



## Original article

## The <sup>13</sup>C-Aminopyrine Breath Test Predicts Advanced Fibrosis in Patients with Chronic Hepatitis C: A Pilot Study

Soliman, R.<sup>1</sup>, Shiha, G.<sup>1,2</sup>, El-Basiouny, M.<sup>1,2</sup>, Mikhail, NNH.<sup>1,3</sup>,

<sup>1</sup>Egyptian Liver Research Institute and Hospital (ELRIH), Sherbin, El-Mansoura, Egypt

<sup>2</sup>Hepatology & Gastroenterology unit, Internal Medicine Department, Faculty of Medicine, El-Mansoura University, Egypt

<sup>3</sup>Department of Biostatistics, South Egypt Cancer Institute, Assiut University, Egypt

\* E-mail: [g\\_shiha@hotmail.com](mailto:g_shiha@hotmail.com)

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### Abstract:

*13C Aminopyrine breath test (13C-ABT) is a dynamic function test based on the use of a labelled substrate that selectively metabolized by the liver which is useful in the evaluation of hepatocyte function providing a quantitative information on liver function activity. A significant inverse correlation between C13-ABT results and liver fibrosis was found. In this study we investigate the accuracy of 13C-ABT as non-invasive method for identification of liver fibrosis compared to other non-invasive methods. Thirty-six chronic hepatitis C patients were recruited from the outpatient clinic of the Egyptian Liver Research Institute and Hospital (ELRIAH). Percutaneous liver biopsy and routine blood sampling were performed at the same day. FibroScan and 13C-ABT were done. Liver fibrosis showed strong correlation with FibroScan ( $r=0.717$ ,  $p<0.001$ ) and significant inverse correlation with 13C-ABT results, especially at 30 min. ( $r= -0.371$ ,  $p=0.026$ ). Significant correlation was found with FIB-4 ( $r=0.350$ ,  $p=0.039$ ) and not significant correlation was found with APRI ( $r=0.261$ ,  $p=0.240$ ). The cut-off value of 3.15% (dose/hr at 30 min) was associated with high prediction (positive predictive value, 60%; negative predictive value, 92.3%) yielding an overall sensitivity of 75% and specificity of 85.7%. As regards the 13C-ABT % cum. dose at 120 min, a cut-off value of 4.35 was also associated with high accuracy (PPV=47.1%, NPV=100%, Sensitivity=100% and Specificity=67.9%). These results of high accuracy is comparable to FibroScan and FIB-4, and is better than the results of APRI. 13C-aminopyrine breath test is a potential biomarker for advanced fibrosis that warrants further validation.*

### Keywords:

<sup>13</sup>C-Aminopyrine Breath

Test

Chronic hepatitis C

FibroScan, FIB-4

APRI

**Abbreviations:** 13C-ABT; <sup>13</sup>C-Aminopyrine Breath Test. APRI; Aspartate aminotransferase to platelet ratio index. AST; Aspartate Transaminase. AUROC; Area Under Receiver Operator Curve. BMI; Body Mass Index. CHC; Chronic Hepatitis C. HCV; Hepatitis C Virus. kPa; Kilopascals. LB; Liver Biopsy. NPP; Negative Predictive Value. Plt; Platelets. PPV; Positive Predictive Value. ROC; Receiver Operator Characteristic curve. SD; Standard Deviation. SPSS; Statistical Package for Social Sciences. WBCs; White Blood Cells.

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## 1. Introduction

Prognosis and management of chronic hepatitis C (CHC) patients is influenced by the degree of liver fibrosis, which is an important parameter to assess the risk of disease progression and immediate antiviral therapy [1,2]. Liver biopsy (LB) is the gold standard for evaluation of hepatic fibrosis and provides information about inflammation, necrosis and steatosis, however, LB has limitations being invasive and lead to complications as bleeding, pain, infection and injury of nearby organ making it unsuitable for patient monitoring [3-5]. Therefore there is a need for accurate non-invasive methods for assessment of liver fibrosis stage. Non-invasive approach can be identified by direct markers of fibro genesis or indirect markers of fibrosis and imaging methods. Indirect markers are obtained by combining routinely performed blood tests to calculate scores as aspartate aminotransferase (AST) to platelet ratio index (APRI) or FIB-4. Imaging methods refer to sonography and transient elastography (FibroScan) [6]. Liver function can be evaluated using <sup>13</sup>C breath tests that explore liver Cytochrome P450 activity. Aminopyrine is one of the first compounds used in liver function testing [7]. <sup>13</sup>C Aminopyrine test (13C-ABT) is a dynamic function test based on the use of a labelled substrate that selectively metabolized by the liver which is useful in the evaluation of hepatocyte function providing a quantitative information on liver function activity .The use of <sup>13</sup>C breath tests for non-invasive assessment of liver function in patient with chronic liver disease is not new. Giannini et al [8] compared <sup>13</sup>C-ABT measurements of CHC Child-Pugh class A cirrhosis patients to normal subjects and <sup>13</sup>C-ABT was able to distinguish different degrees of liver function impairment. Giannini et al [9] found that in patients

with chronic liver disease, <sup>13</sup>C-ABT is useful for the diagnosis of cirrhosis. A significant inverse correlation between <sup>13</sup>C ABT results and liver fibrosis was found [10]. In this study we investigate the accuracy of <sup>13</sup>C-ABT as non-invasive method for identification of liver fibrosis compared to other non-invasive methods.

## 2. Methods

Thirty-six chronic hepatitis C patients were recruited from the outpatient clinic of the Egyptian Liver Research Institute and Hospital (ELRIAH) during the period from September 2014 to September 2015 after having obtained informed consent form. Chronic hepatitis C was defined by the presence of serum anti-hepatitis C virus (HCV) antibodies and serum HCV RNA. Patients with other etiologies of chronic hepatitis, such as chronic hepatitis B, NASH, auto immune hepatitis, alcoholic liver disease and hemochromatosis were excluded. Percutaneous liver biopsy and routine blood sampling were performed at the same day. FibroScan and <sup>13</sup>C Aminopyrine breath tests were done.

### 2.1. Liver histology and quantification of liver fibrosis

Liver biopsy was carried out using a 16-gauge Tru-Cut needle biopsy. Tissue specimens obtained by liver biopsy were fixed immediately in 10% formalin solution and sent to the pathologist at the same day. The stage of fibrosis and grade of inflammatory activity in the liver were determined according to the METAVIR scoring system [11]. All biopsy specimens were examined by experienced pathologists who were blinded to the clinical data and the measurements of liver stiffness.

### 2.2. Transient Elastography

Liver stiffness was also measured by transient elastography (FibroScan; Echosens SA, Paris, France). Ten successful acquisitions were performed on each patient. The results that obtained

ten valid measurements with a success rate of at least 60% and an interquartile range under 30% were considered successful. A median of 10 valid measurements was regarded as the liver stiffness for a given subject, and expressed in kilopascals (kPa).

### **2.3. 13C-Aminopyrine Breath Test**

The patient was fasted for 12 hours prior to the test. Smoking, carbonated water or soft drinks are prohibited the day before the test. Zero (basal) breath sample is collected then the patient takes 13C-Aminopyrine (75 mg) dissolved in warm water (100 ml). Additional breath samples were collected every ten minutes for one hour and every twenty minutes for another hour. Patients were asked to exhale for 10 seconds through a small plastic tube directly into a vial that was immediately sealed. All 10 breath samples were analyzed with Infra-red Isotope analyzer (IRIS), the results were expressed as the percentage of the administered dose of 13C recovered per hour (% dose/h) and the cumulative percentage of the administered dose of 13C recovered over time (% dose cum).

### **2.4. APRI**

The APRI was calculated as follows [12]: (Aspartate Aminotransferase / AST Upper limit of normal)  $\times$  10/ Platelet count ( $\times 10^4/\text{mm}^3$ )

### **2.5. FIB -4**

The FIB-4 score was calculated as follows [13]: [Age (year)  $\times$  AST (IU/L)]/ [platelet count ( $\times 10^9/\text{L}$ )  $\times$  ALT (IU/L)<sup>1/2</sup>].

### **2.6. Statistical analysis**

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0 for Windows (IBM Corp., USA). P value <0.05 was considered statistically significant. The quantitative data was described with mean and standard deviation (SD), and the qualitative data was described by frequency and percent. Spearman's correlation was conducted to correlate results of different diagnostic tests with METAVIR score. To know how well the different diagnostic tests can predict that a patient has non severe

fibrosis (F0-2) or severe fibrosis (F3-4), the positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity were used. Efficiency is an overall estimate of a test's ability to classify patients correctly. It is estimated by adding the numbers of the two correct classifications (true positive and true negative) and dividing by the total number of patients assessed. ROC (receiver operator characteristic) curve(s) were constructed to assess area under the curve (AUROC). Patients were classified into two groups (below and above the cutoff values). Best cut-off values for the independent variables were determined by maximizing the Youden index (Se+Sp-1).

## **3. Results**

The demographic characteristics of the patients are reported in Table (1). The characteristic curve of the mean percentage dose per hour in patients with non- advanced fibrosis (F0-2) and advanced fibrosis (F3-4) is shown in fig. (1). The 13C-ABT result (% dose/h) starting from 30 min was significantly different among the two groups (P < 0.009). Mean values of liver stiffness, FibroScan, 13C-ABT, APRI and Fib-4 categorized according to fibrosis METAVIR score are reported in tab. (2). Liver fibrosis showed strong correlation with FibroScan (r=0.717, p<0.001) and significant inverse correlation with 13C-ABT results, especially at 30 min. (r= -0.371, p=0.026). Significant correlation was found with FIB-4 (r=0.350, p=0.039) and not significant correlation was found with APRI (r=0.261, p=0.240). Figure (2) shows the area under the curve (AUROC) of 13C-ABT result (% dose/h) at 30 min and cumulative percentage dose recovered (%) over time at 120 min. Figure (3) shows AUROC of FibroScan (A), APRI (B) and FIB-4 (C). Cut offs, ROC curves, p values, sensitivity, specificity, negative predictive value and positive predictive values were shown in tab. (3).

Table (1) Patients' characteristics

<i>Characteristic</i>	<i>Value</i>
Number (n)	36
Age (years, mean ± SD)	41.6 ± 9.4
Males / Females (n)	33/3
BMI (kg/m <sup>2</sup> , mean ± SD)	26.8 ± 4.4
Overweight <sup>#</sup> , n (%)	11 (30.6%)
Obese <sup>##</sup> , n (%)	10 (27.8%)
Bilirubin	0.9 ± 0.3
Albumin	4.3 ± 0.4
Hemoglobin	14.5 ± 1.3
WBCs	6.6 ± 2.3
Plt	193.3 ± 70.8
Patients with advanced fibrosis, n (%)	8 (22.2%)
Fibrosis score (METAVIR)	
- F0	- 1 (2.8%)
- F1	- 21 (58.3%)
- F2	- 6 (16.7%)
- F3	- 8 (22.2%)
- F4	- 0 (0.0%)

Data are shown as mean and SD or number and percent. # BMI >24.9 kg/m<sup>2</sup> <29.9 kg/m<sup>2</sup>. ## BMI >30.0 kg/m<sup>2</sup>

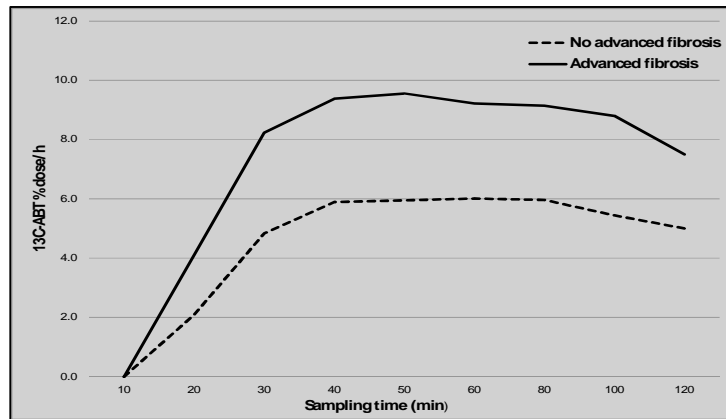


Figure (1) 13C-ABT % dose/h in patients with advanced fibrosis and without advanced fibrosis

Table (2) Mean values and standard deviations of 13C-ABT, FS, APRI and FIB-4 according to METAVIR score

	<i>Fibrosis Score (METAVIR)</i>					
	F0	F1	F2	F3	r <sup>#</sup>	P <sup>#</sup>
13C-ABT dose/hr (%) at 30 min	8.2	5.6 ± 3.3	6.4 ± 2.8	3.2 ± 1.7	-0.371	0.026
13C-ABT % cum. dose at 120 min	10.1	9.7 ± 3.8	10.6 ± 2.8	5.7 ± 2.9	-0.329	0.050
FibroScan (KPa)	9.5	8.4 ± 4.2	13.1 ± 8.9	28.8 ± 16.9	0.717	<0.001
APRI	0.4	0.8 ± 0.4	0.7 ± 0.3	1.1 ± 0.6	0.261	0.240
FIB-4	1.0	1.5 ± 0.7	1.6 ± 0.7	2.9 ± 1.2	0.350	0.039

# Spearman correlation

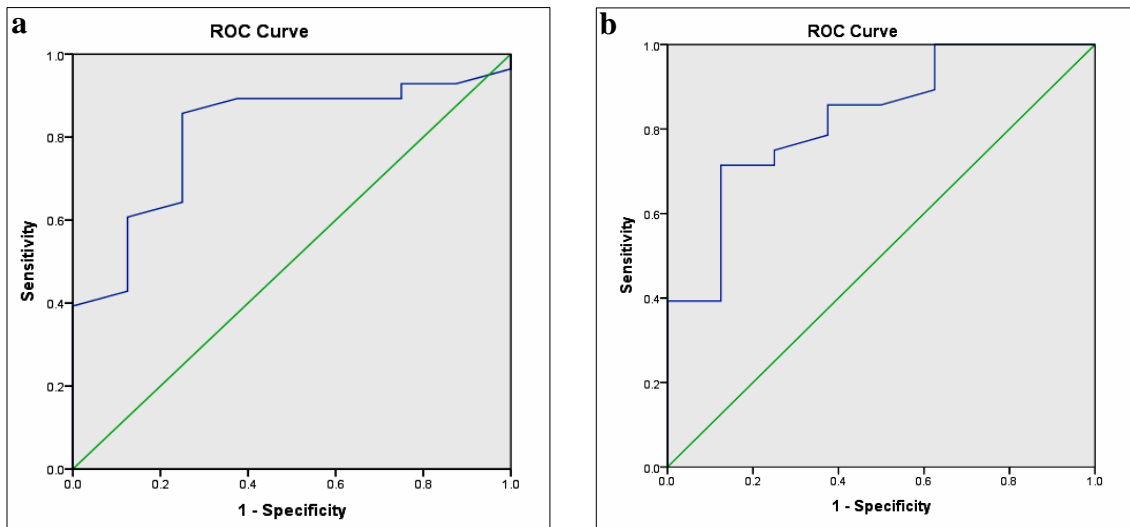


Figure (2) ROC curve for 13C-ABT, **a.** after 30 min and **b.** after 120 min

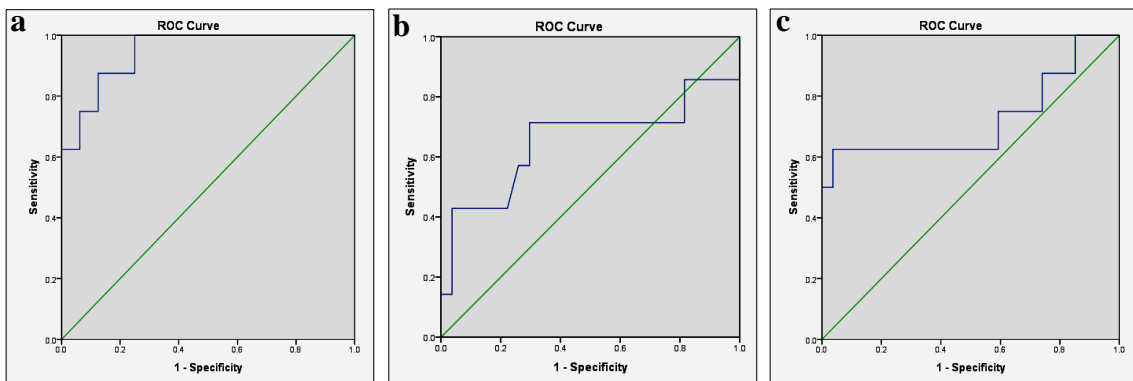


Figure (3) ROC curve for **a.** FibroScan, **b.** APRI and **c.** FIB-4

Table (3) Diagnostic test evaluation of the non-invasive methods studied for the diagnosis of severe fibrosis ( $F \geq 3$ )

--	Cut off	AUROC	95% CI	P value	Se	Sp	PPV	NPP	Accuracy
<b>13C-ABT (30)</b>	3.15	0.808	0.647-0.969	0.009	75.0%	85.7%	60.0%	92.3%	83.3%
<b>13C-ABT (30) [14] ‡</b>	2.48	--	--	--	71.4%	79.2%	50.0%	90.5%	77.4%
<b>13C-ABT (120)</b>	4.35	0.826	0.668-0.984	0.005	100%	67.9%	47.1%	100%	75.0%
<b>13C-ABT (120) [14] ‡</b>	3.62	--	--	--	37.5%	88.0%	50.0%	81.5%	75.3%
<b>FibroScan</b>	10.95	0.945	0.860-1.000	<0.001	100%	75.0%	66.7%	100%	83.3%
<b>FibroScan [15] ‡</b>	10.2	--	--	--	100%	75.0%	66.7%	100%	83.3%
<b>APRI</b>	1.02	0.653	0.368-0.939	0.217	71.4%	70.4%	38.5%	90.5%	70.6%
<b>APRI [12] ‡</b>	1	--	--	--	71.4%	70.4%	38.5%	90.5%	70.6%
<b>FIB-4</b>	2.4	0.722	0.468-0.976	0.059	62.5%	96.3%	83.3%	89.7%	88.6%
<b>FIB-4 [13] ‡</b>	3.25	--	--	--	75.0%	87.1%	42.9%	96.4%	85.7%

# Standard cut-off

The cut-off value of 3.15% (dose/hr at 30 min) was associated with high prediction (positive predictive value, 60%; negative predictive value, 92.3%) yielding an overall sensitivity of

75% and specificity of 85.7%. Compared to the standard cut-off value of 2.48%, this new cut-off value performed better. As regards the 13C-ABT % cum. dose at 120 min, a new cut-off value of 4.35 also

performed better than the standard one of 3.62 (PPV = 47.1%, NPV = 100%, Sensitivity = 100% and Specificity = 67.9% compared to PPV = 50%, NPV = 81.5%, Sensitivity = 37.5% and Specificity = 88%). For FibroScan, both the standard cut-off value of 10.2 kPa and the new cut-off value of 10.95 kPa performed the same (PPV = 66.7%, NPV = 100%, Sensitivity = 100% and Specificity = 75%). As regards FIB-4, a new cut of value of 2.4 performed better than the standard one (PPV = 83.3%, NPV = 89.7%, Sensitivity = 62.5% and Specificity = 96.3% compared to PPV = 42.9%, NPV = 96.4%, Sensitivity = 75% and Specificity = 87.1%). For APRI, both the standard cut-off value of 1 and the new cut-off value of 1.02 performed the same (PPV=38.5%, NPV = 80.5%, Sensitivity = 71.4% and Specificity = 70.4%).

#### 4. Discussion

Several studies have investigated the use of the 13C-ABT. Giannini et al. [8], assessed the use of the breath test to evaluate the severity of the disease in patients with chronic hepatitis C and found that the test was able to differentiate between normal subjects, chronic hepatitis and cirrhosis patients. Schneider et al. [16] found that both fibrosis score and age were found to be independent predictors for the 13C-recovery in patients with chronic hepatitis C. No difference was found between smokers and nonsmokers. Similar to our study, 13C-ABT differed significantly between patients without relevant fibrosis and cirrhosis, beginning after 30 min of sampling [16]. Studies showed that 13C-ABT is able to distinguish between patients with various degrees of liver disease from normal subjects, as well as distinguishing patients with compensated cirrhosis from those with decompensated cirrhosis [9,17,18]. Forestier et al. [19] assessed 13C-ABT in 296 patients with chronic liver disease and showed a negative predictive value of 84% and a

positive predictive value of 90% for the diagnosis of cirrhosis [19]. We observed that 13C-ABT %dose/h was significantly different between patients with advanced fibrosis (F3-4) and those without advanced fibrosis (F0-2). The fact that all 13C-ABT sampling times were significantly different between these two groups may enhance the reliability of the test results and suggests that the test can be shortened to 30 minutes only. Furthermore, we confirmed that have important diagnostic accuracy, thus suggesting that in this setting it can be used without the risk of missing any information. The new suggested cut-off value of 3.15% performed better than the standard one (2.48%), and gave results comparable to FibroScan and FIB-4. Laboratory indexes may offer a simple alternative, because analysis is standardized and references are clearly defined. Currently, several tests are under investigation which largely differ in the choice of the parameters used for index calculation. APRI utilizes two simple laboratory values (thrombocyte count, aspartate aminotransferase) [12]. For this reason, the APRI would be a straight forward alternative for the non-invasive evaluation of liver fibrosis. However, in our study, APRI performance was less accurate than FIB-4, FibroScan and 13C-ABT. FIB-4 performance was the best of studied non-invasive methods. The use of the new cut-off value (2.4) enhanced its performance. FIB-4 has a better AUROC compared to APRI and is the preferred noninvasive fibrosis score to discriminate between F0-F2 and F3-F4. This is similar to results of other studies [21,22]. The majority of studies which involved 13C-labelled hepatic breath test substrates comprised heterogeneous patient groups with a variety of hepatic diseases. All patients we studied had chronic viral hepatitis C, and this could be considered one of the strengths of this study compared to other studies [9,17,19,20],



since chronic infection by hepatitis viruses is the leading cause of liver disease in many countries. Further studies are needed to evaluate the generalizability of results to patients with disease causes other than viral hepatitis.

## 5. Conclusion

*13C-aminopyrine breath test is a potential biomarker for advanced fibrosis that warrants further validation. 13C-ABT is able to identify a clinical condition such as advanced fibrosis cirrhosis, which is not easy to discriminate on a clinical basis alone.*

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