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Review Article

Role of Serological Biomarker Testing (GastroPanel®) in Diagnosis of Symptomatic Dyspepsia and in Screening of the Risks of Stomach Cancer

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Abstract

The two major risk factors of gastric cancer (GC) are *Helicobacter pylori* (HP) infection and atrophic gastritis (AG). It is currently possible to diagnose HP-infection and AG reliably by using serological testing with a panel of biomarkers (GastroPanel®, Biohit Oyj, Finland): pepsinogen I (PGI), pepsinogen II (PGII), gastrin-17 (G-17) and HP-antibodies. In this review, an introduction is made to the GastroPanel® test as the first non-invasive diagnostic tool for i) dyspeptic symptoms, and for ii) screening of asymptomatic subjects for the risks of GC.

GastroPanel® test is based on stomach physiology both in health and disease. Accordingly, pepsinogen levels and their ratio is decreased in corpus atrophy (AGC), accompanied by elevated G-17b (basal). G-17b level also sensitively responds to gastric acid output, being low with high acid output and high when the stomach is acid-free (due to PPI-treatment or AGC). In antrum atrophy (AGA), G-17b is low and, importantly, does not respond to a protein stimulation (G-17s), because the G-cells are disappeared.

The results of GastroPanel® test are interpreted by a specially designed software (GastroSoft®) identifying 8 diagnostic marker profiles (Table 1). Of those, four (profiles 1,2,3 and 8) represent purely functional disorders (of acid output), while three others specify structural abnormalities (profiles 5,6, and 7 for AGC, AGA, and AGpan, respectively). The remaining (profile 4) is typical to HP-infection, with three possible outcomes: a) active HP-infection, b) successful eradication, and c) failed eradication.

During the past 10 years while on the market, GastroPanel® test has been validated in an increasing number of studies both in clinical and screening settings. This published literature was recently subjected to systematic review and meta-analysis, including 27 eligible (biopsy-confirmed) studies that comprise almost 9.000 patients examined with the GastroPanel® test. Studies were eligible, if i) GastroPanel® test (instead of stand-alone biomarkers) was used to diagnose biopsy-confirmed AGC or AGA, and ii) exact numbers were available to enable calculating the sensitivity (SE) and specificity (SP). GastroPanel® was shown to perform better in diagnosis of AGC than AGA, with 70.2% vs. 51.6% pooled SE, and 93.9% vs. 84.1% pooled SP, respectively.

This first meta-analysis corroborates the recently launched statement of 16 international experts, advocating the use of this non-invasive serological test as the first-line diagnostic tool for dyspeptic symptoms and for screening of the risks of GC among asymptomatic subjects. In addition to its high specificity for both AGA and AGC, GastroPanel® test has an extremely high longitudinal negative predictive value (NPV); a normal test result precludes a significant gastric pathology for several years ahead. Similarly, GastroPanel® profiles implicating AGC are powerful independent predictors of an incident GC in a long-term longitudinal setting.

Keywords: *Biomarkers, Pepsinogen I; Pepsinogen II; Gastrin-17; Helicobacter pylori; Serological Testing; Marker Panel; GastroPanel; Gastric Cancer Risk; Atrophic Gastritis; Antrum; Corpus; Dyspepsia; Diagnosis; Screening; Primary Prevention*

Introduction

Gastric cancer (GC) continues to be one of the most common cancers and causes of global cancer mortality; nearly one million new cases and 736.000 annual cancer deaths worldwide [1]. In many Western countries, GC incidence has been steadily declining, however, attributed to major changes in the life-style factors and to a reduced exposure to the known risk factors of GC [2]. These known risk factors for GC include smoking, use of alcohol, dietary factors, occupational exposures, exposure to radiation and/or radiotherapy, as well as genetic predisposition in certain rare inherited syndromes [3,4]. According to the current thinking, the different distribution of these risk factors among different populations explains the large geographic variation in the incidence of GC. It is estimated that nearly 80% of GC cases among males and 70% in women are due to different life-style and environmental factors [1,3,4]. The Mediterranean type of diet has been considered as particularly healthy and clearly linked to a low risk of GC [5].

In addition to the above common-type of risk factors, there are two specific risk factors that far exceed in importance of all the others in pathogenesis of GC: *Helicobacter pylori* (HP) infection and atrophic gastritis (AG) [3,6,7]. As early as in 1994, the International Agency for Research on Cancer (IARC, Lyon; a WHO agency) concluded that the accumulated scientific evidence is sufficient to declare HP as a human carcinogen [8]. This bacterium primarily infects the gastric mucosa, and if uneradicated, develops AG in about half of the affected patients. Although HP itself is not directly carcinogenic, AG is the single most potent risk factor of GC [3,7,9]. In some 5 - 10% of the patients with HP infection, mucosal atrophy is moderate or severe, and the risk of GC increases in parallel with the severity of AG: compared with healthy stomach, the risk is 2 - 5 times higher in those with only chronic HP gastritis but up to 90-fold in patients with severe AG both in the corpus and antrum (pan-gastritis; AGpan) [3,6,7,10].

The other main histological type of GC (intestinal type) develops in atrophic mucosa through various degrees of dysplasia (mild, moderate, severe), which are often accompanied by intestinal metaplasia (IM). This pathogenetic chain of events is known as the Correa cascade [3]. It is important to recall that this cascade can often (but not invariably) be interrupted by appropriate early treatment of HP infection [3,4,9,11]. AG is the single most important risk condition for GC [3,7,12,13]. Based on the Updated Sydney System classification (USS), AG is classified by its topographic location in the stomach (antrum, corpus, or both) as AGA, AGC or AGpan, respectively [14]. In addition to being the key risk factor of GC, HP-infection also plays a causative role in the development of peptic ulcer disease [8-10]. Similarly, both AG and HP can be responsible for the symptoms known as dyspepsia; organic or functional [15]. Debate still continues on the value of systematic HP eradication in relieving the dyspeptic symptoms, however [9,10,15].

The diagnosis of AG has traditionally been made using histological biopsies on gastroscopy. However, gastroscopy is an invasive diagnostic tool, which requires expensive equipment and considerable professional experience. Like other endoscopies, also gastroscopy is a subjective diagnostic method, which is not suitable for population-based screening of GC. As to the diagnosis of HP infections, attempts have been made to standardize the management (diagnosis included) since 1996 by the Maastricht Consensus Conferences publishing their consensus statements at regular intervals [9,10]. However, far too often in daily practice, only the merits of the commonly used HP tests are being emphasized while there is a common tendency to neglect the limitations of their use in special clinical settings, although clearly discussed in all European Consensus Reports [9,10]. This applies to both of the two most widely used HP tests; the ^{13}C-Urea Breath Test (UBT) and Stool Antigen test (SAT), the limitations of which were recently discussed in timely reviews [16-18].

GastroPanel® Test (Serological Biopsy)

Because of these obstacles in diagnosis of both AG and HP-infection, the need to develop a simple and reliable diagnostic blood test has increased in parallel with the increasing understanding of the importance of HP and AG as the key risk factors of GC, as established by long-term follow-up studies conducted e.g. in Finland [7,13]. To meet the increasing demand, the GastroPanel® test was designed in the late 1990's by Biohit Oyj (Helsinki, Finland), representing the first non-invasive diagnostic test for stomach health and disease [19-22]. With GastroPanel®, both these key risk factors of GC can be identified in a simple blood test, which is based on the simultaneous measurement of four stomach-specific biomarkers that characterize the structure and function of the gastric mucosa. This same marker panel is

equally applicable as the first-line diagnostic test in patients with dyspeptic symptoms, with potential to replace the invasive gastroscopy in this diagnostic algorithm [21,22].

Introduction to GastroPanel® test

GastroPanel® is the first-line diagnostic test for HP-infection (5 - 80% of the world population), for the examination of all patients with dyspepsia (20 - 40% of the Western population), as well as for the screening of AG with related risks, such as stomach and esophageal cancer [22-24]. As well known, AG also enhances the risk of malabsorption of vitamin B12, calcium, iron, magnesium, zinc, and some medicines [20,21].

GastroPanel® consists of four stomach-specific biomarkers representing the key regulators of normal stomach physiology. These four biomarkers include pepsinogen I (PGI), pepsinogen II (PGII), amidated gastrin-17 (G-17), and HP antibodies, designed to give information on both the structure and function of the stomach mucosa [20-27]. Most importantly, this panel gives accurate estimates of the capacity of the corpus and antrum mucosa to produce gastric acid and G-17, respectively, as well as of important gastric pathologies, like inflammation, as well as the grade and topography of AG [28-30].

Normal plasma levels of all four biomarkers indicate that the stomach mucosa has normal structure and function, whereas abnormal levels are signs of a non-healthy stomach, reflecting disturbances in the feedback mechanisms between the acid output of the corpus, PGs and G-17. For G-17 assessment, there are two options; G-17 basal (G-17b) values and G-17 stimulated (G-17s) values, the latter being particularly important in distinguishing between functional disturbance of the antrum (G-17s normal) and AGA (G-17s does not increase upon protein stimulation) [31,32].

Being the first non-invasive diagnostic test for stomach mucosal health, GastroPanel® is unique in that the results are interpreted by a software application (GastroSoft®) (http://www.GastroPanel.com), specifically designed for this purpose. GastroPanel® results are classified into one of five possible diagnostic categories related to stomach morphology: 1) normal mucosa, 2) superficial or non-atrophic (HP) gastritis, 3) AG in the corpus, 4) AG in the antrum, and 5) AG in both antrum and corpus (pan-gastritis) [13,32]. Thus, GastroPanel® is optimized for use together with the Updated Sydney System (USS) for the classification of gastritis, which is based on these same five diagnostic categories [33]. In addition, there are three other marker profiles specific to functional disturbances of the stomach where morphology is normal (details to follow).

GastroPanel® has been validated in several large trials based on biopsy-confirmed gastroscopies [23,34,35], all included in a recent meta-analysis [36]. These studies have been exploited to establish the validated reference (cut-off) values for each individual biomarker of the panel for the histological endpoints. These studies also confirm the high accuracy of GastroPanel® in detecting the most important endpoint, moderate-to-severe AG [36]. Thus, normal values of PGI, PGII and their ratio (PGI/PGII) preclude AGC with NPV of over 95% [23]. In turn, the values of PGI and PGII as well as their ratio below the established cut-off levels predict moderate-to-severe AGC with area under ROC curve (AUC) values of above 0.950 in adequately-powered and USS-validated series [34,36].

In brief, the levels of PGI decrease in AGC and in AGpan, but remain within the normal range in all other conditions. Elevated PGII levels reflect mucosal inflammation, the highest values being detected in HP-associated non-AG. The G-17b values are highest in AGC, because of the missing negative feedback by the acid output from an atrophic corpus, resulting in uninhibited secretion of G-17b by the normal antral mucosa. The same applies to the situation where acid output is inhibited by prolonged use of PPI medication. By definition, when antral mucosa is atrophic and the G cells are depleted, G-17 secretion remains very low even after protein stimulation (G-17s) [19]. HP IgG antibodies provide significant added diagnostic value to the three biomarkers. IgG antibody level for HP measures two potentially different conditions: 1) an ongoing HP-infection, or 2) a previous exposure to HP. As the only abnormal marker, HP implicates an HP-associated superficial gastritis (non-AG), while associated with abnormalities in the other three markers, elevated HP antibody levels confirm the diagnosis of HP-associated AG (AGA or AGC) [16-18,37,38].

Biomarkers of the GastroPanel® Test

Pepsinogen I (PG I)

This biomarker is included in GastroPanel® to identify patients who have mucosal atrophy (AG) in the gastric corpus, for which the plasma PGI is a highly specific biomarker [36,38-42]. Pepsinogen I (PGI) is a precursor enzyme (zymogen) of pepsin, synthesized by the chief cells and neck cells of the gastric corpus (in the so-called oxyntic glands). As a pepsin precursor, the major part of PGI is secreted into the gastric lumen but a minor fraction is excreted into the blood. The circulating PGI concentration closely correlates with the quantity of the chief cells in the corpus mucosa, and any loss of these cells (due to mucosal atrophy) results in a linear decrease in plasma levels of PGI [38-42].

For as yet unknown reasons, AG increases the risk of GC [3,6,7,9,10]. Compared with a healthy stomach, this risk is 5-fold among patients with advanced AGC, but up to 90-fold in patients with advanced AG in both the antrum and corpus (i.e., AGpan) [7]. In the screening of middle-aged (50 - 69 years) males in Finland, the circulating PGI level was low (< 25 µg/l) in 9.8% of the subjects, of whom 4.7% revealed either a GC or a precancer lesion on endoscopy [13]. Similar results have also been published in several previous studies included in a recent meta-analysis of Dinis-Ribeiro., *et al* [43].

Pepsinogen II (PG II)

Pepsinogen II is produced by the chief cells and mucous neck cells of the gastric corpus, in pyloric glands of the gastric antrum, and in Brunner's glands of the proximal duodenum. The ratio of pepsinogen I (PGI) to PGII plasma levels in normal subjects is about 3 - 20 [30]. The PGI/PGII ratio decreases linearly with increasing grade of AGC [36,38,39,44]. The ratio falls below 3.0 when AGC is advanced (moderate or severe) [39]. It has been shown that the risk of GC is increased (5-fold) when the PGI/PGII ratio is low [22,26,29,42,45-50]. This test is intended as an additional diagnostic tool for AGC. The Pepsinogen II assay is designed for use concomitantly with the Pepsinogen I assay to determine the PGI/PGII ratio, alongside G-17 to confirm the diagnosis of AGC (G-17 is up-regulated) [36,38]. An elevated PGII level reflects mucosal inflammation, the highest values being detected in HP–associated non-AG. Since HP antibody levels can remain elevated for several months after successful eradication, PGII is a useful marker for the confirmation of positive eradication results [22,36,38].

Gastrin-17 (G-17)

Gastrins are linear peptide hormones produced by the G cells in the duodenum, in the pyloric part of the gastric antrum, as well as in the pancreas [22]. The main function of gastrins is to stimulate the secretion of gastric acid (HCl) by the parietal cells in the gastric corpus, as well as to increase the motility of antrum [51]. In addition, gastrins are known to stimulate gastric chief cells to secrete pepsinogens (PGI, PGII), and also induce the contraction of the lower esophageal sphincter (LES). Like most of the peptide hormones, different molecular weight gastrins are synthesized as a result of post-translational modifications from preprogastrin. The G cells release a mixture of different molecular weight gastrins into the circulation, including gastrin-71, -52, -34, -17, -14, and -6, all of which are carboxy-amidated and circulate in an O-sulfated and non-sulfated form [52]. In healthy humans, the dominant forms of gastrin in plasma/serum are amidated gastrin-34 (G-34) and G-17 [53].

G-17 is a predominant and the most potent form in healthy antral tissue, and it is almost exclusively produced by the antral G cells. The G-17 included in this test is a direct biomarker of antral structure and function, and through a negative feedback loop, an indirect biomarker of gastric corpus. G-17 plasma levels within the normal range implicate a normal structure and function of the antrum, whereas low or high values of G-17 also reflect abnormal functions of the corpus. The maximum information is obtained when G-17 testing is done separately for fasting (G-17b) and stimulated (G-17s) levels, and combined with pepsinogen I (PGI) and pepsinogen II (PGII) measurement as well as with HP antibody testing [7,19,44,54-56].

The measurement of plasma G-17b may also be used for the monitoring of patients who have undergone gastric surgery; secretion of G-17b is practically zero after successful radical antral resection (antrectomy). In HP-negative subjects, a low fasting level of G-17 can indicate high acid output. This in turn may increase the risk of gastroesophageal reflux disease (GERD) and Barrett's esophagus (up to

3-4–fold risk), whereas a normal or elevated G-17b excludes the presence of Barrett's esophagus with high probability [57,58]. The G-17 biomarker in the GastroPanel® test is specific for amidated gastrin-17 in the plasma [38,56,57].

Helicobacter pylori Antibody (IgG)

Helicobacter pylori (HP) infection is the most important cause of chronic gastritis leading to mucosal atrophy. A much more infrequent cause of AG is an autoimmune mechanism [59,60]. In GastroPanel® test, this ELISA is intended for diagnosis of HP-infection in the plasma sample, based on IgG antibody detection.

HP is a spiral-shaped, gram-negative bacterium that colonizes in the human stomach [61]. The organism is found within the mucous layer overlying the gastric epithelium, and also within the mucosal glands, but it does not appear to invade the epithelial cells. However, the mucosa underneath and surrounding the areas of the HP colonization is invariably inflamed; this condition is referred to as chronic superficial or non-atrophic gastritis which, if untreated, persists for life [22,60,61]. Without adequate eradication of the bacteria, this chronic inflammatory process leads to AG [9,10]. AG in turn increases the risk of peptic ulceration and GC, two important sequels of HP-infection [62-65]. The presence of antibodies to HP strains have been linked to the development of AGC [9,10,66]. The epidemiological evidence indicates a link between HP-infection and gastric adenocarcinoma, as well as a mucosa-associated lymphatic tissue (MALT) lymphoma [9,17,18,19,67,68].

Interpretation of the GastroPanel® Results

GastroPanel® is optimized for use in context with the Updated Sydney System (USS) classification of gastritis [14,38]. Both the USS and the GastroSoft® software use five diagnostic categories to classify the biopsies and the GastroPanel® results, respectively. These include: 1) normal mucosa, 2) superficial (HP) gastritis, 3) AGA, 4) AGC, and 5) AG in both antrum and corpus (AGpan) [14,33]. In addition to these five categories related to stomach morphology, three other marker profiles are possible in GastroPanel®, being specific for defined functional disturbances, with normal stomach morphology. All eight diagnostic categories are depicted in Table 1, and explained in the following.

Normal profile (healthy stomach)

With all four biomarkers within the normal reference range, gastric mucosa functions normally. Given that the function of gastric mucosa is critically dependent on the specific cells responsible for acid output (parietal cells), pepsinogens (chief cells) and G-17 (G cells), normal function necessitates the presence of these cells in normal quantities [22,24,29,32,38]. Thus, stomach function and mucosal structure go hand-in-hand, and by definition, a normal GastroPanel® result is a surrogate marker of a healthy stomach. A normal marker profile does not exclude, however, minor abnormalities like non-specific inflammation, mild irritation or micro-erosions that do not impact on the marker profiles [24,38].

High acid output

Gastric acid (HCl) is produced by the highly specialized parietal cells in the corpus. Acid output is controlled, among other things, by the secretion of G-17 in the antrum as a result of a positive feedback loop stimulating acid output after a meal [22,38,51,52]. Acid output results in progressively lower pH in the corpus (stomach contents), and the threshold of pH 2.5 triggers a negative feedback to antral G cells, signaling them to down-regulate the output of G-17 [51-54]. As a result, G-17 output decreases in parallel with the increasing acid output of the corpus [19,22,24,34]. When, due to any reason(e.g. other stimulatory mechanisms), acid content in the corpus remains abnormally high, the end result is abnormally low G-17b output from the antral G cells. Using GastroPanel®, this condition is best diagnosed after a test medication with PPI, when the G-17b should normalize within approximately 2 weeks of therapy. Under these high-acidic circumstances (with low G-17b), however, the postprandial (stimulated) G-17s will be within normal limits, because the G cells are intact and capable of G-17 secretion when properly stimulated (e.g. by a protein powder; Biohit Cat. No. 601038) [38].

	GastroPanel® Biomarkers						
Marker Profile	**Pepsinogen I (30 - 160 mg/l)@**	**Pepsinogen II (3 - 15 mg/l)**	**PGI/PGII Ratio (3 - 20)**	**Gastrin-17b (1 - 7 pmol/l)**	**Gastrin-17s (3 - 30 pmol/l)**	***H. pylori* IgG Antibody titer (< 30 EIU)**	**Interpretation**
1	N	N	N	N	N	N	Healthy mucosa (no atrophy, no HP- infection)
2	N	N	N	L*	N	N	Healthy mucosa. High acid output in the corpus
3	N or H^	N or H^	N	H**	N	N	Healthy mucosa. Low acid output due to e.g. PPI medication
4a	N or H^	N or H^	N	N or H^	ND	N or H†	Active HP-infection, not treated
4b	N	N	N	N	ND	H	HP- infection successfully eradicated
4c	N	H	N	H	ND	H	HPeradication failed
5	L	L	L	H	ND	N^^ or H	Atrophic gastritis in the corpus (AGC)
6	N	N	N	L	L	H	Atrophic gastritis in the antrum (AGA)
7	L	L	L	L	L	N^^ or H	Atrophic gastritis in the antrum and corpus (AGpan)
8	H	H	N	H	ND	N	Short (4-10d) break in PPI treatment

Table 1: *The diagnostic categories of GastroPanel® test results.*

*N=normal; L=low; H=high; *Test PPI medication for two weeks, G17b should normalize; **Stop PPI medication, G-17b should normalize within two weeks; ND, no need for testing; ^PGI, PGII and G-17 can be elevated due to mucosal inflammation; ^^HP antibodies can disappear in mucosal atrophy with protracted clinical course; @Pepsinogen I cut-off value 30 µg/l is consonant with moderate/severe AG; †HP antibody levels can remain elevated for months after successful eradication of HP.*

Low acid output due to proton pump inhibitor (PPI) medication

The regulation above also works in the other way round. When acid output in the corpus is reduced (for any reason), the positive feedback loop triggers antral G cells to increase their G-17b secretion, resulting in elevated serum levels of G-17b [19,24,38]. The two prime conditions leading to low acid output are: 1) AGC, and 2) long-term use of PPI medication (or to a lesser extent, H2-receptor blockers). The former is excluded by the normal (or even elevated) values of PGI, PGII, and normal PGI/PGII ratio [24,38], while the latter is best diagnosed by discontinuing the PPI medication. In that case, the antral G-17b should be normalized within two weeks [24,38,51-54].

Superficial (non-atrophic), *Helicobacter pylori*-associated gastritis

Like all bacteria, HP will also induce acute inflammation in the gastric mucosa, with the usual onset in the antrum [9,10,22,24,28,61,67,68]. Three different marker profiles can be encountered in association with HP-infection (Table 1).

Active HP-infection

In an active HP-infection, HP antibody levels are raised above the cut-off value (30 EIU), which can be the only abnormal finding in GastroPanel® test, with all other markers falling within a normal range. Not infrequently, however, an active ongoing HP-infection causes a severe inflammatory reaction which, due to increased cell permeability, can lead to increased leakage of PGI, PGII and even G-17 from the secretory cells and result in elevated serum levels of any or all of these three biomarkers, as depicted in Table 1 (4a) [9,10,24,28,38].

Successful HP eradication

Successful HP eradication by active treatment should result in normalized values of HP antibodies as well as the three ("inflammatory") markers (PGI, PGII, G-17) (Table 1; 4b). For the latter, this is known to take place with a delay of some weeks [24,38]. In contrast, HP antibody levels can remain elevated for a longer period of time which is subject to individual variation and limits the usefulness of GastroPanel® as an accurate diagnostic test in the immediate post-treatment control of HP eradication. Because a marked individual variation exists in the dynamics of these marker profiles, an accurate record of timing of the HP eradication therapy is mandatory while making the re-testing with GastroPanel® [2,21,38,56].

Failed HP eradication

In cases where HP eradication attempt fails, HP antibody levels remain elevated (usually slightly), while PGI and PGI/PGII ratio usually fall within a normal range, whereas PGII and/or G-17b may remain slightly elevated as a sign of an ongoing inflammatory process Table 1, profile 4c). The result can be confirmed after 5 - 6 months, followed by a new treatment attempt if indicated [9,10,24]. An option is to use another test for the control of HP eradication, e.g. the *Helicobacter pylori* Quick Test (fast) or *Helicobacter pylori* UFT300 Quick test (ultrafast) [69].

Atrophic gastritis of the corpus (AGC)

By definition, the loss of specific cells (chief cells) in the oxyntic glands of the corpus mucosa as a result of mucosal atrophy will lead to a progressively reduced output of PGI and (to a lesser extent) PGII, which is also produced by the same cells in the antral mucosa [24,38]. This disproportionate reduction of these two markers will result in a reduced PGI/PGII ratio, which is another excellent signature of AGC [22,24,26-28,30,34,36,38,43]. This reduction in the PGI and PGI/PGII ratio is progressive and closely correlated with the severity of corpus atrophy, with total atrophy and acid-free stomach as the end point [56,59]. In the case of intact (normal) antral mucosa, this leads to markedly increased output and serum levels of G-17b [19,24,38] (Table 1, profile 5). There is no need to test G-17s in such a situation. In chronic AGC cases with a protracted course over decades, *Helicobacter pylori* itself may disappear from the stomach mucosa, resulting in gradual normalization of the HP antibody levels [70-72].

Atrophic gastritis of the antrum (AGA)

When the mucosal atrophy only affects the antrum, all corpus-specific markers will remain within the normal range (Table 1). By definition, AGA is caused by HP-infection, and HP antibodies are invariably elevated in the GastroPanel® testing. As a result of AGA, the G cells are reduced in number and finally disappear, leading to progressively reduced plasma levels of G-17b. In severe AGA, there is no response in G-17 secretion to protein stimulation (G-17s), because of the lack of (target) G cells in the antral mucosa (Table 1, profile 6) [19,22,24,34,35,37,38,40,56,58]. Thus, the distinction between the two potential causes of low G-17b: i) high acid output (profile 2) and ii) AGA (profile 6), is neatly done by using the G-17 testing after protein stimulation (G-17s)(24,38). As pointed out, G-17s will react normally only in the former, but fails to react in severe AGA.

Atrophic gastritis of the antrum and corpus (AGpan)

The most severe form of AG is known as atrophic pan-gastritis (AGpan), affecting both the antrum and corpus [24,38]. As an end result, the specified cells (chief cells) in the corpus and antrum (G cells) disappear, leading to a biomarker expression profile where both pep-

sinogens (PGI, PGII) and G-17 are substantially reduced (Table 1, profile 7) [19,22,24,34,35,37,38,40,59,60]. This applies to both G-17b and G-17s, which remain low even after protein stimulation because of the missing G cells. Like in AGC, (profile 5), HP antibody levels can be within a normal range or elevated. This is because in chronic AG, HP itself can disappear from an atrophic mucosa, and in the absence of antigen stimulus, a normal decay of IgG antibodies will revert the HP antibody levels below the 30 EIU cut-off [70-72].

Panel profile in context of PPI medication

Any gastric acid suppressive medication (PPI, H_2 blockers) will inevitably interfere with the profile of the GastroPanel® markers because of an altered acid output, as explained above. To enable the assessment of the biomarker profile without such an interference, the manufacturer recommends that the patient discontinues any acid-suppressive treatment 7 days before the sampling (24,38). It is appreciated that because of severe symptoms, this withdrawal of PPI- or H_2-blocker medication is not always possible. Because of this fact, the new version of the software (GastroSoft®) was modified so as to take into account the eventually continued use of these drugs. Import is an accurate record of the PPI/H_2-medication, the fact whether or not discontinued, and if so, for how many days before the sampling. With this information accurately recorded, the GastroSoft® is capable of interpreting the test results correctly, defined as profile 8 in Table 1, based on the following rational.

PPI and H_2-blockers effectively reduce gastric acid production in the parietal cells of the gastric corpus [24]. This increases the production of G-17, and also increasing the output of pepsinogens. Once the PPI/H_2-treatment is discontinued, it takes approximately 4 – 10 days for HCl production and G-17 levels to normalize. However, pepsinogens respond more slowly, and PGI and PGII levels may remain above the cut-off values for a relatively long period (up to 2-3 weeks) [24,38]. Furthermore, an abrupt termination of a long-term PPI medication is typically followed by rebound acid hypersecretion, frequently accompanied by heartburn (and other) symptoms and extremely low levels of G-17b [19,22,24,32,38].

Clinical Performance Confirmed in a Formal Meta-Analysis

To provide an unbiased estimate of the accumulated evidence, we recently performed a systematic review and meta-analysis (with meta-regression) of all studies published on GastroPanel® test since its introduction in the early 2000's [36]. Studies were eligible, if i) GastroPanel® test (instead of stand-alone markers) was used to diagnose biopsy-confirmed AGC or AGA, and ii) exact numbers were available to enable calculating the sensitivity (SE) and specificity (SP). Altogether, 27 studies were eligible, comprising 8.654 tested patients from different geographic regions. Significant heterogeneity between studies reporting AGC (n = 27) or AGA (n = 13) warranted random effects (RE) model for the summary statistics. GastroPanel® was shown to perform better in diagnosis of AGC than AGA, with 70.2% vs. 51.6% pooled SE, and 93.9% vs. 84.1% pooled SP, respectively [36]. Limited number of studies erodes the Q test's power to detect true heterogeneity in meta-analysis stratified by geographic origin of the studies. Few hypothetical missing studies had only marginal effect on the pooled estimates of SE and SP. The results of this first meta-analysis of GastroPanel® literature corroborates the above cited statement of the international experts [22]. Due to its high specificity for both AGA and AGC [36] as well as its extremely high longitudinal negative predictive value [34], GastroPanel® is truly a test for stomach health. In other words, testing GastroPanel-negative at any time point during one's life-time precludes (with over 95% probability) a significant gastric pathology for several years ahead [34]. At the meantime, however, an abnormal GastroPanel® arker profiles implicating AGC are powerful independent predictors of an incident GC in a long-term longitudinal setting [73].

Conclusions

GastroPanel® test has been on the market for roughly 10 years by now. The test is based on long-term natural history studies on gastritis patients run since the 1960's in Finland and Estonia [7,13,14,19,39,40,44,55,58-60,65,70-72]. This test is the first non-invasive diagnostic tool based on physiology of 3 stomach-specific biomarkers of both health and disease. The test also includes testing for HP-infection, the key etiological factor in pathogenesis of peptic ulcer disease and GC [9,10]. In its current version, the Unified GastroPanel®

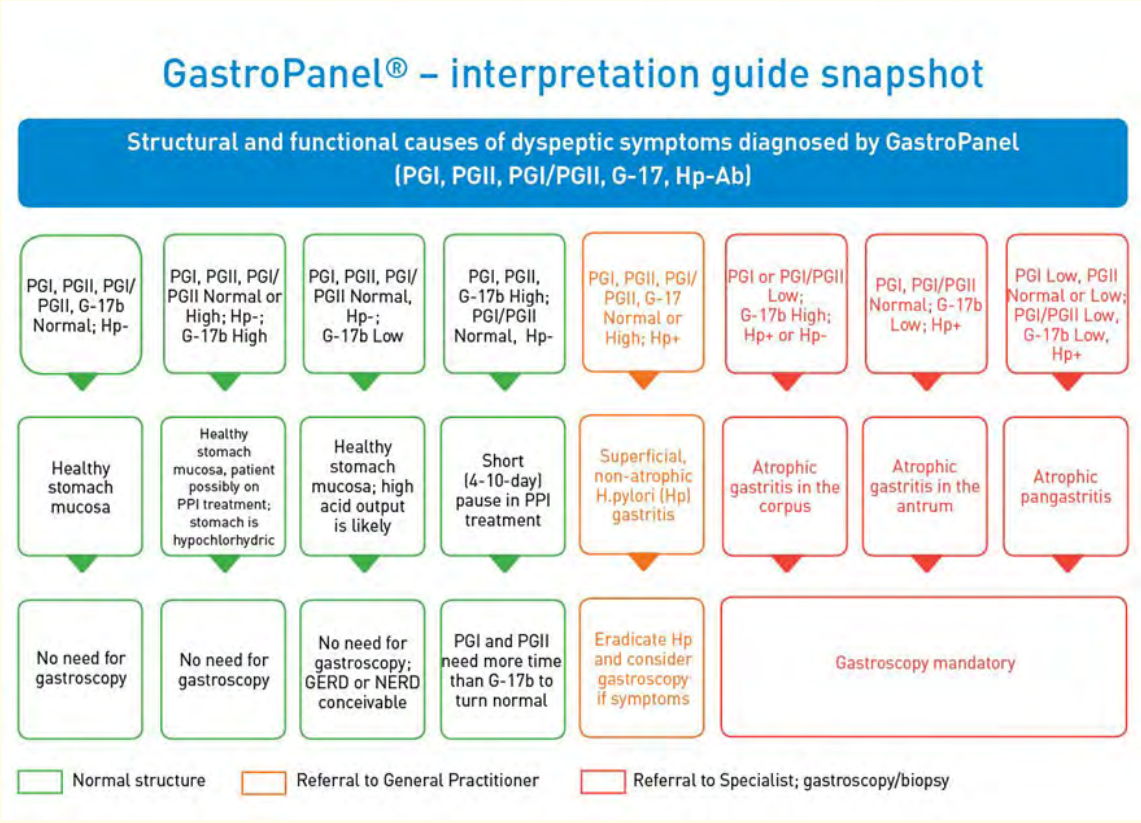

Figure 1: *GastroPanel Interpretation Guide.*

test is fully automated, all 4 biomarkers being processed under identical conditions. The test will be soon available in the quick test version as well, particularly suitable for the POC (point-of-care) testing in doctors' offices with meager facilities for blood sample processing. With the refined diagnostic algorithm of the GastroSoft®, the results are classified into 8 specific marker profiles [38], of which 4 represent functional disturbances (in acid output), 3 indicate AG (and its topographic location), and 1 is specific for HP-infection.

In GastroPanel® test, the HP antibody measurement is complemented by the other 3 biomarkers (PGI, PGII, G-17) which are sensitive indicators of mucosal inflammation. This 4-marker panel makes GastroPanel® test the most comprehensive HP test, devoid of the known shortcomings (false negative and false positive results) of the conventional HP tests [16-18]. Given that this bacteria is the single most important risk factor of GC, it is time to move a step forward towards a flawless diagnosis of *Helicobacter pylori* infections, using the test that is i) free from the shortcoming of the conventional HP tests, and ii) provides an added value by detecting (with high precision) [36] also the other key risk factor of GC, i.e., atrophic gastritis.

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