

Screening of the Patients with Autoimmune Thyroid Disease (AITD) and Type 1 Diabetes Mellitus (DM1) for Atrophic Gastritis (AG) by Serological Biomarker Testing (GastroPanel®)

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Abstract

Background: The high prevalence (10 - 30%) of autoimmune atrophic gastritis (AAG/AG) in patients with autoimmune thyroid disease (AITD) or type 1 diabetes mellitus (DM1) advocates a screening of these patients by gastroscopy. An alternative to invasive gastroscopy is offered by a non-invasive serological biomarker test; GastroPanel® (Biohit Oyj, Helsinki, Finland).

Objective: To establish the prevalence of AAG/AG in AITD- and DM1 patients by the new-generation GastroPanel® test in a clinical validation study.

Material and Methods: A cohort of 244 patients (207 with AITD and 37 with DM1) was enrolled at the Outpatient Department of Internal Medicine, Oulu University Hospital (OUH, Oulu, Finland). A written consent was obtained from all patients, and the study was approved by the joint Ethical Committee of OUH/Oulu University (Dno 28/2017). All patients underwent GastroPanel® examination and those with AG/AAG biomarker profile were referred for gastroscopy and biopsy confirmation (reference test).

Results: Of the 244 patients, biopsy-confirmed AG was found in 12.0% (25/207) of the AITD- and in 10.8% (4/37) of the DM1 patients. The adjusted overall agreement between GastroPanel® and the USS classification was 73.3% (95%CI 60.4 - 86.2%), and the adjusted weighted kappa: $\kappa_w = 0.881$ (95%CI 0.783 - 0.935). In ROC analysis for AGC2+ (moderate/severe AGC) endpoint, the ROC curves have AUC = 0.939 (95%CI 0.870 - 1.000) and AUC = 0.945 (95%CI 0.879 - 1.000) for PGI and PGI/PGII, respectively.

Conclusion: AG/AAG is far more prevalent among the AITD- and DM1 patients as compared with general population. The new generation GastroPanel® is an ideal test for non-invasive screening of these patients for early diagnosis of AG/AAG.

Keywords: Autoimmune Thyroid Disease (AITD); Type 1 Diabetes Mellitus (DM1); Autoimmune Atrophic Gastritis (AAG); Atrophic Gastritis (AG); Serological Biomarker Panel; GastroPanel; Non-Invasive; *Helicobacter pylori*; Pepsinogen I; Pepsinogen II; Gastrin-17; Pernicious Anemia; Parietal Cell Antibody

Introduction

Autoimmune atrophic gastritis (AAG) and pernicious anemia (PA) are common autoimmune diseases with respective prevalence of 2% and 0.15 - 1% in the general population [1-3]. In patients with autoimmune thyroid disease (AITD) [4,5] and those with type 1 diabetes (DM1) [6,7] this prevalence is 3- to 5-fold increased. AAG is characterized by the presence of circulating autoantibodies to parietal cells (PCA) and their secretory product, intrinsic factor (IF) [8-11]. In up to 10% of these patients, AAG may predispose to gastric neuroendocrine tumors or gastric cancer (GC) [12-18].

In DM1 patients, PCAs are found in 10 - 15% of the children and in 15 - 25% of the adults [4,5,19-21], with the respective prevalence of AAG and PA of 5 - 10% and 2.6 - 4% [2,3,7,22-26]. In patients with AITD, AAG is detected in up to one third of the patients [27-31], being associated with iron deficiency anemia in 20 - 40% [10,32] and PA in 15 - 25% [33,34]. Finally, gastric neuroendocrine tumors are observed in 4 - 9% of the patients with AAG/PA, which is 13-times more frequent than in controls [12,35-37]. Patients with AAG/PA also have a 3- to 6-fold risk of GC, ranging from 0.9 - 9% [12,35,37-40]. Another important causative agent of AG is *Helicobacter pylori* (HP)-infection, suspected to be a trigger of AAG as well [41-44].

AG/AAG is the single most powerful independent risk factor for non-cardia GC [45-48]. It is estimated that 50% of all GCs develop through the "Correa cascade" [46,49-53], leading from HP-infection or autoimmune-type gastritis to mucosal atrophy (AG), intestinal metaplasia (IM), dysplasia, and invasive GC. This process takes decades, leaving ample of time for early detection of the cancer precursor lesions [54], but the problem has been the lack of a suitable screening test [55,56].

It is under debate whether the AITD and DM1 patients with AAG/PA should be screened by repeated gastroscopies and biopsies [7]. In many clinics, endoscopy with biopsies remains the gold standard diagnostic tool for this purpose [45,57]. However, this invasive method is uncomfortable, distressing and costly, emphasizing the need for inexpensive non-invasive diagnostic tests [55,56,58]. Such non-invasive methods have been available since the 1980's, when Miki, *et al.* [59] and Samloff, *et al.* [60] introduced assays measuring pepsinogen (PG) concentrations in the blood. The latest development in this field represents a biomarker panel combining serum pepsinogen I (PGI) and II (PGII), gastrin-17 (G-17) and HP IgG antibodies (IgG-HP), known as GastroPanel® test (Biohit Oyj, Helsinki, Finland). This biomarker panel is designed as the first-line diagnostic test for dyspeptic patients [61-63], as well as for screening of the risk conditions (HP, AG) of GC [64,65].

Disclosing AAG/AG as early as possible in DM1 and AITD patients is important to prompt appropriate surveillance measures to prevent the serious clinical sequels of AAG/AG [7,12,35,37-48]. The present study was designed to establish the true prevalence of AAG/AG in AITD- and DM1 patients by targeted screening with the new generation GastroPanel® test providing information on the functional state of the gastric mucosa as well as on the type of chronic gastritis [61-63]. The design was exploited as the clinical validation study of this new-generation GastroPanel® test, a technically advanced test version with uniform processing conditions for all four biomarkers.

Materials and Methods

Patients

The patients were enrolled at Oulu University Hospital (OUH) among the consecutive AITD and DM1 patients attending their regular monitoring at the outpatient department of Internal Medicine. Patient enrollment took place in a single step. The potentially eligible patients were identified among the DM1 and AITD outpatients, and every patient was asked to participate in the study by signing a written consent. All enrolled patients were interviewed using previously validated questionnaires [66]. Eligible were considered all AITD and DM1 patients above 18 years of age, with or without upper abdominal symptoms. The following patients were considered non-eligible: 1) the patients whose treatment required surgery, or immediate follow-up treatment for major symptoms, as well as 2) those who refused to consent. The study protocol followed the Declaration of Helsinki and was approved by the joint Ethical Committee of Oulu University and Oulu University Hospital (DNo 028/2017).

During the study, a cohort of 244 eligible patients were enrolled and completed the study. The key characteristics of the patients are summarized in table 1. Of the 244 patients, 37 had DM1 and 207 had AITD. The mean age of the patients was 55.4 years (SD 13.4 years).

Variable	Diabetes Mellitus type I (DM1)	Autoimmune thyroiditis (AITD)	Total Series
	No. (Per Cent)	No. (Per Cent)	No. (Per cent)
No. Patients	37 (15.2)	207 (84.8)	244 (100.0)
Age (M ± SD)	50.8 (14.9) years*	56.3 (12.9) years*	55.4 (13.4) years
Gender			
Women	22 (59.5**)	194 (93.7**)	216 (88.5)
Men	15 (40.5)	13 (6.3)	28 (11.5)

Table 1: Characterization of the patients included in the study cohort.

*Mean age in the two series; $p = 0.023$; **Gender distribution between DM1 and AITD; $p = 0.0001$.

Methods

Questionnaire of the symptoms

GI-symptom questionnaires on i) functional dyspepsia and IBS according to the Rome III criteria and on ii) reflux disease symptoms according to the Montreal classification were completed prior to blood sampling [66]. The detailed analysis of these questionnaires will be the subject of a separate report.

Patient preparation for GastroPanel® sampling

After consenting to participate, the patients were scheduled for an appointment to GastroPanel® testing at the hospital laboratory, with given instructions for preparatory measures. Apart from the recommended 10h-fasting, they were instructed to discontinue their eventual PPI-medication, preferably one week before GastroPanel® sampling. If not possible due to intractable dyspeptic symptoms, a notice of PPI use must be included in the GastroPanel® request form, including the information whether interrupted or not, and for how many days [67].

Sample collection and processing for GastroPanel® test

For correct interpretation of the test results by the GastroSoft® application (Biohit Oyj, Helsinki), it is essential to complete the GastroPanel® request form, as detailed before [67,68]. A minimum of 2 ml EDTA plasma from a fasting blood sample was taken into an EDTA tube. Because not used for on-site testing, the EDTA plasma samples were frozen instantly (-70°C). Using G-17 stabilizer (Biohit Oyj) (5% of the sample volume) enables a temporary storage in the refrigerator (at 2 - 8°C), for up to 3 days, but immediate freezing at -70°C was the preferred method of storage. This is most critical for G-17, to avoid decay at too high temperature [67].

Stimulated G-17 (G-17s)

Apart from the fasting sample for all 4 biomarkers, another blood sample was needed to measure the level of stimulated G-17s [65,67,68] collected 20 min after intake of a protein drink with average protein content of 77%.

GastroPanel® testing

The frozen plasma samples were delivered to the laboratory of Biohit Oyj (Helsinki) for GastroPanel® analysis, using the new test version and following the instructions for use (IFU) of the test kits [67,68].

Evaluation of GastroPanel® results

The results of the GastroPanel® test were evaluated using the GastroSoft® software application. As repeatedly emphasized [65,67,68], GastroPanel® test is optimized for the Updated Sydney System (USS) for classification of gastritis [69], both including 5 diagnostic categories: a) normal mucosa, b) HP-induced gastritis with no atrophy, c) atrophic gastritis of antrum (AGA), d) atrophic gastritis of corpus (AGC) and e) atrophic gastritis in both antrum and corpus (AGpan) [67-69].

Gastroscopy and biopsy procedures

As per the study design, only those patients who tested GastroPanel®-positive with the biomarker profile consistent with AG/AAG were referred for gastroscopy and biopsies. In addition, a small number of randomly selected test negatives were invited for gastroscopy to enable calculation of the GastroPanel® indicators, using the gastroscopic biopsies as the gold standard reference test.

Gastroscopy referrals were made to the outpatient department of Gastroenterology, OUH Gastro Center. Taking of gastric biopsies followed the USS system, with separate biopsies from the antrum and corpus [67-69]. On gastroscopy, all observed abnormal mucosal lesions were noted and photographed, and if necessary subjected to additional biopsy. Endoscopic findings were classified into one of the following categories: 1) normal; 2) inflammation; 3) suspected atrophy; 4) definite atrophy; 5) ulcer; and 6) other abnormality. At the time of examination, the endoscopists were blinded to the questionnaires and the GastroPanel results. All gastroscopy biopsies were examined by expert pathologists at the Department of Pathology, OUH, and the diagnoses were classified using the USS for classification of gastritis [69]. The grade of AGA and AGC was graded into three categories (mild, moderate, severe) and in atrophic pan-gastritis both components (AGA, AGC) were graded separately.

Statistical analyses

All statistical analyses were performed using the SPSS 26.0.0.1 for Windows (IBM, NY, USA) and STATA/SE 16.1 software (STATA Corp., Texas, USA). The descriptive statistics was done according to routine procedures. Performance indicators: sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV) and their 95%CI, of GastroPanel® test biomarkers (separately for AGA and AGC) were calculated using the algorithm introduced by Seed., *et al* [70]. GastroPanel® is a quantitative ELISA test, and ROC (Receiver Operating Characteristics) curves were used to identify the optimal sensitivity/specificity balance for the biomarkers to detect the two endpoints (AGA and AGC). Significance of the difference between AUC values was estimated using the roccomb test. The agreement between i) GastroPanel® test and ii) the USS or iii) gastroscopy (and between the latter two) was calculated by using the conventional means for overall agreement (OA)(both crude and adjusted) as well as by weighted kappa (κ_w) using the intra-class correlation coefficient (ICC) test, with the following defaults: parallel model, two-way random effects, absolute agreement. All tests were interpreted significant at the level of $p < 0.05$. In addition, Fagan’s nomogram was constructed to give the post-test predictions for AGC at a population level, based on the indicators calculated for the AGC endpoint in this study: i) the pre-test probability; ii) positive likelihood ratio (LR+) and iii) negative likelihood ratio.

Results

The five GastroPanel® diagnostic profiles in AITD and DM1 patients are depicted in table 2. There is no statistically significant difference in the GastroPanel® profile distribution between the DM1 and AITD patients ($p = 0.157$). Because of this similarity, these two patient groups (DM1 and AITD) were treated as a single cohort in all analyses of the GastroPanel® results.

GP Profile	Diabetes Mellitus type I (DM1)	Autoimmune thyroiditis (AITD)	Total Series
	No. (Per Cent)	No. (Per Cent)	No. (Per cent)
Normal	30 (81.1)	172 (83.1)	202 (82.8)
HP-gastritis	4 (10.8)	11 (5.3)	15 (6.1)
AGA	0 (0.0)	2 (1.0)	2 (0.8)
AGC	2 (5.4)	22 (10.6)	24 (9.8)
AGPan	1 (2.7)	0 (0.0)	1 (0.4)
Total*	37 (100.0)	207 (100.0)	244 (100.0)

Table 2: GastroPanel® profiles in the two series (DM1 and AITD).

AGA: Atrophic Gastritis in the Antrum; AGC: Atrophic Gastritis in the Corpus; AGPan: Atrophic Gastritis in the Antrum and in the Corpus; *GP profiles in the two conditions; $p = 0.157$ (Fisher’s exact test).

Table 3 summarizes the biomarker values (M ± SD) in the five diagnostic categories of GastroPanel® results. The biomarker levels in each category are as expected by the inherent design of the GastroPanel® test.

GP Profile	PGI (M ± SD)	PGII (M ± SD)	PGI/PGII (M ± SD)	G-17b (M ± SD)	G-17s (M ± SD)	HpAb (M ± SD)
Normal	84,4 (35,9)	10,0 (4,7)	8,8 (2,3)	2,2 (3,5)	7,5 (8,2)	14,3 (2,9)
HP gastritis	132,0 (74,4)	30,2 (16,9)	4,8 (1,8)	8,7 (10,2)	20,8 (23,3)	227,4 (189,3)
AGA	87,6 (63,7)	13,1 (9,3)	6,6 (0,1)	0,8 (0,1)	1,4 (0,8)	353,8(466,2)
AGC	16,4 (14,1)	11,5 (6,0)	1,4 (0,9)	28,4 (19,3)	32,4 (8,8)	24,2 (48,7)
AGPan	*20,5	*3,0	*6,8	*0,9	*3,2	*14,5

Table 3: Biomarker levels stratified by GastroPanel® profiles.

*Only one case.

Biomarker levels stratified by the five diagnostic categories of the USS classification are summarized in table 4. As compared with the data in table 3, there are both similarities and interesting dissimilarities in the biomarker levels between the five diagnostic categories of the GastroPanel® and the USS. The most visible differences are found in HP antibody titres that did not accurately follow the values of the five GastroPanel® profiles.

USS Grade	PGI (M ± SD)	PGII (M ± SD)	PGI/PGII (M ± SD)	G-17b (M ± SD)	G-17s (M ± SD)	HpAb (M ± SD)	Number of Cases
Normal	87,6 (48,9)	14,7 (9,4)	6,4 (2,3)	7,8 (6,5)	19,5 (11,6)	78 (130,8)	8
HP-gastritis	147,9 (99,1)	32,0 (21,8)	5,0 (1,5)	9,6 (11,0)	26,5 (31,1)	391 (347,1)	8
AGA	132,3 (31,8)	30,0 (12,2)	4,8 (1,3)	10,6 (13,2)	15,9 (10,2)	217 (225,9)	4
AGC	16,7 (16,8)	11,7 (6,4)	1,3 (0,8)	28,0 (9,6)	30,9 (6,8)	103 (267,0)	19
AGPan	37,2 (41,0)	14,4 (7,6)	2,5 (2,4)	23,4 (15,8)	28,5 (20,7)	146 (231,0)	6

Table 4: Biomarker levels stratified by the USS grades of gastritis.

Only the patients testing GastroPanel® positive (AG/AAG) were referred for gastroscopy (n = 45). Altogether, 4 biopsy-confirmed AG cases were found in 37 DM1 patients (10.8%) and 25 AG cases among the 207 AITD patients (12.0%), table 5 illustrates the agreement between the GastroPanel® test and the USS classification. The unadjusted overall agreement (OA) between the two tests is 0.622 (i.e. 62.2%). However, the AGC component (corpus atrophy) of the AGPan was correctly diagnosed by GastroPanel® in 5/6 cases, and adjustment for this correct AGC verification increases the OA to 73.3% (95%CI 60.4-86.2%). Using the weighted kappa test (κ_w) to measure the agreement between GastroPanel® and the USS, $\kappa_w = 0.852$ (non-adjusted). When adjusted for the correct verification of the AGC component in the AGPan cases, the κ_w increases to 0.881 (95%CI 0.783 - 0.935).

The agreement between GastroPanel® test result and gastroscopic diagnosis (AG+/AG-) is shown in table 6. Altogether, 36/45 cases were concordantly diagnosed by both tests: OA = 80.0% (95% CI 68.3 - 91.6%). The level of regular (Cohen's) kappa ($\kappa = 0.593$) (i.e. moderate agreement), is being discounted by the relatively small sample size. The likelihood for diagnosing AG on gastroscopy after a positive (AG+ any type) GastroPanel® test has OR = 15.75 (95%CI 3.61 - 68.65) (p = 0.0001).

Table 7 shows the agreement between gastroscopic examination and the USS classification. In this setting, 31/45 samples are similarly diagnosed by the two tests, with OA = 68.8%. The likelihood of diagnosing AG in the biopsies after gastroscopic diagnosis of AG has OR = 4.88, with 95%CI ranging between 1.30 - 18.26 (p = 0.014).

GastroPanel	The Updated Sydney System (USS)					Total
	Normal	HP-gastritis	AGA	AGC	AGPan	
Normal	4	0	0	0	0	4
HP-gastritis	3	6	4	1	1	15
AGA	0	2	0	0	0	2
AGC	1	0	0	18	5	24
AGPan	0	0	0	0	0	0
	Overall agreement (OA): 28/45; 0.622 (95%CI 0.480 - 0.763); *Adjusted OA: 33/45; 0.733 (95%CI 0.604 - 0.862)					45
	**Weighted kappa (k_w): ICC = 0.852 (95%CI 0.733 - 0.919); *Adjusted (k_w): ICC = 0.881 (95%CI 0.783 - 0.935)					

Table 5: Agreement between GastroPanel® test and the USS classification.
 *: Adjusted for the correctly diagnosed AGC among AGPan cases; **Weighted kappa (ICC; parallel model, two-way random, absolute agreement).

GastroPanel	Gastroscopy		Total No of Cases
	*Atrophy	No atrophy	
AG (AGA or AGC)	21	5	26
No AG**	4	15	19
	Overall agreement (OA): 36/45; 0.800 (95%CI 0.683 - 0.916). (Cohen's kappa) $k = 0.593$ (95%CI 0.472 - 0.714)		45
	Odds Ratio (OR) = 15.75 (95%CI 3.61 - 68.65) (p = 0.0001).		

Table 6: Agreement between GastroPanel® test and gastroscopic diagnosis.
 *Clinically definite or strongly suggestive; **: Includes categories: i) Normal and ii) HP-gastritis.

Gastroscopy	The Updated Sydney System		Total No of Cases
	AG (AGA, AGC, AGPan)	**No AG	
*Atrophy	20	5	25
No atrophy	9	11	20
	Overall agreement (OA): 31/45; 0.688 (95%CI 0.553 - 0.824). Cohen's kappa $k = 0.357$ (95%CI 0.318 - 0.496)		45
	Odds Ratio (OR) = 4.88 (95%CI 1.30 - 18.26) (p = 0.014).		

Table 7: Agreement between gastroscopy and the USS classification.
 *Clinically definite or strongly suggestive atrophy; **: Includes categories: i) Normal and ii) HP-gastritis.

The accuracy of the GastroSoft® AGA- and AGC-profiles in predicting the AGA and AGC in the biopsies is summarized in table 8, separately for all AGA/AGC grades and moderate/severe AG (AGA2+ and AGC2+). Unfortunately, the small number of AGA cases precluded any meaningful analysis for the GastroSoft®- AGA profile. The GastroSoft® AGC-profile predicts the biopsy-confirmed AGC with 92.0% SE and 95% SP, with AUC = 0.940 (95%CI 0.860-1.000). GastroSoft® AGC profile did not miss any of the AGC2+ cases (sensitivity 100%).

USS Grade	Sensitivity	Specificity	PPV	NPV	AUC
*AGA	0 (0 - 32.0)	94.1 (80.3 - 99.3)	0 (0 - 84.2)	76.2 (60.5 - 87.9)	0.471 (0.430 - 0.511)
AGA2+	0 (0 - 97.5)	95.3 (84.2 - 99.4)	0 (0 - 84.2)	97.6 (87.4 - 99.9)	0.477 (0 - 1.00)
**AGC	92.0 (74.0 - 99.0)	95.0 (75.0 - 99.9)	95.8 (78.9 - 99.9)	90.5 (69.6 - 98.8)	0.940 (0.860 - 1.000)
AGC2+	100 (80.5 - 100)	75.0 (55.1 - 89.3)	70.8 (47.9 - 87.4)	100 (83.9 - 100)	0.880 (0.790 - 0.960)

Table 8: GastroPanel® profiles® in diagnosis of biopsy-confirmed AGA and AGC.

®Diagnosis made by the GastroSoft AGA and AGC profiles; *AGA of any grade; AGA2+ (moderate/severe AGA);

**AGC of any grade; AGC2+ (moderate/severe AGC).

Figure 1 illustrates the ROC curve for PGI for the AGC2+ state variable (moderate/severe AGC present or not). The resulting ROC curve has AUC = 0.939 (95%CI 0.870 - 1.000). Similar ROC analysis for the PGI/PGII ratio in diagnosis of AGC2+ endpoint is shown in figure 2. The resulting ROC curve is even more impressive, with the AUC = 0.945.

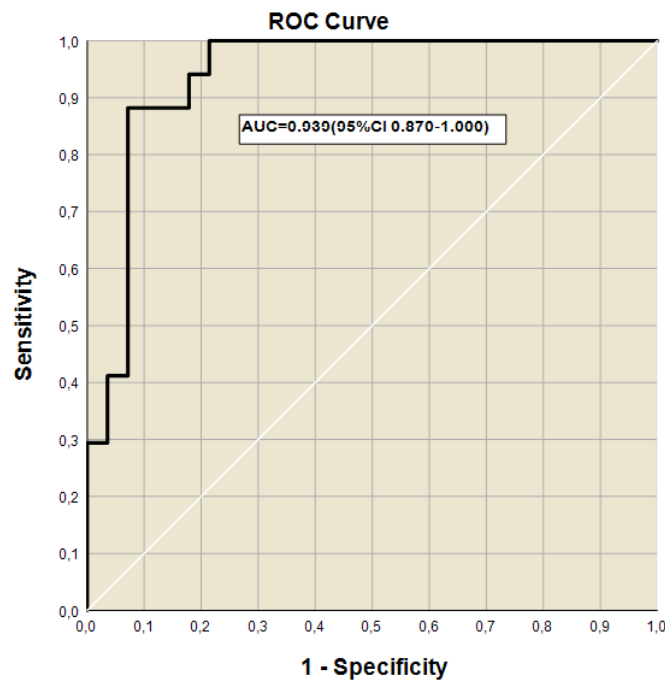


Figure 1: PGI biomarker in detecting biopsy-confirmed moderate/severe AGC in ROC analysis.

Figure 3 shows the Fagan’s nomogram obtained by using the data derived from calculation of the AGC endpoint indicators in table 8, i.e. i) the pre-test probability 0.56; ii) positive likelihood ratio (LR+) 18.4 and iii) negative likelihood ratio (LR-) 0.0800. The Fagan’s nomogram gives the post-test predictions of AGC, implicating that a GastroPanel® result positive for AGC predicts the diagnosis of AGC with the likelihood exceeding 95%. On the other hand, such a likelihood is only 9% if GastroPanel® test result is negative for AGC.

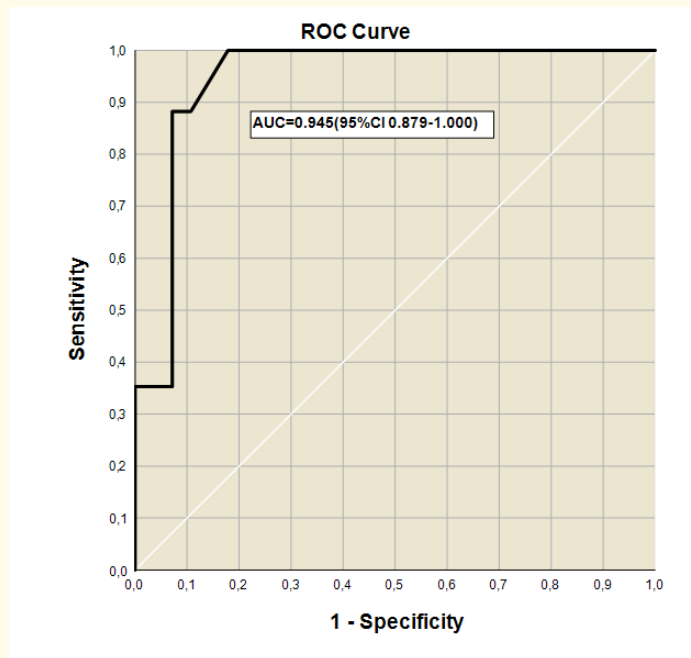


Figure 2: PGI/PGII ratio in detecting biopsy-confirmed moderate/severe AGC in ROC analysis.

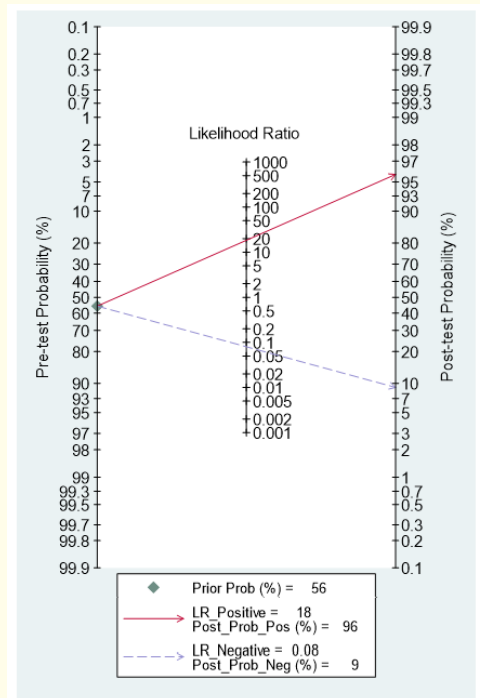


Figure 3: Fagan's nomogram for GastroPanel® as predictor of AGC in a population.

Discussion and Conclusion

The present study had two main aims: i) to establish the prevalence of AG/AAG among the patients with two autoimmune-related diseases (DM1 and AITD) and 2) to perform the clinical validation of the new GastroPanel® test version. AG/AAG in the two groups of patients is a far more common manifestation, as compared with the general population, being detected in 5.4% of those with DM1 and in 10.6% of the AITD patients using the serological assay (Table 2). Given that only the GastroPanel AG-positive cases were referred for gastroscopy, the serological AG diagnoses were enriched among the patients who underwent gastroscopy; 24/39 (61.5%) and 2/6 (33.3%) of the AITD and DM1 patients, respectively. The respective numbers in the USS classified biopsies were: 25/39 (64.1%) and 4/6 (66.7%) for AITD and DM1 patients. Of the 29 biopsy-confirmed AG cases, HP-infection was detected in 8/29 (27.6%) only, suggesting that the majority (72.4%) of the detected AG cases are of autoimmune type (AAG), associated with DM1 and AITD. This cannot be firmly confirmed, however, because HP detection is based only on HP serology. It is also well established that during the protracted course of AG, *Helicobacter* can disappear, leading to decreased prevalence of HP antibodies in chronic AG [65,67,68]. This issue is made even more complex by the fact that HP is suspected as a trigger of AAG as well [41-44,76,77,79]. This is supported by the detection of PCA in 20 - 50% of HP-infected patients and correlation between PCA and HP Ab in patients with AAG/PA [71,72], suggesting that chronic HP-infection is linked with gastric autoimmunity.

In the published literature, the prevalence of AAG in DM1 patients is estimated to vary between 5 - 10% [2-5,7,19-26], while among AITD patients, AAG is detected in up to one third of the patients [27-31]. The present series with four biopsy-confirmed AG cases in 37 DM1 patients (10.8%) and 25 AG cases among 207 AITD patients (12.0%), does not confirm such a 3-fold difference in AG prevalence between the DM1 and AITD patients. Suffice it to conclude that both groups of patients with these autoimmune diseases are at markedly elevated risk of developing AG/AAG, as compared with the general population [1-7]. Importantly, however, there seems to be no significant difference in this risk between the DM1 and AITD patients.

Before entering into a detailed discussion of the GastroPanel® validation data, a few points deserve to be mentioned here [65,67,68,74]. First: GastroPanel® test is based on measurement of the serum levels of the biomarkers that reflect the function and structure of both antrum (G-17b, G-17s) and corpus (PGI, PGII, PGI/PGII ratio), separately but interdependent on each other [65,67,74]. Second: Atrophic gastritis (AG) may affect either antrum (AGA), corpus (AGC) or both (AGPan), and GastroPanel® biomarker profile is dependent on this topographic location of AG. These two conditions should never be combined as a single endpoint (chronic atrophic gastritis) while validating the diagnostic performance of GastroPanel® test [67,68,74]. Third: The biomarker profile of AGPan is contributed by both AGA and AGC, and in the validation of GastroPanel® this is best addressed by splitting the AGPan cases into its both components (AGA and AGC), and analyzing them separately.

Fourth: Because mild AGC and AGA are poorly reproducible diagnosis even among experienced pathologists [67-69,71,74], these should never be used as the endpoint in calculating the performance indicators of the PGI and PGI/PGII and G-17, respectively, but the only valid endpoint should be moderate/severe AG (AGC2+, AGA2+) [65,67,68,73,74]. Fifth: The most common cause of low G-17b values is the high acid output of the corpus, whereas AGA as the cause of low G-17b is uncommon [62,63,65,71,74]. Because of this dual origin, G-17b can never be a highly sensitive or specific biomarker of AGA [67,68,73]. GastroPanel® test offers a solution by measuring the G-17 levels after protein stimulation (G-17s) [67,74]. G-17s values accurately measure the capacity of antral G-cells to synthesize G-17 after protein stimulation, and failure to do so is an accurate indicator of antral atrophy (missing G-cells). Antral G-cells have a remarkable capacity of maintaining their G-17s output along the protracted course of progressing AGA and truly low levels of G-17s are encountered only when G-cells are practically absent or extremely scarce in moderate/severe AGA (AGA2+).

GastroPanel® test was developed as a non-invasive diagnostic tool to replace the invasive and expensive gastroscopic examinations, and the agreement between GastroPanel® results and gastroscopic examination is of major clinical interest (Table 6). Indeed, the agreement between these two diagnostic tools is very good, 36/45 cases being identically diagnosed by both tests: OA = 80%. The likelihood for diagnosing AG in gastroscopy after a positive (AG+) GastroPanel® test has OR = 15.75 (p = 0.0001), which warrants to conclude that

a GastroPanel® test positive for AG is a distinct clinical indication for gastroscopic examination. This provides strong support to the policy adopted since the introduction of GastroPanel® test; any patient with a test result suggesting AG should be referred for gastroscopy [65,67,68,74].

The most important part of this validation is to demonstrate the accuracy of the new GastroPanel® test version in diagnosing the endpoints confirmed by the reference test (the USS classification) [68,73]. Both tests include 5 diagnostic categories, and the inter-test agreement can be calculated using overall agreement (OA) and weighted kappa analyses. The unadjusted OA between GastroPanel and the USS classification is 0.613 (61.3%) (Table 5). Using the adjustment for the correctly diagnosed AGC component (5/6 cases) of the AGPan cases increases the OA to 72.7% (95%CI 57.2 - 85.0%). The results obtained by calculating the weighted kappa test are even more impressive: $\kappa_w = 0.850$ (non-adjusted), and $\kappa_w = 0.879$ (adjusted) (Table 5). Both values are falling within the category “almost perfect” (0.800 - 1.000) used by all tests for inter-observer variation, which justifies the statement that GastroPanel® test has an almost perfect agreement with the USS classification of gastritis.

GastroPanel® test has distinct biomarker profiles for AGA (low G-17b and G-17s, HP+) and AGC (low PGI, PGI/PGII ratio, high G-17b) [67,73] and these can be used to test the diagnostic agreement with biopsy-confirmed AGA and AGC (Table 8). Unfortunately, this analysis was hampered by the low number of AGA cases (n = 4), of which only one was classified as AGA2+. This is not unusual, because AGA is a very rare lesion as compared with AGC [57-63,67,69,73]. The results for AGC are convincing. The AGC-profile of GastroSoft® profile detects the biopsy-confirmed AGC with 91.7% SE and 95% SP, translating to AUC = 0.933 (95%CI 0.859 - 1.000). This corroborates (and even exceeds) the pooled SE and SP estimates reported in the two recent meta-analysis for GastroPanel® test in diagnosis of AGC [68,73].

As a quantitative ELISA test, GastroPanel® performance can be readily analysed using the ROC test for AGA (G-17b, G-17s) and AGC (PGI, PGI/PGII), separately [67,68]. Unfortunately, the rarity of AGA cases in the present cohort also precluded a reliable ROC analysis for the AGA2+ endpoint. This handicap did not affect the ROC analysis for AGC, run separately for PGI and PGI/PGII ratio. Despite the relatively small number of cases, ROC analysis of both PGI and PGI/PGII ratio demonstrate AUC = 0.935 and AUC = 0.942, respectively (Figure 1 and 2). These extremely high AUC values are clearly superior to the HSROC (hierarchical summary ROC) presented by Zagari, *et al.* (2017) in their meta-analysis, with the pooled SE of 0.747 and pooled SP of 0.956 (AUC = (SE+SP)/2; AUC = 0.851) [73]. Taken together, in this validation study, the accuracy of GastroPanel® PGI and PGI/PGII ratio for diagnosing AGC2+ assessed by ROC far exceeds the pooled summary estimates of the two recent meta-analysis covering the entire published literature on GastroPanel® [67,73].

It is also of interest to determine, how these data from a targeted clinical study would be generalizable at a population level. This is important because the population-based screening of GC risks is one of the two main indications of GastroPanel® use [62-65,67,73]. The probability of a patient having a condition of interest, given the evidence collected, is referred to as the posterior (post-test) probability of having the condition, i.e. the predictive value (PV) [75,76]. In 1975, Fagan integrated Bayes' theorem into a nomogram to quantify this post-test probability (PV) that an individual is affected by a condition, based on the probability of the individual having the condition before the test (pre-test probability) [77]. Indeed, the Fagan's nomogram is the simplest of the Bayes' theorem calculators to help practitioners determine the probability of a patient truly having a condition of interest given a particular test result [78]. Assuming that the study samples are representative of the entire population, an estimate of the pre-test probability reflects the global prevalence of this disorder, and in this way, the likelihood ratios (LRs) are clinically even more meaningful than Se and Sp [77,78].

The likelihood ratios (LR+, LR-) generated from the present data for AGC detection can be used to calculate the post-test probabilities of AGC in a population [77,78]. In interpreting the Fagan's nomogram (Figure 3), the post-test predictions of AGC in a population similar as that in the present study implicate that a GastroPanel® AGC profile predicts true AGC with the likelihood exceeding 95%. In contrast, this likelihood is only 9% if GastroPanel® profile is negative for AGC. The present study was focused on patients at high risk for AGC, with a high pre-test prevalence (0.56) of AGC (i.e. a 56% prevalence of AGC in the biopsied cases; 25/45), but Fagan's nomogram is readily applicable to any population with a known AGC prevalence. When translated to a hypothetical screening setting of a population (n = 10.000) with 5% pre-test prevalence of AGC (n = 500), and keeping the test SE (92%) and SP (95%) as in table 8, Fagan's nomogram shows that GastroPa-

nel AGC+ result would predict AGC with a likelihood exceeding 70% while those with an AGC-negative profile have a minimal likelihood (< 1%) of having true AGC (data not shown).

To conclude, the present clinical validation study of the unified GastroPanel® test confirms the performance indicators of the new test version, which in many aspects exceeds those reported for the current test version in two recent meta-analyses [68,73]. AG/AAG is far more prevalent in the screened high-risk (AITD, DM1) patients as compared with the general population. However, in contrast to what has been reported in the literature [2-5,7,19,20-31], the prevalence of AG/AAG in AITD and DM1 patients is practically equal, i.e. around 10 - 12%. This high prevalence of AG/AAG justifies a screening of these patients who are at clearly increased risk for severe clinical sequels of AG. A non-invasive biomarker test (GastroPanel®) is an ideal tool for this follow-up, because a biomarker profile implicating AG predicts biopsy-confirmed AG with high precision. When this AG-profile is used as an indication for gastroscopy in the screening for AG/AAG in DM1 and AITD-patients, substantial cost savings are achieved while avoiding the gastroscopies that are unnecessary in this context.

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