European Review for Medical and Pharmacological Sciences 2013; 17(Suppl 2): 82-89

# Potential use of liver function breath tests in the clinical practice

L. MIELE, G. MARRONE, C. CEFALO, S. D'ACHILLE, G.L. RAPACCINI, A. GASBARRINI, A. GRIECO

Institute of Internal Medicine, School of Medicine, Catholic University of the Sacred Heart, Rome, Italy

**Abstract. – BACKGROUND:Assessment of hepatic functional reserve in acute and chronic liver disease is a discriminating factor for prognostic and therapeutic reasons. For this reason dynamic liver function tests have been developed.**

**AIM: To review the breath method with stable carbon isotopes in hepatological setting.**

**MATERIALS AND METHODS: We conducted a literature review to analyze the experimental evidence about the diagnostic potential of breath tests of liver function.**

**RESULTS: Liver breath tests are able to discriminate between healthy subjects and patients with liver cirrhosis. The use for the assessment of liver fibrosis seems to be still burdened with less capability to discriminate between intermediate stages.**

**CONCLUSIONS: Liver breath test are a promising tool for the evaluation of hepatic functional reserve, but the use of such methods in clinical practice is limited to specialized or research centers. Most extensive studies are necessary to facilitate the spread of these methods in clinical practice.**

*Keywords:*

*Breath tests, Liver function tests, Dynamic function tests, Aminopyrine, Methacetin.*

**Abbreviations:** AAR = Aspartate aminotransferase to alanine aminotransferase ratio; ABT = Aminopyrine breath test; APRI = Aspartate aminotransferase to platelet ratio; AST = Aspartate aminotransferase; Cl = Clearance; CYP = Cytocrome P 450; DOB = Delta over baseline; E = Extraction ratio; GEC-test = Galactose elimination capacity test; GSP test = Galactose single point test; HBV = Hepatitis B virus; HCV = Hepatitis C virus; IC = Inflow substance concentration; IRMS = Isotope ratio mass spectrometry; LiMAx test = Maximal liver function capacity test; MBT = Metacetin breath test; MELD = Model for end stage liver disease; MGEC test = Modified galactose elimination capacity test; NAFLD = Non alcoholic fatty liver disease; NASH = Non alcoholic steato-hepatitis; OC = Outflow substance concentration; PBC = Primary biliary cirrhosis; PBT = Phenylalanine breath test; Q = Hepatic blood flow; SER = smooth endoplasmic reticulum.

## Introduction

The liver is the crossroad of several metabolic pathways involving biosynthetic functions, detoxification of drugs and xenobiotics, nutrition and carry out endocrine and immune functions.

Multiple factors may affect the normal functions of the liver. Viral hepatitis are among the leading causes of chronic liver disease. It is estimated that almost 350-400 million people are chronically infected with HBV with different degrees of disease activity and progression[1,2] and that 130-210 million people are chronically infected with HCV[3,4]. Alcohol is considered the leading cause of advanced liver disease in Europe, causing about a third of liver cirrhosis cases, even if accurate epidemiological data are lacking[5,6]. Non alcoholic fatty liver disease (NAFLD) is probably the first cause of chronic liver disease worldwide and is closely related to diabetes and metabolic syndrome. Simple steatosis may have favorable prognosis but its complicated form, non alcoholic steato-hepatitis (NASH) may evolve to fibrosis, cirrhosis and hepatocellular carcinoma[7,8].

In the natural history of chronic liver diseases, necro-inflammatory activity leads to liver damage and hepatic fibrosis development. The development of liver fibrosis involves structural and functional disruption of the liver and is the necessary condition for the development of liver cirrhosis and hepatic dysfunction. Necroinflammatory activity can be assessed by measuring serum transaminases levels but none of the common liver function tests can assess hepatic fibrosis extent. Fibrosis



*Corresponding Author:* Luca Miele, MD; e-mail: luca.miele@rm.unicatt.it

stage assessment has prognostic value and is required for therapeutic decision in the course of chronic HBV and HCV hepatitis[9,10]. In the current medical practice the gold standard technique for the assessment of fibrosis staging is percutaneous liver biopsy. Although routinely performed this procedure is invasive with a risk of almost 1% of complications, including the risk of death. The major diagnostic limitations of liver biopsy are constituted by sampling errors and inter-individual variability in sample interpretation[11,12].

The assessment of hepatic functional reserve is a critical step in the clinical management of patients with chronic liver disease. Classical static liver function tests provide information on hepatic functional status but are unable to explore on the whole hepatic functional reserve or on the extent of hepatic fibrosis. The combination of these tests with clinical findings or other laboratory test, such as in the CHILD and MELD score, are today used in the clinical practice for patient's functional and prognostic assessment[13,14].

Over the last decades, the idea to explore the metabolic pathways by using focused probes has lead to the development of dynamic function tests. Such methods are based on the principle of hepatic clearance (Cl) of substances whose metabolism is dependent on an hepatic rate limiting step allowing to analyze specific enzyme activities and to detect liver dysfunction not yet clinically apparent[15,16].

Among liver dynamic function tests breath tests have been proposed as a promising non invasive tools for liver functional assessment in acute and chronic liver disease.

## Hepatic breath tests

The carbon element has three isotopes: $^{12}C$; $^{13}C$ and $^{14}C$. $^{12}C$ and $^{13}C$ are stable isotopes while $^{14}C$ has radioactive decay. When the breathing methodology was introduced, almost 4 decades ago, $^{14}C$ radioactive isotope labeled substrates were used but over the years the increased awareness of radiations risk and the need for testing even in the pediatric population or in women at childbearing age, has led to the introduction of carbon stable isotopes[17,18], such as $^{13}C$.

Breath tests actually used in liver functional assessment are conducted evaluating the hepatic Cl of $^{13}C$ labeled substrates. These tests are based on the principle of the "rate limiting step". The metabolism of the administered probe requires a complex series of enzymatic processes whose rate limiting reaction is controlled by hepatic enzymes. In the breath method the probe substance is administered per os and is absorbed in the mesenteric circulation reaching the liver through the portal vein. The hepatic metabolism of the probe leads to the formation of labeled carbon dioxide which diffuses into the systemic circulation and is exhaled in lungs. $^{13}C$ concentration in the exhaled air is determined via isotope-ratio mass spectrometry (IRMS) or infrared spectrometry. The $^{13}C$ isotope represents the 1,11% of all natural carbon atoms so substrate metabolism leads to an increase in the amount of $^{13}C$ usually present in exhaled air. According to this the increase over baseline values of $^{13}C$ exhaled in seriated breath samples (Delta over baseline – DOB) after substrate administration reflects the activity of the enzymatic pathway investigated.

The rate limiting reaction of labeled probe in liver breath tests may occur both in the smooth endoplasmic reticulum (SER), involving enzymes of microsomal cytochrome P-450 (CYP), both at cytosolic or mitochondrial level allowing to explore various hepatocyte functions (Table I).

In the evaluation of labeled $CO_2$ excretion it is necessary to estimate or measure the total carbon dioxide production, which is about 300 mmol per $m^2$ body surface area. $CO_2$ basal production is increased in physical activity, fever, thyroid dysfunction or food ingestion while is reduced during hypothermia, hypothyroidism and during sleep[19,20].

The shape of labeled $CO_2$ excretion curve is characterized, in healthy subjects, by an early peak, usually within the first ten minutes after substrate administration, followed by a slow and progressive reduction in the later stages of the test. Some authors have suggested an influence of bicarbonate plasma kinetics in the excretion of labeled $CO_2$ that may affect test results. Studies on larger populations are needed to understand the real role of this factor in liver breath tests[21].

From DOB measurement are derived the percentage of the administered dose recovered (PDR), cumulative percentage of the dose recovered over time (cPDR), peak excretion and time to peak. PDR is expressed as percent per hour and reflects the rate at which the substrate is metabolized while cPDR is PDR integral and represents the total percent of the substrate metabolized at any given time.

Hepatic Cl of exogenous substances depends on hepatic perfusion (Q) and substance extraction ratio (E) which is the ratio of the difference between inflow (IC) and outflow concentration (OC) and IC of the probe, according with the following formulas:

$$Cl = Q \times E; \quad E = (IC-OC)/IC.$$

**Table I.** Hepatic breath tests.

| Substrate | Enzyme | Hepatic localization |
|---|---|---|
| Aminopyrine | P450s (CYP1A2, 2C9) | Microsomial (SER) |
| Phenacetin | CYP1A2 (CYP2E1) | Microsomial (SER) |
| Metacetin | CYP1A2 | Microsomial (SER) |
| Caffeine | CYP1A2 (CYP2E1, 2B6) | Microsomial (SER) |
| Diazepam | CYP2C19 (CYP3A4) | Microsomial (SER) |
| Erythromycin | CYP3A4 | Microsomial (SER) |
| Galactose | Galactokinase | Cytosolic |
| Phenylalanine | Phenylalanine hydroxylase | Cytosolic |
| Methionine | Krebs cycle | Mitochondrial |
| A-ketoisocaproic | Acid Branched-chain alpha-keto acid dehydrogenase complex | Mitochondrial |

When the E is above 0,7 the Cl of the substrate is mainly dependent on liver blood flow while when E is below 0,3 the Cl is mostly dependent on hepatic metabolic capacity[24].

## Microsomial liver function tests

### Aminopyrine breath test

Aminopyrine breath test (ABT) was historically the first breath test introduced for the study of hepatocyte function and is currently among the most used in the evaluation of CYP activity. Aminopyrine is a low E (0.2) substance so its metabolism is independent of liver blood flow and is not affected by hepatic vascular shunts. Aminopyrine metabolism requires a two-step N-demethylation through the P450 monooxygenase complex with production of formaldehyde and amminoantipyrine. Formaldehyde is then oxidized to bicarbonate which mixes with the circulating pool and is exhaled as $^{13}CO_2$ by a percentage of 30%[22].

Over the years numerous experimental studies evaluated the ability of the ABT to provide an estimate of the "hepatic functional mass". Already in the 70s Hepner and Vesell used aminopyrine labeled with the radioactive isotope $^{14}C$ to evaluate the hepatic functional reserve in patients with different types and degrees of liver disease comparing them with healthy subjects. Patients with liver cirrhosis, non alcoholic fatty liver disease without cirrhosis and hepatitis from different etiology sowed a labeled $CO_2$ excretion which was significantly lower than healthy subjects while patients with benign cholestasis showed values comparable to controls[22]. Merkel et al[21] showed the prognostic power of ABT and that the combined assessment of Child-Pugh score and ABT results improves prognostic stratification.

In the course of HCV and HBV infection the identification of clinically unapparent hepatic dysfunction may represent a crucial factor for the start of an anti-viral therapy. For this reason, many studies have been performed to assess the reliability of ABT in the non-invasive evaluation of the extent of hepatic fibrosis in patients with chronic hepatitis[24,25].

In a comparative study with histological data, ultrasound and laboratory tests, ABT results were able to discriminate between patients without significant fibrosis and subjects with advanced fibrosis or cirrhosis with good specificity and sensitivity, presenting, however, a poor performance in patients with intermediate fibrosis stages. The authors also reported a strong correlation between patient's age and ABT result with an age-dependent decrease in amnopyrine metabolism suggesting the need for age-adjusted reference values[26].

Recently Rocco et al showed the capability of ABT to predict fibrosis progression in a cohort of 50 patients with chronic HCV infection in a long-term follow-up. All the patients in this study executed paired liver biopsy, at baseline and after a mean period of 86 months, ABT at baseline and every three years during follow up. Baseline ABT results revealed a metabolic capacity that was significantly lower in progressor than non-progressor patients and the cumulative probability of fibrosis progression resulted significantly higher in patients with baseline altered ABT test than in patient with a normal test. In non-progressors ABT values remained stable in the course of the follow up while in patient with progressive fibrosis showed a progressive and significant decrease[27].

The usefulness of the ABT is reduced in cholestatic liver diseases because substrate metabolism does not provide a biliary phase nor enterohepatic recycling and is therefore not affected by changes in bile flow. Abnormalities in aminopyrine metabolism have been reported in acute gallstones or drug related cholestasis and in advanced

stages of Primary biliary cirrhosis (PBC). In the latter condition the altered probe metabolism appears to be related to the presence of significant fibrosis that to the extent of cholestasis[28,29].

For the interpretation of ABT results some limitations and potential confounding factor have to be taken into account. The P450-dependent N-demethylation appear to depend on the age of the subject with a minimal activity in the elderly and in childhood. Moreover, the possible concomitant use of drugs with enzyme inductive effect, such as phenobarbital, glutethimide, steroids or spironolactone or drugs inhibiting the microsomal enzymatic activity, such as cimetidine, disulfiram, allopurinol or interferon, must be considered. Finally the female sex hormones seem to have a negative effect on aminopyrine metabolism[30].

There are reports in the literature of aminopyrine induced agranulocytosis[31] but no reports of adverse events with the low dose administered for the execution of the ABT are available. Pharmaceutical formulations of $^{13}$C-aminopyrine specific for the execution of the ABT are available in commerce and, in our opinion, should be preferred in performing the test.

### *Methacetin breath test*

Metacetin or N-4-methoxyphenyl acetamide, is a derivative of phenacetin that has been proposed as an alternative to aminopyrine thanks to the absence of potential toxic effects and for the minor induction by cigarette smoke and anticonvulsant drugs. Its metabolism requires a O-demethylation process in the microsomal mixed function oxidases system, mainly involving the CYP1A2, with formation of acetaminophen and labeled $CO_2$. Metacetin is a high E substance (0,8) so its metabolism may be affected by hepatic blood flow alterations and by hepatic "first-pass" effect.

In a study conducted in a cohort of healthy adult and elderly subjects, metacetin breath test (MBT) showed that the metacetin metabolizing capacity decreases with age. Following metacetin oral administration the maximum percentage of the administered dose is recovered in the first 15-30 min in both elderly and adult but the peak excretion and the percentage cumulative excretion a 120 min in elderly subjects is significantly reduced. The correction of the results taking into account the age-related variations in $CO_2$ production made more evident the reduction of metabolising capacity in the elderly[32].

$CO_2$ labeled excretion during MBT was reduced and delayed in time in patients with overt liver cirrhosis, chronic viral hepatitis and advanced PBC. In the setting of chronic hepatitis a significant difference between healthy subjects and subjects with histologically proven chronic active hepatitis or cirrhosis was reported[33]. In an Asiatic work by Liu et al MBT also demonstrated a good correlation with the common static function indices such as albumin, prothrombin time, bilirubin levels and Child-Pugh functional class. $^{13}CO_2$ excretion rate, $CO_2$ peak excretion and cumulative excretion percentage a 30 min were significantly different in the different Child-Pugh classes but cumulative excretion percentage at 120 minutes was not statistically significant between Child A and B patients[34]. Zipprich et al used MBT to evaluate the effect of $O_2$ supplementation on the enzymatic activity of the microsomal CYP system in patients with liver cirrhosis. This study confirmed the correlation between Child-Pugh classes and $^{13}CO_2$ breathing elimination during MBT and reported an improvement in metabolic capacity by $O_2$ supplementation. The authors suggest that liver microsomal metabolic activity could be affected by anemia and altered $O_2$ transport to the liver that may occur in patients with liver cirrhosis[35].

As for ABT, also for MBT, the field of greater interest is the assessment of functional status and the estimate of the extent of hepatic fibrosis in patients with chronic hepatitis. In patients with chronic HCV infection MBT is more reliable in predicting advanced fibrosis and cirrhosis than the simple biochemical parameters such as aspartate aminotransferase (AST) to platelet ratio (APRI), aspartate aminotransferase to alanine aminotransferase ratio (AAR) or Fibroindex (a score derived from platelet count, AST, and gamma globulin)[36,37]. Goetze et al reported a statistically significant difference in $CO_2$ excretion in the course of MBT between HCV subjects with mild fibrosis and those with severe fibrosis with a reduction in diagnostic accuracy in intermediate stages in the same way as reported for ABT. In the same study these authors compared two different systems of breath testing equipment: the standard IRMS and an automated continuous analysis system by nasal cannulas. No significant differences have been found between the two systems, but the authors[38] suggest a better technical performance of the automated system by reducing the risk of non-diagnostic samples. Braden et al[39] confirmed the ability of MBT to identify the presence of liver cirrhosis, even in asymptomatic Child A class patients but failed to differentiate between non-cirrhotic patients with mild fibrosis and healthy controls. Similar results have been reported by Razlan et al[40] in a popula-

tion of patients with chronic liver disease from different aetiologies. The ability in identifying subjects with or without cirrhosis was confirmed, in particular in the advanced stage (Child B and C) but in non-cirrhotic patients no correlation was found with the extent of liver fibrosis.

In 2007 Schneider et al[41] proposed a simplified test by performing a two points determination of delta over baseline of $^{13}CO_2/^{12}CO_2$ isotope ratio in exhaled air. Performing ROC analysis DOB at 15 minutes proved to be able to appropriately predict the presence of liver cirrhosis. Using a cut-off value < 14.6 δ%0 for the DOB 15 minutes, the simplified test reached 92.6% sensitivity (79.2% to 99.2%) and 94.1% specificity (88.8% to 97.5%) in identifying liver cirrohosis compared with the presence of cirrhosis on liver histology. The area under the curve was 0.974.

A new system of hepatic functional reserve evaluation based on metacetin metabolism was proposed by Stockmann et al[42] to evaluate postoperative outcome in patients undergoing liver resection. The preoperative evaluation of hepatic functional reserve is essential to prevent post-operative liver failure which is the most serious hepatectomy related complication. In this variant of MBT, called LiMAx test, the substrate is administered intravenously as a bolus to minimize potential inter-individual differences determined by variations in gastrointestinal absorption. The expired air is collected through a face mask for a real-time analysis by a modified nondispersive isotope-selective infrared spectroscopy based device. To validate the test the authors compared post surgical LiMAx results and residual liver volume finding a statistically significant correlation. LiMAx results and indocyanine green plasma disappearance rate at first post-operative day were significantly associated with liver failure and death in univariate analysis and LiMAx results at first post-operative day was associated with severe general complication. Moreover LiMAx outcome predictive power obtained by ROC analysis showed the superiority of the LiMAx respect to residual liver volumetry. The authors also showed the existence of a linear correlation between LiMAx pre and post surgical values and demonstrated the ability to estimate LiMAx results at first post-operative day on the basis of resected liver volume.

## Citosolic liver function tests

### *Galactose BT*

Galactose is an hexose sugar which is transformed into glucose by the liver. Its metabolism requires the ATP-dependent phosphorylation of galactose to galactose 1-phosphate by the galactose kinase enzyme. This cytosolic enzyme catalyzes the rate limiting steps so galactose breath test (GBT) is a cytosolic function test. When galactose blood concentration is below 50 mg/dl the extraction fraction of liver is particularly high (E> 0.8), and the information obtained is mainly dependent on liver perfusion. To evaluate the functional capacity of the liver is necessary to administer higher doses, above 50 mg/dl, to saturate the metabolic pathway.

Several liver function tests based on galactose administration are available in the clinical practice. Among these the best known is the galactose elimination capacity test (GEC-test). This test is based on the intravenous administration of a 0,5/g/kg of a galactose solution and on the subsequent determination of substrate plasma concentration through seriated blood samples. Constructing an excretion/time curve it is possible to get an estimate of the hepatic metabolizing capacity. There are also variants of this test such as the modified galactose elimination capacity test (MGEC test) which uses a different substrate dose and the galactose single point test (GSP test), which provides a single blood sample 60 min after substrate infusion[43].

In order to limit the invasiveness of the test and improve patients tolerance, GBT has been introduced. Already in studies performed in the 70's galactose metabolizing capacity was found to be reduced in cirrhotic subjects when compared with healthy controls[44]. Recently it was shown that GBT is able to distinguish between healthy subjects and cirrhotic patients and, among cirrhotic patients, between subjects in Child-Pugh class A and subjects in class B and C but it is not able to distinguish between patients in class B and C[45].

In patients with chronic HCV hepatitis GBT results are altered in the early stages and inversely correlate with the extent of liver fibrosis but no correlation was found between GBT results and histological activity[46].

In a study conducted in a small cohort of non cirrhotic patients with PBC, GBT and MBT were able to distinguish between healthy controls and PBC patients. GBT (but not MBT) correlated with histological stage assessed by liver biopsy[47].

GBT has also proved to be useful in identifying patients with galactosemia, a rare genetically determined error of galactose metabolism[48].

Galactose metabolism can be influenced by hyperglycemia and alcohol intake so these conditions have to be taken into account[49].

### *Phenylalanine BT*

Phenylalanine is an aromatic amino acid metabolized mainly by the liver in the cytosol of hepatocytes. Its metabolism requires substrate hydrolysis into tyrosine that is subsequently converted by tyrosine aminotransferase in hydroxyphenyl pyruvic acid. This substance is finally deoxygenated to omogestinic acid with $CO_2$ release.

The metabolism of branched chain amino acids is impaired in liver cirrhosis and the alteration of plasmatic levels of aromatics and non-aromatics amino acids is considered one of the factors contributing to porto-systemic encephalopathy. For these reasons phenylalanine breath test (PBT) was proposed as a dynamic liver function test[24].

Ishii et al. have shown that PBT is able to distinguish between cirrhotic patients and healthy controls. The cumulative% $^{13}C$ dose/h at 30', 45', 60', 75' and 90' were significantly different between cirrhotic and non-cirrhotic patients and the % dose/h at 30', 45', and 60' and % $^{13}C$ cumulative excretion at 45 min and later were strongly correlated with phenylalanine hydroxylase activity in the whole liver[50]. The same authors have shown a correlation between PBT results, histological activity and fibrosis staging according to METAVIR pathological score. Statistically significant differences were observed between healthy controls and patients with chronic liver disease. Among patients with liver disease significant differences were found between F1, F4 stage and other fibrosis stages suggesting a change in phenylalanine hidroxylase activity in the early stage of liver fibrosis. No significant differences were found between F2 and F3 fibrosis stages[51].

Recently Zhang et al[52] confirmed the diagnostic potential power of PBT in a geriatric cohort. % $^{13}C$ dose/h at 20' and% $^{13}C$ dose/h at 30' combined with the cumulative excretion at 60' and 120' showed significant correlation with static liver function tests and Child-Pugh score.

Hepatic encephalopathy is a clinical event with a strong impact on survival in cirrhotic patients. For this reason it has been suggested a prognostic role of PBT as an indicator of branched chain amino acids metabolism. Gallardo et al. have reported that PBT is an independent indicator of survival in patients with chronic liver disease but the prognostic role of PBT is still under debate[53].

## Liver BT in the clinical practice

Liver breath test are a promising tool for the evaluation of hepatic functional reserve in acute and chronic liver disease but the use of such methods in daily clinical practice is limited to specialized or research centers. Unlike common hepatic functional indexes and scores currently used in clinical practice, which often provide semi-quantitative information and that are sometimes based on clinical findings that may be affected by subjectivity (e.g. encephalopathy in Child-Pugh score), the liver BT provide quantitative and objective data.

All the tests described above have proved to be able to distinguish between healthy subjects and patients with liver cirrhosis, even in the early stage[22,27,38]. Therefore, liver BT have been proposed as a potentially useful method for screening in high risk populations, such as patients with viral or metabolic hepatitis, to identify the presence of clinically unapparent liver cirrhosis.

Despite numerous studies have shown the correlation between BT and liver fibrosis, the usefulness of such methods in the clinical practice is limited. Liver BT in most cases are able to discriminate stages of mild fibrosis from advanced forms but lose diagnostic power in the intermediate stages[31,43]. Intermediate stages are those in which a therapeutic decision, based on fibrosis staging, is often necessary in the course of chronic viral hepatitis. New studies are required to improve the diagnostic potential of such tests in identifying the extent of liver fibrosis. It is possible to hypothesize an improvement in diagnostic accuracy with the introduction of specific age and sex adjusted cut-off and of the analysis of plasma bicarbonate kinetics.

The major limitations to the diffusion of these methods are represented by the long sampling period and the high costs for samples analysis. The standard time sampling is 120-180 minutes for aminopyrine, metacetin and galactose and 60-120 minutes for phenylalanine. New studies are needed to overcome these limitations.

Recently some alerts on the safety of probe substances used in BT have been reported. Our opinion is that dedicated preparations for clinical diagnostic purposes should be used, preferably in pre-packaged single-dose preparations.

## Conclusions

Over several decades the diagnostic potential of liver BT is known but their use in daily clinical practice is still limited. New clinical trials able to improve the reproducibility and the interpretation of the tests can facilitate a wider and more fruitful use of such methods.

## References


1) Fattovich G. Natural history and prognosis of hepatitis B. Semin Liver Dis 2003; 23: 47-58.
2) McMahon BJ. The natural history of chronic hepatitis B virus infection. Semin Liver Dis 2004; 24(Suppl 1): 17-21.
3) Lavanchy D. The global burden of hepatitis C. Liver Int 2009; 29(Suppl 1): 74-81.
4) Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005; 5: 558-567.
5) WHO, European Status Report on Alcohol and Health 2010. Copenhagen: WHO Regional Office for Europe; 2010.
6) Roulot D, Costes JL, Buyck JF, Warzocha U, Gambier N, Czernichow S, Le Clesiau H, Beaugrand M. Transient elastography as a screening tool for liver fibrosis and cirrhosis in a community-based population aged over 45 years. Gut 2011; 60: 977-984.
7) Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology 2011; 140: 124-131.
8) Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in nonalcoholic fatty liver disease. J Hepatol 2008; 49: 608-612.
9) Schuppan D, Ruehl M, Somasundaram R, Hahn EG. Matrix as a modulator of hepatic fibrogenesis. Semin Liver Dis 2001; 21: 351-372.
10) European Association For The Study Of The Liver. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol 2012; 57: 167-185.
11) Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology 2003; 38: 1449-1457.
12) Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med 2001; 344: 495-500.
13) Malinchoc M, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. Hepatology 2000; 31: 864-871.
14) Cholongitas E, Papatheodoridis GV, Vangeli M, Terreni N, Patch D, Burroughs AK. Systematic review: The model for end-stage liver disease—should it replace Child-Pugh's classification for assessing prognosis in cirrhosis? Aliment Pharmacol Ther 2005; 22: 1079-1089.
15) Grieco A, Barone C, Coletta P, Castellano R, Ragazzoni E, Cassano A, Astone A, Gambassi G. Antipyrine metabolism in patients with liver metastases from colorectal cancer. Cancer 1992; 70: 1477-1482.
16) Merkel C, Bolognesi M, Bellon S, Bianco S, Honisch B, Lampe H, Angeli P, Gatta A. Aminopyrine breath test in the prognostic evaluation of patients with cirrhosis. Gut 1992; 33: 836-842.
17) Galizzi J, Long RG, Billing BH, Sherlock S. Assessment of the (14C) aminopyrine breath test in liver disease. Gut 1978; 19: 40-45.
18) Hepner GW, Vesell ES. Quantitative assessment of hepatic function by breath analysis after oral administration of [14C ]-aminopyrine. Ann Int Med 1975; 83: 632-638.
19) Braden B, Lembcke B, Kuker W, Caspary WF. 13C-breath tests: current state of the art and future directions. Dig Liver Dis 2007; 39: 795-805.
20) Armuzzi A, Candelli M, Zocco MA, Andreoli A, De Lorenzo A, Nista EC, Miele L, Cremonini F, Cazzato A, Grieco A, Gasbarrini G, Gasbarrini A. Breth testing fot human liver function. Aliment Pharmacol Ther 2002; 16: 1977-1996.
21) Lock JF, Taheri P, Bauer S, Holzütter HG, Malinowski M, Neuhaus P, Stockmann M. Interpretation of non-invasive breath tests using (13)C-labeled substrates—a preliminary report with (13)C-methacetin. Eur J Med Res 2009; 14: 547-550.
22) Reichen J. Assessment of hepatic function with xenobiotics. Semin Liver Dis 1995; 15: 189-201
23) Merkel C, Morabito A, Sacerdoti D, Bolognesi M, Angeli P, Gatta A. Updating prognosis of cirrhosis by Cox's regression model using Child-Pugh score and aminopyrine breath test as time-dependent covariates. Ital J Gastroenterol Hepatol 1998; 30: 276-238.
24) Giannini E, Fasoli A, Chiarbonello B, Malfatti F, Romagnoli P, Botta F, Testa E, Polegato S, Fumagalli A, Testa R. 13C-aminopyrine breath test to evaluate severity of disease in patients with chronic hepatitis C virus infection. Aliment Pharmacol Ther 2002; 16: 717-725.
25) Herold C, Berg P, Kupfal D, Becker D, Schuppan D, Hahn EG, Schneider HT. Parameters of microsomal and cytosolic liver function but not of liver perfusion predict portal vein velocity in noncirrhotic patients with chronic hepatitis C. Dig Dis Sci 2000; 45: 2233-2237.
26) Schneider AR, Teuber G, Paul K, Nikodem A, Duesterhoeft M, Caspary WF, Stein J. Patient age is a strong independent predictor of 13C-aminopyrine breath test results: a comparative study with histology, duplex-Doppler and a laboratory index in patients with chronic hepatitis C virus infection. Clin Exp Pharmacol Physiol 2006; 33: 300-304.
27) Rocco A, de Nucci G, Valente G, Compare D, D'Arienzo A, Cimino L, Perri F, Nardone G. 13C-aminopyrine breath test accurately predicts long-term outcome of chronic hepatitis C. J Hepatol 2012; 56: 782-787.
28) Herold C, Ganslmayer M, Deyet C, Hahn EG, Shuppan D. Quantitative testing of liver function compared to prognostic scores in patients with primary biliary cirrhosis. Liver 2002; 22: 159-165.
29) Burstein AV, Galambos JT. 14C aminopyrine breath test in chronic liver disease. Dig Dis Sci 1981; 9: 493.
30) Opekun AR, Klein PD, Graham DY. 13C aninopyrine breath test detects altered liver metabolism caused by low dose oral contraceptives. Dig Dis Sci 1995; 40: 2417-2422.

31) Andersohn F, Konzen C, Garbe E. Systematic review: agranulocytosis induced by nonchemotherapy drugs. Ann Intern Med 2007; 146: 657-665.

32) Ciccocioppo R, Candelli M, Di Francesco D, Ciocca F, Taglieri G, Armuzzi A, Gasbarrini G, Gasbarrini A. Study of liver function in healthy elderly subjects using the 13C-methacetin breath test. Aliment Pharmacol Ther 2003; 17: 271-277.

33) Matsumoto K, Suehiro M, Iio M, Kawabe T, Shiratori Y, Okano K, Sugimoto T. [13C] methacetin breath test for evaluation of liver damage. Dig Dis Sci 1998; 32: 344-348.

34) Liu Yun-xiang, Huang Liu-ye WU Cheng-rong, Cui Jun. Measurement of liver function for patients with cirrhosis by 13C-methacetin breath test compared with Child-Pugh score and routine liver function tests. Chinese Medical J 2006; 119: 1563-1566.

35) Zipprich A, Meiss F, Steudel N, Sziegoleit U, Fleig WE, Kleber G. 13C-Methacetin metabolism in patients with cirrhosis: relation to disease severity, haemoglobin content and oxygen supply. Aliment Pharmacol Ther 2003; 17: 1559-1562.

36) Koda M, Matunaga Y, Kawakami M, Kishimoto Y, Suou T, Murawaki Y. Fibroindex, a practical index for predicting significant fibrosis in patients with chronic hepatitis C. Hepatology 2007; 45: 297-306.

37) Dinesen L, Caspary WF, Chapman RW, Dietrich CF, Sarrazin C, Braden B. 13C-methacetin-breath test compared to also noninvasive biochemical blood tests in predicting hepatic fibrosis and cirrhosis in chronic hepatitis C. Dig Liver Dis 2008; 40: 743-748.

38) Goetze O, Selzner N, Fruehauf H, Fried M, Gerlach T, Mullhaupt B. 13Cmethacetin breath test as a quantitative liver function test in patients with chronic hepatitis C infection: continuous automatic molecular correlation spectroscopy compared to isotopic ratio mass spectrometry. Aliment Phamacol Ther 2007; 26: 305-311.

39) Braden B, Faust D, Sarrazin U, Zeuzem S, Dietrich CF, Caspary WF, Sarrazin C. 13C-methacetin breath test as liver function test in patients with chronic hepatitis C virus infection. Aliment Pharmacol Ther 2005; 21: 179-185.

40) Razlan H, Marzuki NM, Tai ML, Shamsul AS, Ong TZ, Mahadeva S. Diagnostic value of the C methacetin breath test in various stages of chronic liver disease. Gastroenterol Res Pract 2011; 2011: 235796.

41) Schneider A, Caspary WF, Saich R, Dietrich CF, Sarrazin C, Kuker W, Braden B. 13C-methacetin breath test shortened: 2-point-measurements after 15 minutes reliably indicate the presence of liver cirrhosis. J Clin Gastroenterol 2007; 41: 33-37.

42) Stockmann M, Lock JF, Riecke B, Heyne C, Martus P, Fricke M, Lehmann S, Niehues SM, Schwabe M, Lemke AJ, Neuhaus P. Prediction of postoperative outcome after hepatectomy with a new bedside test for maximal liver function capacity. Ann Surg 2009; 250: 119-125.

43) Sakka SG. Assessing liver function. Curr Opin Crit Care 2007; 13: 207-214.

44) Shreeve WW, Shoop JD, Ott DG, McInteer BB. Test for alcoholic cirrhosis by conversion of 14C or 13C galactose to expired CO2 Gastroenterology 1976; 72: 98-101.

45) Saadeh S, Behrens PW, Parsi MA, Carey WD, Connor JT, Grealis M, Barnes DS. The utility of the 13C-galactose breath test as a measure of liver function. Aliment Pharmacol Ther 2003; 18: 995-1002.

46) Mion F, Rousseau M, Scoazec JY, Berger F, Minaire Y. 13C galactose breath test: correlation with liver fibrosis in chronic hepatitis C. Eur J Clin Invest 1999; 29: 624-629.

47) Holtmeier J, Leuschner M, Schneider A, Leuschner U, Caspary WF, Braden B. 13C-methacetin and 13C-galactose breath tests can assess restricted liver function even in early stages of primary biliary cirrhosis. Scand J Gastroenterol 2006; 41: 1336-1341.

48) Berry GT, Singh RH, Mazur AT, Guerrero N, Kennedy MJ, Chen J, Reynolds R, Palmieri MJ, Klein PD, Segal S, Elsas LJ. Galactose breath testing distinguishes variant and severe galactose-1-phosphate uridyltransferase genotypes. Pediatr Res 2000; 48: 323-328.

49) Perri F, Marras RM, Ricciardi R, Quitadamo M, Andriulli A. 13C-breath tests in hepatology (cytosolic liver function). Eur Rev Med Pharmacol Sci 2004; 8: 47-49.

50) Ishii Y, Suzuki S, Kohno T, Aoki M, Kohno T, Ito A, Takayama T, Asai S. L-[1-13C] phenylalanine breath test reflects phenylalanine hydroxylase activity of the whole liver. J Surg Res 2003; 112: 38-42.

51) Ishii Y, Suzuki S, Kohno T, Aoki M, Kohno T, Ito A, Takayama T, Asai S. L-[1-13C] phenylalanine breath test reflects histological changes in the liver. J Surg Res 2003; 114: 120-125.

52) Zhang GS, Bao ZJ, Zou J, Yin SM, Huang YQ, Huang H, Qiu DK. Clinical research on liver reserve function by 13C-phenylalanine breath test in aged patients with chronic liver diseases. BMC Geriatr 2010; 10: 23.

53) Gallardo-Wong I, Moran S, Rodriguez-Lean G, Castaneda-Romero B, Poo J, Uribe M, Dehesa M. Prognostic value of 13C breath test on predicting survival in patients with chronic liver failure. World J Gastroenterol 2007; 13: 4579-4585.