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Radosław Kempiński[1, A–F], Katarzyna Neubauer[1, A–F], Szymon Wieczorek[2, A–F], Robert Dudkowiak[1, A–F], Maria Jasińska[1, B], Elżbieta Poniewierka[1, A–F]

^{13}C-Methacetin Breath Testing in Patients with Non-Alcoholic Fatty Liver Disease*

[1] Department of Gastroenterology and Hepatology, Wroclaw Medical University, Poland
[2] Department of Internal and Occupational Diseases and Hypertension, Wroclaw Medical University, Poland

A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. Non-alcoholic fatty liver disease (NAFLD) is a very common chronic liver condition which may potentially develop into fibrosis and cirrhosis. Liver biopsy is still the gold standard for liver fibrosis detection in these patients. However, non-invasive tools for liver assessment in NAFLD patients, like the ^{13}C-methacetin breath test, may be useful.
Objectives. The aim of the study was to evaluate the utility of the ^{13}C-methacetin breath test in NAFLD patients, especially in predicting significant fibrosis.
Material and Methods. Thirty three patients (24 male and 9 female (average age 47.9)) with histologically proven NAFLD had the 13C-methacetin breath test performed.
Results. Different forms of NAFLD were found during the histology phase, from simple steatosis to advanced fibrosis. Simple steatosis (SS) was found in 18 subjects (54.5%), in another 15 (45.5%) signs of inflammation and fibrosis (NASH) were observed. However, more than half of the patients with liver fibrosis had only minimal changes described (0/1). The sensitivity of the test was highest for cumulative recovery after 10 min of the test and for a combination of two parameters (the cumulative recovery after 40 min and the time of maximal momentary recovery). The positive predictive value was low for all the parameters under consideration, but the negative predictive value was over 0.8 in significant fibrosis detection.
Conclusions. The ^{13}C-methacetin breath test could be a promising noninvasive tool for excluding at least F1 fibrosis in NAFLD patients (**Adv Clin Exp Med 2016, 25, 1, 77–81**).

Key words: NAFLD, liver fibrosis, methacetin breath test.

Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver disease and a serious, rapidly growing health problem in developed countries, where it affects approximately 30% of the population [1]. The increasing prevalence of NAFLD is strictly related to the increase of obesity. Recently, obesity and liver disease have been named the epidemics of the 21st century [2].

NAFLD consists of several liver conditions: simple steatosis, non-alcoholic liver steatohepatitis (NASH), hepatic cirrhosis, and hepatocellular carcinoma. NAFLD is strictly associated with metabolic syndrome, which includes obesity and insulin resistance. The course of NAFLD differs from one patient to another and most of them do not develop more advanced forms of the disease. However, 20% of patients with NAFLD develop NASH and 30% of patients with NASH have fibrosis, cirrhosis and liver failure [1]. Being older than 50 years old and having already present fibrosis are factors that increase the risk of cirrhosis in the course of NAFLD [3]. The symptoms are rare and are generally nonspecific. Only in advanced cases of liver cirrhosis patients may show symptoms and laboratory abnormalities typical for portal hypertension and liver failure.

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The diagnosis of NAFLD does not always require liver biopsy but it is necessary for confirmation of NASH, fibrosis detection and assessment of advanced liver damage [4, 5]. Moreover, the presence of fibrosis during the biopsy is crucial for prognosis in NAFLD patients. The histopathology of NASH is characterized by the presence of balloon degeneration of hepatocytes, Mallory corpuscles, fibrosis and inflammation [6]. Because liver biopsy is a time-consuming, expensive and invasive method with rare but serious complications [7], it is not always accepted by patients; as a result, it cannot serve as a commonly used diagnostic tool for liver evaluation.

The diagnostic value of liver biopsy is also limited because of an irregular distribution of abnormalities as well as inter- and intra-observer reading variability. Therefore, searching for both noninvasive and more representative methods of liver fibrosis staging is crucial. The ^{13}C-labeled methacetin breath test (MBT) is one such method under consideration. MBT is a dynamic breath test that enables quantitative evaluation of the function of microsomal cytochrome P450 1A2 (CYP1A2).

^{13}C-labeled methacetin, after easy absorption in the gastrointestinal tract, undergoes a first-pass metabolism in the liver. In cytochrome P450, ^{13}C-methacetin is O-demethylated and converted to acetaminophen and ^{13}C-labeled carbon dioxide. $^{13}CO_2$ is exhausted and becomes the subject of analysis. The remaining acetaminophen and methacetin are excreted in the urine. The utility of MBT has been suggested for different hepatic diseases. Liver assessment with MBT in NASH patients has also been the subject of several studies; however, data is scarce [8, 9].

The aim of the study was to evaluate MBT's utility in differentiating patients with different forms of NAFLD, especially in predicting significant fibrosis.

Material and Methods

The study group consisted of 33 patients, 24 male and 9 female (average age 47.9), with NAFLD. The diagnosis of NAFLD was established based on clinical features (overweight/obese patients with typically abnormal ultrasound features and abnormal liver enzymes) and confirmed by a histological examination obtained during liver biopsy (> 5% of hepatocytes containing fat droplets). All of the patients had had elevated transaminases for at least 6 months. Patients with viral hepatitis, alcohol abuse (alcohol consumption higher than 30 mg/day for men and 20 mg/day for women was exclusion criteria), hepatotoxic drugs, and cholestatic and similar diseases (hemochromatosis, Wilson disease, autoimmune liver disease) were excluded. None of the patients had a history of any kind of liver decompensation. A complete clinical examination with anthropometric measurements and standard laboratory sampling was performed on all subjects at the beginning of the study. The anthropometric data collected was: height, weight, body mass index (BMI) calculated as weight (kg) divided by height squared (m^2), waist circumference (WC), hip circumference and waist-to-hip ratio (WHR). Standard morphology, liver biochemistry, serum lipids, fasting glucose and insulin were measured. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated, and a history of concomitant diseases/medication was taken. The diagnosis of metabolic syndrome was established according to Adult Treatment Panel III criteria.

None of the patients were taking drugs that could affect the CYP1A2 activity or anticoagulants. All the patients had a needle blind biopsy and MBT performed. Both tests were performed during the same hospitalization.

Liver Biopsy

The histological examination of the sample was always performed by the same pathologist. The following parameters were evaluated: level of steatosis, presence of inflammation and/or fibrosis (grading and staging). The most common complication after the procedure was transient pain in the right, upper abdomen. No severe complications occurred.

^{13}C-Methacetin Breath Test

All of the isotopic breath tests were performed in the morning after at least 12 hours of fasting and a 30-min rest in a sitting position. Subjects were asked to avoid eating pineapple and kiwi fruit or drink sparkling water in the 48 h before the test. 75 mg of ^{13}C-methacetin was dissolved in unsweetened tea and administered orally. Air samples were collected in aluminum bags before the administration of methacetin and, then, in 10-min (in the first hour of the test) or 20-min intervals (in the second hour of the test). No side effects were reported. Measurement of the ^{13}C-labeled CO_2 in the exhaled air was made with an IRIS 2 spectrometer (Infra Red Isotope Analyzer), Wagner Analysen Technik GmbH. The following parameters were analyzed: cumulative recovery after 10 (Cum10), 20 (Cum20), 30 (Cum30), 40 (Cum40) and 120 (Cum120) min of the test [% cum dose], maximal momentary recovery (MVmax) [dose/h] and the time to its

occurrence (Tmax) [min]. All of the parameters mentioned were analyzed in accordance with their histology.

Statistical Analysis

Continuous variables, normally distributed, are shown as mean ± SD. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated with a formula for discrete variables in the Statsoft Inc. STATISTICA 10.0 program.

The study protocol was approved by the local ethics committee in compliance with the Helsinki Declaration. Informed, written consent was obtained from each patient before beginning the study.

Results and Discussion

Selected clinical data of the patients is shown in Table 1.

Different forms of NAFLD were found during the histology phase, from simple steatosis to advanced fibrosis. Simple steatosis (SS) was found in 18 subjects (54.5%), in another 15 (45.5%), signs of inflammation and fibrosis (NASH) were observed. However, more than half of the patients with liver fibrosis had only minimal changes described (0/1). The results of the liver biopsies are shown in Table 2.

Table 1. Selected clinical data of patients in the study group (33 patients)

Age (years)[a]	47.9 ± 11.2 (26–69)
Sex (female/male)	9/24
BMI (kg/m^2)[a]	30.7 ± 3.7
Waist circumference (cm)[a]	107.0 ± 9.8
WHR[a]	0.99 ± 0.44
ALT (< 35 U/I)[a]	73.3 ± 33.6
AST (< 31 U/I)[a]	53.0 ± 26.5
GGT (< 38 U/I)[a]	82.8 ± 47.6
Alkaline phosphatase (30–120 U/I)[a]	96.1 ± 35.9
TG (< 150 mg/dL)[a]	194.0 ± 74.5
PLT	251.6 ± 101.7
HOMA-IR[a]	2.2 ± 1.35
Arterial hypertension[b]	20 (60.6%)
Diabetes[b]	13 (39.4%)
Dyslipidemia[b]	19 (57.6%)
Metabolic syndrome[b]	17 (51.5%)

BMI – body mass index; ALT – alanine transaminase; AST – aspartate transaminase; GGT – gamma-glutamyltransferase; HOMA-IR – homeostasis model assessment of insulin resistance; TG – triglycerides; PLT – platelet count; a – mean ± SD; b – N and percentage.

The mean Cum10, Cum20, Cum30, Cum40 and Cum120 values were 1.2, 4.1, 7.4, 10 [% cum dose], and 21.4 [%cum.dose], respectively. The mean MVmax was 16.35 [dose/h]. Tmax was 20 in most cases. No significant differences between the mean values of any MBT parameters were found between the SS and NASH groups. No significant differences were found when patients with minimal fibrosis were incorporated into the SS group either.

Next, the sensitivity and specificity of the MBT parameters for distinguishing between simple steatosis or minimal fibrosis and more advanced stages (1 or more) were counted. The cut-off values were 1.05 for Cum10, 4.15 for Cum20, 7.15 for Cum30, 9 for Cum40 and 20 for Cum120 [%cum.dose]. The cut-off value for MVmax was established at 10.5 [dose/h]. Every peak shift over 20 min (Tmax) of the test was recognized as a sign of decrease in liver function. Some combinations of the parameters mentioned were considered. The results are shown in Table 3.

The sensitivity of MBT was highest for Cum10 and the combination of peak shift and Cum40. The positive predictive value (PPV) was low in all cases, but the negative predictive value (NPV) was at least 0.8 in every parameter under consideration.

Table 4 shows the results obtained when MBT parameters were used for detection of any (even borderline) fibrosis.

In this part of the analysis, the sensitivity of MBT was highest for Cum10 and Cum20. Specificity was highest for Cum40. Both PPV and NPV were low, reaching 0.75 for the combination Cum40 and Tmax.

Table 2. Fibrosis in liver biopsies

Staging	No. of patients
F0	18
F0/1	7
F1	5
F1/2	0
F2	2
F2/3	0
F3	1
Total	33

Table 3. ^{13}C-methacetin breath test for at least F1 fibrosis detection in NAFLD patients

	Sensitivity [%]	Specificity [%]	PPV	NPV	cut-off
Cum10	75	28.6	0.37	0.88	1.05
Cum20	62.5	27.8	0.29	0.81	4.15
Cum30	62.5	26.3	0.31	0.82	7.15
Cum40	62.5	25	0.33	0.83	9
Cum120	62.5	23.8	0.36	0.84	20
MVmax	50	17.4	0.4	0.83	10.5
Tmax	62.5	20.8	0.45	0.86	20
Cum40 or Tmax	87.5	33.3	0.39	0.93	9 or 20

PPV – positive predictive value; NPV – negative predictive value.

Table 4. ^{13}C-methacetin breath test for any fibrosis detection in NAFLD patients

	Sensitivity [%]	Specificity [%]	PPV	NPV	cut-off
Cum10	69.2	47.4	0.53	0.71	1.05
Cum20	60	50	0.5	0.6	4.15
Cum30	53.3	44.4	0.5	0.59	7.15
Cum40	53.3	40	0.57	0.63	9
Cum120	46.7	41.2	0.47	0.56	20
MVmax	40	30	0.6	0.6	10.5
Tmax	33.3	25	0.62	0.6	20
Cum40 or Tmax	73.3	47.8	0.65	0.75	9 or 20

PPV – positive predictive value; NPV – negative predictive value.

Discussion

Previous studies on MBT have shown its potential utility in fibrosis detection in different chronic liver pathologies. Most of the studies with MBT were conducted on HCV patients [10], yet MBT has also been evaluated in primary biliary cirrhosis [11] and in transplantology for graft function assessment [12]. Detection of NASH and fibrosis in the course of NAFDL have been the subject of several studies with MBT.

Fierbineteanu–Braticevici et al., in a study on 64 subjects, showed 95% sensitivity and 75% specificity of MBT in identifying NASH [8].

Another study on NAFLD patients was conducted with MBT and ^{13}C-ketoisocaproate. In this study, patients with NASH had a higher cumulative recovery of CO_2 than the controls. However, the authors excluded patients with advanced fibrosis and cirrhosis [9].

In our study, none of the parameters of MBT reached a satisfying sensitivity for fibrosis detection in NAFLD patients. However, the high negative predictive value for at least F1 fibrosis could be valuable and helpful in identifying patients with fatty liver disease who may qualify for liver biopsy. Taken together, the exact time of maximal momentary recovery and the cumulative recovery after 40 min strongly suggest a lack of significant fibrosis in liver biopsy. Incorporating MBT into the diagnostic algorithm of NAFLD patients could help to avoid an unnecessary liver biopsy in selected patients.

The authors concluded that the ^{13}C-methacetin breath test could be a promising noninvasive tool for excluding at least F1 fibrosis in NAFLD patients.

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Address for correspondence:

Katarzyna Neubauer
Department of Gastroenterology and Hepatology
Wroclaw Medical University
Borowska 213
50-001 Wrocław
Poland
Tel. +48 733 21 20
E-mail: kasianeu@gmail.com

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