

Helicobacter pylori Infection, Gastric Cancer and Gastropanel

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Gastric cancer (GC) is one of the most widespread types of cancer worldwide. *Helicobacter pylori* infection has been clearly correlated with gastric carcinogenesis. At present and in the near future, the most important challenge is and will be the significant reduction of mortality due to GC. That goal can be achieved through the identification of higher-risk patients, such as those with atrophic gastritis, intestinal metaplasia and dysplasia. In this review we intend to discuss the importance of diagnosing *H. pylori* infection and chronic atrophic gastritis in preventing gastric cancer, using a new non-invasive test called GastroPanel. This test is a classification algorithm including four biochemical parameters pepsinogen I and II (PGI and PGII), gastrin-17 (G17), and anti-*Helicobacter pylori* antibodies (Ig G anti-Hp) measured in fasting sera, which allows to classify patients as having atrophic or non-atrophic gastritis and to find whether gastritis is associated or not with *H. pylori* infection. GastroPanel is not a “cancer test”, but it can and should be used in the screening and diagnosis of subjects with a high cancer risk; still, a careful diagnostic made by superior digestive endoscopy is compulsory to find possible precancerous or cancerous lesions at an early and curable stage.

Key words: gastric cancer, *Helicobacter pylori*, gastropanel.

HELICOBACTER PYLORI INFECTION, CHRONIC ATROPHIC GASTRITIS AND GASTRIC CANCER

Helicobacter pylori, a curved bacillus, previously called *Campylobacter pyloridis*, is a Gram negative pathogen found in the stomach. It was discovered by Marshall and Warren in the 1980s. From the beginning, *Helicobacter pylori* was classified as a higher class I carcinogen. Although over 80% of people are asymptomatic, chronic infection can lead to gastritis, gastric and duodenal ulcer, gastric adenocarcinoma, and MALT lymphoma [1].

It has been estimated that 17.8% of cancers worldwide are due to infectious agents, and *H. pylori* is estimated to be responsible for 5.5% of all cancer cases and more than 60% of gastric cancer cases. Gastric cancer still remains one of the most common causes of cancer-related deaths, although in the last decades its incidence has slowly decreased. In 2008, approximately one million cases were documented, most of them in less developed regions [2].

It has been established that early precancerous process starts with the colonization of the gastric mucosa. When *Helicobacter pylori* is in contact with the epithelium lining, it initiates an inflammatory response and an increased production of cytokines and chemokines. These biochemical mediators will attract inflammatory cells to the

mucosa. In most cases, the immune response is unable to eliminate the infecting agent, and unless the bacterium is eradicated by treatment the infection may last for decades. The long-term inflammation may cause damage to the epithelial cells and, over time, loss of glands, which may be replaced by glands lined by epithelium with intestinal phenotype. A sustained injury to the gastric mucosa may lead to dysplastic changes and malignant transformation. These are the following recognized steps for the natural history of gastric adenocarcinomas of intestinal type: a chronic active inflammation induces a multifocal atrophy (gland loss), which in time leads to intestinal metaplasia that is finally followed by dysplasia [3].

Most *H. pylori*-infected individuals have chronic active “superficial gastritis”, which, in most cases, is asymptomatic. Around 20% of subjects infected with *H. pylori* will develop clinical manifestations of the infection, including peptic ulcer disease and gastric neoplasia. Around 10% of patients with *H. pylori* induced chronic active gastritis progress to severe atrophic changes of the gastric mucosa. Around 5% of those with severe atrophic gastritis develop intestinal-type gastric cancer [4].

The first study indicating that *H. pylori* eradication is beneficial in reducing the incidence of gastric cancer (GC) only in subjects without precancerous conditions of the gastric mucosa was published in 2004 by Wong *et al.* [5].

HOW TO DIAGNOSE *HELICOBACTER PYLORI* INFECTION?

In the past 40 years since *Helicobacter pylori* was discovered numerous detection methods for the presence of the bacterium have been developed. Noninvasive tests such as serology, C urea breath test, stool test, antigen tests are usually preferred by clinicians. Serology has its own limitation especially in endemic areas, because the active or cured infection of *H. pylori* cannot be differentiated, antibody levels persist for a long time even after cure. C urea breath test is believed to be specific, but with present revelation of the fact that the stomach is colonized by many other urea producing bacteria makes it questionable. Antigen stool test has a low sensitivity because antigen excretion may vary over time and antigen may degrade while passing through the intestine. Histology, the first method used for *Helicobacter pylori* detection, is an invasive one, often influenced by the site, number and size of the bioptic material collected. Also, diagnosis by histological methods can be obtained in 2-3 days at the earliest, while the detection rate definitely depends on the expertise of examiners. Another downside of this method is the fact that prior antibiotics and PPI may transform the typical shape of *H. pylori* from spiral to coccoid and therefore becomes undetectable. Culture from bioptic samples is a tedious, time-consuming procedure with a low sensitivity (50 up to 70%). PCR method used not only for the detection of the bacterium, but also for characterization of pathogenic genes, bacterial virulence and specific mutations associated with antimicrobial resistance has a high cost and a high risk of contamination [6].

WHAT IS GASTROPANEL?

In the past few years medical research has focused on identifying a non-invasive screening procedure able to identify subjects at high or low risk for gastritis in order to avoid unnecessary gastroscopies and histological examinations especially among dyspeptics. Therefore, a serological panel called "GastroPanel" was designed, combining pepsinogen I and II (PGI and PGII), gastrin-17 (G17), and anti-*Helicobacter pylori* antibodies (Ig G anti-Hp) using an ELISA technique for detection. This test uses a software, called GastroSoft which is based on the Updated Sydney System (USS) for the classification of gastritis. Based on the clinically validated cut-off values for each biomarker, the

software classifies the test results into one of the five categories: 1) Normal result, 2) HP infection (without atrophy) 3) atrophic gastritis in the corpus, 4) atrophic gastritis in the antrum, and 5) atrophic pangastritis (Figure 1). The reference ranges (normal values) of the four biomarkers have been determined from multiple clinical studies on human stomachs with normal structure and function, incorporating endoscopic mucosal biopsy assessment. The latest version of GastroSoft® is based on a stochastic algorithm (not probabilistic as the previous versions), giving an electronic (and printed) report, including the test results, cut-off values and verbal interpretation, classifying the GastroPanel® result into one of these five categories.

The Pepsinogens are gastric acid protease zymogens. They are divided into two distinct immunochemical groups: Pepsinogen I and II.

Pepsinogen I is produced by the main gastric cells from the stomach body (corpus). When the stomach body mucosa is atrophied (as it happens in the autoimmune disease or in *Helicobacter pylori* infection) there is a drop of pepsinogen I level below 30 µg/L, and also a drop of hydrochloric acid. Instead, in a situation in which there is no atrophy of the mucosa, only pepsinogen I inflammation, its blood concentration will tend to increase. Thus, the plasma changes of these biomarkers indicate the state of health of the body of the stomach.

Pepsinogen II is produced primarily in the oxyntic gland mucosa of the stomach, the gastric antrum and the duodenum. It is secreted mainly into the gastric lumen and into circulation. Pepsinogen II has little or no biological activity, but in acid it is converted to the active enzyme pepsin which exhibits proteolytic actions. Pepsinogen II blood concentration reflects the structure and function of whole stomach mucosa, and its concentration will increase in case of gastritis (stomach mucosa inflammation) as it happens in *Helicobacter pylori* infection, viral or parasitic infection, biliary reflux, strong spice or alcohol ingestion. Values bigger than 10 µg/L are correlated with inflammation.

The Pepsinogen I/II ratio is used together with Pepsinogen I value for the diagnosis of atrophy of the gastric body mucosa (atrophic gastritis of corpus). In the case of atrophic gastritis of the corpus, the ratio decreases below 3.

Pepsinogen I blood concentration and pepsinogen I *versus* pepsinogen II ratio reflects truthfully glandulocytes mass and the principal glands from the corpus region of the stomach and hence the atrophy degree of the stomach mucosa.

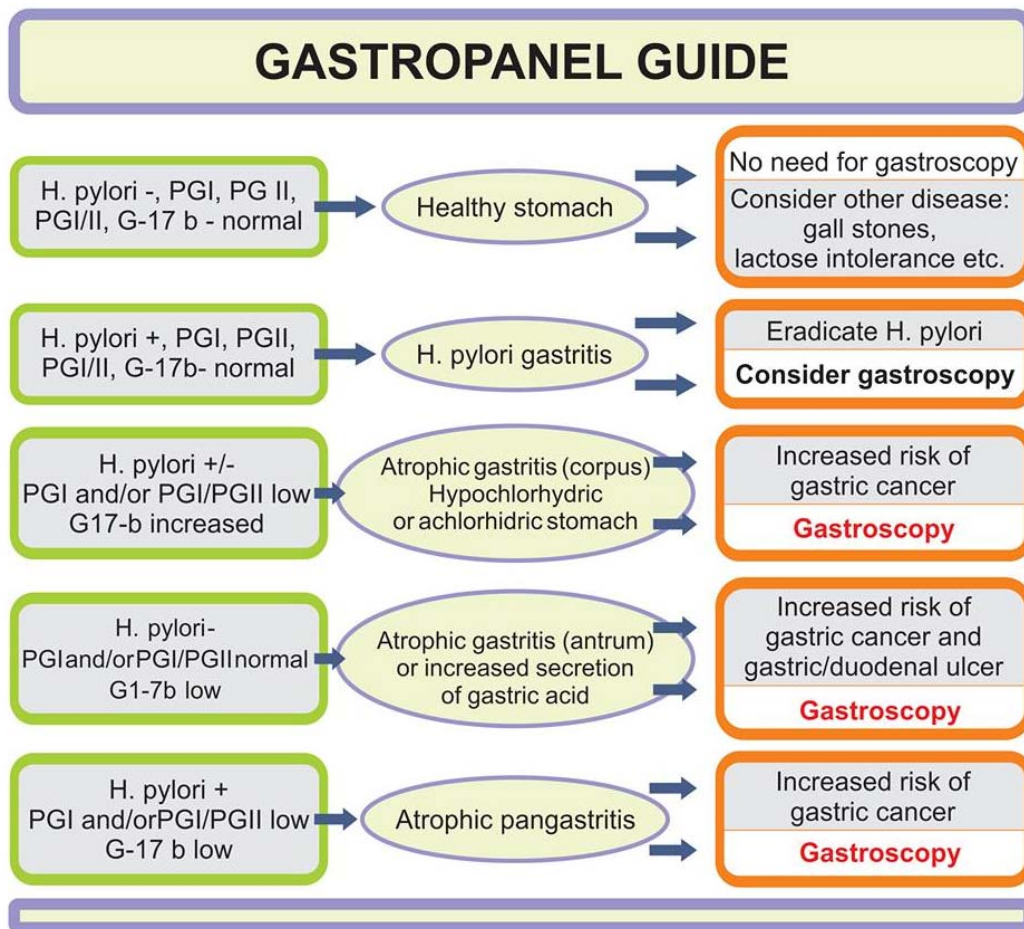


Figure 1. Gastropanel guide.

Basal Gastrin-17 (G-17b) is exclusively produced by the G cells from the antral region of the stomach and duodenum. The GastroPanel monoclonal antibody only measures the biologically active amidated Gastrin-17 peptide (sulphated and non-sulphated), having a specific receptor on the surface of enterochromaffin-like cells (ECL) (cholecystokinin receptor, CCK2R). As a result, Gastrin-17 stimulates the ECL cells to release histamine into the blood circulation, which seeks the histamine receptor on the surface of the parietal cell and stimulates them to secrete hydrochloric acid. Gastrin-17 concentrations in blood (during fasting) decrease when the acidity of gastric contents rises (pH below 2.5) and also when there is an antrum mucosa atrophy, in which situation G-cells disappear. In hypochlorhydria Gastrin-17 concentration increases more than 10 pmol/L and in achlorhydria more than 20 pmol/L.

We also have to mention the stimulated Gastrin-17. Its secretion can be increased by elevated vagal tonus, gastrin-releasing peptide hormone, the stretching of gastric antrum and protein-rich food.

The distinction between antrum mucosal atrophy caused by *Helicobacter pylori* infection and increased secretion of hydrochloric acid (Gastrin-17 basal < 1 pmol/L) can be performed by gastroscopy (preferred) or by measuring the response of Gastrin-17 after protein stimulation. A low level of the stimulated gastrin-17 (less than 3 pmol/L), after protein stimulation, indicates the presence of antrum atrophic gastritis (Figure 2).

Helicobacter pylori IgG antibodies are produced after the infection occurs. IgG anti-Hp give significant added value to the three biomarkers mentioned before. IgG serology for Hp shows two different conditions: an ongoing infection or a previous exposure. It is known that high levels of HP antibodies may persist in plasma months after the eradication. The IgG antibodies levels > 30 EIU of *Helicobacter pylori* antibodies are correlated with the infection.

To conclude, gastric corpus mucosal atrophy is associated with reduced Pepsinogen levels < 25 µg/L, Pepsinogen I/Pepsinogen II ratio < 3 and increased G17 > 10 pmol/L [7].

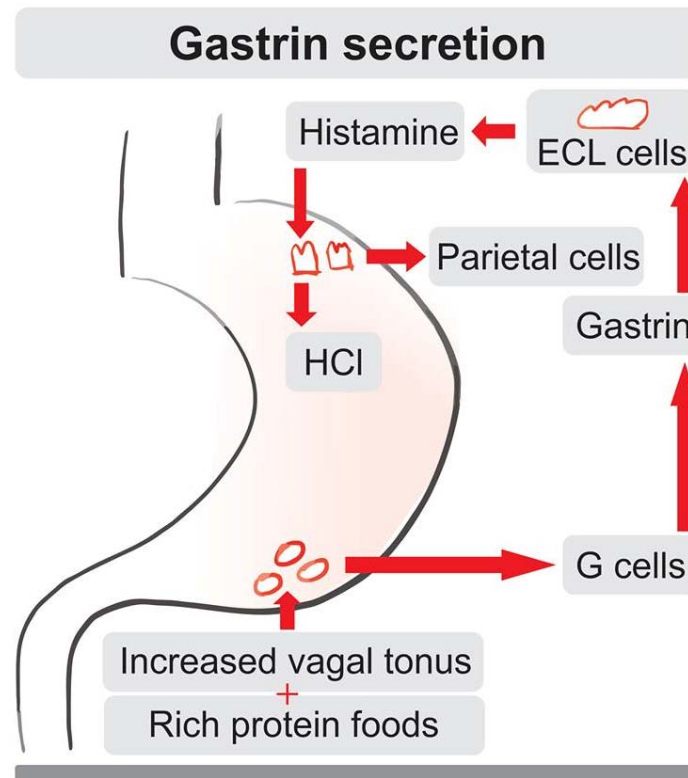


Figure 2. Gastrin secretion.

GASTRO PANEL A RELIABLE NON-INVASIVE TEST

Medical literature includes several studies with good results regarding the accuracy of Gastro Panel that encourage us to use this non-invasive test as a screening method for pre-neoplastic condition for gastric cancer such as chronic atrophic gastritis. Therefore, it is possible to determine which patient has high risk of developing gastric cancer and needs more expensive and invasive specific investigations such as endoscopy.

Noah D. and his colleagues examined the efficacy of GastroPanel Blood kit, in diagnosing and scoring gastritis associated to *Helicobacter pylori* infection. The results showed that the prevalence of *H. pylori* infection based on histology did not differ from that based on GastroPanel 81.4% versus 84.9% [8].

The sensitivity and specificity of the biomarker test panel to diagnose normal and "healthy" (no *H. pylori* gastritis, no AG) stomach mucosa in the population-based sample of the 1000 subjects were 89% (95% CI 86–92%) and 92% (90–95%), respectively [9].

In 2009, in Padova, Italy, Venerito M and his team [4] showed that gastric corpus mucosal atrophy is associated with reduced PGI levels ($< 25 \mu\text{g/dL}$),

reduced PGI/PGII ratio (< 3) and increased G17 ($> 10 \text{ pmol/L}$) with a high sensitivity (80-90%) and specificity (90-100%).

Lombardo L. and his colleagues compared the results of histology and GastroPanel in chronic atrophic gastritis patients (confirmed by histology). PGI levels are significantly lower in patients with corpus fundus atrophic gastritis than in controls without atrophic gastritis, and G17 levels are significantly lower in antral atrophic gastritis than in normal controls. The agreement between GastroPanel and histology for corpus-prevalent chronic atrophic gastritis was 94%, results which encourage us to use GastroPanel as a reliable non-invasive test for diagnosis of chronic atrophic gastritis [10].

GastroPanel test is not a "cancer test", but it can be used in the screening and diagnosis of subjects with a high cancer risk, such as subjects with atrophic gastritis in which a careful diagnostic gastroscopy is mandatory in order to find possible cancerous lesions at an early and curable stage. Another study based on 162 subjects from Tohoku University from 2006 to 2008 obtained a very good accuracy, sensitivity and specificity, 94%, 95% and 93% respectively for GastroPanel test in diagnosing healthy stomach mucosa [10]. In countries with a high incidence of gastric cancer, such as Japan and

Korea, the Cancer Control Program started in 2012 was conducted in conjunction with *H. pylori* eradication and also accepted the measurement of serum PG as a noninvasive screening test of gastric cancer to identify individuals at high risk [11].

A meta-analysis based on 9 prospective cohorts from Eastern Asia reported in 12 publications found that adults with a positive pepsinogen test, as a stand alone test, had an approximately four-fold higher risk of gastric cancer than those with a negative test [12]. Another study based on 3328 participants concluded that a combination of serum PG levels and *H. pylori* antibody test is useful for detecting gastric neoplasms based on Correa's cascade, with a slow gastric carcinogenesis pathway progressing from gastric adenoma with low grade dysplasia to Lauren's intestinal-type GC. The sensitivity and specificity of these two biomarkers have been reported to be 90–95% and 84.6–89.7%, respectively [13].

A longitudinal study conducted in China, a high-risk of gastric cancer zone, has established that increased levels of PGI, PGII, anti-*H. pylori* IgG and a decreased PGI/II ratio is associated with the progression of gastric precancerous lesions [14].

In 2014 in the US the first simulation model-based analysis was designed to evaluate the clinical benefits and economic consequences of serum pepsinogen screening. Among a cohort of 10 million 20-year-old men the model estimated that serum pepsinogen screening would prevent 27.0% of the projected 19.014 intestinal-type gastric carcinoma deaths and that serum pepsinogen screening may have similar benefits to mammography screening among 50–59-year-old women [15].

A retrospective case-control study aimed at determining whether hypergastrinemia was associated with tumor growth or advanced tumor pathology and also to evaluate the effectiveness of G-17 in predicting precancerous or cancerous gastric lesions. The outcomes showed that the mean serums G-17 level was significantly higher in patients with HP infections, gastric adenoma with low-grade dysplasia, and gastric adenoma with high-grade dysplasia than in controls ($p < 0.001$), but higher G-17 levels in each disease category were not associated with increased tumor size, synchronicity, invasiveness, presence of lymph node metastasis, or a higher cellular proliferation index. Thus, hypergastrinemia is a useful predictive marker for precancerous lesions (eg. HP infection, gastric adenoma) [16].

After we reviewed these studies which have used these four biomarkers (PGI, PGII, G-17, IgG HP) together or separately we can sustain that GastroPanel is a reliable test in diagnosing pre-neoplastic conditions for gastric cancer: *Helicobacter pylori* infection and chronic atrophic gastritis.

We have a work in progress in which we study several biomarkers for pre-neoplastic lesions among dyspeptics. One of these biomarkers is GastroPanel. We shall publish the results when the study is ready.

CONCLUSIONS

GastroPanel should be used as a screening non-invasive test to detect the high-risk patients (*Helicobacter pylori* infection, chronic gastric atrophy) for gastric cancer.

Cancerul gastric este unul dintre cele mai răspândite tipuri de neoplazii din lume. Infecția cu Helicobacter pylori reprezintă un factor important în carcinogeneza gastrică. Cea mai mare provocare în momentul de față este reducerea mortalității legată de cancerul gastric. Acest lucru se poate obține prin identificarea pacienților cu risc crescut, cum ar fi cei cu gastrită atrofică, metaplazie intestinală sau displazie. În acest referat se va discuta despre importanța diagnosticării infecției cu Helicobacter pylori și a gastritei cronice atrofice utilizând un test non-invaziv numit GastroPanel. Acest test reprezintă o clasificare algoritmică care utilizează patru parametri biologici, Pepsinogen I și II (PGI și PGII), gastrina-17 (G-17) și anticorpii anti-Helicobacter pylori (Ig G anti-Hp). Dozarea acestor parametri permite clasificarea pacienților ca având sau nu gastrită atrofică și stabilește dacă gastrita este sau nu asociată cu infecția cu Helicobacter pylori. GastroPanel nu este un „test al cancerului” dar, poate, și ar trebui utilizat în screening-ul pacienților cu risc crescut; totuși un diagnostic atent stabilit pe baza

endoscopiei digestive superioare este necesar pentru a depista posibilele leziuni preneoplazice sau neoplazice într-un stadiu precoce în care se poate aplica un tratament curativ.

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