by hepatocytes can be reduced in inflammatory states because of the effect on the expression of transporting polypeptides.[7,8]

### Galactose Elimination Capacity

Galactose elimination capacity quantifies the metabolic function of the liver. Essentially, an intravenous load of 0.5 mg/kg galactose is administered, which undergoes phosphorylation within hepatocytes. The elimination capacity is measured with serial serum samples between 20 and 50 minutes postinjection. Galactose elimination capacity has been correlated with clinical outcomes in chronic liver disease and fulminant hepatic failure. However, a number of shortcomings exist, particularly during liver regeneration where there is an increased requirement for galactose in membrane glycoproteins. Furthermore, a prolonged fasting state may result in galactose being converted into glucose and ultimately lead to false results.[6]

## OTHER FUNCTIONAL TESS

### Breath Tests

Breath tests are infrequently used and involve an oral or intravenous load of a radiolabeled substance (e.g., $^{13}C$- or $^{14}C$-aminopyrine,[9] $^{14}C$-galactose) that is predominately metabolized in the liver. Exhaled carbon dioxide ($CO_2$) is collected in an alkaline medium at serial intervals and the activity of $^{14}CO_2$ is measured. This provides a semiquantitative value when compared with the endogenous production of $CO_2$. These tests required specialized equipment and have little additional value over routine liver biochemical assessment.[10]

### Molecular Imaging

$^{99m}$Tc-diethylenetriamine-pentaacetic acid-galactosyl human serum albumin scintigraphy and $^{99m}$Tc-mebrofenin hepatobiliary scintigraphy are two molecular imaging techniques that are able to assess the regional variability of hepatic blood flow and functional hepatocyte mass. These methods are of particular use in the assessment before hepatic resection and provide more information than computed tomography volumetry or ICG clearance alone. However, the uptake of $^{99m}$Tc-mebrofenin is affected by chronic cholestasis, and $^{99m}$Tc-diethylenetriamine-pentaacetic acid-galactosyl human serum albumin scintigraphy is only approved for use in Japan thus far.[6]

## FUTURE DIRECTIONS

To maximize utility, the ideal "liver function test" should not only assess function but should also predict the clinical outcomes of patients. Functional tests should be correlated with severity of portal hypertension and hard clinical endpoints such as clinical decompensation and mortality to achieve this. ICG clearance has already been investigated in the detection of esophageal varices.[11] Other sequelae of cirrhosis, such as the risk for hepatocarcinogenesis, may be more difficult to correlate with liver function. However, combining noninvasive fibrosis assessment techniques with functional testing may be an innovative way forward.

## CONCLUSION

The routine assessment of liver function is still primarily based on basic biochemical and coagulation tests. Although clearance-based functional tests have been used in the hepatic surgical assessment of patients, these are yet to translate to the standard evaluation of patients with liver disease. The discovery of a reliable biomarker for liver function that correlates with clinical outcomes is yet to be found, but certainly is a "holy grail" worth searching for.

### CORRESPONDENCE

Massimo Pinzani, M.D., Ph.D., F.R.C.P., UCL Institute for Liver and Digestive Health and Sheila Sherlock Liver Centre Royal Free Hospital, London, United Kingdom. E-mail: m.pinzani@ucl.ac.uk

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