TURNOVER OF CARBON IN THE $^{13}$C-UREA BREATH TEST FOR THE DETECTION OF *Helicobacter pylori* INFECTION

Vladimir E. Costa$^{1,2}$, Mariana Andreazzi$^2$, Caio S. Cury$^2$, Carlos A. Z. Bassetto Júnior$^2$, Maria A. M. Rodrigues$^3$ and Carlos Ducatti$^{1,2}$

$^1$ Centro de Isótopos Estáveis - Instituto de Biociências de Botucatu, Universidade Estadual Paulista, Distrito de Rubião Jr, s/n, 18618-970 Botucatu, SP. vladimir@ibb.unesp.br, ducatti@ibb.unesp.br

$^2$ Departamento de Física e Biofísica - Instituto de Biociências de Botucatu, Universidade Estadual Paulista, Distrito de Rubião Jr, s/n, 18618-970 Botucatu, SP. mariana.andreazzi@gmail.com, caiocury@hotmail.com, juniorbassetto@hotmail.com

$^3$ Departamento de Patologia - Faculdade de Medicina de Botucatu, Universidade Estadual Paulista, Distrito de Rubião Jr, s/n, 18618-970 Botucatu, SP. mariar@fmb.unesp.br

ABSTRACT

To obtain a standard protocol for the application of $^{13}$C-urea breath test ($^{13}$C-UBT) analyzed by Isotope Ratio Mass Spectrometer (IRMS) to detect *Helicobacter pylori* infection in the population is necessary to know the behavior of the turnover of $^{13}$C during the test in different individuals. The aims of this study was to find out a pattern for the turnover of the $^{13}$C in the $^{13}$C-UBT, analyzed by IRMS, in patients infected with *H. pylori*, in a Brazilian population, to define a protocol test application. We found that the isotopic ratio $^{13}$C/$^{12}$C in expired CO$_2$ from patients infected with *H. pylori* and subjected to $^{13}$C-UBT does not follow a single pattern of behavior. However this behavior can be similar in subjects having the same maximum values following an inverse proportional relationship between the maximum value and the time of appearance in the curve.

1. INTRODUCTION

After the discovery of *Helicobacter pylori* at 1979, which yielded the Nobel Prize for Medicine and Physiology in 2005 for B. J. Marshall and j. R. Warren, there was a revolutionary change in understanding the process of cause, diagnostic and treatment of various pathological process of the upper digestive tract [1]. This discovery contributed to numerous scientific publications about the subject that concluded that the bacterium infects the gastric mucosa of most patients with duodenal ulcer disease or gastric and antral gastritis.

Eradicating of bacteria cure gastritis and duodenal ulcers and decreases the relapse of this disease. Contagion with *H. pylori* is not well stated, but it is believed that the main way of
acquisition of the bacteria either by feed unwashed. Treatment with antibiotics has shown effectiveness in most cases and diagnostic methods to identify the bacteria as divided into two groups: invasive and non-invasive [2].

The invasive diagnostic methods are performed, in most cases, by upper endoscopy, where you can perform a biopsy for histopathological examination and culture, or apply the urease rapid test. Of these, histopathological exam is the one with higher sensitivity and specificity for detection of infection by *H. pylori*. Diagnostic centers in Brazil this exam predominates due to low cost and utilization of infrastructure for gastric endoscopy.

The non-invasive diagnostic methods are labeled urea breath test in \(^{13}\text{C} - {^{13}\text{C}}\text{-UBT}\) and fecal test. Of these, the \(^{13}\text{C}\)-UBT is widely accepted because it has higher sensitivity and specificity when compared to the fecal test [2-4]. The \(^{13}\text{C}\)-UBT uses the carbon-labeled urea that is ingested orally. When present in the stomach *H. pylori* degrade urea, liberating carbon labeled urea, which is absorbed by the body and excreted as carbon dioxide by respiration. If the carbon dioxide does not contain the labeled carbon at doses higher than the baseline, the patient has not infection with *H. pylori* [5-7].

Initially we used the \(^{14}\text{C}\)-UBT, which is a radioisotope or radioactive isotope to make urea and a radiation detector to analyze the breath [6]. Currently the use of the markings \(^{14}\text{C}\) urea in this test was banned and replaced by the \(^{13}\text{C}\)-UBT that is not radioactive and therefore a stable isotope [8]. The disused \(^{14}\text{C}\) happened due to our strict control for use of radioactive material and exposure to radiation. In the \(^{13}\text{C}\)-labeled urea, analyzes of the breath of the patient is made by the \(^{13}\text{C}/^{12}\text{C}\) isotope ratio. If increase ratio, the patient infected by *H. pylori* [6].

The \(^{13}\text{C}/^{12}\text{C}\) isotope radio is expressed in ‰ and exhibit in DOB – delta over baseline value – that is the difference of sample value minus baseline [6]. The \(^{13}\text{C}/^{12}\text{C}\) isotope ratio analyzes can be performed by four types of equipment: Isotope Ratio Mass Spectrometer – IRMS; Non-Dispersive Isotope-Selective Infrared Spectroscope – NDIRS [9-11]; Laser-Assisted Ratio Analyser - LARA [12]; and Fourier Transform Infrared Spectroscopy - FTIR [13], but these latter two are not as widely used as the NDIRS and IRMS.

The NDIRS is longer used due the low initial cost, but has a lower sensitivity and specificity making only a quantitative analyze, and takes longer to realize analyzes than the IRMS [14,10]. On the other hand, the IRMS, besides more accurate, has the ability to analyze large number of samples equaling or decreasing the cost of NDIRS long term. Both have the ease of not having to move the patient to a specific location and can be performed even in a hospital bed.

The dose used in the urea breath test has been subject of controversy, but today the literature has reached a consensus that the minimum necessary for the examination in adults and children is 75mg of urea and 1.4g of citric acid [14,15,3,7], and almost all manufacturers use these doses regardless of the type of analyzer.

The time interval for collecting the breath after ingestion of \(^{13}\text{C}\)-UBT is still subject of controversy [14,16,11], and most research has been conducted in population of countries in the first world. Another important tissue and controversy regarding the procedure of \(^{13}\text{C}\)-UBT is the cut off that characterizes the test as positive or negative [17,16,11]. This value differs
between scientific publications on the subject by creating uncertainty about the correct value to use in the test, so as to NDIRS for IRMS [17].

The $^{13}$C-UBT using the NDIRS is not able to quantify the analysis; only qualify the result as positive or negative. On the other hand, the test using the IRMS is able to qualify and quantify the test for density colonization of gastric mucosa by *H. pylori*. This methodology is not well defined and needs further research [18,10].

To obtain a standard protocol for the application of $^{13}$C-UBT analyzed by IRMS in other populations is necessary to know the behavior of the turnover of $^{13}$C during the test in different individuals, and from this, identify the best time to collect, the cutoff and some relationship of the value of the test with the density of colonization of the gastric mucosa. The aim of this study was to find a pattern for turnover in $^{13}$C on $^{13}$C-UBT in patients infected by *H. pylori* to define protocol test application.

2. MATERIALS AND METHODS

We selected 53 patients who presented at the time at the Endoscopy Section Hospital of Botucatu to conduct the examination of upper endoscopy pre-scheduled. At selection were observed four contraindications for $^{13}$C-UBT: 1) do not want to participate; 2) be fasting less than 4 hours; 3) has less than eighteen years old; 4) have administered proton pump inhibitors (PPI or omeprazole) or antibiotics (ABS) in the last four weeks.

The $^{13}$C labeled urea used in the study was the composite liquid marketed to the pharmaceutical from and content for oral solution in 10ml vial containing the following specifications: active 75mg $^{13}$C-urea (stable isotope, not radioactive carbon); excipient (inactive substance used as a vehicle to complete the active mass or volume specified) citric acid monohydrate in purified water sq. 10ml [7].

The $^{13}$C-UBT was performed in selected patients at the Endoscopy Section of the University Hospital of Botucatu and consisted of three steps: 1) collection of breath in duplicate before the intake of urea and fasting; 2) oral ingestion of urea dissolved in 200ml of water, the record start time and rinsing the mouth with water without ingestion [3,11]; 3) collection of duplicate breath after ingestion of urea from 5.0 minutes to 30.0 minutes intervals 2.5 minutes; 4) collection of the breath in duplicate from 30.0 minutes to 45.0 minutes at intervals of 5.0 minutes.

Each collection is made by blowing through a straw which the patient breathes into a 12ml tube with screw cap which is closed immediately after the blow [6]. The straws and tubes are disposable and supplied in a urea with quite marked. Analyses blows properly stored in the tubes were held in a IRMS in Stable Isotope Center, Institute of Biosciences, UNESP, Botucatu campus.

After the $^{13}$C-UBT patients underwent endoscopy where biopsies were performed in the gastric body and antrum for histology. This study was submitted and approved by the local Research Ethics, Faculty of Medicine of Botucatu, 292.236.
3. RESULTS

Of the 52 patients selected for application of $^{13}$C-UBT, 23 were and 29 were not infected by *H. pylori* considering the time for air collect in 10 minutes and the cut-off value 3,0‰ [7]. The results of 13C-UBT agreed in all results of histological examination, with 100% of specificity and sensitivity.

The $^{13}$C turnover in the infected patients did not follow an uniform behavior and presented within an interval of analyzed time of 45 minutes some behaviors, like: more or less accentuated crescent; crescent and stable; and more or less accentuated crescent and decrescent, as observed in figure 1.

![Figure 1](image)

**Figure 1** $^{13}$C turnover in the $^{13}$C-UBT tests at 52 patients analyzed.

The different behaviors of $^{13}$C turnover is not associated at genre or age of the patients that are shown in table 1. In the possibility to find groups with different behaviors, the patients were divided in three groups.

The maker of $^{13}$C labeled urea used in this study recommends the collect of breath in 10 minutes for a conventional test. In this interval of time, we divided the turnover in groups: low (with DOB until 20‰); medium (with DOB from 20‰ to 40‰); and high (with DOB more than 40‰), to facilitate our interpretation of results showed in table 1.

The maximum value of DOB for each turnover also has a close relationship with the groups division and the interval of time that each turnover reaches the maximum value of DOB, in table 1.

Observing individually the low, medium and high groups in figures 2, 3 and 4, respectively, the behavior of turnover seems to have a pattern for each other group. The low group presents a slow growth in its DOB value that could persist in all of analyzed interval of time. The other turnover patients on this group took a maximum value followed by a decrease in DOB value.
Table 1 Characteristics of the 23 patients infected by *H. pylori* and divided between low (≤ 20‰), medium (from 20‰ to 40‰) and high (≥ 40‰) groups, referring to DOB in 10 minutes and the maximum DOB value of $^{13}$C turnover during the $^{13}$C-UBT.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>DOB ‰ (10min.)</th>
<th>Group (High, Middle and Low)</th>
<th>Maximum DOB ‰</th>
<th>Maximum DOB %</th>
</tr>
</thead>
<tbody>
<tr>
<td>P01</td>
<td>M</td>
<td>37</td>
<td>23,53</td>
<td>M</td>
<td>31,29</td>
<td>17,5</td>
</tr>
<tr>
<td>P02</td>
<td>F</td>
<td>53</td>
<td>26,55</td>
<td>M</td>
<td>37,96</td>
<td>27,5</td>
</tr>
<tr>
<td>P08</td>
<td>M</td>
<td>45</td>
<td>15,69</td>
<td>L</td>
<td>15,99</td>
<td>15,0</td>
</tr>
<tr>
<td>P11</td>
<td>F</td>
<td>31</td>
<td>10,85</td>
<td>L</td>
<td>26,41</td>
<td>45,0</td>
</tr>
<tr>
<td>P12</td>
<td>M</td>
<td>44</td>
<td>82,81</td>
<td>H</td>
<td>91,58</td>
<td>15,0</td>
</tr>
<tr>
<td>P13</td>
<td>F</td>
<td>36</td>
<td>3,15</td>
<td>L</td>
<td>6,57</td>
<td>45,0</td>
</tr>
<tr>
<td>P14</td>
<td>F</td>
<td>34</td>
<td>20,45</td>
<td>M</td>
<td>20,45</td>
<td>10,0</td>
</tr>
<tr>
<td>P17</td>
<td>F</td>
<td>32</td>
<td>95,43</td>
<td>H</td>
<td>103,79</td>
<td>12,5</td>
</tr>
<tr>
<td>P18</td>
<td>F</td>
<td>49</td>
<td>30,38</td>
<td>M</td>
<td>30,38</td>
<td>10,0</td>
</tr>
<tr>
<td>P21</td>
<td>F</td>
<td>62</td>
<td>11,77</td>
<td>L</td>
<td>20,33</td>
<td>45,0</td>
</tr>
<tr>
<td>P22</td>
<td>F</td>
<td>47</td>
<td>8,91</td>
<td>L</td>
<td>12,67</td>
<td>27,5</td>
</tr>
<tr>
<td>P27</td>
<td>F</td>
<td>58</td>
<td>22,16</td>
<td>M</td>
<td>36,93</td>
<td>30,0</td>
</tr>
<tr>
<td>P29</td>
<td>F</td>
<td>58</td>
<td>98,25</td>
<td>H</td>
<td>105,62</td>
<td>15,0</td>
</tr>
<tr>
<td>P30</td>
<td>F</td>
<td>53</td>
<td>33,08</td>
<td>M</td>
<td>50,56</td>
<td>27,5</td>
</tr>
<tr>
<td>P31</td>
<td>M</td>
<td>59</td>
<td>26,88</td>
<td>M</td>
<td>38,68</td>
<td>27,5</td>
</tr>
<tr>
<td>P35</td>
<td>M</td>
<td>36</td>
<td>15,19</td>
<td>L</td>
<td>39,05</td>
<td>45,0</td>
</tr>
<tr>
<td>P38</td>
<td>M</td>
<td>64</td>
<td>51,90</td>
<td>H</td>
<td>54,67</td>
<td>12,5</td>
</tr>
<tr>
<td>P39</td>
<td>M</td>
<td>52</td>
<td>37,19</td>
<td>M</td>
<td>52,77</td>
<td>30,0</td>
</tr>
<tr>
<td>P42</td>
<td>M</td>
<td>48</td>
<td>68,68</td>
<td>H</td>
<td>80,61</td>
<td>17,5</td>
</tr>
<tr>
<td>P43</td>
<td>M</td>
<td>59</td>
<td>12,16</td>
<td>L</td>
<td>13,09</td>
<td>17,5</td>
</tr>
<tr>
<td>P45</td>
<td>M</td>
<td>56</td>
<td>14,01</td>
<td>L</td>
<td>17,04</td>
<td>30,0</td>
</tr>
<tr>
<td>P46</td>
<td>F</td>
<td>50</td>
<td>28,87</td>
<td>M</td>
<td>39,93</td>
<td>30,0</td>
</tr>
<tr>
<td>P50</td>
<td>M</td>
<td>62</td>
<td>9,26</td>
<td>L</td>
<td>18,79</td>
<td>45,0</td>
</tr>
</tbody>
</table>

Figure 2 $^{13}$C turnovers during the $^{13}$C-UBT in the patient infected by *H. pylori* of low groups with DOB until 20‰ in the time of 10 minutes.
The medium group also presents a characteristic behavior of $^{13}$C turnover, being a moderate growth of DOB at beginning until a little more of half of the time interval analyzed and a decrease also moderate. In this group, it is observed that some behaviors of turnover with decrease are more accentuated and begun before of half of the time interval analyzed.

**Figure 3** $^{13}$C turnovers during the $^{13}$C-UBT in patients infected by *H. pylori* of the medium groups with DOB from 20‰ to 40‰ in 10 minutes.

The high group, even with fewer patients than the other groups, presents other pattern of behavior for turnover, being a quickly growth at the beginning followed by an abrupt decrease of DOB, but with high values in the DOB maximum.

**Figure 4** $^{13}$C turnovers during the $^{13}$C-UBT in patients infected by *H. pylori* of the high group with DOB more than 40‰ in the time of 10 minutes.
The maximum DOB value of $^{13}$C turnover of the 23 patients infected is divided in groups in function of time and showed in figure 5. It is observed that the highest maximum DOB value appears earlier and faster than other groups.

**Figure 5** Maximum DOB values of $^{13}$C turnover during the $^{13}$C-UBT in patients infected by *H. pylori* of the high, medium and low groups.

4. **DISCUSSION**

The present study was undertaken to investigate if there is a pattern for the $^{13}$C turnover in $^{13}$C-UBT, in patients infected by *H. pylori*, to define a protocol of test application. We have found that the isotopic ratio $^{13}$C/$^{12}$C in CO$_2$ expired by infected patients and submitted to $^{13}$C-UBT does not follow an unique pattern, but this behavior can be similar in infected patients with the maximum values of DOB too close, and also follows, approximately, an inversely relationship between the maximum DOB value and the time of it appearance in the curve.

This turnover $^{13}$C behavior in $^{13}$C-UBT could be due to different densities of *H. pylori* colonization in the gastric mucosa. Considering the hypotheses that the patients of low, medium and high groups of DOB may also have low, medium and high densities of *H. pylori* colonization in the gastric mucosa this would justify that the quickly increase of DOB would be caused by activity of several bacteria, as proposed by Suto et al. [18].

A little variation of $^{13}$C turnover could be related to several factors, such as the fasting state less than 4 hours, differences in metabolism for each patient, the use of PPI or antibiotics in the last 4 weeks. The absence of fasting can influence the $^{13}$C turnover behavior by slowing the increase of DOB. This pattern of behavior was seen in patients P11, P13, P35, P50 from the low group, as show in figure 2, probably due to the dilution of urea with the stomach contents [19,20].

Patients with faster metabolism can influence the $^{13}$C turnover, not at the beginning of the test, but latter, with a quick decreases of DOB, as observed in patients P01, P14 and P18, as
show in figure 3, and P12, P17, as show in figure 4. This behavior pattern might be related to the influence of medications, such as PPI or ABS, not mentioned by the patients [21].

The description presented of $^{13}$C turnover behavior can be useful to set the best time and cut-off value in different populations. The results of the present study add important information for the best time to collect the samples for the $^{13}$C-UBT, and for the cut-off values in the population under study. This is the first study on the investigation of $^{13}$C turnover in $^{13}$C-UBT in a Brazilian population.

In conclusion, our finding show that the $^{13}$C turnover in $^{13}$C-UBT performed with IRMS in patients with H. pylori infection does not follow an unique behavior pattern, but the maximum DOB value presents an inversely proportional relations hip with time of it appearance in the turnover curves.

REFERENCES


