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Serum Biomarker Panel (GastroPanel®) and Slow-Release L-cysteine (Acetium® Capsule): Rational for the Primary Prevention of Gastric 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. Severe AG leads in acid-free stomach colonized by HP and other bacteria, producing acetaldehyde (Group I human carcinogen; IARC). Together with other conditions leading to a) acid-free stomach (e.g. chronic users of PPI medication, autoimmune AG) or b) those exposing the subjects to increased concentrations of acetaldehyde (e.g. cigarette smokers, alcohol intake, ALDH2 enzyme mutations), these subjects are at high-risk for gastric and esophageal cancer.

This document has a dual purpose: First, to give an introduction to the GastroPanel® as the first non-invasive diagnostic tool for i) dyspeptic symptoms, and for ii) screening of asymptomatic subjects for the risks of GC. Second, to describe a novel formulation (Acetium® Capsule, Biohit Oyj) based on slow-release L-cysteine, designed to protect the stomach mucosa in these high-risk subjects by its capacity to eliminate carcinogenic acetaldehyde.

GastroPanel® 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 (Figure 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, GastroPanel® has been validated in an increasing number of studies both in clinical and screening settings, and its excellent clinical performance was validated in two recent meta-analysis. In addition to its high specificity for both AGA and AGC, GastroPanel® test has excellent longitudinal predictive values (NPV and PPV) for incident GC as shown in two recent cohort studies.

Acetium® Capsule is a novel formulation of slow-release L-cysteine, being a unique medical device designed to elimination of carcinogenic acetaldehyde in the stomach. A regular use of Acetium® Capsule is indicated for all those who have acid-free stomach, irrespective of its cause. This formulation effectively protects the stomach against the exposure to acetaldehyde. The most common high-risk groups include the following: 1) AG associated with HP infection; 2) AG caused by autoimmune mechanisms; 3) cigarette smokers; 4) alcohol consumers; 5) chronic users of PPI medication, and 6) those (500 million) people in Asia who have a mutation

of the aldehyde dehydrogenase (ALDH2) enzyme, failing to metabolize acetaldehyde to acetic acid, and exposed to higher local concentrations of acetaldehyde.

The efficacy of Acetium® Capsule in acetaldehyde elimination has been documented in controlled clinical experiments. In all studies, L-cysteine concentration in the gastric juice remained elevated for up to 3 hours, suggesting that an insignificant amount of L-cysteine is absorbed from the stomach or transported into the small intestine. It was demonstrated that slow-release L-cysteine effectively (by 60 - 80%) eliminates carcinogenic acetaldehyde in patients with acid-free stomach caused by either AG or PPI treatment. This was the case in individuals with either active or deficient ALDH2 enzyme. This capacity of slow-release L-cysteine to eliminate acetaldehyde persisted for up to three hours after ingestion of two Acetium® Capsules. L-cysteine was combined with acetaldehyde locally in the stomach, forming a stable and non-carcinogenic 2-methylthiazolidine carboxylic acid (MTCA). MTCA concentrations in the gastric juice remained significantly elevated for up to three hours after the administration of alcohol and Acetium® Capsules.

With a rational use of these two medical devices, one can diagnose the gastric high-risk conditions and subsequently protect the stomach against acetaldehyde exposure.

Keywords: *Serum Biomarker; GastroPanel®; L-cysteine; Acetium® Capsule; Gastric Cancer*

Background

Gastric cancer (GC)

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].

Key risk factors

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.

In Japan, where gastric cancer has ranked as the most common cancer by incidence and mortality for the last several decades, the prevalence of HP-infection has dramatically declined by birth cohort effect, mainly due to improvements in the general hygiene environment in childhood [9]. Older generations born before around 1950 still show a high HP prevalence of around 80-90%, decreasing with age to reach around 10% or less in those born around the 1990's, and less than 2% for children born after the year 2000 [9]. This change will have generational effects on GC prevention strategies, both primary and secondary. The risk-stratified approach to gastric cancer prevention should be considered in Japan and other countries which have similarly experienced rapid economic development [9,10].

Pathogenesis (Correa cascade)

Although HP itself is not directly carcinogenic, AG is the single most potent risk factor of GC [3,7,11]. 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) [3,6,7,12]. 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 [3,4,11,13]. AG is the single most important risk condition for GC [3,7,14,15]. 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 [16]. In addition to being the key risk factor of GC, HP-infection also plays a causative role in the development of peptic ulcer disease [8,11,12]. Similarly, both AG and HP can be responsible for the symptoms known as dyspepsia; organic or functional [17]. Debate still continues on the value of systematic HP eradication in relieving the dyspeptic symptoms, however [11,12,17].

Carcinogenic agent in common (acetaldehyde)

HP and AG are the two most powerful independent risk factors of GC. The carcinogenic agent in common to both HP and AG is acetaldehyde, determined as class I human carcinogen by IARC in 2009. Apart from HP itself which is capable of synthesizing acetaldehyde, the other bacteria colonizing in acid-free stomach of AG are an abundant source of this carcinogenic substance [18-20]. Other groups subjected to high exposure to acetaldehyde are i) cigarette smokers, and ii) alcohol consumers, both being abundant sources of acetaldehyde. The same applies to iii) AG caused by autoimmune disease, and iv) chronic users of PPI medication. Yet, another special group of people at high risk for acetaldehyde exposure are those 500 million people in Asia, who have a mutation of the aldehyde dehydrogenase (ALDH2) enzyme, and who fail to metabolize alcohol to acetic acid, resulting in higher local concentrations of acetaldehyde [18-20]. Similarly, a recent meta-analysis provided evidence that chronic use of PPI medication increases the risk of GC [21], plausibly explained by the PPI-induced acid-free stomach that will be colonized by acetaldehyde-producing microbial flora [22,23].

Diagnosis of the gastric high-risk conditions

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 [11,12]. 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 [11,12]. 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 a series of timely reviews [22-26].

These caveats of the conventional HP-tests are entirely avoided by the BIOHIT GastroPanel® innovation, where the HP antibody (Ab) measurement is complemented by 3 other biomarkers (PGI, PGII, G-17) which are sensitive indicators of mucosal inflammation. This 4-marker panel makes GastroPanel® the most comprehensive HP test, devoid of the known shortcomings (false negative and false positive results) of the conventional HP tests [22-26]. 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 HP-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) also the other key risk factor of GC, i.e., atrophic gastritis (AG) with all its potential clinical sequels (http://www.biohithealthcare.com/additional-information).

Minimizing the gastric exposure to carcinogenic acetaldehyde

Because acetaldehyde is ubiquitous, the exposure to it is impossible to avoid completely [18-20]. The only realistic option is to reduce acetaldehyde exposure to the absolute minimum. This can be achieved by a very simple and inexpensive means based on the biochemical characteristics of a natural amino acid L-cysteine, patented by Biohit Oyj (Helsinki): Acetium® capsule and Acetium® lozenge. The

patented slow-release L-cysteine formulation reacts covalently with carcinogenic acetaldehyde to form a stable and inert compound, 2-methylthiatsolidne-4-carboxyl acid (MTCA) [27]. By so doing, Acetium® preparations reduce the concentration of acetaldehyde in the stomach and in saliva, thus protecting these two mucosal sites against the harmful effects of this carcinogenic compound [28-30].

The Combined Use of GastroPanel® and Acetium® Capsule is Rational

The combined use of GastroPanel® test and Acetium® capsule is rational as will be explained in this document. The former is for diagnosis of the high-risk conditions in the stomach, whereas the latter provides an effective and unique means for minimizing the exposure of the stomach to carcinogenic acetaldehyde. With regard to gastric carcinogenesis, the most important high-risk conditions of the stomach include HP infection and AG, readily diagnosed by GastroPanel® as explained in the following sections.

These very same conditions (HP, AG) are also the two prime indications for use of Acetium® capsules to eliminate carcinogenic acetaldehyde in acid-free stomach. Two other important groups at high risk for acetaldehyde exposure in their stomach include chronic users of PPI-medication and those bearing the mutation in their ALDH2 enzyme. For the other indications, see section (1.4.).

This document discusses the evidence-based rational for the combined use of these two unique medical devices; one for diagnosis and the other for prevention/protection.

GastroPanel® Examination

Because of these obstacles in diagnosis of both AG and HP-infection, the need to develop a simple and reliable diagnostic blood examination 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 [7,15]. To meet the increasing demand, the GastroPanel® was designed in the late 1990's by Biohit Oyj (Helsinki, Finland), representing the first non-invasive diagnostic test for stomach health and disease [31-34]. With GastroPanel®, both these key risk factors of GC can be identified in a simple blood examination, 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 examination in patients with dyspeptic symptoms, with potential to replace the invasive gastroscopy in this diagnostic algorithm [33,34].

Introduction to GastroPanel®

GastroPanel® is the 1) first-line diagnostic examination for HP-infection (5 - 80% of the world population), for 2) the examination of all patients with dyspepsia (20 - 40% of the Western population), as well as 3) for the population-based screening of AG with related risks, such as stomach and esophageal cancer [34-36]. As well known, AG also increases the risk of malabsorption of vitamin B12, calcium, iron, magnesium, zinc, and some medicines [32,33]. It is essential to realise that GastroPanel® is not a test for gastric cancer itself. This is because of the fact that the biomarkers included in the panel are not specific markers of gastric malignancy but subject to changes upon functional disturbances as well as in mucosal pathologies (HP and AG), and as such their serum levels are poor markers of GC [22-26,34,37,55].

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 [32-39]. Most importantly, this panel gives accurate estimates of 1) the capacity of the corpus and antrum mucosa to produce gastric acid and G-17, respectively, of 2) important gastric pathologies, like inflammation, as well as of 3) the grade and topography of AG [40-42].

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 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) [43,44].

Being the first non-invasive diagnostic test for stomach mucosal health, GastroPanel® is also unique in that the results are interpreted by a specific software (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) [15,44]. 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 [45]. 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 [35,46,47], all included in a recent meta-analysis [48]. 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 [48]. Thus, normal values of PGI, PGII and their ratio (PGI/PGII) preclude AGC with NPV of over 95% [35]. 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 [46,48].

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 long-term 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) [31]. 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) [22-24,49,50].

Biomarkers of the GastroPanel®

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 [48,50-54]. Pepsinogen I (PGI) is a precursor enzyme (zymogen) of pepsin, synthesized by the chief cells and neck cells of the gastric corpus (in 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 [50-54].

For as yet unknown reasons, AG increases the risk of GC [3,6,7,11,12]. 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 [15]. Similar results have also been published in several previous studies included in a recent meta-analysis [55].

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 between 3 - 20 [42]. The PGI/PGII ratio decreases linearly with increasing grade of AGC [48,50,51,56]. The ratio falls below 3.0 when AGC is advanced (moderate or severe) [51]. It has been shown that the risk of GC is increased (5-fold) when the PGI/PGII ratio is low [34,38,41,54,57-62].

This parameter 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) [48,50]. 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, i.e., lack of active inflammation [34,48,50].

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, and in pancreas [34]. 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 [63]. 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 pre-progastrin. 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 [64]. In healthy humans, the dominant forms of gastrin in plasma/serum are amidated gastrin-34 (G-34) and G-17 [65].

G-17 the most potent form in healthy antral tissue, and it is almost exclusively produced by the antral G cells. The G-17 included in GastroPanel® 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 [7,31,56,66-68].

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 [69,70]. The G-17 biomarker in the GastroPanel® test is specific for amidated gastrin-17 in the plasma [50,68,69].

***Helicobacter pylori* Antibody**

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 [71,72]. 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 [73]. 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 [34,72,73]. Without adequate eradication of the bacteria, this chronic inflammatory process leads to AG [11,12]. AG in turn increases the risk of peptic ulceration and GC, two important sequels of HP-infection [74-77]. The presence of antibodies to HP strains have been linked to the development of AGC [11,12,78]. The epidemiological evidence indicates a link between HP-infection and GC as well as a mucosa-associated lymphatic tissue (MALT) lymphoma [11,22,23-26,79,80].

Interpretation of the GastroPanel® Results

GastroPanel® is optimized for use in context with the Updated Sydney System (USS) classification of gastritis (16,50). 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) [16,45]. 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 figure 1.

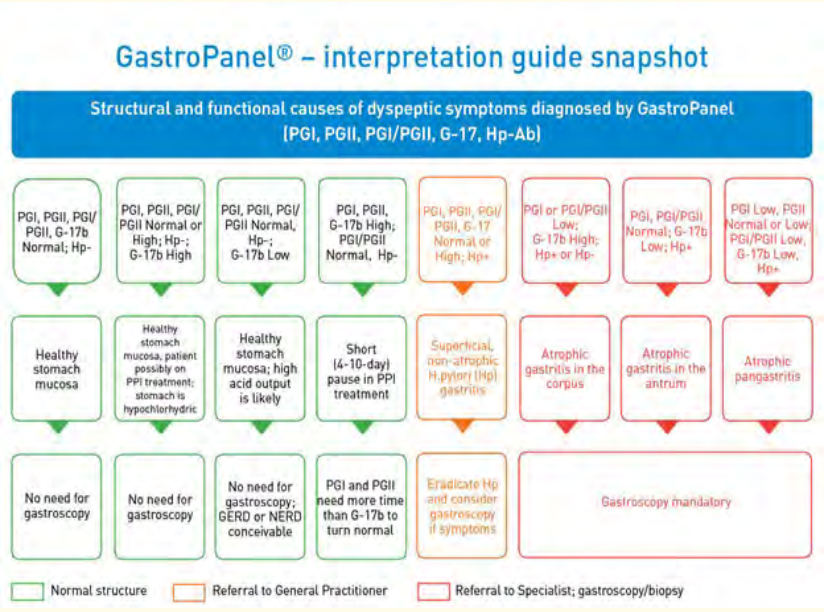

Figure 1: *GastroPanel Interpretation Guide.*

Normal profile

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 [34,36,41,44,50]. 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 [36,50].

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 [34,50,63,64]. 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 [63-66]. As a result, G-17 output decreases in parallel with the increasing acid output of the corpus [31,34,36,46]. 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) [50].

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 [31,36,50]. 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 [22-26,36,50], while the latter is best diagnosed by discontinuing the PPI medication. In that case, the antral G-17b should be normalized within two weeks [36,50,63-66].

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 [11,12,24,28,34,61,67,68]. Three different marker profiles can be encountered in association with HP-infection.

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®, 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 [11,12,36,40,50].

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). For the latter, this is known to take place with a delay of some weeks [22-26,36,50]. 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® 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® [2,33,50,68].

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. The result can be confirmed after 5 - 6 months, followed by a new treatment attempt if indicated [11,12,22-26,36]. An option is to use another test for the control of HP eradication, e.g. the *Helicobacter pylori* Quick Test (fast) or *Helicobacter pylori* UFT 300 Quick test (ultrafast) [81].

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 [34,36,38-40,42,46,48,50,55]. 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 [68,71]. In the case of intact (normal) antral mucosa, this leads to markedly increased output and serum levels of G-17b [31,36,50]. 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 [82-84].

Atrophic gastritis of the antrum (AGA)

When the mucosal atrophy only affects the antrum, all corpus-specific markers will remain within the normal range. 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 [31,34,36,46,47,49,50,52,68,70]. Thus, the distinction between the two potential causes of low G-17b: i) high acid output, and ii) AGA, is neatly done by using the G-17 testing after protein stimulation (G-17s) [22-26,36,50]. 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 [22-26,36,50]. 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 pepsinogens (PGI, PGII) and G-17 are substantially reduced [31,34,36,46,47,49,50,52,71,72]. 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, 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 [82-84].

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 [22-26,36,50]. 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 GastroSoft® was modified so as to take into account the eventually continued use of these drugs. Important is an accurate record of i) the PPI/H_2-blocker medication, ii) the fact whether or not discontinued, and if so, for iii) how many days before the sampling. With this information accurately recorded, the GastroSoft® is capable of interpreting the test results correctly, based on the following rational.

PPI and H2-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/H2-blocker 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) [22-26,36,48,50]. 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 [22-26,31,34,36,44,50].

Clinical Performance Confirmed in Two Recent Meta-Analysis

To provide an unbiased estimate of the accumulated evidence, we recently performed a systematic review and meta-analysis of all studies published on GastroPanel® test since its introduction in the early 2000's [48]. 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 [48]. Limited number of studies eroded 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 consensus statement of international experts [34].

Another meta-analysis was recently published by a group of gastroenterologists in Italy [85]. Similar to the first meta-analysis [48], also these authors assessed the diagnostic performance of the GastroPanel® test in the diagnosis of AG. The extensive literature search covered all the key databases (PubMed, Embase, Scopus, Cochrane Library) between 1995 and December 2016. Studies were eligible if they assessed the accuracy of the GastroPanel® test for the diagnosis of AG using the USS [45] as the reference. The authors identified 20 eligible studies with a total of 4.241 subjects tested with the biomarker panel for AG, regardless of its topographic location (antrum or corpus) in the stomach [85]. The pooled SE was 74.7% (95%CI 62.0 - 84.3) and the pooled SP was 95.6% (95%CI, 92.6 - 97.4). The authors concluded that BIOHIT GastroPanel® test appears to be a reliable tool for the diagnosis of AG, and applicable for both screening of the subjects or populations at high-risk of GC [85].

GastroPanel® has an Excellent Longitudinal Predictive Value for GC

In addition to the great value of GastroPanel® in the diagnosis of dyspeptic symptoms [22-26,34,48] as well as in screening of asymptomatic subjects for the risk conditions of GC [34,36,39-43,48,49,50,86,87], GastroPanel® has been recently studied also in longitudinal settings to assess the value of its biomarkers as long-term predictors of GC [88,89].

The first of these studies assessed the predictive value of GastroPanel® biomarkers in a case-control setting nested within a cohort of Caucasian population in Western Siberia [88]. Both the cases and controls for this study were derived from a population-based cohort of 45-69-year-old subjects (n = 9.360) in the HAPIEE (Health, Alcohol and Psychosocial Factors In Eastern Europe) study, enrolled in Novosibirsk (Siberia) during 2003 - 2005. Cases represent all GCs reported to the Cancer Registry until 2012, being matched (1:2) with healthy controls (COs). Altogether, 156 (52 GCs and 104 COs) serum samples collected at study entry were available for GastroPanel® analysis. Conditional logistic regression models (uni- and multivariate) were used to analyse this matched case-control setting. The biomarker levels below cut-off at baseline predicted the development of GC as follows (OR; 95%CI): PGI (2.9; 95%CI: 1.3 - 6.4), PGII (9.0; 95%CI: 1.8 - 44.3), PGI/PGII (3.3; 95%CI: 1.5 - 7.3); G-17 (1.8; 95%CI: 0.7 - 4.8), and HP-Ab (0.4; 95%CI: 0.1 - 1.3). In a multivariate model adjusted for sex, age, and all four biomarkers, PGI/PGII ratio was the most powerful independent predictor of GC (OR = 2.9; 95% CI: 1.01 - 8.0). Indeed, this was the first time in a Caucasian population, where PGI, PGII and PGI/PGII ratio were shown to be reliable longitudinal predictors of incident GC [88].

The second longitudinal study was completed in Northern China (90). The authors analysed the role of GastroPanel® biomarkers in identifying high-risk individuals and predicting the risk of developing (GC). Among 12,112 participants with prospective follow-up from an ongoing population-based screening program using both serology and gastroscopy, the authors conducted a multi-phase study involving a cross-sectional analysis, a follow-up analysis, and an integrative risk prediction modeling analysis [89]. In the follow-up analysis, low PGI levels and PGI/II ratios were associated with higher risk of developing GC, and both low (< 0.5 pmol/l) and high (> 4.7 pmol/l) G-17 levels were associated with higher risk of developing GC. In their risk prediction modeling, the five biomarkers combined yielded a C statistic of 0.803 (95%CI = 0.789 - 0.816) and improved prediction beyond traditional risk factors (P < 0.001) for identifying precancerous lesions at enrollment. Similarly, higher serological biopsy scores based on the five biomarkers at enrollment were associated with higher risk of developing GC during follow-up (p for trend < 0.001). Accordingly, GastroPanel® test could be used to identify the high-risk individuals for further diagnostic gastroscopy, and to stratify the individuals' risk of GC, thus guiding a targeted screening and tailored prevention [89].

Together with the published meta-analyses [48,85], these two studies [88,89] implicate that due to its high specificity for both AGA and AGC as well as its extremely high longitudinal predictive value, GastroPanel® is truly a test for stomach health and disease. In other words, testing GastroPanel-negative at any time point during one's life-time precludes (with > 95% probability) a significant gastric pathology for several years ahead [88,89]. At the meantime, however, a GastroPanel® marker profile implicating AGC is a powerful independent predictor of an incident GC in a long-term longitudinal setting [88,89].

Acetium® Capsule for Protection of the Stomach in High-Risk Subjects

Subjects at high-risk for exposure to carcinogenic acetaldehyde

Whenever GastroPanel® is revealing an abnormality suggesting AG (any topography), the subject should be referred for gastroscopy to confirm the diagnosis. By targeted mucosal biopsies, gastroscopy is considered as the gold standard to classify the grade and topography of gastritis, using different classifications, e.g. the USS [45]. It is estimated that HP-infection is involved in > 90% of all GC cases that develop through the "Correa cascade", from intermediate steps of AG, intestinal metaplasia (IM), and dysplasia. HP and AG are currently considered the two most powerful independent risk factors of GC (16,51,55,73). The carcinogenic agent in common to both HP and AG is acetaldehyde, determined as Group I human carcinogen by IARC in 2009 [20]. Apart from HP itself which is capable of synthesizing acetaldehyde, the other bacteria colonizing in acid-free stomach of patients with AG, are an abundant source of this carcinogenic substance.

Accordingly, acid-free stomach due to any cause predisposes the subject to acetaldehyde exposure and thus increases the risk of developing GC and esophageal cancer [18-21]. Accordingly, AG caused by HP infection is a well-known cause of acid-free stomach, and as an intermediate step in Correa cascade, is a high-risk lesion for GC. The same applies to AG caused by autoimmune disease, which is another well-known causative factor of AG [90-92]. Acetaldehyde is the major carcinogenic substance in cigarette smoke, predisposing cigarette smokers to acetaldehyde exposure in oral cavity and the upper gastrointestinal tract [18-20,93,94]. The same applies to alcohol intake, because acetaldehyde is the first metabolite of alcohol, also produced in the stomach endogenously from ethanol, e.g. by local microbial or mucosal oxidation of ethanol to acetaldehyde [18-20,28,95].

Another well-known condition that increases the risk of acetaldehyde exposure is a point mutation in ALDH2-gene resulting in deficient activity of the main acetaldehyde metabolizing mitochondrial enzyme (ALDH2), and provides conclusive evidence for the causal relationship between local acetaldehyde exposure and upper digestive tract cancer [96,97]. It has been estimated that some 500 million people (mainly in Asian populations) are affected. When drinking alcohol, the upper digestive tract mucosa of ALDH2-deficients is exposed via saliva to about 2-times and via gastric juice, up to 5-6 times higher acetaldehyde concentrations than in persons with active ALDH2-enzyme [96-98]. Parallel to increased local acetaldehyde exposure, the risk of ALDH2-deficient alcohol drinkers for oral, pharyngeal, esophageal and gastric cancer is many-fold compared to alcohol drinking ALDH2-actives. Thus, ALDH2-deficiency provides a unique human cancer model for local acetaldehyde exposure in the upper digestive tract. These and other evidence prompted the International Agency for Research on Cancer (IARC) to reclassify acetaldehyde associated with the consumption of alcoholic beverages as a Group I human carcinogen [20].

Yet, another well-defined risk group are the chronic users of PPI-medication. Two recently published meta-analyses suggest that the use of acid-suppressive (PPI) drugs is associated with an increased risk of GC [21,99]. A denominator in common with AG is acetaldehyde endogenously formed from ethanol [18,19,20,93-98]. An acid-free stomach secondary to either AG or PPI treatment is colonized by oral microbes, which effectively produce acetaldehyde from ingested alcohol via their ADH enzymes. This was convincingly demonstrated in recent experiments, where PPI- treatment for 7 days significantly increased gastric juice acetaldehyde levels in ALDH2-active subjects after intra-gastric infusion of a moderate dose of alcohol. However, the highest gastric juice acetaldehyde concentrations were measured in PPI-treated ALDH2-deficient subjects [98].

AG and IM - are these conditions always irreversible?

Whether gastric carcinogenesis progressing through the stepwise manner from HP-infection, through AG and IM to invasive GC, can be arrested or even reverted, is a contradictory subject [11,12]. This issue is of crucial importance from the clinical point of view, because according to our current thinking, both AG and IM are irreversible conditions once established, and without adequate monitoring, inevitably lead to invasive GC [3,100].

However, there are some recent implications that an early eradication of HP-infection can slow down or even revert this cascade [11-13], suggesting that HP eradication has the potential to prevent GC [11]. In a recent study in patients with premalignant lesions, it was

shown that HP eradication may prevent their progression [101]. It is thought that a so-called 'point of no return' may exist in the histological cascade from chronic gastritis to GC after which eradication is unlikely to prevent GC [11-13,101]. It appears that once IM has become established, eradication, although retarding the progression of IM, cannot completely prevent GC [6,102]. This is not necessarily true for AG, however, for which there appears to be a discrepancy between the effect of eradication in the corpus and in the antrum. Thus, according to a recent meta-analysis of 12 studies comprising 2.658 patients, eradication of HP-infection results in significant improvement in AG of the corpus but not in the antrum, and, importantly, had no effect on IM [103].

Acetium® Capsule Effectively Eliminates Acetaldehyde in the Stomach

L-cysteine is a non-essential amino acid, which was shown (in 1975) to be capable of eliminating the toxicity of acetaldehyde by reacting covalently with it to form a stable 2-methylthiazolidine-4-carboxylic acid (MTCA) [27]. This binding results in inactivation of the reactive aldehyde group. MTCA is a stable and inert compound that is eliminated from the body mainly through feces. This principle was used in the recent innovation of Biohit Acetium® Capsule, which contains 100 mg L-cysteine. The novelty of this slow-release L-cysteine capsule (Acetium®) is based on the local elimination of carcinogenic acetaldehyde in the stomach.

Acetium® Capsule

The Acetium® Capsule contains 100 mg of L-cysteine as an active ingredient. L-cysteine is bound with matrix granules including Eudragit® RS-PO, Hypromellose (HPMC), calcium hydrogen phosphate (CaHPO4) and titanium dioxide. This special formulation causes L-cysteine to be released at a sustained rate, locally in the stomach. The use of a multi-particle system ensures that the formulation spreads to as large a part of the stomach as possible, even when the stomach contains solid or semi-solid content. Granules can also be assumed to remain longer in gastric mucosal folds, even in an upright position. The Acetium® capsule is a CE-marked product and the formulation is registered in many countries as a medical device (class IIa). This classification is based on the nature of the active ingredient, L-cysteine, as a natural amino acid and on the mode of action of this formulation [28-30].

After the proof-of-concept work at laboratory, the mode of action and efficacy of this novel Acetium® formulation has been conclusively documented in three carefully controlled clinical studies in Finland [28], Sweden [104], and Japan [98].

Elimination of acetaldehyde in acid-free stomach

In a placebo-controlled study, slow-release L-cysteine capsules effectively eliminated ethanol-derived acetaldehyde in the gastric juice of patients with AGC [28]. In this study, 7 volunteers with acid-free AGC were given either slow-release L-cysteine or placebo capsules in a double-blinded randomized trial. Volunteers served as their own controls. The volunteers ingested placebo or 200 mg of L-cysteine capsules, and ethanol 0.3 g/kg body weight (15 vol%), infused through a nasogastric tube. Five-milliliter samples of gastric contents were aspirated at 5-minute intervals [28]. During the follow-up period, the mean acetaldehyde level of gastric juice was 2.6-times higher with placebo than with L-cysteine (13 vs. 4.7 μM, $p < 0.05$, $n = 7$) [28]. These results implicate that Acetium® Capsule can be used to decrease acetaldehyde concentration in an acid-free stomach during alcohol exposure.

Elimination of acetaldehyde in patients with AG

In the second study, patients with AGC received 15% ethanol (0.3g/kg) through a nasogastric tube on two separate days, accompanied by randomized Acetium® Capsule (2 x 100mg) or placebo. Gastric juice samples were drawn every 20 min for 4 hours to measure pH, acetaldehyde-, ethanol-, L-cysteine- and MTCA concentrations [104]. After intra-gastric infusion of 15% ethanol with placebo, gastric juice acetaldehyde levels increased from zero to mean 36 μM (peak) and remained elevated up to 100 minutes [104] (Figure 2). Slow-release L-cysteine decreased gastric juice acetaldehyde by a mean of 68% (max. 87% at 60 min) and the decline persisted for up to two hours. Peak gastric juice L-cysteine level (8370 μM) was seen at 40 min and gastric juice L-cysteine remained elevated up to 160 min (220 μM at 160 minutes) [104]. Furthermore, the peak gastric juice MTCA level (233 μM) was seen at 80 min and gastric juice MTCA remained

elevated for up to 180 min (36 µM at 180 minutes) (Figure 3). The authors concluded that: a) an exposure of gastric mucosa to acetaldehyde is decreased by a mean of 68% ($p < 0.0001$) with slow-release L-cysteine capsule [104], and this acetaldehyde-eliminating effect continues for at least two hours; b) with L-cysteine, intra-gastric acetaldehyde is inactivated to inactive MTCA [27] that remains stable in the gastric juice for up to three hours [104].

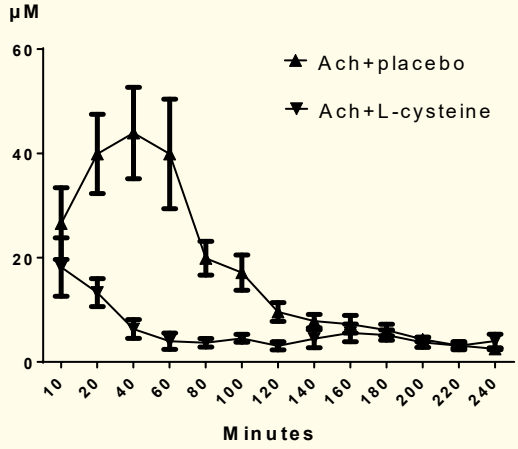

Figure 2: *The effect of slow-release L-cysteine (100mg x2) capsule on gastric juice acetaldehyde levels in patients with AG following intra-gastric infusion of 15% ethanol (0.3g/kg) (mean ± SEM).*

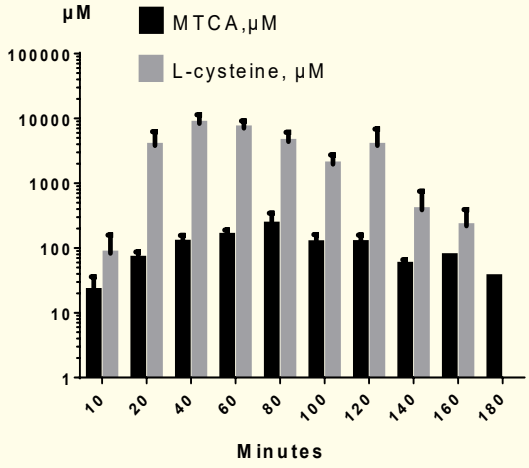

Figure 3: *L-cysteine and MTCA-concentrations in gastric juice of patients with AG following intra-gastric infusion of 15% ethanol (0.3g/kg) (mean ± SEM). At zero time point, the subjects received two Acetium Capsules® (2 x 100mg).*

Acetaldehyde elimination, ALDH2 mutation and PPI treatment

Yet another clinical trial confirmed that slow-release Acetium® Capsule (2 x 100 mg) reduced markedly the gastric juice acetaldehyde levels also in PPI-treated individuals with either active or deficient ALDH2 enzyme [98]. This study was conducted in Japan, showing that acetaldehyde eliminating capacity of slow-release L-cysteine was effective even after a moderate dose (0.5 g/kg, 3 doses) of alcohol and persisted for 2 hours. In ALDH2-active subjects, L-cysteine administration markedly reduced the gastric juice acetaldehyde concentration

from the mean peak value of 26.4 ± 3.7 μM (range 9.3 - 43.0 μM) at 30 min to a mean peak of 8.4 ± 3.7 μM (range 0.2 - 35.5 μM) at 30 minutes [98]. The acetaldehyde-eliminating effect of L-cysteine persisted almost the entire follow-up period of 120 minutes. Quantitatively, L-cysteine resulted in a mean of 67% (3-fold) decrease in gastric juice acetaldehyde (p = 0.001) (Figure 4a and 4b).

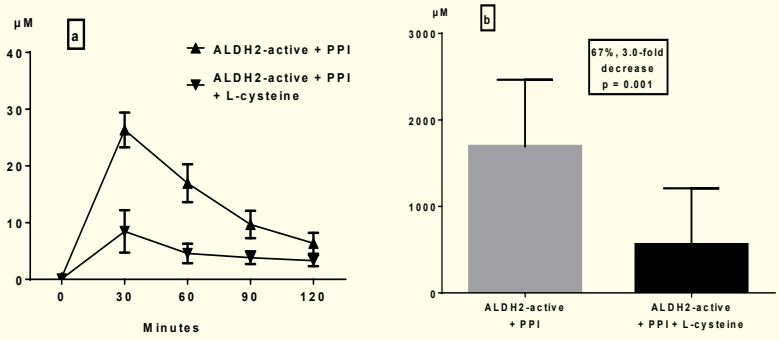

Figure 4: *The effect of Acetium Capsules® (2 x 100mg) on gastric juice acetaldehyde levels in PPI-treated ALDH2-active individuals (a), and areas under the curve (b) after intra-gastric infusion of ethanol (15 vol%, 0.5 g/kg) (means ± SEM).*

Similarly, L-cysteine greatly reduced the gastric juice acetaldehyde concentration in ALDH2-deficient subjects from a mean peak value of 63.9 ± 7.7 μM (range 32.0 - 96.7 μM) at 30 minutes without L-cysteine to a mean peak of 26.7 ± 8.1 μM (range 3.8 - 51.2 μM) at 30 minutes with L-cysteine [98]. Again, this effect persisted throughout the follow-up period of 120 minutes. In this study, L-cysteine resulted in a 60% (2.5-fold) reduction in the mean AUC of gastric juice acetaldehyde in ALDH2-deficient subjects (p = 0.0027) [98].

Conclusions

GastroPanel® innovation

GastroPanel® has been on the market for roughly 10 years by now. The test design was based on long-term natural history studies on gastritis patients run since the 1960's in Finland and Estonia [7,15,16,31,51,52,56,67,70-72,77,82-84] (http://www.biohithealthcare.com/additional-information). This examination is the first non-invasive diagnostic tool based on physiology of three 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 [11,12,73]. In the current test version (the Unified GastroPanel®), all 4 biomarkers are being processed under similar conditions. GastroPanel® 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 [22-26,50], 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®, the HP antibody measurement is complemented by the other three biomarkers (PGI, PGII, G-17) which are sensitive indicators of mucosal inflammation. This 4-marker panel makes GastroPanel® the most comprehensive HP test, devoid of the known shortcomings (false negative and false positive results) of the conventional HP tests [22-26]. 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) [34,48] also the other key risk factor of GC, i.e., atrophic gastritis.

Acetium® Capsule

Acetium® Capsule is a unique medical device designed to eliminate carcinogenic acetaldehyde in the stomach contents. A regular use of

this device is indicated for all those who have acid-free stomach, irrespective the cause of their achlorhydria. This formulation effectively protects the stomach against the exposure to acetaldehyde, classified as Group I carcinogen by IARC [20]. The most common high-risk groups include the following: 1) AG associated with HP infection; 2) AG caused by autoimmune mechanisms; 3) cigarette smokers; 4) alcohol consumers; 5) chronic users of PPI medication, and 6) those (500 million) people (mostly) in Asia who have a mutation of the ALDH2 enzyme, failing to metabolize acetaldehyde to acetic acid and thus exposed to higher local concentrations of this carcinogenic substance [18-20].

The efficacy of Acetium® Capsule in acetaldehyde elimination has been documented in three controlled clinical experiments [28,98,104]. In all studies, L-cysteine concentration in the gastric juice remained elevated for up to 3 hours, suggesting an insignificant absorption of L-cysteine or its transport into the small intestine. It was demonstrated that slow-release L-cysteine effectively (by 60 - 80%) eliminates acetaldehyde in patients with acid-free stomach caused by either AG or PPI treatment, both in individuals with active or deficient ALDH2 enzyme. This capacity of slow-release L-cysteine to eliminate acetaldehyde persisted for up to three hours after ingestion of two Acetium® Capsules [28,98,104]. L-cysteine bound with acetaldehyde locally in the stomach, forming a stable MTCA compound [27]. MTCA persisted in the gastric juice for up to three hours after the administration of alcohol and Acetium® Capsules, indicating a sustained effect [104].

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