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Serological Biomarker Test (GastroPanel®) in the Diagnosis of Functional Gastric Disorders, *Helicobacter pylori* and Atrophic Gastritis in Patients Examined for Dyspeptic Symptoms

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Abstract. *Background/Aim: The GastroPanel® test (Biohit Oyj) is interpreted by the GastroSoft® application distinguishing eight biomarker profiles, of which five profiles have a morphological equivalent in the Updated Sydney System (USS) classification of gastritis, and 3 others specify functional disorders of the stomach: 1) high acid output, 2) low acid output, and 3) effects of proton pump inhibitor (PPI) medication. This study evaluated the prevalence of these biomarker profiles in dyspeptic patients. Patients and Methods: A cross-sectional study was designed to assess the point prevalence of these biomarker profiles in a random sample of 500 subjects derived from our archives of GastroPanel® samples. Results: Reflux symptoms were reported by 35.2% and use of PPI medication by 36.8% of the study subjects. Biomarker profile 2 (high acid output) was the second most common GastroPanel® profile in this cohort; 31.2%, second only (33.6%) to profile 1 (healthy stomach). Hp-infection was detected in 25.0% of the subjects. Profiles related to use of PPI (low acid output, PPI effect) were found in 7.4% of the cases. AG was uncommon, diagnosed in 14 patients only (2.8%). Conclusion: These data are derived from the population with the highest frequency of dyspepsia, and the results might have widespread implications in diagnostic and screening practices.*

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To circumvent the shortcomings of the conventional *Helicobacter pylori* (Hp) tests (13urea breath test; UBT and stool antigen test; SAT) (1-15), as well as to reduce the extensive and (mostly) unnecessary use of invasive diagnostic tools (gastroscopy) in the clinical management of patients with dyspeptic symptoms (14, 15), a Finnish biotechnology company Biohit Oyj (Helsinki, Finland) designed a non-invasive serological biomarker assay, launched on the market in the early 2000's with the brand GastroPanel® (16, 17). The test is composed of enzyme-linked immunosorbent assays (ELISA) of three biomarkers: pepsinogen I (PGI), pepsinogen II (PGII) and gastrin-17 (G-17), combined with an ELISA assay of Hp (IgG) antibodies, all being measured from the same serum/plasma sample (16-20).

GastroPanel® uses specific monoclonal antibodies to detect stomach-specific biomarkers that regulate the normal stomach physiology. These biomarkers provide information on both the structure and function of the stomach mucosa (both antrum and corpus) (21-28), thus disclosing the major gastric pathologies and their topography, including the extent of atrophic gastritis (AG) and the activity of mucosal inflammation, *e.g.* due to Hp-infection (29-31). This marker panel is the only test that measures the state of gastric acid output (1, 17). The two prime indications of GastroPanel® use include: 1) non-invasive diagnosis of dyspeptic patients, and 2) screening of asymptomatic subjects for the latent risk conditions of gastric cancer (GC), *i.e.*, Hp-infection and AG (16, 17, 19, 20, 22-25). Because of its unique properties, GastroPanel® is also the most comprehensive Hp-test on the market, free from some of the shortcomings of the UBT and SAT tests (1-10, 14, 15, 19, 20).

Since its introduction, GastroPanel® has been extensively used in different clinical settings, including large-scale gastroscopy-referral studies (symptomatic patients), screening settings (asymptomatic subjects) as well as in longitudinal (prospective) studies and risk group analyses (18, 21, 32). The world literature accumulated until 2016-2017 was

subjected to two systematic reviews and meta-analyses (20, 33). In both of these meta-analyses, GastroPanel® test showed pooled sensitivity >70% and pooled specificity close to 95%, when moderate/severe AG was used as the endpoint (20, 33). The recently (in 2018) introduced the new-generation (Unified) GastroPanel® test, especially developed ELISA-automation in mind, recently passed the clinical validation studies (34, 35), confirming the outstanding clinical performance of the new test version; the indicators (sensitivity, specificity) even exceeded those calculated in the two meta-analyses (20, 33).

One of the features of the GastroPanel® test is in that the results can be interpreted by the GastroSoft® application (17, 19, 20, 36), classifying the biomarker profiles into five categories that have a morphological equivalent in the USS (Updated Sydney System) (37) classification of gastritis: i) normal mucosa, ii) non-atrophic (Hp) gastritis, iii) AGC, iv) AGA, and v) AG in both the antrum and corpus (14-17, 20, 21, 36, 37). In addition, GastroSoft® distinguishes three other biomarker profiles that specify functional disorders of the stomach: 1) high acid output, 2) low acid output, and 3) effects of PPI (proton pump inhibitor) medication (34-36).

Only the "morphological" biomarker profiles can be validated in settings where gastroscopy and biopsies are used as the reference test (20, 34-36), but because of the lack of any technically/clinically feasible reference test for the three "functional" biomarker profiles, a different approach needs to be used to cast light on these functional profiles of the GastroPanel® test. The present study is the first population-based survey reporting the point prevalence of the eight biomarker profiles in a random sample of patients examined by this non-invasive serological assay due to dyspeptic symptoms.

Patients and Methods

Study setting. The present study exploited the unique test material accumulated in the archives of the service laboratory of Biohit Oyj (Helsinki, Finland), to assess the point prevalence of all GastroPanel® biomarker profiles in a random sample of patients. The design is a cross-sectional study, where the study material is derived from the serum and plasma samples archived (frozen) in our service laboratory, accumulated during the whole period when GastroPanel® test has been offered to the national health care in Finland. Out of >10,000 samples, only those patients (around 2,000) who were tested using the new-generation (Unified) GastroPanel® test since 2016 were used as the targets for case selection. Furthermore, only those patients (around 1,000) who had given their written consent of using their GastroPanel® results for research purposes (signed on the original test referral form), were eligible for inclusion in the present study. A random number generator was used to derive a random sample of 500 patients, whose test results were then selected from the archived data base of GastroSoft® reports for detailed analysis.

New-Generation GastroPanel® test. The details on the GastroPanel® test, including its technical details and performance have been detailed in a series of recent reviews (14-17, 20, 21, 36). The latest test version, *i.e.*, the new-generation (unified) GastroPanel® test contains the same four biomarkers as the original test version (16-25), maintaining its basic design as an ELISA test. This new test version successfully passed two clinical validation studies recently (34, 35). It should be re-iterated, that the present study exclusively includes the patients who were tested using this latest version of the GastroPanel® test. The patient preparation, sample storage and processing for the new test are basically similar as described lately (34, 36), making redundant to repeat the details here.

GastroSoft® application. GastroPanel® results are interpreted by the GastroSoft® application, and a correct interpretation has two important prerequisites: i) accurate sample processing, storage and delivery to the laboratory (14-17, 20, 21, 34, 35), and ii) precise recording of the pertinent clinical information on the test request form (36). The latter is essential because these data are assimilated with the biomarker values by the diagnostic algorithm of the GastroSoft® application, to generate the correct diagnostic report. The final printed GastroSoft® report includes the patients age and gender, clinical information, written consent of the patient to use the results for research purposes, the values (and reference range) of the four biomarkers, as well as interpretation of the result (14, 15, 17, 36). The clinical information recorded in the request form includes the following specific inquiry: 1) Has an Hp-infection been eradicated or not? If yes, was the eradication completed less or more than a year ago? 2) Use of PPI medication: None, occasionally, frequently? If the latter, how many days before GastroPanel® sampling did you discontinue using PPI? 3) Do you have reflux symptoms due to high acid output? (no, frequent); and 4) Use of non-steroidal anti-inflammatory drugs (NSAIDs) medication? (not, frequently) (36). With all this information accurately recorded, GastroSoft® is capable of providing interpretation of these aforementioned diagnostic profiles.

GastroPanel® Biomarker profiles. The biomarker profiles and their diagnostic equivalents have been detailed in a recent comprehensive review (36), which the reader is being referred to for additional details. The biomarker profiles distinguished in the present study are summarized in Table I.

Profile 1: Normal biomarker levels. With all four biomarkers within the normal reference range, the gastric mucosa functions normally and the mucosal structure is normal (14, 15, 17, 36). The function of the gastric mucosa is dependent on the presence or absence of specific cells responsible for acid output (parietal cells), and for output of pepsinogens (chief cells) or G-17 (G-cells), and a normal gastric function necessitates the presence of these cells in normal quantities (36). Thus, stomach function and mucosal structure go hand-in-hand, and by definition, a normal GastroPanel® biomarker profile is a surrogate of a healthy stomach.

Profile 2: 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 output of G-17 from antral G-cells as a result of a positive feedback loop stimulating acid secretion after a meal (18, 22, 23, 36). Acid output results in a progressively lower pH in the stomach, and the threshold of pH 2.5 triggers a negative feedback to antral G-cells, signaling them to down-regulate their G-17 secretion. In the GastroPanel® test, low G-17 as a single aberration is a sign of increased acid output.

Profile 3: Low acid output (non-atrophy related). When acid output in the corpus is reduced (for any reasons), the positive

Table I. *The GastroPanel® biomarker profiles and their diagnostic equivalents.*

	GastroPanel® Biomarker Levels[1]						Diagnostic equivalent
Marker profile	PI (30-160 µg/l)[a]	PII (3-15 µg/l)	PGI/PGII ratio (3-20)	G-17b (1-7 pmol/l)	G-17s (3-30 pmol/l)	Hp IgG ELISA titer (<30 EIU)	
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.
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	H	Active Hp-infection, not treated
4b	N	N	N	N	ND	N or H[†]	Hp-infection successfully eradicated
4c	N	H	N	H	ND	H	Hp eradication failed
5	L	L	L	H	ND	N^^ or H	Atrophic gastritis in corpus and fundus (AGC)
6	N	N	N	L	L	H	Atrophic gastritis in antrum (AGA)
7	L	L	L	L	L	N^^ or H	Atrophic gastritis in both antrum and corpus (AGpan)
8	H	H	N	H	ND	N	Short (4- to 10-day) pause in continuous PPI treatment. Rebound in gastric acid output.

[1]Values in parenthesis indicate the normal reference range; N: Normal; L: low; H: high; Hp: *Helicobacter pylori*; ND: no need for testing; PG: pepsinogen; PPI: proton pump inhibitor. *Test PPI medication for 2 weeks. G-17b should normalize. **Stop medication. G-17b should normalize within 2 weeks; ^Can be elevated due to mucosal inflammation; ^^can disappear in mucosal atrophy with protracted clinical course. [a]PGI cut-off value 30 µg/l is consonant with moderate/severe atrophic gastritis (AG); [†]*Helicobacter pylori* antibody levels can remain elevated for months after successful eradication.

feedback loop triggers antral G-cells to increase their G-17b secretion, resulting in an elevated blood level of G-17b (17, 21, 22, 36). The two prime conditions leading to low acid output are AG of the corpus, and long-term use of PPI medication. The former is excluded by normal values of PGI, PGII, and PGI/PGII ratio (36), while the latter is best diagnosed by discontinuing PPI medication. In this case, G-17b should be normalized within 2 weeks (17, 22-25, 36).

Profile 4: Non-atrophic Hp-associated gastritis. Like bacteria in general, also Hp induces an acute inflammation in the gastric mucosa, usually stating from the antrum (36). With GastroPanel®, three distinct biomarker profiles are representative of Hp-infection (Table I).

Profile 4a: Active Hp-infection. In an active Hp-infection, Hp IgG antibody level is raised above the cut-off value (30 EIU), which may be the only abnormal finding in the biomarker profile. Not infrequently, however, an active Hp-infection causes a severe inflammatory reaction which, due to increased cell permeability, can lead to increased leakage of PGI, PGII and G-17 from the secretory cells; another characteristic feature of Profile 4a (16, 29, 36).

Profile 4b: Successful Hp eradication. Successful Hp eradication results in normalized values of the three ("inflammatory") markers (PGI, PGII, G-17), usually with a delay of some weeks (17, 37). This makes the distinction to Profile 4a. However, Hp IgG antibody levels remain elevated for several months, subject to individual variation (28-33).

Profile 4c: Failed Hp eradication. Whenever Hp eradication attempt fails, i) Hp IgG antibody levels remain elevated (usually slightly), ii) PGI and the PGI/PGII ratio usually fall within the normal range, whereas iii) PGII and/or G-17b may remain slightly elevated as a sign of an ongoing inflammatory process (35, 36). If this profile persists for months, a new treatment attempt is indicated.

Profile 5: Atrophic corpus gastritis (AGC). Loss of chief cells in the corpus along with the loss of parietal cells as a result of mucosal atrophy, results in a progressively reduced production of PGI and (to a lesser extent) PGII, which is also produced by glandular cells in the antral mucosa (17, 21-25). The unbalanced reduction of PGI and PGII results in a lower PGI/PGII ratio, which is another excellent signature of AGC (36). The degree of this reduction in PGI and PGI/PGII ratio closely correlates with the severity of AGC (34, 36). In the case of a normal antral mucosa, AGC leads to a markedly increased serum level of G-17b, which is another typical feature of Profile 5 (Table I).

Profile 6: Atrophic antrum gastritis (AGA). By definition, AGA is caused by Hp-infection, and Hp antibodies are invariably elevated. The G-cells are reduced in number and finally disappear, leading to progressively reduced plasma levels of G-17b in AGA. In severe AGA with missing G-cells, there is no response in G-17 output to protein stimulation (G-17s) (17, 36). 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 made by using G-17s testing with protein stimulation (17, 21-25, 36). G-17s will increase normally only in the former, but fails to increase in severe AGA.

Profile 7: Atrophic gastritis of the antrum and corpus (AGpan). The most severe form of AG is affecting both the antrum and corpus (23, 37). In severe AGpan, the chief cells in the corpus and G-cells in the antrum disappear, leading to the unique biomarker profile, with PGI, PGII and G-17 substantially reduced (Table I) (36). This applies to both G-17b and G-17s, which remain low even after protein stimulation. If Hp has disappeared during the protracted clinical course of AGpan, Hp IgG levels can be within normal range (35, 36).

Profile 8: Panel profiles due to PPI effects. Any gastric acid-suppressive medication will inevitably interfere with the profile of the GastroPanel® markers. Because of this, it is recommended that the patient discontinues any acid-suppressive treatment 7-10 days before sampling (17, 36). This withdrawal of PPI or H2-blocker medication is not always possible because of intractable symptoms, and to cope with this, the latest version of GastroSoft® was designed to take into account also the continued use of PPI medication (Profile 8).

PPI and H2-blockers effectively reduce gastric acid production by parietal cells (17, 22, 36). This increases the output of G-17b, which stimulates the output of PG I and PGII. Once the PPI/H2-blocker treatment is discontinued, it takes 4-10 days for acid output and G-17b levels to normalize. However, PGs respond more slowly, and PGI and PGII levels may remain above the cut-off values for up to 2-3 weeks (Table I) (17, 34, 36). An abrupt termination of long-term PPI medication is typically followed by rebound acid hypersecretion, frequently accompanied by symptoms of hyper-acidity and extremely low levels of G-17b (36).

Statistical analysis. The archived GastroPanel® test results were first classified with the help of GastroSoft®, transferred to an Excel file and converted to SPSS for final statistical analysis. All statistical analyses were performed using the SPSS 27.0.1.0 for Windows (IBM, NY, USA) software. The descriptive and analytical statistics were performed according to routine procedures. All tests were two-sided and interpreted significant at the level of $p<0.05$.

Results

The key characteristics of the study subjects are summarized in Table II. Of the 500 randomly selected patients examined by the Unified GastroPanel® test in the laboratory of Biohit Oyj during 2016-2020, 353 (70.6%) were women and 147 (29.4%) were men. The mean age of the patients was 59.9 years (SD±14.8 years), and the median age was 63 years (range=14-88 years). No age difference was detected between the genders ($p=0.862$).

According to the registered clinical information, 389 patients (77.8%) did not report Hp eradication, 10 did not remember or did not know this, while 3 patients had undergone eradication less than 1 year ago and 98 did so more than a year ago. Altogether, 314 patients reported no use of PPI, 2 patients did not know, while the rest 184 reported regular, irregular or occasional use. Reflux complaints [gastroesophageal reflux disease (GERD)] were reported by 176 patients as continuous symptoms, while 320 subjects did not experience GERD symptoms. Use of non-steroid anti-inflammatory drugs (NSAIDS) was uncommon, reported only by 9.4% of the subjects. Altogether, 214 (42.8%) confirmed having undergone gastroscopy.

As to the registered clinical information stratified by gender (data not shown), there was no difference in the proportion of subjects who had experienced Hp eradication ($p=0.257$, Fisher's exact test). Similarly, the use of PPI was equally frequent among males and females; 35.4% and 37.4%, respectively ($p=0.850$). GERD symptoms were slightly more frequently experienced by males (38.8%) than female patients (33.7%) ($p=0.311$). On the other hand, women reported slightly more frequently (10.5%) than men (6.8%) the use of NSAIDS medication regularly ($p=0.416$). Finally, women had undergone gastroscopy significantly more often than men (OR=1.54, 95%CI=1.016-2.348) ($p=0.044$); 56.6% *vs*. 45.8%, respectively.

Table II. *Characteristics of the 500 study subjects*.

Characteristics	Number	Percent
Gender		
Women	353	70.6
Men	147	29.4
Age (M±SD)	59.9±14.8	
Women	59.9±14.5	$p=0.862$
Men	59.7±15.7	
Hp eradication		
Not known	10	2.0
No	389	77.8
<1 year ago	3	0.6
>1 year ago	98	19.6
PPI medication		
Not known	2	0.4
No	314	62.8
Yes (regular or irregular)	184	36.8
Reflux symptoms		
Not known	4	0.8
No	320	64.0
Regularly	176	35.2
Use of NSAIDS		
Not known	3	0.6
No	450	90.0
Regularly	47	9.4
Ever done gastroscopy		
Not remember	5	1.0
No	189	37.8
Yes	214	42.8
Data missing	92	18.4

NSAIDS: Non-steroid anti-inflammatory drugs.

The point prevalence of the 8 biomarker profiles in this random sample of 500 patients is summarized in Table III. The two most frequent profiles were profile 1 and 2, in that order. These two were followed by profile 4b (Hp successfully eradicated) and profile 4a (active Hp-infection). In this sample, the profiles specifying AG were rare; AGC (2.6%), AGA (0%) and AGpan (0.2%). Taken together, 31.2% of these randomly selected 500 subjects presented with high acid output (profile 2) in their GastroPanel® test.

Table IV depicts the 8 biomarker profiles stratified by gender. The overall distribution of the biomarker profiles was significantly different between women and men ($p=0.001$). Most importantly, normal profile was less frequent among males, mostly compensated by their higher

Table III. *The biomarker profiles among the 500 random patients examined with the Unified GastroPanel® test.*

Biomarker profile	Diagnostic equivalent	Number	Percent
1	Healthy mucosa (no atrophy, no Hp infection)	168	33.6
2	Healthy mucosa. High acid output	156	31.2
3	Healthy mucosa. Low acid output due to *e.g.* PPI medication	17	3.4
4a	Active Hp infection. Not treated	37	7.4
4b	Hp-infection successfully eradicated	82	16.4
4c	Hp eradication failed	6	1.2
5	Atrophic gastritis in the corpus and fundus (AGC)	13	2.6
6	Atrophic gastritis in the antrum (AGA)	0	0.0
7	Atrophic gastritis in both the antrum and corpus (AGpan)	1	0.2
8	Short (4- to 10-day) break in continuous PPI treatment. Rebound in gastric acid output	20	4.0
Total sample		500	100.0

frequency of profile 2 (high acid output); 44.9% and 25.5%, respectively. Differences between the genders was less dramatic as to the other biomarker profiles.

As to the mean age of the subjects with different biomarker profiles, the overall difference was highly significant (p=0.0001; Figure 1). Not unexpectedly, the mean age increased along with the abnormal biomarker profiles in that the highest mean age was encountered among the subjects with a biomarker profile consistent with AG (AGC and AGpan).

Table IV. *GastroPanel® biomarker profiles stratified by gender.*

Profile	Women		Men	
	No.	Percent	No.	Percent
1	132	37.4	36	24.5
2	90	25.5	66	44.9
3	16	4.5	2	1.4
4a	24	6.8	13	8.8
4b	64	18.1	18	12.2
4c	5	1.4	1	0.7
5	8	2.3	5	3.4
6	0	0.0	0	0.0
7	1	0.3	0	0.0
8	13	3.7	6	4.1
Total	353	100.0	147	100.0

p=0.001; Fisher's exact test

Discussion

The recently introduced new-generation (Unified) GastroPanel® test recently passed the clinical validation studies (34, 35). Because examined in different clinical settings (high-risk groups and gastroscopy-referral patients, this validation was performed in two separate studies; one focused on the assessment of the performance of PGI, PGI/PGII ratio and G-17 in the diagnosis of AGC and AGA, respectively (34), and the other one evaluated the accuracy of Hp IgG ELISA in the detection of biopsy-confirmed Hp-infection (35). Common to both these studies is that only the five biomarker profiles of the GastroPanel® with a morphological equivalent in the USS classification (37) could be evaluated. In both studies (34, 35), gastroscopy and biopsies were used as the reference test (=gold standard), with moderate/severe AG (AG2+) as the endpoint, following the manufacturer's recommendations (14-25, 36). Both these clinical validation studies confirmed the outstanding clinical performance of the Unified GastroPanel® test, the performance indicators (sensitivity, specificity) far exceeding (34, 35) those reported by the two meta-analyses based on the published world literature (20, 33).

Unfortunately, a similar approach (gastroscopic biopsy as the reference test), as used in the validation of the five biomarker profiles with morphological equivalent in the USS (34, 35, 37), is not possible for validation of the three biomarker profiles that specify the functional disturbances of the stomach; profiles 2, 3 and 8 (Table I). This is because of the lack of any technically/clinically feasible reference test to confirm high- (profile 2) or low acid output (profile 3), or PPI effects (profile 8). As repeatedly emphasized, these profiles are based on firmly established physiological principles of the acid output in the stomach (17-25, 36), and as such, do not necessitate any "validation" in the usual strict sense of this concept. At conceptual level, however, it is of interest to estimate the prevalence of these different functional disorders of the stomach in a general population, because such information is extremely difficult to obtain. This would necessitate a cross-sectional population-based cohort to be analysed by methods that measure the level of stomach acid output at any time point. In practice, however, any invasive technique based on collection of gastric juice is not suitable for a systematic analysis of any sizeable cohort, thus a non-invasive test is the only feasible option. Based on its unique capabilities as a non-

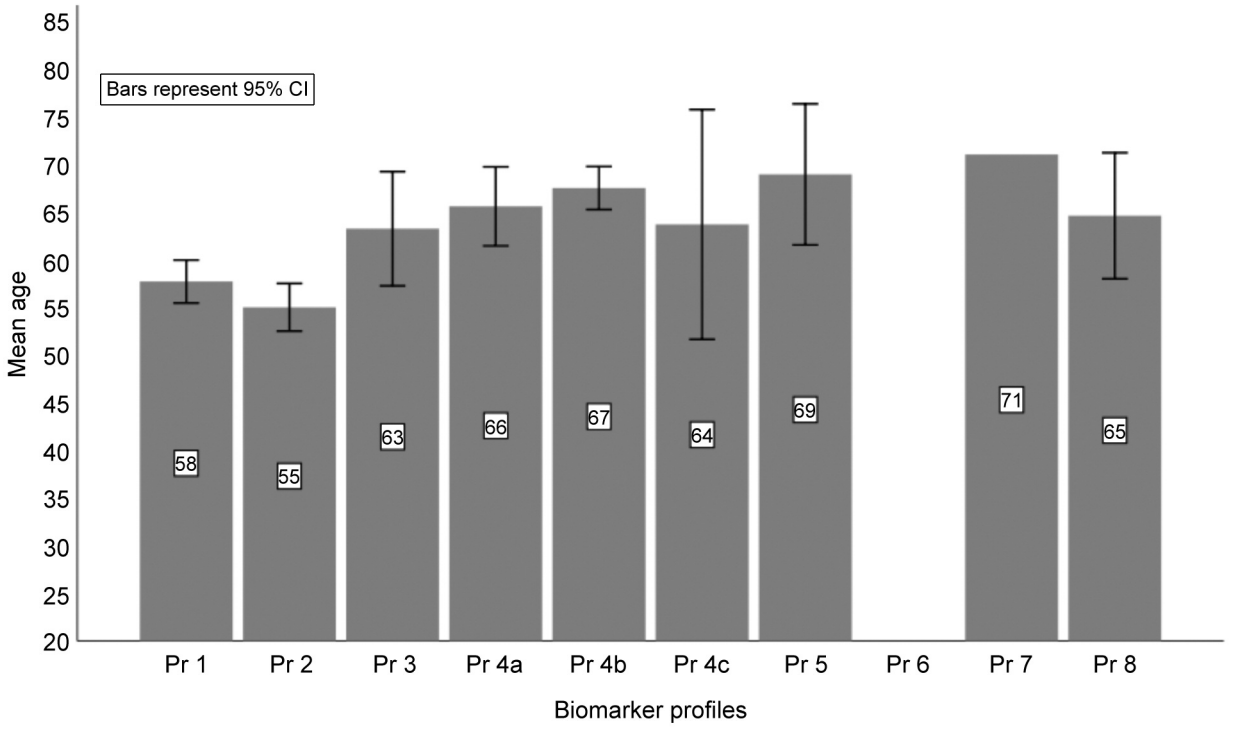

Figure 1. *Mean age of the patients with different biomarker profiles.*

invasive serological biomarker assay, GastroPanel® is an ideal test to provide this type of information, *i.e.*, the population prevalence of functional gastric disorders. In fact, GastroPanel® is the only test capable of analysing high acid output, low acid output and PPI effects at a population level.

The present cross-sectional study is the first population-based study on the point prevalence of these distinct gastric disorders diagnosed by this non-invasive serological assay. Given that the test samples were continuously submitted to the service laboratory of Biohit Oyj (Helsinki, Finland) by different health care providers from all over the country, these samples are highly representative of the Finnish population. "Representative" in this sense must be interpreted to include symptomatic people who a) are being examined for abdominal complaints (dyspepsia) within the primary health care, and b) asymptomatic subjects examined as a part of their annual health check-ups within the occupational health care sector, as well as c) those enlightened asymptomatic subjects who seek for GastroPanel® examination to rule out the occult risk conditions of gastric cancer (GC)(AG, Hp). Accordingly, the present cohort of 500 patients was derived from a population, which is the most common target of these gastric disorders, thus giving a representative view of these disorders in the population with a similar age coverage (mean age: 59 years, median age: 63 years). This "population-derived" GastroPanel® test material clearly does not include pediatric patients and young adults (only few cases), among whom the indications for this test are rare if non-existent (Table II; age distribution).

The results of this cross-sectional study unveil a representative view on the point prevalence of these distinct gastric disorders in our country. These data are derived from dual sources: i) the subject-reported clinical information (on the test referral form) (Table II) and ii) results of the stomach-specific biomarker analysis by GastroPanel®/GastroSoft® combination (Table III). In this cohort, 20.2% (n=101) of the patients had undergone Hp eradication; 19.6% more than a year ago and 0.6% less than a year ago. In addition, 2% (n=10) of the responders did not know or remember whether they had undergone Hp eradication. The vast majority (77.8%), however, clearly had not experienced Hp eradication. When interpreted with caution, these data suggest that over 20% have been exposed to Hp-infection in this population. This estimate closely coincides with the results of the biomarker profile analysis, where 88 (17.6%) subjects presented with a profile indicating successful or failed Hp eradication, while ongoing Hp-infection was detected in 7.4% only (Table III). With this point prevalence of Hp-infection, Finland clearly belongs among the low Hp-prevalence countries (35, 38).

More alarming than these sensible Hp prevalence rates is the high proportion of the study subjects reporting the use of PPI medication; 36.8% using regularly or irregularly (Table II). A few (0.4%) of the responders were uncertain or unaware

of their PPI use. These numbers closely coincide with the reported prevalence of GERD symptoms; 35.2% of all patients having these symptoms regularly. Of those who reported reflux symptoms, 56.8% were PPI users, compared with 26.3% of those with no reflux (OR=3.68, 95%CI=2.49-5.42) (p=0.0001). There is a close match with the reported frequency of a previous gastroscopy (42.8%) (Table II), being an indication that gastroscopy is still frequently used in this country as the primary diagnostic tool for patients complaining of reflux symptoms. Another conclusion from these data suggests that, the subjects who undergo gastroscopy (42.8%), are more likely to receive PPI medication (43.5%) (OR=2.13, 95%CI=1.40-3.25; p=0.0001).

With these figures, one would expect to see at least the same proportion of the subjects presenting with a biomarker profile indicating high acid output in the GastroPanel® test. Indeed, this was exactly the case in the present study, where profile 2 (high acid output) was encountered in 31.2% of the subjects (Table III). Given that gastroscopy is an invasive diagnostic tool, failing to detect any abnormality in patients with high acid output as the only aberration, these data also clearly confirm that gastroscopy for 31.2% of the subjects was unnecessary, as pointed out repeatedly before (17-25, 36).

Given that the GastroPanel® test can be used in risk stratification at three levels: i) healthy mucosa, ii) Hp-infection, and iii) AG, the rational conclusion is that gastroscopy is only indicated for patients whose GastroPanel® detects atrophic gastritis (17, 36). This indication is absolute to confirm the severity of AG and to exclude malignancy/cancer precursor (18-22, 36, 37). Hp-infection without concomitant AG is not an indication for gastroscopy, but appropriate eradication therapy (with control) should be instituted first (1-16, 35, 36). Another, albeit relative, indication for gastroscopy is a long-term symptomatic high acid output, to exclude the potential esophageal complications (*e.g.* Barrett's esophagus, erosive esophagitis) (17-25, 38, 39). However, weighting all this information as a whole (*i.e.*, reported Hp prevalence, reflux symptoms, PPI-medication, gastroscopy, detected AG), one cannot exclude the possibility that in this particular patient sample (covering the age groups with the highest prevalence of dyspeptic symptoms), most of the above listed indications for gastroscopy have been fulfilled for each individual patient.

As to the point prevalence of the individual biomarker profiles (Table III), the most prevalent profile seems to be profile 1 (healthy stomach). This dual profile, reflecting both i) the structure (no abnormality in the USS) and ii) function (no disorder in acid output) of the stomach, notifies the clinician that the stomach is healthy. The fact that this normal profile was seen in 33.6% of these subjects who were referred for GastroPanel® test with different indications (mostly dyspepsia; Table II), further emphasizes that the correct diagnosis of dyspepsia cannot be made on the basis of symptoms alone (14-24, 34-36). This also makes clear that among these subjects with a normal function and structure of the stomach, invasive gastroscopy does not provide any added diagnostic value and would be unnecessary also for 33.6% of the patients.

Of interest are also the profiles related to Hp-infection (Table III). Of these 500 subjects, 7.4% had an ongoing Hp-infection, obviously diagnosed for the first time. In 16.4%, Hp had been eradicated successfully, while in a small minority (1.2%), Hp eradication had evidently failed. These numbers add to 25.0% of all the study subjects, which is in alignment with anamnestic data reported on Hp eradication by the patients before GastroPanel® testing (Table II). At large, one can conclude that i) Finland belongs among the low-prevalence countries as regards to Hp-infection, and ii) failure of Hp eradication is quite rare in this country (35, 40).

It is well known that the prevalence of AG increases in parallel with advanced age, its prevalence exceeding 10% among 70-year-old people also in this country (22, 23, 41). In the present population sample with a median age of 63 years, AG was disclosed in 2.8% of the subjects only (Table III). As expected, AG was confined to older age groups (Figure 1). This AG prevalence in the general population sharply contrasts with AG prevalence in distinct high-risk groups, *e.g.*, those with other autoimmune diseases (AITD, DM1), recently analysed by GastroPanel® test (34). However, AG is associated with a wide variety of potentially serious clinical complications (14, 15, 25, 36), and even these low numbers (n=14) of detected AG cases among these 500 subjects, are not negligible (22, 23, 41). When translated to the population level, this would signify that a minimum of 100,000 people bear AG (mostly asymptomatic) in this country. This clearly substantiates a systematic screening (42) of certain age groups of asymptomatic subjects by GastroPanel® to disclose subjects with AG and at increased risk for GC and other clinical sequels (17, 36). Given that AG seems to affect equally both women and men (Table IV), this systematic screening would be similarly justified to both genders (42).

Conclusion

Taken together, this cross-sectional study based on a random sample of 500 subjects referred for GastroPanel® examination, is the first population-based study on the point prevalence of distinct gastric disorders diagnosed by this non-invasive serological assay. While including the pertinent clinical information recorded from the study subjects, these data give valuable information on the prevalence of their key symptoms, the practices of using medications for these symptoms (PPI, Hp eradication), as well as the diagnostic procedures (gastroscopy).

As to the GastroPanel® biomarker profiles, those with a morphological equivalent in the USS system (37) have been extensively validated in studies where test results have been verified by gastroscopy and biopsies (20, 33-35). In the lack of a suitable reference test for those biomarker profiles that

specify a functional disorder, however, this is the first study reporting the point prevalence of i) high acid output (profile 2), ii) low acid output (profile 3), as well as iii) the effect of PPI medication (profile 8). These profiles closely correlate with the results of invasive methods used for measurement of gastric acid output (40). Recent evidence suggests that high acid output (low G-17) is associated with an increased risk of GERD and Barrett's esophagus (38, 39). Given that the present data are derived from the population with the highest frequency of dyspepsia, these results show the possible widespread clinical implications of GastroPanel® testing in the diagnostic and screening practices at the population level (43).

Conflicts of Interest

The Authors are current or retired employees of Biohit Oyj, the manufacturer of the GastroPanel test.

Authors' Contributions

LP (Lea Paloheimo) as retired Director of R &D, had the overall responsibility for organizing the service laboratory activities, on which the current test sample was derived. She has actively participated in study design, and revision of the manuscript. TT (Tapani Tiusanen), as the technical director is the person-in-charge of the technical aspects of the GastroPanel diagnostics in the laboratory, including the maintenance of the cloud services. Actively participated in the study design and personally performed the random sampling of the patients. OS (Osmo Suovaniemi), Chairman of the Board of Directors, has the overall responsibility of the company. As an MD, he has provided valuable insights in the study design and its execution, as well as insightful comments on the manuscript. KS (Kari Syrjänen) as retired Chief Medical Director, contributed to study design, analysed the data and wrote the first draft of the manuscript.

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