INDEX COPERNICUS INTERNATIONAL

Can urea breath test (UBT) replace rapid urea test (RUT)?

Authors' Contribution:
A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection

Łukasz Nawacki[1,2ABCDEF], Agata Czyż[2BD], Piotr Bryk[1BD], Dorota Kozieł[2BGF], Renata Stępień[2BGF], Stanisław Głuszek[1,2ABCDEF]

[1] Clinic of General, Oncological and Endocrine Surgery, Regional Polyclinical Hospital, , Kielce, Poland
[2] Faculty of Medicine and Health Sciences, Jan Kochanowski University, , Kielce, Poland

Article history: Received: 05.12.2017 Accepted: 07.05.2018 Published: 30.09.2018

ABSTRACT:

Background: Helicobacter pylori (Hp) is classified by the International Agency for Research on Cancer (IARC) as a Group 1 carcinogen. Its influence on the carcinogenesis of gastric cancer has been confirmed in many research studies. The conclusion is obvious - early detection and eradication of Hp can prevent the development of the disease.

Methods: The objective of the study was to analyze the clinical and practical value of Carbon-13 urea breath test (UBT) in patients hospitalized due to pain complaints in the upper abdomen and dyspeptic symptoms. Fifty patients were enrolled in the study. Each patient underwent urea breath test according to the instruction included by the producer. Thereafter, each patient included in the study group was subjected to endoscopy of the upper gastrointestinal tract with the biopsy of the mucosa to determine the urease activity with rapid urease test (RUT).

Results: In the study group, 14 patients (28%) achieved a positive urease test result which was confirmed in RUT. Four (8%) patients, despite a positive breath test, did not have a positive result in urease activity test from gastric mucosa. In 2 cases (4%) despite negative result of UBT, urease activity was confirmed in gastroscopic sections. The remaining 30 patients (60%) had a negative result in both studies.

Conclusions: The limited availability of the gold standard for diagnostics of upper gastrointestinal tract diseases (gastroscopy) is the basis for the search for new methods of detection of Helicobacter pylori infections. The urea breath test is a method of high sensitivity and specificity. The positive result of urea breath test may be the basis for inclusion of eradication therapy.

KEYWORDS: helicobacter pylori, urea breath test, rapid urea test, gastric cancer

INTRODUCTION

Gastric cancer is the fourth most common cancer, and the second most frequent cause of cancer-related deaths worldwide. Every year, approximately 990,000 new cases are registered globally, and in 2015 the mortality was 840,953 patients [1, 2]. In the management course, surgical and endoscopic treatment should be primarily considered, as well as neoadjuvant and adjuvant chemoradiotherapy and palliative chemotherapy. The procedures of gastric resection with curative intent provide the greatest possibilities for obtaining a good result of oncologic treatment.

Despite considerable progress in the treatment of gastric cancer, mortality still remains on a high level, with the exception of Japan, where the 5-year survival period is more than 50% [3]. The high survival rate results from the population screening program and early diagnosis. Therefore, one of the basic tasks of medicine is an early detection and elimination of the risk factors for the development of the disease, and consequently, the prevention of cancer development [4].

There is a constant need for finding a test which would be most helpful and simple, as an instrument for early diagnosis of gastric cancer and pre-cancerous conditions. Helicobacter pylori (Hp) is classified by the International Agency for Research on Cancer (IARC) as a Group 1 carcinogen [5]. The diagnostic methods in case of Hp infection are divided into two types: invasive, including endoscopic examination with the cultivation of bacteria on a medium, rapid urea test, histopathological examination or molecular diagnostics, and non-invasive methods, including breath tests, examination of stool for the presence of antigens, serologic biomarkers. We need to find out whether and to what degree the urea breath test is able to detect Helicobacter pylori infection.

OBJECTIVE

The objective of the study was to anlyze the clinical and practical value of Carbon-13 urea breath test (UBT) in patients hospitalized due to pain complaints in the upper abdomen and dyspeptic symptoms.

MATERIALS AND METHOD

The study included 50 patients with complaints typical for the diseases of the upper part of the gastrointestinal tract: heartburn, empty belching, and epigastric pain. From the study were excluded patients who had:

- applied antibiotics for 4 weeks before the examination;
- applied proton pump inhibitors for 7 days before the examination;
- applied antacids or H2 antihistamines for 3 hours before the examination.

The examination consisted of the following stages:

- exhaling the air into the first collection bag;
- swallowing C^{13} labelled urea dissolved in orange juice (200 mL);
- exhaling the air into a second collection bag 30 minutes after drinking urea.

The samples collected in this way were subjected to analysis using a FANci2 - Fischer Analysen Instrumente GmbH Breath Analyzer.

Helicobacter pylori produces urea, which causes decomposition

DOI: 10.5604/01.3001.0012.0669

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{urease}} CO_2 + 2NH_3$$

Ryc. 1. Urea decomposition reaction.

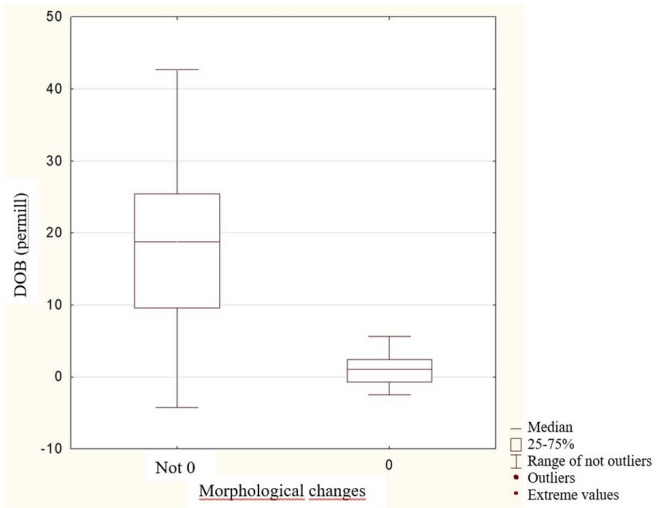

Fig. 2. Distribution of DOB value according to the presence or absence of morphological changes in endoscopic examination.

of C^{13} labelled urea. Thus, the exhaled air contains $^{13}CO_2$ produced according to the formula (Figure 1).

The principle of measurement is based on the specific absorption of infrared light in the middle of the infrared spectrum of the wavelength of 2 and 8 µm (NDIR: non-dispersive infrared spectroscopy). A high selectiveness of infrared detectors of the analyzer is obtained by filling it with proper components: $^{13}CO_2$ and $^{12}CO_2$. The $^{13}CO_2/^{12}CO_2$ ratio, and comparison with the first collection sample reflect the current state of metabolism of the examined person (DOB- delta over baseline). A DOB value above 4.0‰ evidences an infection with Helicobacter pylori.

Subsequently, each patient had an endoscopic examination of the upper part of the gastrointestinal tract performed with full assessment of the endoscopic image and with the collection of specimens from the fundus, corpus, gastric antral region, and from the duodenal bulb. The specimens were examined using the rapid urea test (RUT).

The results of the breath test and rapid urea test from specimens collected during gastroscopy were compared. In addition, we determined the grade of the intensity of changes in the endoscopic appearance of the gastric and duodenal mucosa in accordance with the following categories:

- Grade 0 - lack of changes in endoscopic assessment;
- Grade 1- gastritis;
- Grade 2 - gastritis and duodenitis;
- Grade 3 - peptic ulcer.

The patients (n) were divided into the following groups:

- positive result RUT and positive UBT - patients true positive (TP);
- positive result RUT and negative UBT - patients false negative (FN);
- negative result RUT and positive UBT - patients false positive (FP);
- negative result RUT and negative UBT - patients true negative (TN).

The UBT test was evaluated from the aspect of the 'gold standard' adopted as a result of the RUT test. In the assessment of diagnostic properties of UBT test, sensitivity, specificity, predictive value of positive result and predictive value of negative result of the test were used.

The normality of variables was assessed using Shapiro-Wilk test. Differences between groups for quantitative variables were examined using Mann-Whitney U test, whereas the differences for 2x2 tables were assessed using Fisher's exact test.

The p values < 0.05 were considered statistically significant. All statistical calculations were performed using the software Statistica (StatSoft, Inc. 2014, version 12).

RESULTS

We included 21 males and 29 females aged 18 - 78 (mean 43, standard deviation = 17.5 years) in the study. In endoscopic examination the intensity of changes of Grades 0, 1, 2 and 3 was observed in 30 (60%), 6 (12%), 4 (8%), and 9 (15%) patients, respectively, while in one patient (2% of the total number of patients) adenocarcinoma was diagnosed.

Division of the total number of patients (n= 50) into groups was as follows:

- TP: 16
- FN: 2
- FP: 4
- TN: 28

The precise division of patients is presented in the Tables (at the end of the article).

A positive result of RUT test was observed in 18 patients (36%).

Table VIII. presents the results of UBT test (cut-off level 4‰) and RUT test.

Conformity of the results of both tests was 88%.

Adopting the result of RUT test as a 'gold standard', Table IX. demonstrates an assessment of the diagnostic properties of UBT test.

Conclusion: UBT shows a highly statistically significant capability for differentiation between the conditions 'positive' and 'negative' determined by the results of RUT test.

A change of cut-off value in UBT test at 9.5‰ improves the conformity of the results of UBT test and RUT test (table X).

Conformity of the results of both tests was 96%. The only non-conformity concerned two women with Grade 1 changes in gastroscopy (in one of those women DOB = 1.5‰, while in the other DOB = - 0.8‰).

Tab. I. Males true positive (UBT positive; RUT positive).

AGE (YEARS)	DOB VALUE (PER THOUSAND)	GRADE OF INTENSIFICATION OF CHANGES IN GASTROSCOPY
24	9,6	1
74	12,7	1
31	18,3	2
50	42,4	3
62	18,9	2
52	18,6	2
32	20,4	3

Tab. II. Males false positive (UBT positive; RUT negative).

AGE (YEARS)	DOB VALUE (PER THOUSAND)	GRADE OF INTENSIFICATION OF CHANGES IN GASTROSCOPY
38	5,6	0
62	4,5	0
30	4	0

Tab. III. Males true negative (UBT negative; RUT negative).

AGE (YEARS)	DOB VALUE (PER THOUSAND)	GRADE OF INTENSIFICATION OF CHANGES IN GASTROSCOPY
44	-4,3	1
60	0,6	gastric tumour (hist-path: adenocarcinoma)
58	1,4	0
53	-1,9	0
28	3,9	0
41	1,8	0
58	1,2	0
58	0,7	0
78	-0,3	0
27	-1,4	0
21	-2,4	0

The DOB values in the analyzed group ranged from -4.3‰ - 42.7‰, while the first, second (=median) and the third quartiles were -0.2‰, 1.95‰ and 18.3‰, respectively. The distribution of DOB values differed statistically significantly ($p<0.0001$) between groups '0' (lack of morphological changes, n=30) and 'not 0' (presence of morphological changes [Grades 1, 2 or cancer], n=20) (see Fig. 2).

A positive result of UBT test (with cut-off value 4 ‰) was significantly more frequently observed in the group with morphological changes, compared to the group with no such changes (80% [16/20] vs. 13% [4/30], $p<0.0001$).

If the value 9.5‰ was adopted as the cut-off value in the UBT test, a positive result of this test would be significantly more often noted in the group with morphological changes than in the group without such changes (80% [16/20] vs. 0% [0/30], $p<0.0001$).

DISCUSSION

Helicobacter pylori was discovered in 1875 by German resear-

Tab. IV. Females true positive (UBT positive; RUT positive).

AGE (YEARS)	DOB VALUE (PER THOUSAND)	GRADE OF INTENSIFICATION OF CHANGES IN GASTROSCOPY
51	20	3
44	42,7	3
60	29,5	3
21	21,2	3
56	18,5	2
28	23,9	3
37	9,5	1
73	39,1	3
21	-2,4	0

Tab. V. Females false negative (UBT negative; RUT positive).

AGE (YEARS)	DOB VALUE (PER THOUSAND)	GRADE OF INTENSIFICATION OF CHANGES IN GASTROSCOPY
22	1,5	1
74	-0,8	1

Tab. VI. Females false positive (UBT positive; RUT negative).

AGE (YEARS)	DOB VALUE (PER THOUSAND)	GRADE OF INTENSIFICATION OF CHANGES IN GASTROSCOPY
22	4,4	0

Tab. VII. Females true negative (UBT negative; RUT negative).

AGE (YEARS)	DOB VALUE (PER THOUSAND)	GRADE OF INTENSIFICATION OF CHANGES IN GASTROSCOPY
41	2,4	0
57	-1,4	0
63	2,1	0
18	1,5	0
24	1,8	0
73	-2,5	0
32	2,4	0
52	0,5	0
20	1,5	0
22	-0,7	0
32	-0,7	0
42	3,6	0
54	-0,1	0
26	-0,3	0
28	-2	0
20	0,9	0
31	-0,2	0

Tab. VIII. Results of UBT test and RUT test.

UBT TEST	RUT TEST		TOTAL
	POSITIVE	NEGATIVE	
positive	16	4	20
negative	2	28	30
Total	18	32	50

Tab. IX. Diagnostic properties of UBT test evaluated in relation to the results of RUT test.

MEASURE	VALUE
Sensitivity	88,9%
Specificity	87,5%
Positive predictive value (PPV)	80%
Negative predictive value (NPV)	93,3%
AUC (95%CI, p-value)*	0,94 (0,84–1, p < 0,0001)

AUC: Area under curve, 95%CI: 95% confidence interval

Tab.X Improvement of the conformity of the results after changing the cut- off value.

DOB IN UBT TEST	RUT TEST		TOTAL
	POSITIVE	NEGATIVE	
9.5‰ or above	16	0	16
below 9.5‰	2	32	34
Total	18	32	50

chers; however, it is considered that it has accompanied mankind for more than 100,000 years, with a considerable genetic biodiversity according to the geographic region. The farther from the region of East Africa, the lower the genetic diversity [6]. From the time of discovery by Marshalla and Warren of the influence of H. pylori on the development of acute gastritis, its relation has been confirmed with the development of gastric and duodenal ulcer, atrophic gastritis, gastric cancer, MALT lymphoma and thrombocytopenia. Therefore, medicine focuses on an early detection of the infection and eradication of the bacteria.

The usefulness of the method of diagnostics of the infection varies. Invasive examinations are based primarily on endoscopy of the upper part of the gastrointestinal tract. Apart from the standard viewing of the gastric and duodenal mucosa, the effectiveness of the NBI method (narrow band imaging) has also been confirmed in the diagnostics. In addition, NBI also facilitated the collection of specimens from the changed mucosa for further tests, which is more effective than biopsy under the control of ordinary gastroscopy [7]. Similar effects are also obtained with the use of confocal laser endomicroscopy [8]. When the specimens of mucosa have already been collected, there are several methods of further diagnostics.

The most popular method is the rapid urea test (RUT), which was used in the presented study. This is a quick, simple, and cheap diagnostic method, the sensitivity and specificity of which increases with the number of collected specimens. In this study, the specimens were collected from four sites during white light gastroscopy. While using RUT it should be remembered that proton pump inhibitors decrease the sensitivity of the method, therefore, their application should be inhibited at least 12 days prior to performing the examination.

Bacterial culture is the subsequent diagnostic method, which also enables determination of antibiogram. Determination of the sensitivity to antibiotics is important considering the gradual increase in resistance. Nevertheless, this method is rarely used in routine clinical practice, and the treatment of first choice is often an empirical therapy. Determination of the antibiogram is indispensable if eradication is not obtained by application of the two schemes of treatment.

Histopathological examination, apart from the visualization of the bacteria in the preparation allows also a precise assessment of the mucosa, and consequently, an early detection of the disease.

From among the molecular methods, the method of choice is rtPCR (real-time polymerase chain reaction) targeting 23S rDNA. This enables the detection of strains resistant to clarithromycin. This method enables detection of the pathogen, even if it occurs in a small amount.

The advantage of non-invasive examinations is primarily the lack of necessity for undergoing endoscopy of the upper part of the gastrointestinal tract. One of these is the examination of stool for H. pylori antigens (SAT- stool antigen test). Antigens present in stool were examined and compared with the results of RUT test and breath test. The sensitivity of the method was 90.1%, and the specificity - 92.4% [9].

Despite insignificant recommendations, serologic tests are still being applied. While using this method, its limitations should be borne in mind, which are the differences in the H. pylori strains among populations, e.g. those used in western countries have low efficiency when applied in the countries of the Near East [10].

In the presented study, the urea breath test was applied with ^{13}C labelled urea. In the diagnostics of infection with Helicobacter pylori, two types of urea are used - ^{13}C and ^{14}C - labelled. UBT is used both in the diagnostics of infection, and the control of the effectiveness of eradication of the bacteria. The sensitivity of urea breath tests is 76.2% - 97%, and specificity from 69.2% to 97% [6, 11, 12, 13, 14]. The results depend on the method used for the control of effectiveness of UBT, and time elapsed between collection of both test doses. In the presented study, it was confirmed that there is a relationship between the DOB values and changes in the endoscopic image of the gastric and duodenal mucosa. If the DOB was below 10‰, gastritis was confirmed in endoscopic examination. If DOB was between 10 and 20‰, gastritis and duodenitis was confirmed by endoscopy. When DOB was over 20‰, peptic ulcer was diagnosed. According to the recommendations by the manufacturer, we considered DOB over 4‰ as a positive result of UBT. Originally, DOB above 5‰ was considered as a positive result [15]. In the case of adopting the DOB 5‰ in our study, we would obtain the specificity of the test on the level of 96.86%.

CONCLUSION

Urea breath test has a high sensitivity and specificity for the detection of infections with Helicobacter pylori. Considering the fact that this is an examination which is non-invasive, simple and quick to perform, it constitutes a good alternative, compared to the invasive examinations which require the performance of endoscopy, and other non-invasive examinations, because the sensitivity of the latter, to a high degree, depends on the strain of the bacteria with which a person is infected. However, it should be considered whether the sole positive result of the urea breath test should be a basis for the inclusion of eradication treatment, or a basis for performing endoscopic examination of the upper part of the gastrointestinal tract.

REFERENCES:

1. Gore R. Gastric Cancer; Cambridge University Press 2010
2. Fitzmaurice C, Dicker D, Pain A; The global burden of cancer 2013; JAMA Oncol. 2015 Jul;1(4):505-27
3. Lochhead P, El- Omar E. Gastric cancer; British Medical Bulletin 2008; 85(1): 87- 100
4. Kiełtucki J, Wawrzyniec K. The role of endoscopic ultrasonography (EUS) in contemporary diagnosis of gastrointestinal diseases. Medical Studies/Studia Medyczne 2016; 32 (3): 216–224
5. IARC. A review of Human Carcinogens. B. Biological Agents. Lyon; WHO Press 2012
6. Leja M, Axon A, Brenner H; Epidemiology of Helicobacter pylori infection; Helicobacter 2016; 21: 3-7
7. Megraud F, Floch P, Labenz J, Lehours P; Diagnostic of Helicobacter pylori infection, Helicobacter 2016; 21: 8- 13
8. Goetz M; Characterization of lesions in the stomach: will confocal laser endomicroscopy replace the pathologist? Best Pract Res Clin Gastroenterol 2015; 29: 589- 599
9. Ramirez- Lazaro MJ, Lite J, Lario S et al. Good diagnostic accuracy of a chemiluminnescent immunoassay in stool samples for diagnosis of Helicobacter pylori infection in patients with dyspepsia. J Investig Med. 2016; 64: 388- 391
10. Lee Sy, Moon HW, Hur M, Yun YM; Validation of western Helicobacter pylori IgG antibody assays in Korean adults. I Med Microbiol 2015; 64: 513- 518
11. Honar N, Minazedeh A, Shakibazad N et al. Diagnostic accuracy of urea breath test for Helicobacter pylori infection in children with dyspepsia in comparison to histopathology. Arq Gastroenterol. 2016 Apr-Jun;53(2):108-12
12. Dede F, Civen H, Dane F et al. Carbon- 14 urea breath test: does it work in patients with partial gastric resection? Ann Nucl Med 2015; 29: 786- 791
13. Zhou Q, Li L, Ai Y et al. Diagnostic accuracy of the 14C- urea breath test in Helicobacter pylori infections: a meta analysis. Wien Klin Wochenschr 2017; 129: 38- 45
14. Maity A, Pal M, Som S et al. Natural 18O and 13C- urea in gastric juice: a new route for non- invasive detection of ulcers. Anal Bioanal Chem 2017; 409: 193- 200
15. Logan R P H, Urea breath tests in the management of Helicobacter pylori infection. Gut 1998; 43: S57- S50

Word count: 2420 Page count: 5 Tables: 10 Figures: 2 References: 15

DOI: 10.5604/01.3001.0012.0669 Table of content: https://ppch.pl/issue/11379

Competing interests: The authors declare that they have no competing interests.

Corresponding author: Łukasz Grzegorz Nawacki, Clinic of General, Oncological and Endocrine Surgery, Regional Polyclinical Hospital, Kielce, Poland; Email: lukasznawacki@gmail.com

Cite this article as: Nawacki L., Czyz A., Bryk P., Kozieł D., Stepien R., Gluszek S.; Can urea breath test (UBT) replace rapid urea test (RUT)?; Pol Przegl Chir 2018: 90 (5): 44-48