Long-term dynamics of gastric biomarkers after eradication of *Helicobacter pylori* infection

Ilva Daugule[a], Agnese Ruskule[a], Georgijs Moisejevs[a], Dace Rudzite[a], Laimas Jonaitis[c], Dainius Janciauskas[c], Gediminas Kiudelis[c], Limas Kupcinskas[c] and Marcis Leja[b]

Background Secretion of pepsinogen I (PgI), pepsinogen II (PgII), fasting gastrin-17 (fG-17) and stimulated gastrin-17 (sG-17) changes after *Helicobacter pylori* eradication. Few data are available on the long-term dynamics of gastric biomarkers after *H. pylori* eradication. The aim of this study was to investigate the dynamics of gastric biomarkers in *H. pylori*-positive patients after eradication over a 3-year period and to compare the levels with initially *H. pylori*-negative patients.

Materials and methods Blood samples for the detection of gastric biomarkers were obtained from dyspeptic patients coming for upper gastrointestinal endoscopy. In *H. pylori*-positive patients, after eradication therapy, three follow-up blood samples were drawn after 12, 24 and 36 months; in *H. pylori*-negative patients, two samples were taken – at 12 and after 30 months. Median values of biomarkers in follow-up samples were compared with the baseline sample.

Results The final sample included 110 patients (median age 67 years, M/F ratio 27/83). In patients after *H. pylori* eradication ($n = 83$) PgI, PgII, fG-17 and sG-17 had decreased significantly during a 36-month period, whereas the PgI/PgII ratio had increased significantly from 5.59 to 11.64.

Conclusion In *H. pylori*-positive dyspeptic patients, after eradication therapy, a decrease in PgI, PgII, fG-17 and sG-17 was observed after 36 months whereas an increase in the PgI/II ratio suggested an improvement in gastric atrophy. The median levels of gastric biomarkers in patients after *H. pylori* eradication therapy may become similar to biomarker levels among initially *H. pylori*-negative individuals. Eur J Gastroenterol Hepatol 27:501–505

Introduction

Helicobacter pylori infection is recognized as a risk factor for atrophic gastritis. It has been estimated that the lifetime risk for the development of atrophic gastritis among *H. pylori*-positive individuals is 50%, whereas in 10%, atrophic gastritis will be moderate or severe [1–3]. It has also been recognized that *H. pylori* infection abolishes the inflammatory response and may halt the progression of atrophy or even reverse it [4]. As atrophic gastritis increases the risk of development of gastric cancer [1], it would be reasonable to eradicate the bacteria. However, eradication treatment is not strictly recommended for all *H. pylori*-positive individuals and should be evaluated individually, although the Maastricht IV guidelines state that *H. pylori* eradication could also reduce the incidence of gastric cancer [4]. Moreover, the latest IARC guidelines state that it still remains controversial whether *H. pylori* eradication can reduce or prevent gastric cancer, although data support the association between *H. pylori* infection and gastric cancer [5].

Despite intensive research, we still do not know the long-term consequences of *H. pylori* eradication, especially in dyspeptic patients without ulcer disease. Prevention of progression of atrophy could be an important argument in the decision of eradication of *H. pylori* infection.

Gastric biomarkers are a good diagnostic tool for an indirect investigation of gastric atrophy. Moreover, some authors argue that serological assessment of gastric atrophy could be more precise, avoiding sampling errors and also diagnosing initial changes in gastric acid secretion. Briefly, atrophic gastritis results in a decrease in acid output and a further decrease in pepsinogen I (PgI) and pepsinogen II (PgII), as well as fasting gastrin-17 (fG-17) and stimulated gastrin-17 (sG-17) [1,6]. It has been stated that the levels of PgI and PgII correlate with the degree of gastric atrophy (especially corpus mucosa), whereas G-17 reflects the degree of gastric inflammation and extent of atrophic gastritis: in a case of antral atrophy, not only fG-17 but also the plasma levels of sG-17 will be low [6].

However, secretion of gastric biomarkers changes after *H. pylori* eradication. Generally, PgI and PgII levels in plasma decrease whereas the PgI/PgII ratio increases [7]. Nevertheless, few data are available on the long-term dynamics of gastric biomarkers after *H. pylori* eradication and we do not know whether pepsinogen and gastrin levels return to normal levels as defined by data from *H. pylori*-negative patients with dyspepsia. This will help to assess the mucosal status indirectly.

European Journal of Gastroenterology & Hepatology 2015, 27:501–505

Keywords: atrophic gastritis, gastric biomarkers, *Helicobacter pylori*

[a]Faculty of Medicine, University of Latvia, [b]Digestive Diseases Centre GASTRO, Faculty of Medicine, University of Latvia, Riga East University Hospital, Riga, Latvia and [c]Faculty of Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania

Correspondence to Ilva Daugule, MD, PhD, Faculty of Medicine, University of Latvia, 6 Linezera Street, LV1006 Riga, Latvia

Tel: +371 67040367; fax: +371 67040248; e-mail: ilva_daugule@hotmail.com

Received 14 October 2014 **Accepted** 13 January 2015

Aim

This study aimed to examine the dynamics of gastric biomarkers in *H. pylori*-positive dyspeptic patients after *H. pylori* eradication over a 36-month period and to compare the levels of gastric biomarkers with initially *H. pylori*-negative patients.

Materials and methods

Study design

This study was carried out in two University Clinics in Baltic states (Latvia and Lithuania) in 2006–2010. Consecutive patients scheduled for upper gastrointestinal endoscopy because of their symptoms were included in the study. Blood samples were obtained before the endoscopy for detection of gastric biomarkers. Upper gastrointestinal endoscopy with standardized biopsy sampling was performed: two biopsies were taken from the antral part (one from the greater curvature and one from the lesser curvature), one from the incisura and two biopsies from the corpus part of the stomach (one from the greater curvature and one from the lesser curvature). *H. pylori*-positive patients were offered eradication therapy according to existing guidelines. After eradication therapy, patients were asked to attend follow-up visits after 12, 24 and 36 months for collection of blood samples. Initially, *H. pylori*-negative patients attended the first follow-up after 12 months. As no specific changes were expected, patients were asked to come for the last visit only once after 30 months. Biomarker levels in the initial blood sample were compared with the levels in samples drawn afterwards. Biomarker levels were compared between *H. pylori*-positive patients after eradication therapy and initially *H. pylori*-negative patients at baseline and after a 30-month period.

Patients

Patients older than 55 years of age referred for upper endoscopy because of dyspeptic symptoms were recruited. Patients with peptic ulcer (active or with ulcer disease history), previous upper gastrointestinal surgery, personal or first-degree family history of gastric cancer as well as known or suspected past *H. pylori* eradication therapy were not included. Those being treated with antibiotics and/or proton pump inhibitors (PPIs) for any reason within 1 month before inclusion were also excluded.

Biomarker analysis

Blood samples were collected before the upper endoscopy or 12–48 h after the procedure. The sample drawn after an overnight fast was used for the analysis of PgI, PgII and combined immunoglobulin G (IgG)-type and IgA-type antibodies to *H. pylori* infection; another blood sample was obtained the same day for the evaluation of fG-17 and sG-17; the sample was drawn 20 min after the intake of a protein-rich drink (nutrient concentrate – protein content 8.4 g, fat content 0.1 g, energy 153 kJ/36 kcal), but with no other food intake before the venipuncture.

Blood samples were collected in EDTA vials; within 30 min, the samples were centrifuged, plasma was separated and frozen immediately. Plasma samples were kept frozen (up to 1 week at – 20°C, but for a longer period at – 80°C) until tested. Transportation was arranged on dry ice (CO_2-ice).

Frozen plasma samples from both recruitment centres were transported to a laboratory. PgI, PgII, fG-17 and sG-17 as well as combined IgA and IgG antibodies to *H. pylori* were determined at the Biohit Oyj service laboratory in batches of 43 samples on a microwell plate according to the instructions of the manufacturer using specific enzyme-linked immunosorbent assay (ELISA) tests (PgI ELISA test kit cat. no. 601 010, PgII ELISA test kit cat. no. 601 020, G-17 ELISA test kit cat. no. 601 035, and *H. pylori* IgA/IgG ELISA test kit cat. no. 601 045; Biohit Oyj, Helsinki, Finland). All ELISA techniques were based on measurement of the absorbance after a peroxidation reaction at 450 nm. Between the reaction steps, the plates were washed using a BW50 microplate Strip Washer (Biohit Oyj). The absorbances were measured using a microwell plate reader (BP800 Microplate Reader; Biohit Oyj).

For determination of PgI, PgII and G17 values, second-order fits on the standard concentration were used to interpolate/extrapolate unknown sample concentrations automatically using the BP800 built-in software. The *H. pylori* antibody arbitrary enzyme immunounits were calculated from the semilogarithmic calibrator curve. Double measurements were performed on every sample and the mean result of these two measurements was reported.

Gastric atrophy

Two biopsies from corpus, two from the antrum (one from the lesser curvature and the other from the greater curvature) and one from the arcus, were used to access gastric mucosa atrophy. The slides were stained with haematoxylin and eosin. Biopsies were read according to the updated Sydney classification for atrophy independently by two expert gastrointestinal pathologists and blinded to the serological results. Cases of disagreement for any grade of atrophy were re-evaluated simultaneously and a consensus was achieved.

Detection of H. pylori positivity

The presence of *H. pylori* infection at baseline was detected by three indirect tests: rapid urease test, ^{13}C urea breath test and combined ELISA test for IgG and IgA group antibodies, and two direct tests (histopathology and culture). Patients were considered *H. pylori*-positive if at least three indirect or one indirect and one direct test was positive.

The presence of *H. pylori* infection after follow-up was detected by combined ELISA for IgG and IgA: patients were considered *H. pylori*-negative 30 months after eradication therapy if the antibody titre had decreased at least 65% compared with the baseline level. If available, the rapid urease test and histopathology were used to evaluate *H. pylori* status.

Statistical analysis

Descriptive statistics were used to characterize the groups of patients. A bivariate comparison was carried out using the χ^2-test. Median values between *H. pylori*-positive and *H. pylori*-negative individuals were compared using the

Mann–Whitney *U*-test. The dynamics of biomarkers was analysed using a *t*-test and the Wilcoxon matched paired test for dependent samples and shown as median values (statistical programme STATISTICA; StatSoft, Tulsa, Oklahoma, USA).

Ethics

The local Committee of Ethics approved the study protocol before patient recruitment was started. All the patients signed consent forms before enrolment.

Results

In total, 146 patients participated in the study. Thirty-three patients were excluded because of the following reasons: no *H. pylori* eradication or no data on eradication, and unknown *H. pylori* status at inclusion. Three *H. pylori*-positive patients who had received eradication therapy were positive after 36 months and were excluded from the final sample. Therefore, the sample for analysis included 110 patients (median age 67 years, interquartile range 61–72; range 55–86 years, M/F ratio 27/83).

Of these patients, 83 were *H. pylori*-positive and 27 were *H. pylori*-negative. The characteristics of *H. pylori*-positive and *H. pylori*-negative patients in terms of demographic data, mucosal status and baseline gastric biomarker levels are shown in Table 1. The median age, female/male ratio and presence of antral atrophy did not differ among *H. pylori*-positive and *H. pylori*-negative patients, whereas PgI and PgII levels were significantly higher among *H. pylori*-positive patients compared with *H. pylori*-negative patients. Corpus atrophy was more often observed among *H. pylori*-negative patients.

Among *H. pylori*-positive patients, 82 were available after 12 months, 55 after 24 months and 76 after 36 months. Among *H. pylori*-negative patients, 23 were available after 12 months, whereas 27 came for analysis after 30 months.

The dynamics of the level of biomarkers during the follow-up period is shown in Table 2. Among patients after *H. pylori* eradication, PgI, PgII, fG-17 and sG-17 had decreased significantly during the follow-up period, whereas the PgI/PgII ratio had steadily increased significantly from 5.59 to 11.64.

Among *H. pylori*-negative patients, a significant increase in PgI was observed, leading to a significant increase in the PgI/PgII ratio.

The levels of biomarkers after 30 months of follow-up did not differ significantly between *H. pylori*-positive individuals after eradication and initially *H. pylori*-negative individuals (Table 3).

Table 1. Characteristics of *H. pylori*-positive and *H. pylori*-negative patients at baseline

	H. pylori-positive ($n=83$)	*H. pylori*-negative ($n=27$)
Age: median of age (IQR)	66 (62–71)	68 (63–75)
Sex (M/F) [*n*/*N* (%)]	20/63 (24/76)	7/20 (26/74)
Corpus atrophy [*n*/*N* (%)]		
None or mild	69/77 (90)	15/23 (65)
Moderate or severe	8/77 (10)	8/23 (35)*
Antrum atrophy [*n*/*N* (%)]		
None or mild	69/77 (90)	23/23 (100)
Moderate or severe	8/77 (10)	0/23 (0)
PgI median (IQR)	83.5 (62.7–109.3)	54.5 (22.2–78.4)*
PgII median (IQR)	14.1 (10.1–19.8)	8.2 (6.2–12.4)*
PgI/PgII median (IQR)	5.6 (3.3–6.9)	6.4 (2.1–10.3)
fG-17 median (IQR)	6.3 (3.3–20.7)	5.8 (1.02–23.7)
sG-17 median (IQR)	39.7 (17.3–66.5)	21.5 (12.3–79.4)

fG-17, fasting gastrin-17; *H. pylori*, *Helicobacter pylori*; IQR, interquartile range; PgI, pepsinogen I; PgII, pepsinogen II; sG-17, stimulated gastrin-17.
*$P<0.01$.

Discussion

Our study shows changes in the levels of gastric biomarkers among *H. pylori*-positive patients after eradication therapy and in initially *H. pylori*-negative individuals during a 3-year period.

Generally, it has been accepted that the decrease in PgII is observed already 1 month after *H. pylori* eradication [8–10] whereas the decrease in PgI levels occurs progressively during 6 months, remaining unchanged within a period of 1 year [11]. The decrease in PgI and PgII is further associated with an increase in the PgI/PgII ratio [7,12].

Our study confirmed that the decrease in PgI and PgII after *H. pylori* eradication is stable and is evident in patients even after 36 months. Although a small but significant decrease in the PgII level was evident already at 1 year after eradication therapy, the values continued to decrease and the lowest values of PgI and PgII were observed after 2 years. However, the levels did not indicate gastric atrophy. Similarly, Ohkusa *et al.* [7] have reported a long-term (up to 3 years) decrease in PgI and PgII after *H. pylori* eradication.

A low PgI/PgII ratio has been widely accepted as a marker of gastric atrophy [1]. As gastric acid secretion and consequently both PgI and PgII are affected by *H. pylori* eradication, the correlation between these two parameters could be different before and after eradication. As an increase in the PgI/PgII ratio already 2 months after eradication therapy cannot be caused by improvement in gastric atrophy, serum pepsinogens should be used with caution to evaluate gastric mucosal status soon after *H. pylori* eradication. However, our study also shows a long-term increase in the PgI/PgII ratio in patients after *H. pylori* eradication, reflecting either improvement in gastric atrophy (thus indirectly favouring *H. pylori* eradication) or a more pronounced decrease in PgII after eradication.

A significant decrease in gastrins observed after *H. pylori* eradication could possibly indicate a decrease in inflammation or even development of antral atrophy. However, it could also be because of normalization of secretion of gastrin. As in a case of initial corpus atrophy the feedback mechanism results in an increase in gastrin levels, the decrease in gastrin after the follow-up period could possibly reflect improvement in gastric mucosal status with respect to atrophy, indirectly supported by the increase in the PgI/PgII ratio. Similarly, Ohkusa *et al.* [7] have also reported a long-term decrease in gastrin after *H. pylori* eradication. However, the median level of fG-17 was below three after a 2-year follow-up, thus suggesting development of antral atrophy.

Further, it is interesting to compare the levels of different biomarkers in *H. pylori*-positive patients after

Table 2. Median (interquartile range) biomarker results in different time periods among *H. pylori*-positive patients after eradication and initially *H. pylori*-negative patients

	PgI (IQR)	PgII (IQR)	PgI/PgII (IQR)	fG-17 (IQR)	sG-17 (IQR)
H. pylori-positive patients after eradication therapy					
At baseline ($n=83$)	83.5 (62.7–109.3)	16.12 (10.1–19.8)	5.59 (3.3–6.9)	6.35 (3.3–20.7)	39.71 (17.3–66.5)
12 months ($n=82$)	83.9 (62.9–111.1)	12.81* (9.3–17.8)	6.31* (3.9–7.6)	5.97 (3.1–17.5)	38.85 (17.04–63.4)
24 months ($n=55$)	56.46* (43.8–67.9)	4.97* (4.17–6.4)	11.33* (9.4–13.9)	0.73* (0.4–4.3)	5.94* (3.2–17.9)
36 months ($n=76$)	60.49* (48.5–93.9)	6.7* (4.4–13.4)	11.64* (9.3–13.5)	1.65* (4.1–74.6)	9.93* (3.8–26.2)
Initially *H. pylori*-negative patients					
At baseline ($n=27$)	54.5 (22.2–78.4)	8.25 (6.2–12.4)	6.42 (2.1–10.3)	5.85 (1.02–23.7)	21.53 (12.3–79.4)
12 months ($n=23$)	49.64 (14.8–71.8)	5.9* (4.9–9.1)	7.67* (1.7–10.8)	6.36 (2.4–54.9)	13.81** (9.6–25.4)
30 months ($n=27$)	55.8** (30.5–95.2)	7.07 (5.5–11.6)	10.02* (5.6–12.9)	1.95 (0.78–19.7)	15.12** (5.6–89.5)

fG-17, fasting gastrin-17; *H. pylori*, *Helicobacter pylori*; IQR, interquartile range; PgI, pepsinogen I; PgII, pepsinogen II; sG-17, stimulated gastrin-17.
*$P<0.001$.
**$P<0.05$ (compared with baseline).

Table 3. Median (interquartile range) biomarker results after 30–36 months in *H. pylori*-positive patients after eradication treatment and in initially *H. pylori*-negative patients

	Patients after *H. pylori* eradication ($n=76$)	Initially *H. pylori*-negative patients ($n=27$)	*P*-value
PgI (IQR)	60.49 (48.5–93.9)	55.8 (30.5–95.2)	0.6
PgII (IQR)	6.7 (4.4–13.4)	7.07 (5.5–11.6)	0.06
PgI/PgII (IQR)	11.64 (9.3–13.5)	10.02 (5.6–12.9)	0.06
fG-17 (IQR)	1.65 (4.1–74.6)	1.95 (0.78–19.7)	0.1
sG-17 (IQR)	9.93 (3.8–26.2)	15.12 (5.6–89.5)	0.2

IQR expressed as percentile 25–75.
fG-17, fasting gastrin-17; *H. pylori*, *Helicobacter pylori*; IQR, interquartile range; PgI, pepsinogen I; PgII, pepsinogen II; sG-17, stimulated gastrin-17.

eradication with the levels in initially *H. pylori*-negative individuals.

First, a significant increase in PgI after a 30-month period was observed among initially *H. pylori*-negative individuals. The possible explanation for this could be the intercurrent use of PPI because of dyspepsia, shown to be associated with increased levels of PgI [13], or increase of PgI with age, especially in women, observed in some studies [14]. However, increases in the PgI/PgII ratio in initially *H. pylori*-negative patients might have been observed because of an increase in PgI.

Nevertheless, PgI and PgII levels were only non-significantly higher whereas the PgI/PgII ratio was non-significantly lower among initially *H. pylori*-negative individuals compared with patients after eradication. This corresponds to data reported by Okhusa *et al.* [7] showing that 1–3 months after eradication, serum PgI and PgII levels in the *H. pylori*-positive patients decreased to levels similar to those in the *H. pylori*-negative patients.

At baseline, the median gastrin levels were higher in *H. pylori*-positive individuals compared with *H. pylori*-negative individuals. As *H. pylori* eradication was associated with a significant decrease in G-17 and sG-17, gastrin levels in both patient groups did not differ after a 30-month period. Therefore, our data correspond to the results of Ohkusa *et al.* [7] showing that gastrin levels become similar between the groups at 12–15 months after eradication. However, the low median level of fG-17 in both groups suggests possible antral atrophy that could be associated with age.

Conclusion

In *H. pylori*-positive dyspeptic patients after *H. pylori* eradication therapy, the decrease in PgI, PgII, fG-17 and sG-17 remains stable for 36 months, whereas the increase in the PgI/PgII ratio could reflect either long-term improvement of mucosal atrophy or a more pronounced decrease in PgII after eradication. Median levels of gastric biomarkers in patients after *H. pylori* eradication therapy may become similar to biomarker levels among initially *H. pylori*-negative individuals, indirectly supporting eradication therapy in *H. pylori*-positive dyspeptic patients.

Acknowledgements

This study was supported by the Latvian National Research Programme BIOMEDICINE and in part by the project European Fund for Regional Development No. 2010/0302-/2DP/2.1.1.1.0/10/APIA/VIAA/158 'Development of a genetic/serological biomarker diagnostic method for early identification of autoimmune gastrointestinal lesions with increased malignancy risk in patients with autoimmune disease'.

The authors acknowledge Pentti Sipponen for his involvement in the pathological examination. They also acknowledge the doctors at the Riga East University Hospital, Digestive Diseases Centre GASTRO, Ivars Tolmanis, Ilze Kikuste, Dans Stirna, Aigars Vanags, as well as members of the Baltic-Taiwan atrophic gastritis study group, in particular: Viesturs Putnins, Armands Sivins, Guntis Ancans, Liga Panina, Ingrida Rumba-Rozenfelde, Uldis Vikmanis, Aija Karklina, Agita Vovilova, Ming-Shiang Wu, Yi-Chia Lee. Further, the authors thank Biobank services of Riga East University Hospital/University of Latvia for sample processing and storage. Finally, they are grateful to all the patients who participated in the study.

Conflicts of interest

There are no conflicts of interest.

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