Aliment Pharmacol Ther 2002; 16: 717–725.

^{13}C-Aminopyrine breath test to evaluate severity of disease in patients with chronic hepatitis C virus infection

E. GIANNINI, A. FASOLI, B. CHIARBONELLO, F. MALFATTI, P. ROMAGNOLI, F. BOTTA, E. TESTA, S. POLEGATO, A. FUMAGALLI & R. TESTA

Gastroenterology Unit and Postgraduate School of Gastroenterology and Digestive Endoscopy, Department of Internal Medicine, University of Genoa, Italy

Accepted for publication 2 November 2001

SUMMARY

Background: There are few data on the use of the ^{13}C-aminopyrine breath test to evaluate the severity of disease in patients with hepatitis C virus-related chronic liver disease, although these patients represent one of the most important problems in clinical hepatology.

Aims: To compare ^{13}C-aminopyrine breath test results of patients with hepatitis C virus-related chronic hepatitis and Child–Pugh class A cirrhosis with those of normal subjects, and to evaluate different methods of expressing ^{13}C-aminopyrine breath test results.

Methods: Twenty-four patients with hepatitis C virus-related chronic hepatitis and 17 patients with Child–Pugh class A cirrhosis underwent ^{13}C-aminopyrine breath test. Breath samples were collected every 30 min up to 2 h after ^{13}C-aminopyrine administration. ^{13}C-Aminopyrine breath test results were expressed as a percentage of the administered dose of ^{13}C recovered per hour (% dose/h) and the cumulative percentage of administered dose of ^{13}C recovered over time (% dose cum). Nineteen healthy subjects served as controls. Patients with hepatitis C virus-related chronic hepatitis were divided into subgroups on the basis of histological staging and grading.

Results: The ^{13}C-aminopyrine breath test result (% dose/h) at 30 min was significantly different among the three subgroups of subjects (normal subjects, 11.5 ± 3.5; chronic hepatitis patients, 8.1 ± 4.1; cirrhosis patients, 5.0 ± 3.1; $P < 0.0005$). Moreover, the differences between chronic hepatitis and cirrhosis patients were statistically significant ($P < 0.03$). The fibrosis score showed a significant inverse correlation with the ^{13}C-aminopyrine breath test result (% dose/h) at 30 min ($r_s = -0.409$, $P = 0.05$). The ^{13}C-aminopyrine breath test result (% dose/h) at 30 min also allowed normal subjects and chronic hepatitis patients with low (≤ 2) or high (> 2) fibrosis scores to be distinguished. The ^{13}C-aminopyrine breath test results (% dose cum) at 30, 60 and 90 min allowed discrimination between normal subjects and chronic hepatitis and cirrhosis patients. The ^{13}C-aminopyrine breath test result (% dose cum) was also able to distinguish between normal subjects and chronic hepatitis patients with high but not low fibrosis scores. Both ^{13}C-aminopyrine breath test results (% dose/h and % dose cum) at 120 min allowed the differentiation between normal subjects and chronic hepatitis patients with high (≥ 6) necro-inflammatory activity.

Conclusions: In patients with hepatitis C virus-related chronic liver disease, the ^{13}C-aminopyrine breath test proved to be safe and easy to perform, and was able to evaluate different degrees of liver function impairment which were partly correlated to clinical and histological evaluation. In future studies, ^{13}C-aminopyrine breath test results should be expressed in a standardized fashion to permit comparison.

Correspondence to: Professor R. Testa, Gastroenterology Unit, Department of Internal Medicine (D.I.M.I.), Viale Benedetto XV no. 6, 16132 Genoa, Italy. E-mail: rtesta@unige.it

INTRODUCTION

Liver function tests have been used by clinical hepatologists to refine the diagnosis, determine the severity, foresee the prognosis and monitor the therapeutic efficacy in patients with chronic liver disease. Certain conventional 'static' tests are essential to assess the presence of hepatocellular damage (e.g. aminotransferase determination),[1] and others detect the derangement of more subtle functions (e.g. α-glutathione S-transferase determination).[2] However, a single 'static' test for the global assessment of liver function does not exist due to the multiple functions accomplished by the liver (metabolic, synthetic, detoxifying), which may be affected differently in various phases of disease.[3, 4]

Multiple tests, together with clinical assessment, have proved to be useful in the evaluation of the severity and prognosis of patients with advanced liver disease.[5–7] However, both conventional 'static' tests and biochemical–clinical scores are not useful in the work-up of patients with mild disease. Indeed, chronic hepatitis may take decades to evolve towards liver cirrhosis, and biochemical parameters often do not show significant modifications for years, while, during this period, clinical examination may remain normal. Therefore, 'dynamic' function tests have been proposed to evaluate these patients. Dynamic tests, although usually exploring a specific function, provide information about the global liver function activity, which often means the blood-supplied actively functioning hepatocellular mass.[4, 8]

Among the dynamic tests, breath tests have previously been shown to provide useful information on the prognosis of patients with advanced liver disease of various aetiologies.[9–11] However, studies conducted in patients with less severe disease, such as those with chronic hepatitis, are few, and the majority have been carried out using ^{14}C-labelled substrates.[12, 13] Using non-radioactive, harmless, ^{13}C compounds, Mion *et al.* have demonstrated that galactose breath test results show good correlation with the severity of fibrosis on liver histology in patients with chronic hepatitis related to hepatitis C virus infection, which is the most common cause of liver disease in Western countries.[14] Moreover, the same authors have shown that the ^{13}C-aminopyrine breath test (^{13}C-ABT) is a simple, sensitive tool to evaluate liver function, and its results may discriminate between patients with or without cirrhosis.[15] Lastly, we have previously shown that, in patients suffering from chronic liver disease of various aetiologies, ^{13}C-ABT can discriminate between those with chronic hepatitis and those with cirrhosis.[16] Nevertheless, with regard to ^{13}C-aminopyrine breath testing, some issues still remain to be investigated in patients with chronic liver disease. Firstly, to our knowledge, there are no data in the literature concerning the evaluation of ^{13}C-ABT in patients with chronic hepatitis or cirrhosis associated with hepatitis C virus infection. Because hepatitis C virus infection is a major world-wide health, social and economic problem, tools able to evaluate liver 'status' in this situation would be valuable in order to improve the diagnostic and prognostic assessment of patients. Secondly, the method of expressing breath test results, although previously well described and validated,[17, 18] is still not standardized, thus making the comparison of results obtained in various studies difficult.

Therefore, in this study, our aims were to compare ^{13}C-ABT results between normal subjects and patients with chronic hepatitis or Child–Pugh class A cirrhosis associated with hepatitis C virus infection, in order to identify functional differences between various degrees of severity of chronic liver disease, to evaluate different methods of expressing ^{13}C-ABT results and to maximize the information obtained from the test.

METHODS

Patients

We studied 41 patients referred to our unit for the evaluation of chronic hepatitis C virus infection (serum aminotransferases greater than or equal to 1.5 times the upper normal value for at least 6 months). The anti-hepatitis C virus antibodies were detected by a third-generation enzyme immunoassay that contained hepatitis C virus antigens from the viral core and from areas of the non-structural NS3, NS4 and NS5 regions (Ortho HCV SAVe 3.0, Raritan, NJ, USA). Positivity for anti-hepatitis C virus antibodies was confirmed by a strip immunoblot assay (RIBA HCV 3.0, Chiron Corp., Emeryville, CA, USA). Positivity for hepatitis C virus RNA in serum was assessed by means of nested reverse transcription polymerase chain reaction. Patients with other concomitant causes of liver disease, such as hepatitis B virus infection, autoimmunity and alcohol abuse (alcohol intake of more than 40 g/day), and patients positive for human immunodeficiency virus were excluded from the study. Patients with Child–Pugh

class B and C cirrhosis and those with hepatocellular carcinoma were also excluded. None of the patients had previously received interferon therapy and none were taking drugs known to interfere with liver function.

On admission, all patients underwent biochemical evaluation of serum that included blood cell count, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltranspeptidase, alkaline phosphatase, total bilirubin, albumin and prothrombin activity. Routine biochemical tests were carried out using commercially available kits.

Twenty-four patients had chronic hepatitis and 17 had Child–Pugh class A cirrhosis. The diagnosis of chronic hepatitis was made histologically by means of percutaneous liver biopsy using the Menghini technique. The liver biopsy specimen was then formalin-fixed and paraffin-embedded. Histopathological evaluation was carried out by application of Ishak *et al.*'s histological score[19] by an experienced liver pathologist unaware of the clinical data of the patients. Ten of the 17 Child–Pugh class A cirrhotic patients had undergone a liver biopsy showing the presence of cirrhosis no longer than 2 years before the study. In the remainder, cirrhosis was diagnosed on the basis of clinical signs of portal hypertension, Doppler ultrasonographic measurements and/or the endoscopic presence of oesophageal or gastric varices. Nineteen healthy volunteers (11 males and eight females; mean age, 54 ± 9 years; height, 169 ± 9 cm; weight, 69 ± 17 kg) served as controls (normal subjects). Age, gender and anthropometric measurements were no different between the normal subjects and patients with chronic liver disease. All had normal liver ultrasound results and routine liver function tests (aspartate aminotransferase, alanine aminotransferase, γ-glutamyltranspeptidase, alkaline phosphatase, total bilirubin, albumin and prothrombin activity), and none had a history of previous or active liver disease.

^{13}C-ABT

All patients and normal subjects underwent ^{13}C-ABT as follows: a basal breath sample was collected after an overnight (at least 12 h) fast; then 2 mg/kg of ^{13}C-aminopyrine (*N*,*N*-dimethyl-^{13}C-aminopyrine; Euriso-Top Carbon^{13} Breath Tests Substrates, Saint Aubin, France, supplied by Cortex Italia, Milan, Italy) was dissolved in 200 mL of water and administered orally. Breath samples were collected every 30 min for 2 h after ^{13}C-aminopyrine administration and were obtained as follows: patients were asked to exhale for 10 s through a small plastic tube directly into a vial that was immediately sealed. The ratio of $^{13}CO_2$ to $^{12}CO_2$ was determined for each sample with an isotope ratio mass spectrometer (Breath Mat, Finnigan, Bremen, Germany) and the excess $^{13}CO_2$ was calculated by the increase in the isotope ratio. The δ value obtained was converted to the percentage of ^{13}C and the results were expressed as the percentage of the administered dose of ^{13}C recovered per hour (% dose/h) and the cumulative percentage of the administered dose of ^{13}C recovered over time (% dose cum). Simplified formulae for calculating both % dose/h and % dose cum were used.[20] The production of CO_2 was estimated on the basis of the body surface area, assuming a CO_2 production of 5 mmol.min/m^2.[21] Patients were at rest for 15 min before the test, and remained at rest and fasted during the whole test to minimize their total CO_2 production and to avoid the influence of food intake. The reproducibility of ^{13}C-ABT was assessed weekly on five healthy volunteers (coefficient of variation, 5.6%).[22]

The ^{13}C-ABT results of chronic hepatitis patients were analysed according to the histological staging and grading. Briefly, after excluding patients with histological findings of cirrhosis, a median grading score was used to subdivide chronic hepatitis patients into two subgroups with low (score, < 6) or high (score, ≥ 6) necro-inflammatory activity. The analysis of patients with different staging was carried out by grouping together patients with fibrosis scores of 1 and 2 and those with fibrosis scores in the range 3–6. The reason for subdividing patients into these two groups was that the latter group had histological lesions with different diagnostic and prognostic indications (i.e. the formation of portal-to-portal bridging).

Statistical analysis

Statistical analysis was carried out using the chi-squared test to compare proportions and the Mann–Whitney *U*-test to compare means. The Kruskal–Wallis test was used to compare ^{13}C-ABT results among different subgroups of patients. Correlations between the ^{13}C-ABT results and histological scores were carried out using the Spearman rank correlation (r_s). Data are shown as the mean ± s.d. $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Table 1 shows the main characteristics of the patients with chronic hepatitis and Child–Pugh class A cirrhosis. On average, cirrhotic patients were older than chronic hepatitis patients. No differences were observed in gender or anthropometric measures. There were no differences in common biochemical liver tests between chronic hepatitis and cirrhosis patients. All patients were viraemic at the time of the study. Lastly, none of the chronic hepatitis patients had a fibrosis score of zero. The ^{13}C-ABT results of chronic hepatitis patients were analysed according to histological grading (scores, < 6 vs. ≥ 6) and staging (scores, 1–2 vs. > 2).

^{13}C-ABT results (% dose/h)

The characteristic curves of the mean percentage dose per hour in normal subjects, chronic hepatitis patients and Child–Pugh class A cirrhotic patients are shown in Figure 1. The ^{13}C-ABT result (% dose/h) at 30 min was significantly different among the three subgroups of subjects ($P < 0.0005$). At this sampling time, normal subjects had ^{13}C-ABT results (11.5 ± 3.5) that were significantly higher than those of both chronic hepatitis (8.1 ± 4.1) and cirrhosis (5.0 ± 3.1) patients, and the difference between chronic hepatitis and cirrhosis patients was statistically significant ($P < 0.03$). The difference between the ^{13}C-ABT results of normal subjects and the two subgroups of patients with different degrees of liver disease was maintained at the subsequent sampling times, but the difference between chronic hepatitis and cirrhosis patients was no longer observed.

The fibrosis score showed a significant inverse correlation with the ^{13}C-ABT result (% dose/h) at 30 min ($r_s = -0.409$, $P = 0.05$) in chronic hepatitis patients.

Table 2 shows the mean ^{13}C-ABT results of normal subjects and patients with chronic hepatitis subdivided according to fibrosis score (1–2 vs. > 2). At all sampling times, normal subjects could be differentiated from chronic hepatitis patients with high fibrosis scores. The 30 min sample discriminated between patients with different degrees of fibrosis ($P < 0.02$). Child–Pugh class A cirrhotic patients showed significantly different mean ^{13}C-ABT results (% dose/h) from those of chronic hepatitis patients with low fibrosis scores at sampling times of 30, 60 and 90 min, but not from those with high fibrosis scores (data not shown).

The mean ^{13}C-ABT result (% dose/h) of normal subjects was significantly different from that of chronic hepatitis patients with high necro-inflammatory activity (score, ≥ 6) at 120 min (8.1 ± 2.1 vs. 5.1 ± 2.4; $P = 0.04$; Kruskal–Wallis test). All other differences were not statistically significant.

^{13}C-ABT results (% dose cum)

The curves of the mean cumulative percentage dose of normal subjects and patients with chronic hepatitis and cirrhosis are shown in Figure 2. The 30 min

Table 1. Main characteristics of normal subjects, chronic hepatitis patients and patients with Child–Pugh class A liver cirrhosis

Variable	Chronic hepatitis	Cirrhosis class A	*P*
Age (years)	49 ± 10	60 ± 11	< 0.005
Gender (male/female)	21/3	14/3	N.S.
Height (cm)	168 ± 21	170 ± 8	N.S.
Weight (kg)	79 ± 23	71 ± 7	N.S.
AST (IU/mL)	83 ± 59	112 ± 66	N.S.
ALT (IU/mL)	126 ± 71	130 ± 118	N.S.
GGT (IU/mL)	62 ± 42	99 ± 65	N.S.
AP (IU/mL)	181 ± 43	221 ± 60	N.S.
BIL (mg/dL)	0.7 ± 0.3	1.0 ± 0.4	N.S.
ALB (g/dL)	4.3 ± 0.5	4.1 ± 0.4	N.S.
PA (%)	88 ± 10	84 ± 8	N.S.

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl-transpeptidase; AP, alkaline phosphatase; BIL, total bilirubin; ALB, albumin; PA, prothrombin activity.
Data are shown as the mean ± s.d.
Statistical analyses were carried out by means of the chi-squared test and Mann–Whitney *U*-test.

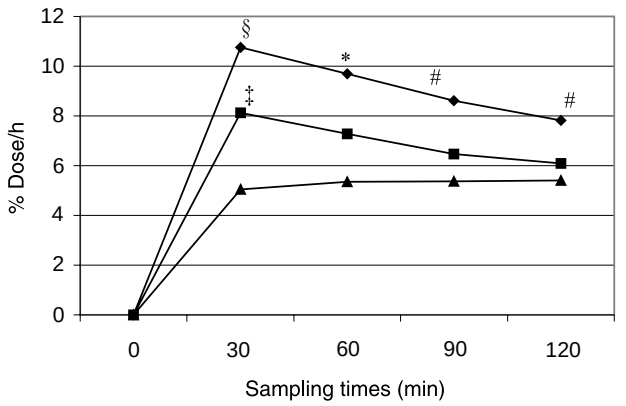

Figure 1. Mean percentage dose per hour in normal subjects (◆), chronic hepatitis patients (■) and Child–Pugh class A cirrhotic patients (▲). Differences were evaluated by Kruskal–Wallis test with internal comparisons. §$P < 0.0005$ compared to chronic hepatitis and cirrhosis. ‡$P < 0.03$ compared to cirrhosis. *$P < 0.0005$ compared to chronic hepatitis and cirrhosis. #$P < 0.005$ compared to chronic hepatitis and cirrhosis.

(normal subjects, 1.6 ± 0.6; chronic hepatitis patients, 1.2 ± 1.1; cirrhosis patients, 0.6 ± 0.4), 60 min (normal subjects, 4.3 ± 1.4; chronic hepatitis patients, 3.1 ± 1.6; cirrhosis patients, 1.9 ± 1.1) and 90 min (normal subjects, 6.7 ± 2.1; chronic hepatitis patients, 4.7 ± 2.2; cirrhosis patients, 3.2 ± 1.6) samples could be used to discriminate between the three groups of subjects, while the 120 min sample was significantly different between normal subjects (8.9 ± 2.6) and both subgroups of patients with liver disease, but not between chronic hepatitis (6.2 ± 2.8) and cirrhosis (4.6 ± 2.2, $P = 0.06$) patients.

Table 3 shows the mean ^{13}C-ABT results (% dose cum) of normal subjects and patients with different degrees of liver fibrosis. All sampling times could be used to differentiate between normal subjects and chronic hepatitis patients with high fibrosis scores, but not from those with low fibrosis scores. Chronic hepatitis patients with low fibrosis scores showed different ^{13}C-ABT results (% dose cum) at 120 min compared to chronic hepatitis patients with high fibrosis scores ($P = 0.001$). Lastly, all sampling times could be used to differentiate Child–Pugh class A cirrhosis patients from chronic hepatitis patients with low fibrosis scores, but not from those with high fibrosis scores (data not shown).

The mean ^{13}C-ABT result (% dose cum) of normal subjects was significantly different from that of chronic hepatitis patients with high necro-inflammatory activity (score, > 6) at a sampling time of 120 min (8.9 ± 2.6 vs. 6.3 ± 2.4; $P = 0.03$; Kruskal–Wallis). All other differences were not statistically significant.

DISCUSSION

This study shows that the analysis of ^{13}C-ABT results can be used to delineate characteristic behaviour in patients with different degrees of chronic liver disease. We confirmed previous results showing that $^{13}CO_2$ excretion in normal subjects has an early peak and a rapid disappearance rate, while in cirrhotics there is a late, blunted peak followed by a slowly declining phase. Moreover, in this study, we showed that chronic hepatitis patients have a $^{13}CO_2$ excretion curve which is shaped similarly to that of normal subjects, although with lower values.

The results of this study indicate a possible role for ^{13}C-ABT in patients with hepatitis C virus-related chronic liver disease. The relationships observed between the ^{13}C-ABT results and the severity of disease suggest that it may be used as a diagnostic tool. Indeed, we observed that the ^{13}C-ABT results are progressively impaired as the severity of disease increases. In our

Table 2. Comparison of ^{13}C-aminopyrine breath test (^{13}C-ABT) results (% dose/h) between normal subjects and chronic hepatitis patients with different degrees of fibrosis

	^{13}C-ABT (% dose/h)			
	30 min	60 min	90 min	120 min
Normal subjects	11.5 ± 3.5*	10.3 ± 3.4†	9.1 ± 3.0†	8.1 ± 2.1†
CH (fibrosis 1–2)	10.2 ± 4.8‡	8.6 ± 4.1	7.6 ± 3.3	6.9 ± 2.5
CH (fibrosis > 2)	6.0 ± 1.6*‡	5.9 ± 2.0†	5.4 ± 2.1†	5.3 ± 1.9†

CH, chronic hepatitis.
Data are shown as the mean ± s.d.
Differences were evaluated by Kruskal–Wallis test with internal comparisons.
*$P < 0.001$ and †$P = 0.004$ compared to CH patients with fibrosis score > 2.
‡$P < 0.02$ compared to CH patients with fibrosis score > 2.

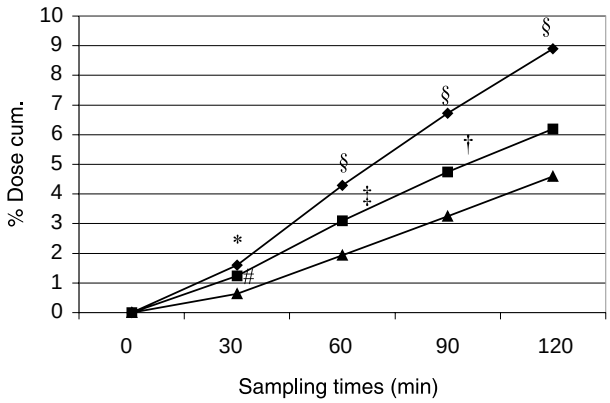

Figure 2. Mean cumulative percentage dose in normal subjects (◆), chronic hepatitis patients (■) and Child–Pugh class A cirrhotic patients (▲). Differences were evaluated by Kruskal–Wallis test with internal comparisons. *$P < 0.0005$ and §$P < 0.001$ compared to chronic hepatitis and cirrhosis. #$P < 0.01$, ‡$P < 0.02$ and †$P = 0.04$ compared to cirrhosis.

series, ^{13}C-ABT was able to discriminate between normal subjects and chronic hepatitis patients, between chronic hepatitis patients with low or high fibrosis scores, and between chronic hepatitis patients and Child–Pugh class A cirrhotics. The latter finding has clinical significance as these two clinical entities are often difficult to distinguish on a clinical–biochemical basis, thus requiring histological or instrumental evaluation to refine the diagnosis. Lastly, in chronic hepatitis patients, the ^{13}C-ABT results are related to the degree of liver fibrosis, and ^{13}C-ABT shows a discrete correlation with histological staging.

Nevertheless, although these initial results are promising, we are aware that the presence of overlap in the ^{13}C-ABT results between different degrees of chronic liver disease may represent a possible drawback of the test. We believe that, at present, it should be used cautiously in the 'first diagnosis' assessment, although the difference observed between the ^{13}C-ABT results of patients with low and high fibrosis scores may suggest its use as a complement to initial biopsy and during the follow-up of patients who are not suitable for, or have not responded to, anti-viral therapy in order to avoid repeated biopsy simply to monitor the evolution of disease. Moreover, it may be a useful tool to stage disease in patients who cannot, or do not want to, undergo liver biopsy.[23]

Patients infected with hepatitis C virus are an increasing health and social burden,[24] and most infected patients have minimal or mild disease. These patients are usually not considered for anti-viral therapy and have a life expectancy which is no different from that of the general population,[25] as the disease follows a long course. Nevertheless, in this study, we have shown that chronic hepatitis patients with minimal fibrosis have a certain degree of liver function impairment compared to healthy subjects. Because, during their lifetime, these patients may need liver-metabolized or hepatotoxic drugs for comorbidities, they could be exposed to a higher risk of adverse reactions or of impairment of liver function. In this field, non-invasive evaluation of liver function can provide useful information in the global assessment of patients, e.g. to adjust the dosage of drugs with a low therapeutic index or to assess the presence of concomitant drug-induced liver damage. Thus, on the basis of previous results obtained using either ^{13}C-ABT or ^{14}C-ABT to monitor liver function during multiple therapy in normal subjects,[22] or specific therapies in patients with chronic liver disease,[26, 27] we suggest that ^{13}C-ABT can play a role in therapeutic drug monitoring.

Table 3. Comparison of ^{13}C-aminopyrine breath test (^{13}C-ABT) results (% dose cum) between normal subjects and chronic hepatitis patients with different degrees of fibrosis

	^{13}C-ABT (% dose cum)			
	30 min	60 min	90 min	120 min
Normal subjects	1.6 ± 0.6*	4.3 ± 1.4§	6.7 ± 2.1‡	8.9 ± 2.6*
CH (fibrosis 1–2)	1.3 ± 0.6	3.6 ± 1.7	5.5 ± 2.6	7.3 ± 3.3†
CH (fibrosis > 2)	1.2 ± 1.6*	2.6 ± 1.4§	3.9 ± 1.5‡	5.0 ± 1.5*†

CH, chronic hepatitis.
Data are shown as the mean ± s.d.
Differences were evaluated by Kruskal–Wallis test with internal comparisons.
*†$P = 0.001$.
‡$P = 0.004$.
§$P = 0.01$.

Lastly, ^{13}C-ABT can be used in patients with chronic liver disease as a surrogate end-point marker in controlled clinical trials.[28]

The results of our study led us to agree partly with Hofmann, who stated that quantitative liver function tests '…cannot indicate the extent of inflammation or the degree of fibrosis'.[29] In fact, we found that ^{13}C-ABT showed good discriminating capability between normal subjects and patients with different degrees of chronic liver disease. However, although we observed that chronic hepatitis patients with mild or severe fibrosis showed different ^{13}C-ABT results, the same finding did not apply to patients with different degrees of necro-inflammatory activity. This result underscores the fact that, in chronic hepatitis patients, liver function as expressed by ^{13}C-ABT only partly reflects the histological severity of disease. The ^{13}C-ABT results are progressively impaired as liver histology worsens, although they express a method of evaluating liver 'status' (i.e. the hepatocellular functioning mass) which is different from, but linked to, histological grading or staging. Therefore, we agree with Scheuer, who states that: 'A grade of 2 for …interface hepatitis is not exactly half way between 1 and 3; it is merely, in the opinion of the pathologist doing the grading, more than 1 and less than 3.'[30] Thus, we suggest that ^{13}C-ABT could be used to fill this gap, and could be employed as a complementary tool in histological or biochemical–clinical evaluations in the assessment of patients with hepatitis C virus-related chronic liver disease.

Lastly, this study allowed us to draw some methodological conclusions. Although, two decades ago, Schneider *et al.* and Schoeller *et al.* suggested and validated an easy method of expressing ^{13}C-ABT results,[17, 18] successive and recent studies using both ^{14}C-ABT and ^{13}C-ABT have evaluated other parameters, thus making it difficult to compare the results. In agreement with Schneider *et al.* and Schoeller *et al.*, we believe that the percentage dose per hour and the cumulative percentage dose should be the parameters used to correctly express ^{13}C-ABT results. In fact, they should represent an index of the first pass metabolic activity (% dose/h) and the global metabolic capacity (% dose cum), thus providing an overall picture of the metabolic function of the liver. With regard to the sampling times, we have shown that a few samples over 2 h are sufficient to discriminate between normal subjects and patients with hepatitis C virus-related chronic hepatitis or Child–Pugh class A cirrhosis, avoiding the prolonged collection of samples. Moreover, we suggest that the test may be shortened so as to facilitate patient compliance. Indeed, we found that the ^{13}C-ABT result (% dose/h) at 30 min: (i) best discriminated between normal subjects, chronic hepatitis patients and Child–Pugh class A cirrhotics; (ii) was able to discriminate between normal subjects and chronic hepatitis patients with minimal or severe fibrosis, and between these two degrees of liver disease; and (iii) showed significant correlation with histological staging. Although Schoeller *et al.* stated that a 60 min sampling time (% dose/h) was preferred due to the possible influence of delayed gastric emptying in some patients,[18] we feel that this influence need not be taken into account in patients fasted for at least 12 h as in our study. This topic has been strongly debated and agreement is hard to find: some authors believe that early breath sampling is adequate for the evaluation of liver function in patients with chronic liver disease,[31, 32] while others believe that shortening of the test results in a loss of information.[33] We believe that quantitative liver function testing should aim to shorten tests, in order to simplify their broader application and maximize patient compliance. Nevertheless, prolonged sample collection (up to 2 h) can be performed when there is a need to evaluate the global drug metabolic capacity of the liver or drug interactions, due to the possible presence of delayed, although preserved, aminopyrine metabolization. Thus, we propose that the percentage dose per hour at 30 min should be used when ^{13}C-ABT is employed for diagnosis, but the cumulative percentage dose at 120 min should be employed when using the test to evaluate global liver metabolic capacity.

To conclude, our findings underscore the fact that clinical–histological and functional evaluations of patients with hepatitis C virus-related chronic liver disease should not be considered to be mutually exclusive; rather, their use should be optimized in order to provide the best information regarding the diagnosis, prognosis and follow-up of patients so as to improve their care. In patients with hepatitis C virus-related chronic liver disease, ^{13}C-ABT proved to be safe and easy to perform; it was able to evaluate different degrees of liver function impairment, which are partly correlated to clinical–histological evaluation. Although liver biopsy remains the gold standard technique for the evaluation of patients with hepatitis C virus-related

chronic liver disease, ^{13}C-ABT can be used in particular subsets of patients as a surrogate to liver biopsy. Lastly, we suggest that, in future studies, ^{13}C-ABT results should be expressed homogeneously so as to permit their comparison.

REFERENCES

1 Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. Clin Chem 2000; 46: 2027–49.

2 Giannini E, Risso D, Ceppa P, *et al.* Utility of alpha-glutathione S-transferase assessment in chronic hepatitis C patients with near normal alanine aminotransferase levels. Clin Biochem 2000; 33: 297–301.

3 Reichen J. MEGX test in hepatology: the long-sought ultimate quantitative liver function test? J Hepatol 1993; 19: 4–7.

4 Jalan R, Hayes PC. Review article: quantitative tests of liver function. Aliment Pharmacol Ther 1995; 9: 263–70.

5 Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973; 60: 646–64.

6 Ricci P, Therneau TM, Malinchoc M, *et al.* A prognostic model for the outcome of liver transplantation in patients with cholestatic liver disease. Hepatology 1997; 25: 672–7.

7 Kamath PS, Wiesner RH, Malinchoc M, *et al.* A model to predict survival in patients with end-stage liver disease. Hepatology 2001; 33: 464–70.

8 Oellerich M, Armstrong VW. The MEGX test: a tool for the real-time assessment of hepatic function. Ther Drug Monit 2001; 23: 81–92.

9 Schneider JF, Baker AL, Haines NW, Hatfield G, Boyer JL. Aminopyrine N-demethylation: a prognostic test of liver function in patients with alcoholic liver disease. Gastroenterology 1980; 79: 1145–50.

10 Urbain D, Muls V, Thys O, Ham HR. Aminopyrine breath test improves long-term prognostic evaluation in patients with alcoholic cirrhosis in Child classes A and B. J Hepatol 1995; 22: 179–83.

11 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: 276–82.

12 Herold C, Berg P, Kupfal D, *et al.* 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: 2233–7.

13 Herold C, Heinz R, Niedobitek G, Schneider T, Hahn EG, Schuppan D. Quantitative testing of liver function in relation to fibrosis in patients with chronic hepatitis B and C. Liver 2001; 21: 260–5.

14 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: 624–9.

15 Mion F, Queneau PE, Rousseau M, Brazier JL, Paliard P, Minaire Y. Aminopyrine breath test: development of a 13C-breath test for quantitative assessment of liver function in humans. Hepato-Gastroenterology 1995; 42: 931–8.

16 Fasoli A, Giannini E, Botta F, *et al.* $13CO_2$ excretion in breath of normal subjects and cirrhotic patients after 13C-aminopyrine oral load. Comparison with MEGX test in functional differentiation between chronic hepatitis and liver cirrhosis. Hepato-Gastroenterology 2000; 47: 234–8.

17 Schneider JF, Schoeller DA, Nemchausky B, Boyer JL, Klein P. Validation of $^{13}CO_2$ breath analysis as a measurement of demethylation of stable isotope labeled aminopyrine in man. Clin Chim Acta 1978; 84: 153–62.

18 Schoeller DA, Baker AL, Monroe PS, Krager PS, Schneider JF. Comparison of different methods of expressing results of the aminopyrine breath test. Hepatology 1982; 2: 455–62.

19 Ishak K, Baptista A, Bianchi L, *et al.* Histological grading and staging of chronic hepatitis. J Hepatol 1995; 22: 696–9.

20 Ghoos Y. $^{13}CO_2$-Breath Tests at the Laboratory 'Digestion–Absorption'. Leuven: University Hospital Gasthuisberg, 1996: 43–54.

21 Haycock G, Shwartz G, Wisotsky D. Geometric method for measuring body surface area. J Pediatr 1978; 93: 62–6.

22 Giannini E, Romagnoli P, Fasoli A, *et al.* Influence of *Helicobacter pylori* eradication therapy on 13C aminopyrine breath test: comparison among omeprazole-, lansoprazole-, or pantoprazole-containing regimens. Am J Gastroenterol 2000; 95: 2762–7.

23 Keeffe EB. Too many liver biopsies in patients with chronic hepatitis C? www.GastroHep.com. http://www.gastrohep.com/freespeech/freespeech.asp?id = 48, 12 July 2001.

24 Kim WR, Gross JB Jr, Poterucha JJ, Locke GR 3rd, Dickson ER. Outcome of hospital care of liver disease associated with hepatitis C in the United States. Hepatology 2001; 33: 201–6.

25 Alter HJ, Seeff LB. Recovery, persistence, and sequelae in hepatitis C virus infection: a perspective on long-term outcome. Semin Liver Dis 2000; 20: 17–35.

26 Reichen J, Bianchi L, Frei PC, Male PJ, Lavanchy D, Schmid M. Efficacy of steroid withdrawal and low-dose interferon treatment in chronic active hepatitis B. Results of a randomized multicenter trial. Swiss Association for the Study of the Liver. J Hepatol 1994; 20: 168–74.

27 Horsmans Y, Brenard R, Geubel AP. Short report: interferon-alpha decreases ^{14}C-aminopyrine breath test values in patients with chronic hepatitis C. Aliment Pharmacol Ther 1994; 8: 353–5.

28 Lotterer E, Hogel J, Gaus W, Fleig WE, Bircher J. Quantitative liver function tests as surrogate markers for end-points in controlled clinical trials: a retrospective feasibility study. Hepatology 1997; 26: 1426–33.

29 Hofmann AF. The role of breath test in liver function testing. In: Perri F, Andriulli A, eds. Clinical Applications of Breath Tests in Gastroenterology. Rome: International University Press, 1998: 47–51.

30 Scheuer PJ. Grading and staging of chronic hepatitis: a trap for the unwary. www.GastroHep.com. http://www.gastrohep.com/freespeech/freespeech.asp?id = 24, 16 March 2001.

31 Horsmans Y, Lejeune D, Geubel AP, Otte JB, Pauwels S. Hepatic [14C]aminopyrine demethylation capacity after portocaval shunting. Comparative study in patients with and without arterialization of portal vein. Dig Dis Sci 1993; 38: 2177–82.
32 Miotti T, Bircher J, Preisig R. The 30-minute aminopyrine breath test: optimization of sampling times after intravenous administration of 14C-aminopyrine. Digestion 1988; 39: 241–50.
33 Perri F, Pastore M, Andriulli A. Aminopyrine breath test. Dig Dis Sci 1994; 39: 2445–7.