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EDITORIAL

Could quantitative liver function tests gain wide acceptance among hepatologists?

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Abstract

It has been emphasized that the assessment of residual liver function is of paramount importance to determine the following: severity of acute or chronic liver diseases independent of etiology; long-term prognosis; step-by-step disease progression; surgical risk; and efficacy of antiviral treatment. The most frequently used tools are the galactose elimination capacity to asses hepatocyte cytosol activity, plasma clearance of indocyanine green to assess excretory function, and antipyrine clearance to estimate microsomal activity. However, a widely accepted liver test (not necessarily a laboratory one) to assess quantitative functional hepatic reserve still needs to be established, although there have been various proposals. Furthermore, who are the operators that should order these tests? Advances in analytic methods are expected to allow quantitative liver function tests to be used in clinical practice.

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Key words: Chronic diseases; Fatty liver; Hepatitis, viral; Liver cirrhosis; Liver function tests; Prognosis

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INTRODUCTION

Liver biopsy is the first-line method for evaluating liver injury. As a result of its limitations and risks, alternative methods have been developed. Many markers and several imaging methods have been evaluated in studies published in peer-reviewed journals. Although the methodological rigor of the design, execution and analysis of the studies proposing new tests has always been ascertained, only their regular use can establish their acceptance among physicians. Probably, nervousness-based medicine plays a role. Fear of litigation is a powerful stimulus to over-investigation, and in an atmosphere of litigation phobia, the only bad test is the test that the physician did not think of ordering[1].

The assessment of liver function has always been considered as important for estimating such things as the severity of any acute or chronic liver disease, and its prognosis and treatment efficacy (Table 1). Generally, methods used to determine cellular damage and related consequences consist of noninvasive and rapid tests, which are easy to perform, e.g. serum enzymes, surrogate serum fibrosis markers, or transient elastography and ultrasound imaging. The first quantitative liver function tests (QLFTs) were suggested almost 50 years ago[2]. Their rationale was based on the fact that a drug or a foreign compound is metabolized primarily by the liver cytochrome P450 system, through sequential oxidative processes, and the major metabolite can be detected. The main characteristic of the proposed drug is its relatively high hepatic extraction ratio, but a QLFT depends not only on hepatic metabolic capacity, but also on hepatic blood flow. A more recent study has addressed the clinical utility of measuring galactose elimination capacity (GEC), aminopyrine breath test (ABT) and indocyanine green (ICG) retention test. The results have shown that all QLFTs are predictors of survival in cirrhosis, and that GEC adds new prognostic information to that already available using the Child-Pugh classification[3]. In spite of this, relatively few centers perform these tests. Hence, they are currently performed only in very few specialized institutions and usually within the setting of clinical research projects.

More recently, preoperative assessment of liver function and prediction of residual postoperative functional liver parenchymal mass and reserve has been ascertained to be of paramount importance to minimize surgical risk, especially in patients with hepatocellular carcinoma, the

Table 1 Frequently used QLFTs in patients with liver cirrhosis, chronic viral hepatitis and non-alcoholic liver disease

Type	Methods/drawbacks
GEC	Intravenous administration of galactose; blood samples at 5, 25 and 45 min; urine collected for 5 h
ICG	Measurement of liver plasma flow; injection of ICG must be performed quickly; blood samples at 3, 6 and 49 min
MEGX	Blood samples at 15 and 30 min after i.v. lidocaine administration; allergy to anesthetics
ABT	Resting period of at least 30 min before the breath test that should be repeated three times at 10-min intervals
13C-C breath test	Subjects ingested 2 mg/kg of [3-methyl-13C]-caffeine sitting quietly for 15 min before and throughout the test; breath samples were collected immediately prior to, and 60 min after, caffeine ingestion
13C-M breath test	Measurement of breathed CO_2 by laser-based technology
SCl	Measurement of liver plasma flow. Sorbitol (500 g/L) was administered *via* a perfusor at 7.5 mL/h. Serum and urinary concentrations of sorbitol were determined at the beginning of the perfusion and after reaching steady-state
TOSCA	Unique sample of saliva to be collected in the morning; rare complaiance to drinks containing caffeine by some patients
LURs	Volumetric information as well as functional assessment; expensive
MRI	Expensive; to be validated

QLFTs: Quantitative liver function tests; GEC: Galactose elimination capacity; ICG: Indocyanine green; MEGX: Monoethylglycinexylidide; ABT: Aminopyrine breath test; MRI: Magnetic resonance imaging; LURs: Liver uptake ratios.

majority of whom have liver cirrhosis as a complication. Incorporation of ICG into the decision tree has enabled patients conventionally classified into Child-Pugh class A to be subdivided into several groups in which various hepatectomy procedures are feasible. Applying this choice to 685 patients, during 10 years, only a single fatality was encountered[4]. Probably, this is the most important area of application of QLFTs. Indeed, new limits have been established to decrease mortality and morbidity rates after liver resection in cirrhotic and non-cirrhotic patients.

Various laboratory data and imaging techniques have been used to complement the Child-Pugh score to predict liver failure after hepatectomy and to assess functional hepatic reserve. The greatest experience has been made so far with the ABT and GEC, which are decreased among hepatic failure patients after liver resection. However, absence of these changes does not totally exclude hepatic failure. The ICG retention test is the most widely used clearance test. Nevertheless, it remains imperfect because it depends on hepatic blood flow and on the functional capacity of the liver.

Nuclear imaging plays a determinant role in assessing liver function, although it is expensive. Nuclear imaging of the asialoglycoprotein receptors with radiolabeled synthetic asialoglycoproteins provides volumetric information as well functional assessment of the liver[5]. Single photon emission computed tomography (SPECT) is superior to the planar method for determining liver uptake ratios (LURs). Evaluation of LURs is a suitable indicator of 99mTc-galactosyl serum albumin clearance from the blood pool and of binding to the asialoglycoprotein receptor, which is a simple and clinically useful indicator for the assessment of hepatic functional reserve in chronic liver diseases[6].

Recent interest toward QLFTs comes from emergency medicine and intensive care physicians. There is no ideal real-time and bedside technique for assessing liver function in critically ill patients. Dynamic tests such as ICG plasma disappearance rate and lidocaine metabolism [monoethylglycinexylidide (MEGX) test], are superior to static tests such as prothrombin time and bilirubin measurement. Recently, the ICG plasma disappearance rate, which nowadays can be measured reliably by a transcutaneous system in critically ill patients at the bedside and provides results within a few minutes, has been confirmed to correlate well with ICG clearance. In general, the ICG plasma disappearance rate is superior to bilirubin, and comparable or even superior to complex intensive care scoring systems in terms of outcome prediction. Furthermore, ICG plasma disappearance rate is more sensitive than serum enzyme tests for assessing liver dysfunction, and early improvement in the ICG plasma disappearance rate after onset of septic shock is associated with better outcome[7].

SOME APPLICATIONS OF QLFTs

Liver cirrhosis

The aim of this report is not to review QLFTs, but to provide a critical appraisal of their extremely selective use. Widespread application of QLFTs as a prognostic tool is controversial. In a recent study, the predictive value of serial evaluations of GEC and MEGX on survival in a cohort of 35 patients was assessed, and secondarily, these tests were compared to Child-Pugh and Model for End Stage Liver Disease (MELD) scores. The end points were patient death or liver transplantation. Statistically significant differences between dead/transplanted patients and survivors were found for basal values of GEC, MEGX, Child-Pugh class and MELD score. Surprisingly, receiver operating characteristic (ROC) curves of Child-Pugh class and MELD score showed a higher prognostic accuracy than GEC and MEGX. On multivariate analysis, neither GEC nor MEGX were independent predictors of survival. Repeated-measures analysis of GEC and MEGX did not increase the prognostic accuracy of these tests, and did not add useful prognostic information on patient outcome during the following 6 mo. These data suggest that neither single nor repeated determinations of GEC and MEGX are superior to Child-Pugh class and MELD score in predicting prognosis of patients with viral cirrhosis[8].

Blood galactose clearance after an intravenous galactose load has been used widely as a QLFT. A novel

QLFT, the galactose single point (GSP) method, has been developed to assess residual liver function in various diseases[9]. The goal of that study was to evaluate the influence of non-hepatic factors such as hyperglycemia on GSP and GEC in rats. Four groups of animal studies were carried out, i.e. normal control (NC), streptozotocin-induced diabetes mellitus (DM), CCl_4-induced hepatotoxicity (CCl_4), and streptozotocin-induced diabetes with CCl_4-induced hepatotoxicity (DM + CCl_4). The serum glucose levels in the diabetic groups (DM and DM + CCl_4) were significantly increased compared with those in the NC and CCl_4 groups. A significant increase of aspartate aminotransferase and alanine aminotransferase was observed in the CCl_4-treated groups (CCl_4 and DM + CCl_4) compared with those in the NC and DM groups. In comparison with the NC group, the values of GSP and GEC in the diabetic groups (DM and DM + CCl_4) were significantly reduced and increased, respectively. GSP had highly significant correlations with GEC. These results suggest that galactose metabolism tests should be interpreted with caution under conditions of significant hyperglycemia[9].

Transplantation

The unique ability of the liver to regenerate quickly after resection makes living donor liver transplantation (LDLT) possible. However, the quality and course of this regeneration process in humans are still unexplored. In a recent study, GEC, ICG and lidocaine half-life as markers for the quality of liver regeneration in the first 3 mo after LDLT were investigated. Twenty-two consecutive living liver donors and their corresponding recipients were analyzed at baseline and at 10 and 90 d after LDLT. Six recipients lost their grafts during the study period. We compared donors and recipients at the different time points. After LDLT, GEC decreased (-42.6%) and ICG increased (+50.6%) significantly in donors. ICG and GEC remained significantly altered over 3 mo in donors with an improvement between days 10 and 90. ICG and GEC improved significantly in recipients between days 10 and 90. The lidocaine half-life showed no significant changes. The donors had better test results and recovered faster than the recipients. In conclusion, after LDLT, the parameters for liver capacity and flow remain altered in donors and recipients despite rapid volume growth[10].

Timing

The key point of the whole problem is not how to test residual liver function but when. The ^{13}C-methacetin (13C-M) breath test enables the quantitative evaluation of cytochrome-P450-dependent liver function. 13C-M is metabolized in the liver by O-demethylation to $^{13}CO_2$ and acetaminophen. The aim of a previous study was to evaluate the 13C-M breath test in comparison to the Child-Pugh class and other QLFTs (MEGX and ICG). 13C-M (2 mg/kg) was given orally to 31 patients with histologically proven liver cirrhosis of different etiology and severity (nine Child-Pugh class A, 13 class B, and nine class C). The increase of exhaled $^{13}CO_2$ was expressed as delta over baseline (DOB; delta/1000). All breath test parameters analyzed provided an excellent discrimination between cirrhotic and non-cirrhotic individuals. The DOB value at 20 min showed a superior correlation with the Child-Pugh class than did MEGX or ICG clearance results. With a cut-off value of $\leqslant$ 25 delta/1000 at 20 min, sensitivity and specificity to discriminate between cirrhotic and non-cirrhotic individuals was 93.5% and 95%, respectively[11].

Pre-cirrhotic stage

To find out whether this breath test is sensitive in non-cirrhotic patients who also have chronic hepatitis C in the early stages of fibrosis, the following study was carried out. Eighty-one patients with chronic hepatitis C underwent a 13C-M breath test. In all patients, a liver biopsy was performed. The liver histology was classified according to the histology activity index-Knodell score. Patients with early fibrosis did not differ in DOB values from patients at 15 min (23.2 ± 7.9 per thousand *vs* 22.6 ± 7.2 per thousand; $P = 0.61$), or cumulative recovery (13.6% ± 3.7% *vs* 13.2% ± 3.8%; $P = 0.45$) from patients with more advanced fibrosis. Conclusively, the noninvasive 13C-M breath test fails to detect early stages of fibrosis in patients with chronic hepatitis C[12].

The 13C-caffeine (13C-C) breath test is a noninvasive, QLFT that is considered to be a valid tool by many authorities. The utility of the 13C-C breath test was measured in 48 patients with chronic hepatitis B and 24 controls, along with its ability to monitor response to lamivudine. In 28 patients on lamivudine, 13C-C breath tests were performed at 1 wk and 1 year after therapy. Patients with Metavir F0-1 fibrosis had a 13C-C breath test similar to the controls. However, patients with F2-3 fibrosis and cirrhosis patients had a decreased 13C-C breath test. Fibrosis correlated best with the 13C-C breath test. The 13C-C breath test independently predicted significant ($F \geqslant 2$) and advanced ($F \geqslant 3$) fibrosis and yielded the greatest area under the ROC curve (0.91 ± 0.04) for predicting advanced fibrosis. The 13C-C breath test was unaltered by 1 wk of lamivudine but improved by 61% ($P < 0.001$) in responders to long-term lamivudine, whereas in those with viremia and elevated alanine aminotransferase, values remained stable or deteriorated. The 13C-C breath test distinguishes chronic hepatitis-B-virus-related fibrosis and detects improvement in liver function in response to long-term lamivudine[13].

Survival studies

Caffeine clearance (CCl) has been suggested as a more exact method than those commonly used. The aim of the following study was to assess the usefulness of CCl in survival prediction of patients with liver cirrhosis. Thirty-four patients with cirrhosis of varying etiology were included: 19 were Child-Pugh class A or B and 15 were class C. CCl was determined from saliva samples. The mean length of follow-up was 33.8 mo. A bivariant

survival analysis was carried out following the Kaplan-Meier method, together with a multivariate analysis using the Cox proportional hazards model. Twelve patients died during follow-up. CCl values < 0.24 mL/kg per minutes, age > 60 years, and non-alcoholic cause of cirrhosis were factors predicting lower survival. CCl was the only independent predictive factor in the multivariate analysis. The authors concluded that that CCl enables hepatologists to predict survival in cirrhotic patients and, considering its harmlessness, simplicity and cost, it can be used as a routine procedure in the assessment of these patients[14].

A simplification of this test, the so-called Total Overnight Salivary Caffeine Assessment (TOSCA), comes from an other study[15] with a further application (patients divided into rapid and slow metabolizers). Furthermore, TOSCA shows near complete safety (patients drink one or two cups of coffee according their habit in the morning). One drawback of QLFTs is the possible occurrence of severe side effects that are sometimes life-threatening (e.g. anaphylaxis).

Magnetic resonance imaging (MRI) offers several advantages. Gadolinium methoxybenzyl diethylenetriamine penta-acetic acid is a newly developed MR contrast agent. Its hepatic extraction fraction is a direct, noninvasive technique for the quantitative evaluation of liver function. It may be a promising alternative, although expensive, for the determination of noninvasive hepatic function in patients with liver disease[16].

Antiviral therapy

Whether and to what extent does antiviral therapy for chronic hepatitis C influence a broad panel of QLFTs? Fifty patients with chronic hepatitis C were treated with interferon (n = 8), interferon/ribavirin (n = 19) or peg-interferon/ribavirin (n = 23). Quantitative testing of liver function, including ABT, GEC, sorbitol clearance (SCl) and ICG clearance was performed before and 3 mo after initiation of antiviral therapy. After 3 mo, 36 patients showed normal transaminases and were negative for hepatitis C virus RNA, and 14 patients did not respond to therapy. ABT and GEC as parameters of microsomal and cytosolic liver function were reduced in all patients before therapy initiation and returned to normal values in the 36 therapy responders after 3 mo. Parameters of liver perfusion (SCl and ICG) were not affected by antiviral therapy. In the 14 non-responders, no changes in QLFT values were observed during the treatment period. ICG and SCl remained unaffected in patients with chronic hepatitis C, while ABT and GEC were significantly compromised. ABT and GEC normalized in responders to antiviral therapy. Early determination of ABT and GEC may differentiate responders from non-responders to antiviral treatment in hepatitis C[17].

Assessing liver regeneration

Improvement of nitrogen balance is desirable in patients with acute or chronic illness. Both growth hormone and insulin-like growth factor-I are promising anabolic agents, and their combined administration has been shown to reverse catabolism more efficiently than each of the peptides alone. The capacity of urea-nitrogen synthesis [μmol/(min × 100 g body weight)] was evaluated in rats, unravelling a neglected QLFT, based on mitochondrial-cytosolic metabolic capacity (M-CMC) for conversion of amino-nitrogen[18]. Following this approach, the authors used GEC to assess hepatocyte cytosol activity, plasma clearance of ICG to assess excretory function, antipyrine clearance to estimate microsomal activity, and functional hepatic nitrogen clearance to assess M-CMC in females with Turner syndrome[19].

Non-alcoholic steatohepatitis (NASH)

Finally, 13C-C, a noninvasive tool for the evaluation of the cytochrome P450 system, which is implicated in the development of NASH, has been proposed in patients with metabolic dysfunction. Up-to-date research has demonstrated that 13C-C can predict reliably severe hepatic fibrosis in patients with the most severe form of non-alcoholic fatty liver disease (NAFLD). Although this test does not quantify the residual functional liver mass, it is safe and easy to perform. Further large-scale studies are needed to verify its reliability[20]. A previous study that tested 13C-M and ^{13}C-ketoisocaproate for microsomal and mitochondrial liver function has demonstrated its usefulness for better and noninvasive characterization of patients with NAFLD[21]. It is worth stressing that every breath test can be affected badly by severe restrictive lung disease, and in elderly patients with chronic heart failure[22].

Unfortunately, MRI findings of liver steatosis and fibrosis in NASH show moderate correlations with histopathological grade of steatosis and stage of fibrosis, but MRI findings of NASH do not demonstrate any significant correlations with MELD score[23]. Liver scintigraphy (SPECT) might be a promising noninvasive tool in the follow-up of NASH patients in therapeutic trials[24].

CONCLUSION

While liver function is absolutely complex, a widely accepted test to assess quantitative functional hepatic reserve still needs to be established, although there are various tests currently available. The diagnostic and prognostic gain has been quantified as modest. A new condition in which it may be useful to test residual liver function is acute liver disease[25]. Focusing on some aspects of controversial conclusions, or those not supported by very positive results, in the context of the current doctrine is always provocative, although it provides scientists and physicians with responsible and balanced information to support experimental and clinical decisions. Future technical advances may lead to a decrease in time, cost and the number of subjects required to perform QLFTs, therefore, their use in clinical practice is expected to increase.

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