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Breath tests with stable isotopes: have they a role in liver transplantation?

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Abstract. – **Evaluation of liver function is crucial in the overall management of patients with liver disease. In particular, patients with end-stage liver disease need accurate prognostic indicators to plan liver transplantation, and in this case, to manage their presence in the waiting list. Availability of predictors of clinical outcome is further essential after liver transplant, mainly to correctly diagnose and adequately treat complications, such as acute rejection, drug toxicity, liver dysfunction. Breath tests using labelled substrates selectively metabolized within the liver may represent an accurate diagnostic and prognostic tool in these clinical conditions, possibly with an adjuntive role to the most commonly used prognostic models (Child-Pugh and MELD scores). Promising results have been in fact recently obtained by the use of different substrates (aminopyrine, methacetin, erythromycin, methionine) which explore different metabolic function of the hepatocyte. The usefulness of breath tests has been documented in liver disease patients both before and after liver transplantation, in the early as well as in the late phase.**

Key Words:

Child-Pugh score, MELD score, Liver cirrhosis, Liver fuction.

Introduction

Liver transplantation represents an accepted treatment modality for end-stage of liver disease, with an excellent long-term survival. However, patient clinical outcome is influenced, either before or after liver transplantation, by critical steps, which greatly affect the overall successful rate of this therapeutic strategy. These steps are mainly represented by the waiting list period, the early postoperative phase and the late one. In order to achieve the best results in the management of these clinical phases, accurate diagnostic and prognostic indicators, in particular scoring systems and tests able to quantify liver function, are mandatory.

Child-Pugh[1] and MELD (model of end-stage liver disease)[2] scores represent the most commonly used prognostic models to assess survival in cirrhotic patients awaiting for liver transplantation, while dynamic functional tests, such as clearance of indocyanin green[3], galactose elimination capacity[4] and monoethylglycinexylidide (MEGX)[5], have been used to evaluate liver function early after transplantation.

However, dynamic tests, as well as the conventional biochemical parameters commonly used in the follow-up of cirrhotic patients, could not provide a satisfactory evaluation of liver functional reserve, while scoring systems may be influenced by the subjectivity of some parameters (i.e., degree of ascites or hepatic encephalopathy) and by modifications induced by concomitant treatments (i.e., albumin infusion). For these reason, it has been suggested[6,7] that quantitative information on hepatic function can easily be obtained from breath tests, based on the use of labelled substrates selectively metabolized within the liver.

In patients with chronic liver disease, different substrates have been proposed, each exploring a specific hepatic function: aminopyrine, which was the first compound to be studied[8], is useful in the evaluation of hepatocyte microsomial function[9], other sub-

strates include phenacetin[10], caffeine[11], lidocaine[12], methacetin[13] and erythromycin[14]. Phenylalanine[15] and galactose[16] are used to explore the cytosolic enzymatic activity, while methionine and ketoisocaproic acid have been proposed in the study of the mithocondrial function[17].

Some of these substrates have been tested in cirrhotic patients, before and after liver transplantation.

The possibility to estimate prognosis of patients with advanced liver disease and, consequently, to define the optimum time for liver transplantation represents an important clinical challenge and different authors have evaluated the existence of clinical and/or biochemical parameters suitable to predict prognosis.

Adler et al[18] evaluated retrospectively in patients with parenchymal cirrhosis different biochemical variables, documenting that the best prognostic index was obtained by two independent variables: ascites and aminopyrine breath test. However, the same authors were unable to confirm these results in a subsequent study, where endogenous tests resulted more discriminant than aminopyrine breath test and lidocaine metabolization test in predicting in cirrhotic patients 1-year mortality in cirrhotic patients[19].

Mortality rate of cirrhotic patients on the waiting list still represents a serious clinical problem; available data indicate that the mortality rate ranges from 15% to 28%[20]. Therefore attempts have been made to identify in these patients predictors for death.

Degre et al[21] analysed the risk factors for death while on the liver transplant list for patients with liver cirrhosis; they confirmed a high mortality rate (10%) and documented that the risk of death correlated with five parameters evaluated at the time of listing: history of infected ascites, aminopyrin breath test, prothrombin time or international normalized ratio (INR), the Child-Pugh score[1] and the moldel of end-stage liver disease (MELD)[2] score. In particular, these authors demonstrated that the use of aminopyrin breath test represented a strong predictor of death while awaiting transplantation, thus suggesting that this test constitutes a non invasive quantitative tool for the assessment of priority on the liver transplant list. In fact, aminopyrine breath test showed an accuracy equal, or even better than, that of other grading systents, such as the Child-Pugh and MELD score.

Severe graft dysfunction represents an important complication of liver transplantation and it might represent a life-threatening condition. Early diagnosis is crucial for the patient survival and several attempts have been performed to identify markers to predict graft function[22].

In has been documented that early postoperative erythromycin breath test may be sensitive and specific in identifying a group of patients with severe graft dysfunction and a high risk of graft loss[23]. Furthermore, since this test represents an in vivo measurement of the graft CYP3A activity, it is able to predict the development of cyclosporine and tacrolimus nephrotoxicity[24].

Aminopyrine breath test was used, together with routine liver function tests and galactose elimination capacity, to monitor the recovery of the liver graft after episodes of acute cellular rejection: the microsomal metabolic capacity was within normal limits for the majority of patients and did not differ significantly between patients with and without previous acute cellular rejection, thus indicating that early rejection episodes do not affect late function of liver allografts in man[25].

The diagnostic and prognostic role of a combination of two breath tests (^{13}C-aminopyrine and ^{13}C methionine breath tests) in the early phase after liver transplant was tested by Di Campli et al[26]; this test combination was used since it has been suggested[27] that graft viability should be related to the energy production of hepatic mithochondria (whose function can be measured by means of 1 ^{13}C-methionine breath test)[17] and to the drug metabolism capability at the microsomal levels (a function that can be explored using ^{13}C-aminopyrine breath test)[9]. This study documented that the cumulative percentage of the dose of ^{13}C progressively increased in patients with a successful transplant, reaching shortly the control values; this did not happened in patients who developed a primary non-function, whose cumulative percentage of the ^{13}C dose remained always lower than controls and unchanged with respect to the pre-transplant period.

Petrolati et al[28] followed cirrhotic patients at the time of listing, at 12-week intervals on

the waiting list and after the surgical procedure. Furthermore, intraoperative measurements were obtained during the liver transplantation procedure, in order to confirm the relationship between hepatic function and methacetin metabolism. These authors used ^{13}C-methacetin as substrate, since it has been documented[29,30] that this substance has a low cost, a rapid clearance and, differently from aminopyrine that may induce side-effects such as agranulocytosis[31], is safe.

Before liver transplantation, cirrhotic patients showed a significant reduced ^{13}C-methacetin cumulative oxidation compared to controls; in those patients who underwent successful liver transplantation, mean oxidation progressively increased to reach normal values at 6 months of follow-up. The mean intraoperative ^{13}C-methacetin cumulative oxidation increased from 0.1% during the anhepatic phase to 3.7 ± 2.0% 2 hours after reperfusion, thus not only confirming the close relationship between hepatic function and methacetin metabolism., but also suggesting that this test may be useful to monitor intra-operative hepatic function in the high-risk phase occurring immediately after liver reperfusion.

Moreover, all the available data suggest that ^{13}C breath-tests represent an useful tool in cirrhotic patients before and after liver transplantation.

Further prospective studies are obviously needed to confirm the diagnostic and prognostic role of ^{13}C breath-test in these patients, as well as to identify the most accurate substrate.

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