CORRESPONDENCE

Phase-1 Evaluation of ^{13}C-Liver Function Breath Tests

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Dear Sir,

We read with great interest the paper by Afolabi et al. [1]. It is true that ^{13}C-liver function breath tests (^{13}C-LFBTs) appear to be attractive both to patients and physicians because of their non-invasive protocol, as opposed to diagnostic procedures infringing the body integrity, which inherently entails a liver biopsy. Unfortunately, after almost three decades during which ^{13}C-LFBTs were available to clinicians, those tests still have not paved their way to become a routine diagnostic tool. The breakthrough made by the quoted paper consists in the clear delineation of targets for future research which, if attained, should gain an objective view upon the clinical usefulness of those tests. Accordingly, phase one of this validation process should involve evaluation of reproducibility, the second one involves the assessment of prognostic utility, and ultimately the third phase should investigate the effect of ^{13}C-LFBTs upon the patients' outcome [1].

One should be aware that the result of a breath test, like in the case of any other quantitative diagnostic method applied in medicine, may contain a certain degree of inexactitude because an immanent feature of any measurement is its proneness to random as well as systematic errors. Therefore it is necessary to identify possible sources of measurement errors and to estimate of their contribution to the overall error of a diagnostic method and, as the ultimate step, to undertake means to possibly minimize it.

In the case of ^{13}C breath tests, the total measurement error will be accounted for by the precision and exactitude of the apparatus used to determine the content of ^{13}CO$_2$ within samples of the expired air, degree of conformity with the recommended protocol of accomplishing the test, and inherent biological variability of the living organism undergoing a diagnostic procedure.

The error introduced by the measurement equipment is relatively easy to estimate, because it will be characterized by sensitivity, linearity range, as well as by within- and between-series consistency of measurement results. Those items are basically addressed by manufacturers, and a daily routine of calibration assures the maintenance of optimum performance of the equipment. Knowledge of the performance of the measuring system is of course necessary to adjust an optimum dosage of the ^{13}C-labeled substrate applied for a given breath test [2].

Minimization of the error associated with the implementation of a breath test is achieved by standardization of the composition and method of preparing a test meal, the time allowed for its consumption, number, time intervals and the method of sampling the expiratory air, as well as the ambiance offered to the examined subjects while undergoing the examination. The set of interventions usually undertaken in this respect comprises advice and recommendations given to subjects with regard to some restrictions that have to be observed before an examination (like remaining fasting, abstaining from smoking cigarettes, withdrawal of use of medication), and the behavior during the test (avoidance of physical activity, maintenance of a recommended body position, refraining from smoking and from taking meals or drinks other than that provided by the laboratory staff) [3–6].

Measures undertaken in an attempt to control the error introduced by biological variability may consist in a strict observation of a constant time of day when the test is performed, or, in the case of women at a reproductive

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age—in taking into account their menstrual cycle status [7, 8].

We totally agree with Afolabi et al. [1] that a valuable tool enabling the assessment of the performance of a quantitative measurement in medicine is the determination of the reproducibility of the results it provides. An estimation of the gross error of the diagnostic method, accounted for by the factors and circumstances described above, can be thus obtained. Consequently, a poor reproducibility of a test will in fact determine its unsuitability for clinical applications, since it means that the results of tests performed in the same person under identical conditions may largely differ from one another [9].

It is our pleasure to provide herein additional data on reproducibility of the ^{13}C-LFBTs, not reported in the paper by Afolabi et al. [1]. In our laboratory we pursued prospective evaluation of the reproducibility of the ^{13}C-methacetin breath test (^{13}C-Meth-BT) [10], the ^{13}C-alpha-ketoisokaproic acid breath test (^{13}C-KICA-BT) [11], and the ^{13}C-phenylalanine breath test (^{13}C-PhenAla-BT) [12]. Thus, insight on the precision of the representatives of three main groups of the ^{13}C-LFBTs has been obtained, since the ^{13}C-Meth-BT evaluates the microsomal liver metabolism, whereas the ^{13}C-KICA-BT and the ^{13}C-PhenAla-BT are dedicated to assess the mitochondrial and the cytosolic metabolic efficiency of the liver, respectively. The results of the short-term (repeat examination were taken 1–3 days apart) and the medium-term (the repeat measurements were separated by a 2–3-week break) reproducibility of the three ^{13}C-LFBTs are assembled in Table 1. The common denominator of those

data is that T_{max}—the time to reach the peak of ^{13}CO$_2$ concentration in expiratory air—is considerably less reproducible than the two other quantitative parameters of the ^{13}C-LFBTs, namely, the maximum momentary elimination (D_{max}) which is characterized by a fair reproducibility, and the best reproducible cumulative elimination of ^{13}C in breath air, conveyed as the area under the ^{13}C elimination curve (AUC). In no instance did the medium-term reproducibility prove any worse than the short-term one (Table 1). Quite strikingly, taking into account the magnitude of the pertinent coefficients of variation for paired examinations (CV_p), the reproducibility of the ^{13}C-PhenAla-BT appears to be remarkably worse than in the case of either the ^{13}C-Meth-BT or ^{13}C-KICA-BT. The latter finding raises concerns whether the precision of the ^{13}C-PhenAla-BT may be sufficient to yield clinically sound conclusions.

Detailed analyses of the reproducibility data referred in this correspondence have been published elsewhere [10, 11]. In summary, we would like to recall some important observations. First, in the case of ^{13}C-Meth-BT it was found that on repeat examinations the exactitude of AUC may be modestly affected by a persistent stimulation of CYP1A2 responsible for a fixed bias which amounted to 8 % [10]. Second, achievement of a necessary reproducibility level of the ^{13}C-KICA-BT requires calculation of the AUC for a time span from within the range between 0 and 90 min or even better, for 0–120 min [11].

We do hope that the data and remarks contained herein supplement and support the idea of systematic validation of ^{13}C-LFBTs outlined in the paper by Afolabi et al. [1].

Table 1 Reproducibility of three liver breath tests

Parameter	D_{max}		T_{max}		$AUC_{0–60}$		$AUC_{0–90}$	
	S_term	M_term	S_term	M_term	S_term	M_term	S_term	M_term
^{13}C-methacetin breath test								
CV_p	16.2 %	16.5 %	30.6 %	32.5 %	10.0 %	10.0 %	9.2 %	8.7 %
RC	15.6 % dose/h	16.8 % dose/h	16.9 min	16.7 min	5.8 % dose	5.9 % dose	7.0 % dose	6.8 % dose
$\Delta_{0.05}$ ($N = 20$)	3.8 % dose/h	3.5 % dose/h	4.0 min	3.8 min	1.4 % dose	1.3 % dose	1.8 % dose	1.4 % dose
^{13}C-alpha-ketoisokaproic acid breath test								
CV_p	11.2 %	13.9 %	20.0 %	24.8 %	12.6 %	12.4 %	8.2 %	9.0 %
RC	7.4 % dose/h	9.5 % dose/h	15.9 min	19.6 min	5.4 % dose	5.4 % dose	5.1 % dose	5.7 % dose
$\Delta_{0.05}$ ($N = 14$)	2.2 % dose/h	2.9 % dose/h	4.3 min	5.2 min	1.6 % dose	1.5 % dose	1.6 % dose	1.6 % dose
^{13}C-phenylalanine breath test								
CV_p	28.5 %	27.2 %	35.2 %	30.0 %	25.9 %	25.9 %	21.6 %	21.1 %
RC	11.6 % dose/h	10.8 % dose/h	17.8 min	15.6 min	5.4 % dose	5.2 % dose	5.9 % dose	5.6 % dose
$\Delta_{0.05}$ ($N = 12$)	3.9 % dose/h	3.6 % dose/h	6.0 min	5.3 min	1.8 % dose	1.7 % dose	2.0 % dose	1.9 % dose

D_{max} maximum momentary ^{13}C elimination, T_{max} time to reach the D_{max}, $AUC_{0–60}$ and $AUC_{0–90}$ 60-min and 90-min cumulative ^{13}C elimination in expiratory air, respectively, *S_term and M_term* short-term and medium-term reproducibility, respectively, CV_p coefficient of variation for paired examinations [13], *RC* repeatability coefficient [14], $\Delta_{0.05}$ = the least difference detectable at $p = 0.05$ level, two-tailed in the case of N paired examinations

Conflict of interest   None.

References

1. Afolabi P, Wright M, Wootton SA, Jackson AA. Clinical utility of (13)C-liver-function breath tests for assessment of hepatic function. *Dig Dis Sci*. 2012. (Epub ahead of print). doi: 10.1007/s10620-012-2340-z.

2. Kasicka-Jonderko A, Jonderko K, Budniok M, Błońska-Fajfrowska B. Comparison of two dosage regimens of the substrate for the ^{13}C-methacetin breath test. *Isotopes Environ Health Stud*. 2012; 48. doi:10.1080/10256016.2012.707979.

3. Kasicka-Jonderko A, Jonderko K, Kamińska M, et al. Breath $^{13}CO_2$ profiles after intake of three naturally abundant in ^{13}C foods rich in carbohydrates. *Ann Acad Med Siles*. 2006;60: 206–212.

4. Jonderko K, Kasicka-Jonderko A, Kamińska M, Błońska-Fajfrowska B. A systematic study on a neutral meal suitable for subjects undergoing $^{13}CO_2$ breath tests. *Med Sci Monit*. 2008; 14: CR543–CR546.

5. Kasicka-Jonderko A, Jonderko K, Chabior E, Błońska-Fajfrowska B. Exact profiles of $^{13}CO_2$ recovery in breath air after per oral administration of ^{13}C-methacetin in two groups of different age. *Isotopes Environ Health Stud*. 2008;44:295–303.

6. Kasicka-Jonderko A, Loska D, Jonderko K, Kamińska M, Błońska-Fajfrowska B. Interference of acute cigarette smoking with [^{13}C]methacetin breath test. *Isotopes Environ Health Stud*. 2011;47:34–41.

7. Kasicka-Jonderko A, Jonderko K, Bizior-Frymus D, Kamińska M, Błońska-Fajfrowska B. Interferencia por los anticonceptivos hormonales con el metabolismo de ^{13}C-metacetina. XXXII Congreso Panamericano de Enfermedades Digestivas, Ecuador, Guayaquil, 2–4 Octubre 2010, CD "Trabajos libres", abstract # HGD064.

8. Kasicka-Jonderko A, Jonderko K, Galas E, Kamińska M, Błońska-Fajfrowska B. Influencia del estado hormonal de las mujeres sobre la actividad mitocondrial del hígado. XXXII Congreso Panamericano de Enfermedades Digestivas, Ecuador, Guayaquil, 2–4 Octubre 2010, CD "Trabajos libres", abstract # HGD063.

9. Jonderko K, Gabriel-Jaśniok A, Szymszal M, Kasicka-Jonderko A, Błońska-Fajfrowska B. Unreliability of breath methane as a candidate indicator of functional bowel disorders. *Gut Liver*. 2008;2:180–185.

10. Kasicka-Jonderko A, Nita A, Jonderko K, Kamińska M, Błońska-Fajfrowska B. ^{13}C-methacetin breath test reproducibility study reveals persistent CYP1A2 stimulation on repeat examinations. *World J Gastroenterol*. 2011;17:4979–4986.

11. Kasicka-Jonderko A, Jonderko K, Kamińska M, Bielecka M, Błońska-Fajfrowska B. ^{13}C-alpha-ketoisocaproic acid breath test revisited—an in-depth reproducibility study advocates an extended breath sampling period. *Dig Dis Sci*. 2007;52:3481–3487.

12. Kasicka-Jonderko A, Jonderko K, Ptaszek K, Kamińska M, Błońska-Fajfrowska B. La reproducibilidad de la prueba del aliento con ^{13}C-fenilalanina. XXXII Congreso Panamericano de Enfermedades Digestivas, Ecuador, Guayaquil, 2–4 Octubre 2010, CD "Trabajos libres", abstract # HGD065.

13. Loo FD, Palmer D, Soergel K, Kalbfleisch JH, Wood CM. Gastric emptying in patients with diabetes mellitus. *Gastroenterology*. 1984;86:485–494.

14. Bland J, Altman D. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;i: 307–310.