

^{13}C -Breath Tests and liver fibrosis

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Abstract. – The evaluation of the presence and degree of liver fibrosis in patients with chronic liver disease is a fundamental diagnostic and prognostic issue. This is mainly due to the repercussions of liver fibrosis on liver function, whose derangement, in turn, is mainly responsible for the negative events of advanced liver disease. ^{13}C -Breath Tests ($^{13/14}\text{C}$ -BTs) for the study of liver function were developed more than twenty years ago in order to non-invasively assess residual liver function in patients with various degrees of liver fibrosis, from minimal stages up to liver cirrhosis. Sequential studies that were performed over the years using various ^{13}C -BT substrates showed that increasing degrees of liver fibrosis are paralleled by concomitant modifications in ^{13}C -BT results. The ^{13}C -BT probes that reportedly obtained interesting results were aminopyrine, galactose, and more recently phenylalanine. As the knowledge in this field evolved, probes for the study of specific functions, such as the ^{13}C -Octanoate Breath Test were sought. Analysis of the published studies would seem to show that ^{13}C -BTs alone, or in combination may provide a non-invasive picture of the functional alterations secondary to liver fibrosis. Further studies are needed to evaluate the diagnostic yield of the ^{13}C -BT in particular clinical situations, such as in patients with normal static parameters of liver function, or after therapy.

Key Words:

Liver, Liver fibrosis, Cirrhosis, Breath Test.

Introduction

Evaluation of the presence and degree of liver fibrosis has always been a key issue in the work-up of patients affected by chronic liver disease. As a reaction to the disease, many chronic liver injuries result in collagen deposition, and this process, together with hepatocellular necrosis and regeneration, and distortion of vascular architecture produces ongoing damage that may lead to cirrhosis. Once cirrhosis develops it is not a static disease, and in-

deed it can worsen, stabilise, and sometimes even improve, depending on the underlying insult leading that lead to this stage. Although these pathologic changes are well documented in patients with chronic liver disease, they are not the real determinants of the patients' stage or prognosis. Indeed, it is not fibrosis or cirrhosis per se, but rather their detrimental effects on liver function that determine, for example, the patients' tolerance to a drug, their capability to undergo surgery, the development of life-threatening sequelae of liver disease, and most importantly, their prognosis. All in all, a progressive decrease in liver function(s) and an increase in portal pressure lead to end-stage liver disease, and while the former is directly dependent upon the hepatocellular functioning mass, the latter is caused by both liver architectural distortion and impaired liver function(s). Despite these well-known shortcomings, however, liver biopsy has always been considered the "gold standard" technique to assess the patients' stage and prognosis, while, on the contrary, it should be considered a "surrogate" for the true status of the patient.

Some thirty years ago, $^{13/14}\text{C}$ -Breath Tests ($^{13/14}\text{C}$ -BTs) started being applied to evaluate liver function in humans. The principle of the $^{13/14}\text{C}$ -BT is simple: the ^{12}C of a functional group is replaced by $^{13/14}\text{C}$ in a compound whose enzymatic, rate-limiting process is carried out within the liver, and is then administered to a subject. Once the $^{13/14}\text{C}$ -labelled functional group has undergone enzymatic cleavage within the liver, it proceeds to further metabolic processes up to $^{13/14}\text{CO}_2$ which is then exhaled by the subject and collected in dynamic breath samples. After appropriate analysis of the samples and elaboration of data, the $^{13/14}\text{CO}_2$ recovery rate, as well as the shape of the appearance curve of $^{13/14}\text{CO}_2$ in the breath provide information on the liver metabolic function(s) that have been explored. Various and complex liver functions can be studied, depending on the extraction ratios of

the administered compound (high extraction ratio → liver blood flow; low extraction ratio → liver metabolic activity) and the location of the rate-limiting metabolic step within the liver cell (cytosol, microsomes, mitochondria). On the basis of these factors, the results of many BTs are considered representative of the global hepatocellular functioning mass, although it must be kept in mind that certain probes do have peculiar limits, and that the ideal substrate for assessing liver function does not exist.

This review will deal with the relationships between histological assessment of liver fibrosis and ^{13}C -BTs for the study of liver function. ^{13}C -labelled compounds are harmless, they can be safely administered to children, women of child-bearing age, and pregnant women and, though more expensive than ^{14}C -labelled compound, they have gained popularity due to the diffusion of mass spectrometers for ^{13}C -Urea Breath Testing. This is the main reason why studies that used ^{13}C -BTs alone will be reviewed.

The message that seems to emerge from reviewing the literature and from our own personal experience is that ^{13}C -BTs with various substrates are able to assess the metabolic changes that are determined by different degrees of fibrosis in patients with chronic liver disease of various aetiology. Moreover, the information provided by ^{13}C -BTs seems to bypass the inconvenient bond to the use of histological scores, thus providing a continuous variable rather than fixed categories. This is of particular importance for patients with chronic viral hepatitis or non-alcoholic fatty liver disease, that currently represent the leading causes of chronic liver disease in the western world, and for which there is growing evidence of the usefulness of ^{13}C -BTs.

Breath Tests and Liver Fibrosis in Chronic Viral Hepatitis

The troubled relationship between ^{13}C -BTs and the severity of virus-related chronic liver disease unwinds over the past twenty years. During the last decades, in fact, a series of studies that used various ^{13}C -labelled compounds attempted to unveil the correlation between what can be invasively assessed (liver histology) and the results of non-invasive ^{13}C -BTs. This history winds its way through consecutive, progressive steps, beginning with the differentiation of larger categories of patients, then

through the functional identification of both patients with compensated cirrhosis and patients with high versus low fibrosis, up to the most subtle distinction among the various degrees of liver fibrosis. ^{13}C -Aminopyrine Breath Test (^{13}C -ABT), ^{13}C -Galactose Breath Test (^{13}C -GBT), and more recently ^{13}C -Phenylalanine Breath Test (^{13}C -PBT) have proven to be the most promising tools to help the physician non-invasively assess the functional derangement associated with liver fibrosis.

In 1982, Monroe and colleagues prospectively evaluated the capability of the ^{13}C -ABT to reflect histological severity in a series of patients with chronic liver disease¹. The severity of liver lesions was scored using the terminology “chronic persistent hepatitis” (CPH), “chronic active hepatitis” (CAH) “with bridging fibrosis” (CAHB) or “with cirrhosis” (CAHC). This pioneering study was the first to report lower ^{13}C -ABT values in CAHB and CAHC as compared to CPH, CAH, or normal subjects. However, it fell short of demonstrating a significant difference in ^{13}C -ABT results between CPH or CAH and normals, or between CAHB and CAHC. Since the histological definitions of CPH and CAH were based on an overall comprehensive evaluation of fibrosis and necro-inflammatory activity, the relationship between ^{13}C -ABT and fibrosis would have been more evident if separate histological assessment had been carried out. Nonetheless, this study was the first to provide evidence that the functional liver cell mass is reduced in patients with milder disease as compared to patients with more severe forms of hepatopathy, and had the pristine intuition that liver function can be well preserved despite the presence of minimal hepatic damage. Later on the same year, the same group published a landmark paper in the field of ^{13}C -BTs establishing the bases for a homogeneous expression of ^{13}C -BTs results². In this study they expanded the results of the previous one and provided a visual analysis of the ^{13}C -ABT curve by representing the characteristic profiles of the ^{13}C -ABT %dose/hour. By means of this analysis they showed that the profile of the ^{13}C -ABT %dose/hour curve progressively flattens and that at its peak the ^{13}C -ABT %dose/hour shifts towards the right as liver disease worsens.

In the '90s, the hepatitis C virus “epidemic” focused the clinicians’ attention on the early phases of the disease, due to the potential prog-

nostic and therapeutic implications connected to their early recognition. Thus, this practical need shifted the researchers' field of interest to the subtle differentiation of minimal changes in liver fibrosis. In the meantime, the usefulness of other ¹³C-labelled compounds was tested in this setting. We demonstrated that in chronic hepatitis C patients, ¹³C-ABT results were able to distinguish chronic hepatitis patients from Child-Pugh class A cirrhosis ($p < 0.03$)³. Then, we showed that ¹³C-ABT results had an inverse correlation with the histological fibrosis score ($r_s = -0.409$, $p = 0.05$), and that ¹³C-ABT allowed us to distinguish normal subjects from high fibrosis score chronic hepatitis patients ($p < 0.001$), and this latter group from patients with low fibrosis score ($p = 0.004$)³. In the same period, Mion and colleagues reported interesting results using the ¹³C-GBT in patients with different degrees of chronic liver disease^{4,5}. The results they obtained were twofold and allowed progressive functional characterisation of various degrees of chronic liver disease. In the first study they preliminarily observed that there was a significant difference in ¹³C-GBT values between patients with chronic hepatitis and those with Child-Pugh class A liver cirrhosis ($p < 0.05$)⁴. These results are of particular interest since these two conditions are extremely difficult to distinguish on clinical grounds alone, and therefore liver biopsy is often required to distinguish the former from the latter. However, the Authors published their study in a preliminary form alone, without providing a detailed analysis of data, and therefore, we deemed it of interest to confirm their preliminary report in a larger series of patients. We found that ¹³C-GBT %dose per hour at 30 minutes had an 80.4% accuracy rate in differentiating patients with chronic hepatitis from patients with Child-Pugh class A liver cirrhosis, with 76.2% sensitivity and 80.0% specificity⁶. Thus, the results by Mion and colleagues, as well as our own, seem to confirm the usefulness of ¹³C-GBT in the non-invasive assessment of chronic hepatitis and compensated cirrhosis. In the second study, Mion and colleagues found that in a series of patients with chronic hepatitis C a progressive impairment of ¹³C-GBT results was associated with increasing degrees of liver fibrosis, as evaluated by the METAVIR score ($p < 0.0001$)⁵. It is noteworthy that they observed how ¹³C-GBT % dose/hour at 60 minutes was able to identify the metabolic impairment asso-

ciated with subtle and minimal changes in liver fibrosis by providing, on the average, significantly different results between each fibrosis score ($p < 0.05$), except between F₃ (extensive fibrosis with septa) and F₄ (cirrhosis). These results set the bases for the use of ¹³C-GBT in patients with mild hepatitis, and for the identification of minimal variations in fibrosis-associated modifications of liver function. Unfortunately, the Authors did not specify whether the ¹³C-GBT results of patients with initial fibrosis were significantly different from those of normal subjects. However, the most recent study concerning ¹³C-BTs and liver fibrosis seems to fill this gap. Ishii and colleagues used ¹³C-PBT to identify the functional changes determined by increasing fibrosis in a series of patients with chronic hepatitis C⁷. They found a close correlation between ¹³C-PBT and the METAVIR fibrosis score, and most importantly they observed that the ¹³C-PBT % dose/hour at 45 minutes as well as % of cumulative dose at 75 minutes of patients with minimal fibrosis were significantly lower than those obtained in healthy controls.

Breath Tests and Liver Fibrosis in Nonalcoholic Fatty Liver Disease

The ¹³C Methionine Breath Test (¹³C-MBT) evaluates the liver mitochondrial function by assessing the end-product of intra-mitochondrial decarboxylation of ¹³C labelled methionine. The peculiar metabolic pathway of methionine makes this dynamic function test an ideal candidate for the evaluation of chronic liver diseases related to fat accumulation and lipid peroxidation, such as non-alcoholic fatty liver disease (NAFLD). Indeed, the most important issue in NAFLD is to distinguish patients with significant liver damage and fibrosis, and therefore prone to progressive disease, from patients with fat deposition alone, with or without minimal inflammation, in whom the disease follows a rather benign course. NAFLD is a disease of recent interest and characterisation, though its prevalence in the western world is indeed, not negligible. In this regard, Spahr and colleagues performed ¹³C-MBT on a series of patients with severe liver steatosis (> 40% of involved hepatocytes, without features of inflammation or fibrosis) before bariatric surgery for morbid obesity⁸. They demonstrated that the ¹³C-MBT values of these patients were significantly reduced as compared to healthy subjects, taking both

%dose per hour at 60 minutes and %cumulative dose at 60 minutes into consideration. These data put forward an important preliminary hypothesis, since the first step in NAFLD is the occurrence of hepatic steatosis, and only a later, "second hit" leads to inflammation and fibrosis. The fact that fat accumulation alone can decrease the activity of α -ketobutyrate decarboxylase is an important issue, although further data are needed to find out whether ^{13}C -MBT results can be progressively impaired throughout the whole spectrum of NAFLD. More recently, Miele and colleagues used the ^{13}C -Octanoate Breath Test (^{13}C -OBT) to try to disclose the relationship between the presence of fibrosis and functional impairment in patients with NAFLD⁹. Their preliminary results showed that the ^{13}C -OBT results of a series of patients with nonalcoholic steatohepatitis (NASH) were significantly lower than those of normal subjects. These results are interesting since the uncoupling of mitochondrial β -oxidation and the subsequent generation of reactive oxygen species seems to be an important mechanism of hepatic damage in NAFLD patients. Future studies are needed to evaluate whether the use of ^{13}C -BTs could be of use in identifying the various degrees of impairment of liver function associated with fibrosis in NAFLD patients.

Conclusions

^{13}C -BTs for the study of liver function have surely acquired popularity over the last few years. The good results obtained in scientific research, and the practical need for a non-invasive tool to stage the disease and assess the patients' prognosis have undoubtedly facilitated their diffusion. ^{13}C -BTs with various substrates proved to be able to evaluate the degree of fibrosis in patients with chronic viral hepatitis and showed promising results in patients with NAFLD. Whether the use of multiple ^{13}C -BTs can provide complementary information regarding the patients' diagnosis and prognosis remains to be established¹⁰. Thus, the field of application of ^{13}C -BTs is broad and can be further broadened by new applications. In fact, a significant proportion of patients with chronic viral hepatitis, and some with NAFLD as well, have persistently normal aminotransferase levels, despite the presence

of significant liver damage. Thus, evaluation of the diagnostic yield of the ^{13}C -BTs in this setting could be an interesting challenge for the future. In this regard, we have started performing ^{13}C -ABT in patients with chronic hepatitis C virus infection and persistently normal aminotransferase levels. Preliminary results of our data seem to suggest that ^{13}C -ABT, alone or combined with other biochemical parameters, could obviate the need for liver biopsy in a substantial percentage of patients. Surely these results need confirmation in larger series of patients, although they have set the basis for future research.

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