13C urea breath test to identify *Helicobacter pylori* infection in patients with upper gastrointestinal bleeding admitted to the Emergency Department


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**Abstract.** – **OBJECTIVE:** Upper gastrointestinal bleeding (UGIB) is a cause of Emergency Department (ED) visits. Peptic ulcer secondary to *H. pylori* (HP) infection and/or to the use of NSAIDs is the most frequent cause. The aim of the study is to evaluate directly in the ED the prevalence of HP infection through Urea Breath test (UBT) in patients admitted to the ED for UGIB.

**PATIENTS AND METHODS:** We enrolled 87 patients (58M/29F) with a mean age of 63.8 ± 11.7 yrs with an active UGIB who performed EGDS and UBT.

**RESULTS:** 34.4% of patients performing EGDS and UBT resulted positive to HP. Peptic ulcer was present in 20/30 (66.7%) of HP+ compared to 20/57 (35.1%) of HP- (p<0.001), and also gastritis and/or duodenitis were mostly present in HP+ (23.3% vs. 15.8%) (p<0.05). A biopsy was performed in only 31% of patients with a positive rate of 33.3%. In 78% we obtained a correspondence between UBT and biopsy results. Compared to biopsy result, we obtained for UBT a positive predictive value (PPV) of 71% and a negative predictive value (NPV) of 80%. Taking the UBT as a gold standard, we obtained for biopsies a PPV of 69% and a NPV of 85%.

**CONCLUSIONS:** Our study confirms that the use of UBT directly in ED in patients with UGIB allows for a rapid, reliable and non-invasive diagnosis of HP infection as a causative agent for bleeding, thus permitting a right etiological treatment.

**Key Words:** Upper gastrointestinal bleeding, *Helicobacter pylori*, Urea breath test, Emergency Department.

**Introduction**

Melena and hematemesis, as an expression of an UGIB, represent among the most common causes of admissions to the Emergency Department (ED). According to recent estimates, the in-hospital management costs are considerably high, reaching about 3’400$ for uncomplicated nonvariceal bleeding, and around 5’600 $ for complicated ones1.

In all cases, peptic ulcer represents the most frequent and important cause of acute UGIB, being responsible for approximately 35 to 60% of episodes. The main risk factors for peptic ulcer development are well known: HP infection and the use of NSAIDs2.

Detection of HP during bleeding can be a challenge, as the accuracy of the diagnostic methods is diminished in this setting. The invasive methods (histology, culture and rapid urease test) need biopsies, and this could be a problem. Some study showed that urease test has a reduced sensitivity (25% to 40%), with many false negatives in case of UGIB3-5. This suggests that blood plasma has a buffering effect and adversely affects the urease activity. Fantry et al6 stated that the position of nasogastric tubes and gastric lavage before the endoscopy may reduce the bacterial load, thus leading to false-negative diagnostic test. Colin et al7 found a low performance, with sensitivity around 30%, for the other invasive tests (i.e., histology and culture), too.

Finally, serology is associated with a high false-positive rate because antibodies persist even after the infection has been eradicated8.

As regards noninvasive methods, both urea breath test (UBT) and the stool antigen test (HP-SA) have an excellent accuracy9, even if UBT requires that the patient be off proton pump inhibitors and antibiotics prior to testing, and the stool
test is not preferred in clinical practice because it involves the collection of feces\textsuperscript{10}.

However, considering that both serologic, histologic tests and HPSA require particularly long technical waiting times, the most indicated test to be conducted in emergency for acute UGIB is undoubtedly UBT\textsuperscript{11-13}. Furthermore, in a systematic review by Calvet et al\textsuperscript{14} evaluating 11 studies with a total of 811 patients, the authors reported for UBT a sensitivity of 93\%, specificity of 88\% and a positive predictive value of 97\%. However, given that false negatives do exist, when there is clinical suspicion of the presence of HP, but the first UBT is negative, this result must be taken with caution and a second UBT should be conducted\textsuperscript{15}.

Nowadays, for a bleeding peptic ulcer, the available strategies are based on a continuous treatment with proton pump inhibitors (PPI) and HP eradication in patients who might have an increased bleeding risk. Therefore, the European Society of Gastroenterology (ESGE) recommends, for patients bleeding from a peptic ulcer, a search for HP directly at acute presentation, so as to promptly start a possible eradication therapy and to reduce the risk of recurrence\textsuperscript{16}. Moreover, the International Consensus Recommendations on the management of nonvariceal gastrointestinal bleeding\textsuperscript{15} stress the importance of HP testing, eradication and successful treatment confirmation. Indeed, a metanalysis by Gisbert et al\textsuperscript{17} underlines how convenient eradication therapy is with respect to performing an EGDS once the bleeding recurrence has occurred.

Patients coming to ED with melena or hematemesis are initially evaluated, stabilized and, in the majority of cases, an esophagogastroduodenoscopy (EGDS) is required in order to establish the etiological diagnosis, treatment and hemostasis. However, in the clinical practice of UGIB, it is uncommon to perform a biopsy for HP identification during endoscopy, due to the presence of blood onto the gastric walls or when the exam is performed in the emergency during night or holidays\textsuperscript{18,19}.

This represents an issue for the future therapy prescription at discharge: indeed, these patients start a high-dose PPI treatment, which delays a possible Urea Breath Test (UBT) for the search of HP, with an important impact on management costs and recurrence risk.

Recent literature clearly shows that in a group of patients with UGIB, where HP was not investigated or the eradication therapy failed, re-bleeding risk at one year was present in 1/3 of the study population\textsuperscript{16}. On the other side, a Japanese study demonstrated that patients with a successful eradication did not show further episodes of bleeding\textsuperscript{20}.

With this background, the aim of our study is to estimate the prevalence of HP infection in patients admitted to the ED for UGIB via UBT, to quickly establish a rapid, reliable and non-invasive diagnosis of the bleeding cause.

**Patients and Methods**

This is a monocentric, prospective study conducted at the ED of Fondazione Policlinico Universitario A. Gemelli IRCCS. All of the enrolled patients, who came with acute UGIB, underwent routine blood tests (full blood cell count, blood chemistry test, coagulation tests), UBT and EGDS when appropriate.

**Study Population**

We enrolled 87 (58M/29F) consecutive patients, from the 1st of June 2019 to the 1st of June 2020, admitted to our ED with UGIB (melena and/or hematemesis).

**Inclusion criteria**

- Melena and/or hematemesis (current/within the previous 48 hours).
- Fasting for at least 6 hours before the test.
- Signature of informed consent.

**Exclusion criteria**

- < 18 years old.
- Suspected or confirmed pregnancy.
- Hemodynamically unstable patients.
- Antibiotics, bismuth compounds and proton pump inhibitors (PPIs) for at least two weeks.
- Patients with a previous gastric resection.
- Unable to swallow or with a nasogastric tube.
- Patients could withdraw from the study upon written request.

The flowchart representing the enrollment process, with the application of exclusion criteria, is shown in Figure 1.

**Urea Breath Test**

The commercial brand of the 13C-UBT that we used was UBT-Kit (Richen Europe, Milan, Italy).

We collected an exhaled air sampling at baseline (before urea administration): the patient is asked to take a deep breath and blow in a sachet exhaling all
the air contained in the lungs. At this point, the patient drinks a solution of 13C labeled urea (75 mgr.) mixed with citric acid (to reduce gastric emptying) in 200 ml of water and waits for thirty minutes without eating or drinking. At the end, the entire procedure is repeated in a second sachet.

Breath tests were collected in twice, with a concordance of 98.5% (Cohen's test).

The exhaled air is then analyzed, within 24 hours, by an infrared spectrometer “IR-force 200” by Beijing Richen-force Science and Technology Co. Ltd., which allows to measure the amount of CO2 13C with respect to the total of expired CO2 12C in three minutes.

IR Force – 200 is an instrument designed to guarantee an efficient and rapid response in the diagnosis of HP infections with an optimal performance for small and medium routines.

The instrument is user-friendly and does not require specialized personnel for its use. The difference in the ratios between the baseline value and the post-urea value is referred to as delta over baseline (DOB). A DOB >3.50 ppm after thirty minutes is commonly considered diagnostic for HP infection.

**EGDS**

All EGDSs were performed within 48 hours from the access to ED by expert endoscopist with a high-resolution double-channel video endoscope (Olympus GIF 2-T scope, Tokyo, Japan).

**Ethics Statement**

The study was conducted in accordance with the Declaration of Helsinki. None of the patients or authors received any honorary or economic benefits for the participation in this work.

The study was approved on 29th March 2019 by the Ethical Committee of the Università Cattolica del Sacro Cuore of Rome, with ID 2302, Protocol No. 14245/19.

**Statistical Analysis**

A descriptive statistical analysis was performed using absolute and relative frequencies, mean and standard deviation (SD), when appropriate, for demographic and clinical characteristics of surveyed patients and clinical outcome parameter. Statistically significant differences in clinical outcome parameter, between hospitalized and non-hospitalized patient, were tested through t-test, Wilcoxon rank-sum (Mann-Whitney test), Chi-square test, as applicable. Association between length of stay and other continuous variables was tested using Spearman correlation. Multi-variate analysis was performed to evaluate the impact of independent variables in predicting length of stay. The statistical significance level was set at \( p < 0.05 \) and all the analyses were carried out by using the software “Stata MP 14 for Mac” (Stata Corp., Lakeway, TX, USA).

**Results**

We enrolled 87 patients (58M/29F) with a mean age of 63.8 ± 11.7 yrs. None of the patients had any problem in completing the UBT. UBT (DOB>3.5) was positive in 30 out of 87 patients (34.4%) with a mean DOB value of 22.37 ± 15.11 ppm. The mean age of positive patients was 64 ±10.4 yrs, meanwhile for negative patients was 63.7 ± 11.9 yrs (\( p = \text{ns} \)). Prevalence of \( HP^+ \) was significantly higher in males compared to females (80% vs. 20%; \( p < 0.001 \)) (Figure 1).

Meanwhile, in negative patients (\( HP^- \)) we observed only a slightly higher prevalence of males 34M/23F (59.7% vs. 40.3%, \( p = \text{ns} \)). Interestingly, females showed a significant higher mean DOB value compared to males (51.70±35.11 vs. 15.7±11.6; respectively \( p < 0.001 \)).

Demographic characteristics are shown in Table I.

**Clinical Features**

Clinical features and statistical significance are summarized in Table I.

As regards clinical presentation such as hematemesis, melena or syncope we did not observe any difference between the two groups and previous GI bleeding occurred with the same frequency.
A history of coronary artery disease was referred in 13.3% of HP+ compared to 8.8% of HP- (p<0.05), meanwhile no difference was observed for cerebrovascular disease. As regards medication history, interestingly we observed that NSAID had been used only in 3/30 (10.0%) of HP+ compared to 14/57 (24.6%) in HP- (p<0.001), also antiplatelet agents were used more frequently in HP- compared to HP+ (21.0% vs. 16.7%) (p<0.05) suggesting a prevalent role of this drugs in the genesis of GI bleeding. Low hemoglobin levels (<9 g/dL) on admission were significantly less prevalent in HP+ compared to HP- (56.7% vs. 43.9%, respectively) (p<0.05) (Table I).

### Endoscopic Findings

Endoscopic findings in HP+ and HP- are summarized in Table II.

As expected, peptic ulcer was present in 20/30 (66.7%) of HP+ compared to 20/57 (35.1%) of HP- (p<0.001), and also gastritis and/or duodenitis were mostly present in HP+ (23.3% vs. 15.8%) (p<0.05). Another interesting result is that in 36% of HP- patients a definite source of bleeding was not detected through upper GI endoscopy, compared to only 3.3% in HP+ (p<0.001), suggesting a bleeding from the small or large bowel.

### Biopsies

A biopsy during endoscopy was performed in only 27 out of 87 (31%) patients with a positive rate of 33.3% (9/27). In 78% (21 out of 27 cases) we obtained a correspondence between UBT and biopsy results, on the contrary in 6/27 (22.2%) the results were discordant. In particular, in 4 patients the biopsy resulted positive and the UBT negative, while in the other 2 patients UBT resulted positive and the biopsy negative. Compared to biopsy result, we obtained for UBT a positive predictive value (PPV) of 71% and a negative pre-

### Table I. General characteristics of overall population, HP positive (HP+) and negative (HP-) patients.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Overall</th>
<th>HP+</th>
<th>HP-</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age</td>
<td>63.8 ± 11.7</td>
<td>64± 10.4</td>
<td>63.7 ± 11.9</td>
<td>ns</td>
</tr>
<tr>
<td>Gender</td>
<td>M 58 (66.7%)</td>
<td>M 24 (80.0%)</td>
<td>M 34 (59.7%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>F 29 (33.3%)</td>
<td>F 6 (20.0%)</td>
<td>F 23 (40.3%)</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Mean DOB by gender (for HP+)</td>
<td>M 15.03 ± 11.6</td>
<td>F 51.70 ± 35.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical presentation</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hematemesis</td>
<td>22 (25.3%)</td>
<td>6 (20.0%)</td>
<td>16 (28.1%)</td>
<td>ns</td>
</tr>
<tr>
<td>Melena</td>
<td>67 (77.0%)</td>
<td>22 (73.3%)</td>
<td>45 (79.0%)</td>
<td>ns</td>
</tr>
<tr>
<td>Syncope</td>
<td>11 (12.6%)</td>
<td>5 (16.7%)</td>
<td>6 (10.5%)</td>
<td>ns</td>
</tr>
<tr>
<td>Hb &lt; 9 g/dL (admission)</td>
<td>42 (48.3%)</td>
<td>17 (56.7%)</td>
<td>25 (43.9%)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Length of stay (LOS) &lt; 3 days</td>
<td>65 (74.7%)</td>
<td>25 (83.3%)</td>
<td>40 (70.2%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Medical history and medications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous bleeding</td>
<td>18 (20.7%)</td>
<td>8 (26.7%)</td>
<td>10 (17.5%)</td>
<td>ns</td>
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<tr>
<td>Coronary Artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease</td>
<td>9 (10.3%)</td>
<td>4 (13.3%)</td>
<td>5 (8.8%)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>3 (3.5%)</td>
<td>1 (3.3%)</td>
<td>2 (3.5%)</td>
<td>ns</td>
</tr>
<tr>
<td>NSAID</td>
<td>17 (19.5%)</td>
<td>3 (10.0%)</td>
<td>14 (24.6%)</td>
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<td>Antiplatelet</td>
<td>17 (19.5%)</td>
<td>5 (16.7%)</td>
<td>12 (21.1%)</td>
<td>&lt; 0.05</td>
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<tr>
<td>Warfarin</td>
<td>3 (3.45%)</td>
<td>1 (3.3%)</td>
<td>2 (3.5%)</td>
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<tr>
<td>DOAC</td>
<td>7 (8.1%)</td>
<td>4 (13.3%)</td>
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### Table II. EGDS findings in HP+ and HP- patients.

<table>
<thead>
<tr>
<th>Endoscopic diagnosis</th>
<th>HP+ (n = 30)</th>
<th>HP- (n = 57)</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Peptic ulcer</td>
<td>20 (66.7%)</td>
<td>20 (35.1%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Gastritis/Duodenitis</td>
<td>7 (23.3%)</td>
<td>9 (15.8%)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Variceal bleeding</td>
<td>0 (0.0%)</td>
<td>1 (1.7%)</td>
<td>ns</td>
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<tr>
<td>Esophagitis</td>
<td>1 (3.3%)</td>
<td>3 (5.3%)</td>
<td>ns</td>
</tr>
<tr>
<td>Gastric polyposis</td>
<td>0 (0.0%)</td>
<td>3 (5.3%)</td>
<td>ns</td>
</tr>
<tr>
<td>Delafox ulcer</td>
<td>1 (3.3%)</td>
<td>0 (0.0%)</td>
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<tr>
<td>Negative EGDS</td>
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dictive value (NPV) of 80%. Taking the UBT as a gold standard, we obtained for biopsies a PPV of 69% and a NPV of 85%.

**Length Of Stay (LOS)**

83% of HP+ patients had a hospitalization of <3 days, compared to 70% of HP-, reaching a statistically significant difference ($p<0.001$), thus suggesting that the research for HP infection in ED and its positivity reached an etiological diagnosis of UGIB.

**Discussion**

Our study showed that the most common clinical manifestation of UGIB is melena, that males are more infected by HP than female, meanwhile, the DOB value observed in females is significantly higher than males. This data is confirmed by Martel et al\(^1\) that found an association between HP infection and male gender with an OR of 1.16; this close association could explain also the higher prevalence of peptic ulcer and gastric cancer in males\(^2\).

The DOB value seems to be linked to the HP density\(^3\). Indeed, some authors found a strong correlation among UBT value, HP load and gastric inflammation\(^4\).

Some authors speculated that gastric emptying rates, bacterial colonization density, or even HP urease production in the host depends on gender difference\(^5\).

The higher level of DOB value observed in females and the consequent HP density could have an implication in the well-known less successful eradication rate.

**HP** prevalence is variable between 19% and 88% and depends on various factors such as geographical location, patient’s age, sanitation, and socioeconomic status\(^6\). We found that one-third of our population with UGIB harbour HP in their stomach\(^7\).

In the early 2000s, some researches were published\(^8,9,10\) on the importance of researching HP infection in patients with acute bleeding through the UBT. Most of them indicate the usefulness of this noninvasive test to diagnose HP infection in patients with bleeding peptic ulcers, suggesting that the test was not affected by the presence of blood in the stomach.

In particular, these studies\(^8,9,10\) evaluated HP infection through UBT in the setting of acute UGIB with reasonably accurate results. In the study by Valayos et al\(^10\), the authors found a positive predictive value of 96%, but a negative predictive value of 54% suggesting that false negatives might often occur. Winiarski et al\(^10\), instead, obtained a much superior negative predictive value.

They all concluded that larger studies are however required prior to advocating a negative UBT test as a sufficiently accurate result in this setting.

To our knowledge, so far, no investigations have been published on this topic. Therefore, we wanted to carry out a study to give an update nowadays on the efficacy of UBT directly in ED patients with UGIB and on the prevalence of HP infection in these patients. Compared to biopsy result, in our study, we obtained, for UBT, a positive predictive value (PPV) of 71%, and a negative predictive value (NPV) of 80%. Some other interesting features emerge from our study: we found an association between recurrence of bleeding and those who had not previously been tested and treated for eradication of HP, hence the need to test it in the ED so as to avoid missing diagnosis. Hypothetically HP infection should be tested once the acute bleed is over. However, this introduces the problem of having to hold acid suppression for 2 weeks prior to testing, definitely not recommended in a patient who recently bled. Regardless of which test one uses, it is important to test for and treat HP infection in a patient with bleeding peptic ulcer.

Our study shows that a higher proportion of patients in the HP+ group had a coronary artery disease, suggesting a role of HP antigens in the atherosclerotic plaque instability via a cross-mimicry mechanism\(^11\).

As expected, 24% of HP- patients used NSAIDs and this was the cause of bleeding, likewise for those HP- patients under antiplatelet.

Our data confirm that treatment with DOAC, rather than warfarin, in patients harboring HP in their stomach favors GI bleeding\(^12-15\).

Concerning endoscopy, our findings support a milestone in literature that is the association of HP with peptic ulcer\(^16-18\), more common in men with a mean age around 60 years\(^19\).

A further proof that HP is one of the most relevant risk factors for upper GI bleeding is the fact that 40% of HP- patients were negative at EGDS, therefore suggesting a lower GI bleeding.

We want to stress the concept that biopsies for HP are rarely performed in acute UGIB\(^20\): in our case, only 27 out of 87 pts performed biopsies and only 9 of these resulted positive. With this in mind, the most important finding in our study is
that if we had not tested all these patients in ED, we would have missed 21 diagnoses of HP. Thus, we increased the HP diagnoses by 233% compared to biopsy. Missing diagnoses would have translated into future recurrences of bleeding\textsuperscript{36,39}, further admissions in the ED and consequent increase in healthcare costs.

The determination HP through UBT directly in ED has also an implication in the LOS of these patients, indeed 83% of HP\textsuperscript{+} patients had a LOS in the BOU of ED of less than 3 days. Having diagnosed HP in the ED as a cause of bleeding allowed a faster management of these patients and they were discharged with the proper diagnosis and treatment directly from ED. On the other hand, 30% of HP\textsuperscript{-} patients had to be hospitalized in a ward in order to reach the correct diagnosis, and, once again, this percentage would have been quite higher if we had not tested these patients in ED.

**Limitations**

The presence of blood onto the gastric walls might have altered stomach’s pH, causing some false negatives at UBT.

Furthermore, the upright position that should be maintained during the UBT was not always possible in the emergency setting (e.g., clinically unstable patients; considerable blood loss with severe anemia). So, we could have underestimated HP infection in these patients.

Finally, considering that many of these patients referred to ED of our hospital for the acute bleeding event but not for follow up in the outpatient clinic, data confirming a successful eradication of HP after proper discharge therapy are missing.

It would have been interesting comparing other methods such as the detection of monoclonal antibodies in the feces or the culture on biopsies with consequent antibiogram. However, these methods require a microbiology laboratory open 24 hours, 7 days and a long time waiting to acquire the results of HP antibiotic sensibility.

**Conclusions**

In the clinical practice with UBT we increased the diagnosis of HP by 233% compared to biopsy results. The methodology utilized was simple, as the test per se is easy to conduct.

The use of this method would be recommended in all EDs, in order to allow the etiologic diagnosis and appropriate treatment, while reducing the LOS.

**Conflict of Interest**

The Authors declare that they have no conflict of interests.

**Authors’ Contribution**

Conceptualization: V Ojetti; methodology and revision: A Saviano; software: G Pignataro, M Covino, C Petruzzello; validation and formal analysis: M Covino, C Petruzzello; investigation: G Pignataro ME Riccioni, F Barone, A Saviano; resources: L Saviano, F Barone; data curation: V Ojetti, C Petruzzello, M Brigida, A Saviano, writing: M Brigida, A Saviano; writing review and editing: V Ojetti, M Candelli; visualization: V Ojetti; supervision: V Ojetti, F Franceschi.

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