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Non-invasive Screening of Autoimmune Atrophic Gastritis in Asymptomatic Subjects by Serological Biomarker Test (GastroPanel®)

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Abstract. *Background/Aim: To estimate the prevalence of autoimmune atrophic gastritis (AAG) in the Russian Federation, a systematic screening of asymptomatic healthy adults by non-invasive biomarker testing was conducted. The aim was i) To test the validity of non-invasive serological screening for AAG; ii) to establish the prevalence of AAG among asymptomatic adults. Patients and Methods: Altogether, 1,283 asymptomatic, healthy adults (mean age: 38 years) were screened by GastroPanel® test. Those with a biomarker profile indicating AG (n=46) were invited for further examinations; 21 consented to gastroscopy with biopsies classified using the Updated Sydney System and Operative Link to Gastric Atrophy. Blood tests included parietal cell, intrinsic factor and thyroid peroxidase antibodies, and analysis of vitamin B12 and iron. Results: Gastroscopy and biopsies confirmed AG in 20 of the individuals. Parietal cell, intrinsic factor and thyroid peroxidase antibodies were present in five, one and eight individuals, respectively. AAG-associated co-morbidities (iron deficiency and pernicious anemia) were diagnosed in 10 out of 21. The final diagnosis of AAG was made in 15 out of 1,283 subjects (1.2%), of whom four were Helicobacter pylori-positive. When corrected for verification bias (non-attendees in the confirmatory tests; n=25), the adjusted prevalence of AAG was 2.6% (33/1,283). Conclusion: AAG prevalence of 2.6% is among the highest reported using non-invasive tests. GastroPanel® is an optimal screening tool, providing the first link in the diagnostic protocol leading to the final diagnosis of this condition. The role of Helicobacter pylori as a trigger of AAG cannot be ruled out.*

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Autoimmune atrophic gastritis (AAG) is characterized by atrophy of the gastric corpus and fundus, and the presence of circulating autoantibodies to parietal cells (PCA) and to their secretory product, intrinsic factor (IFA) (1-5). In the general population, there is an age-related increase in the prevalence of PCA, from 2.5% in the third decade to 12% in the eighth decade (6, 7). The prevalence is markedly higher in those affected by other autoimmune disorders, frequently accompanied by pernicious anemia (PA) (8-10). Thus, in patients with type 1 diabetes mellitus (DM1), PCAs are found in 10-15% of children and in 15-25% of adults (11-13). The respective prevalence of AAG and PA in the general population are 2% and 0.15-1% (14-16), compared with 5-10% and 2.6-4%, respectively, among patients with DM1 (15, 17-19). In patients with autoimmune thyroid disease (AITD), AAG is concomitantly detected in up to one-third of patients (20-24).

AAG is associated with significant co-morbidities, including iron deficiency anemia found in 20-40% of patients (25, 26), and PA is diagnosed in up to 15-25% (3, 4, 27). The progression to PA in patients with AAG is likely to span 20-30 years (28). Finally, gastric carcinoid tumors are observed in 4-9% of the patients with AAG/PA, which is 13 times more frequent than in controls (29-32). Patients with AAG/PA also have a 3- to 6-fold increased risk of gastric cancer, ranging from 0.9-9% (29, 30, 32-35).

In most cases, like AG associated with *Helicobacter pylori* infection, AAG is asymptomatic and diagnosed as incidental finding among patients examined for other conditions, such as iron-deficiency anemia, PA or other autoimmune conditions (2, 36). The diagnosis of AAG is confirmed by gastroscopy and biopsies, demonstrating mucosal atrophy in the corpus but sparing of the antrum (1-5, 8, 9, 37). Being an invasive diagnostic technique, however, gastroscopy cannot be considered as a tool for systematic, population-based screening for asymptomatic AAG, non-invasive diagnostic methods, *i.e.*, serology, are used instead. The most sensitive serum biomarker for AAG is PCA, detected by enzyme-linked immunosorbent assay (ELISA), although detection of IFA is more specific than PCA (1-4, 8-10). The sensitivity of IFA is very low, but rises in parallel with disease progression (1, 3, 4). PCA, targeted against gastric H^+/K^+ ATPase, are detected in 60-85% and IFA in 30-50% of patients with AAG (17, 38).

An interesting proposal was made by Antico *et al.*, who suggested a combination of testing for PCA and IFA with anti-*H. pylori* and serum gastrin as a 'serological biopsy' for diagnosis of AAG, shown to be useful in classification of gastritis (39). The latest development in the field of serological biopsy is a panel combining serum pepsinogen I (PGI) and II (PGII), gastrin-17 and *H. pylori* IgG antibodies using ELISA, the GastroPanel® test (Biohit Oyj, Helsinki, Finland), proposed as a first-line diagnostic test for dyspeptic symptoms, as well as for screening of asymptomatic individuals for AG/AAG (40-42). Since its introduction in the early 2000s (43), GastroPanel® has been extensively tested in different clinical and screening settings worldwide (44-48), and its high accuracy in diagnosis of AG was confirmed in two recent meta-analyses (49, 50).

The present study is the first in which GastroPanel® test was used for systematic screening for AAG in a cohort of asymptomatic healthy volunteers, followed by confirmation of AAG by gastroscopic biopsies and AAG-specific serological tests (PCA and IFA).

Patients and Methods

Study subjects. The study subjects in this screening trial represent asymptomatic, healthy volunteers who consented to participate in the study as a part of the preventive health care program arranged by their employer (AO Werteks, St. Petersburg, Russian Federation). All employees of the company were asked to participate in the study by providing written consent. All those who were asymptomatic and did not report any upper abdominal symptoms were considered eligible. The study protocol followed the Declaration of Helsinki, and the whole trial was approved (April 22, 2020) by the Institutional Review Board (Astarta Ltd. Co, St. Petersburg, Russian Federation), fulfilling the rigorous ethical principles applied to clinical studies in the country (no. 04/2020). Altogether, 1,283 study subjects were enrolled and had completed the first round (GastroPanel® screening) by the end of summer 2021. Their mean age (±standard deviation) was 38 years (±11.2 years) (range=19-79 years). Of the 1,283 participants, 758 (59%) were women and 525 (41%) were men.

Study design. This study was a systematic screening trial targeted to all the employees of a single company (AO Werteks) in St. Petersburg (Russian Federation). All study subjects were healthy adults with no dyspeptic or other gastric symptoms, who volunteered to be screened by GastroPanel® test to reveal asymptomatic AAG.

In the first study round (screening), all participants donated a blood sample for analysis by the new-generation GastroPanel® test. This test categorizes the biomarker profiles into five diagnostic endpoints: i) Healthy stomach, ii) superficial *H. pylori*-related gastritis, iii) AG of the antrum (AGA); iv) AG of the corpus (AGC), and v) AG of the antrum and corpus, pan-gastritis (AGP) (42, 43-48, 51).

In the second round, all those who tested positive for AG in GastroPanel® (profiles iii-v) were contacted personally by the study monitors, asking for their consent to participate in the second phase of the study, which included gastroscopy and biopsies, as well as blood sampling for measurement of the following: Transferrin, iron, PCA, IFA, vitamin B12 (total + active), and TPO (antibodies to thyroid peroxidase).

Figure 1 shows the study flowchart. Of the 1,283 subjects examined by GastroPanel® test, 46 had a test result indicating AGC or AGP. Of the 46 individuals who were invited to participate in the second round, 25 either did not receive an invitation (*i.e.*, were not reached) or refused to participate. This left a total of 21 subjects who completed both study rounds and were analyzed in detail to confirm or exclude AAG.

The GastroPanel® test. The GastroPanel® used in this study is the original test version containing four biomarkers (Pepsinogen I, Pepsinogen II, Gastrin-17, and *H. pylori* IgG ELISA), processed using automated ELISA technique and biomarker-specified processing conditions (43, 46-48).

Sample processing for GastroPanel® test. Test results are interpreted by the GastroSoft® application (Biohit Oyj, necessitating completion of the GastroPanel® request form with pertinent clinical information (43, 46-48, 51). A minimum of 2 ml EDTA plasma from a fasting blood sample was taken into an EDTA tube, frozen instantly (−70°C), as instructed by the manufacturer (43, 46-48, 51).

GastroPanel® testing. All plasma samples were delivered to the laboratory (LabStori Laboratories, St. Petersburg, Russia) for analysis with the conventional GastroPanel® test version following the instructions detailed elsewhere (43, 46-48, 51).

GastroPanel® results were interpreted by the GastroSoft® application. The GastroPanel® test was designed for use with the Updated Sydney System (USS) classification of gastritis (52), both

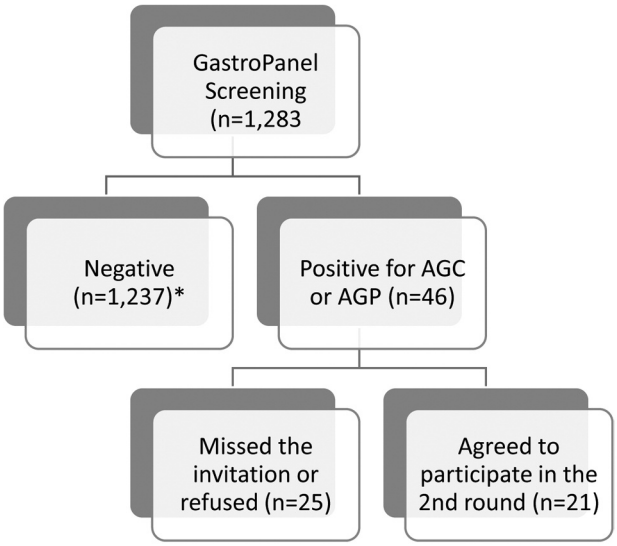

Figure 1. *Flowchart of the study subjects in the two rounds of the study. AGC: Atrophic gastritis of the corpus; AGP: atrophic gastritis in both the antrum and corpus. *Normal, or Helicobacter pylori infection with no atrophy.*

using the same diagnostic categories: a) Normal mucosa, b) *H. pylori*-related gastritis with no atrophy, c) AGA, d) AGC, and e) AGP (40, 41, 44-52).

Gastroscopy and biopsies. In this study, a total of 21 individuals who consented to participate in the second study round underwent gastroscopy and biopsies at the Department of Internal Medicine (St. Petersburg State University Hospital). Macroscopic endoscopy findings were classified using the adopted practice of the clinic, the endoscopist being blinded to the GastroPanel® results. Endoscopic findings were classified into one of the following categories: i) Normal; ii) inflammation; iii) suspected atrophy; iv) definite atrophy; v) atrophy and intestinal metaplasia; and vi) other abnormality.

Gastroscopic biopsies followed the protocol of the USS, targeting both the antrum and corpus (52). All biopsies were examined by expert pathologists at the Department of Pathology (National Center of Clinical Morphological Diagnostics, St. Petersburg, Russia). The diagnoses were classified using the USS classification for gastritis (52) as AGA, AGC or AGP, and all lesions were classified as mild, moderate or severe in degree. In addition, the pathologists also classified all biopsies using the OLGA staging (53).

All biopsies were stained with Giemsa stain for specific demonstration of *H. pylori*, quantified using a semi-quantitative 4-tier grading: 0=absent; 1=*H. pylori* present in small quantities; 2= *H. pylori* present in moderate quantities; and 3=*H. pylori* present in abundance (47, 52). In some statistical testing, a 3-tier grading was also used, where grades 2 and 3 were combined. Immunohistochemical staining for chromogranin was performed to assess the level of enterochromaffin-like cell (ECL) hyperplasia in the atrophic corpus. Conventional immunohistochemistry procedures were followed and chromogranin expression was graded as normal or intense in the biopsies from gastric corpus.

Laboratory tests for AAG. In the second study round, blood was sampled for analysis of the markers that are indirect or direct indicators of AAG. These include the following: ferritin, iron, PCA, IFA, vitamin B12 (total + active), and antibodies to thyroid peroxidase (TPA). Ferritin and iron were analyzed using conventional laboratory tests. Active vitamin B12 was measured using the test for active vitamin B12 (Biohit Oyj) as instructed (54). Also, the total B-12 vitamin was measured using Architect i2000sr (Abbott Laboratories, Abbott Park, IL, USA; reference: 187-883 pg/ml). The levels of PCA were measured by ImmuGlo™ COVI III Rat Liver/Kidney/Stomach IFA kit (IMMCO Diagnostics, Amherst, NY, USA) (reference titer <1:40). The levels of IFA were measured by anti-intrinsic factor (Orgentec Diagnostika GmbH, Mainz, Germany) (reference <6 EIU/ml). TPA were tested using Architect Anti-TPO Reagent (Abbott Laboratories; reference <5.6 EIU/ml).

Statistical analyses. All statistical analyses were performed using the SPSS 27.0.1.0 for Windows (IBM, Armonk, NY, USA). The descriptive statistics were performed according to routine procedures. All tests were two-sided and interpreted as significant at the level of $p<0.05$.

Results

GastroPanel® test was performed for all 1,283 individual who consented to participate in the screening trial (Table I). The most frequent test result was *H. pylori*-associated gastritis with no atrophy, detected in 736/1,283 (57.4%). GastroPanel® test was normal in 38.7% (n=496) of the cases. The disease of interest, *i.e.*, AG was distinguished by its biomarker profile in 51 study subjects (3.97%). Because AAG only affects the gastric corpus leaving the antrum intact (1-5), the five patients with the GastroPanel® profile of AGA were not invited for further examinations.

Of the 46 invited to undergo second round testing, 21 finally consented to participate. The GastroPanel® profiles of these patients are illustrated in Table II. The 20 cases with the AGC biomarker profile, the PGI level was low and the PGI/PGII ratio very low, with concomitant extremely high gastrin-17, and *H. pylori*-positive test with mean values above the cut-off (30.0 EIU). As expected, the gastrin-17 level was very low in the one case classified as AGP.

In the second round of the study, all consenting participants (n=21) attended the clinic and underwent gastroscopic examination with multiple biopsies, as well as a series of laboratory tests for diagnosis of AAG (Table III). Women clearly predominated in this group (at 15:6, *i.e.*, 2.5:1). The mean age of this group was 12 years older than the whole screened cohort. Gastroscopic examination confirmed AG in all 21 patients, accompanied by signs of intestinal metaplasia in seven of them. In 17/21 patients, OLGA stage was far advanced (stage 2 or 3). Histological examination of the biopsies failed to find any atrophy in one case, while AGA was found in one instead of predicted AGC. ECL hyperplasia was present in all but four cases, classified as moderate to

Table I. *Results of the GastroPanel® test in the screened cohort of 1,283 asymptomatic subjects.*

Biomarker profile	No. of cases	PGI, μg/l	PGII, μg/l	PGI:PGII	Gastrin-17, pmol/l	HpA, EIU
Normal	496	86.8±292.5	6.0±3.2	15.5±5.2	3.5±6.6	10.6±8.0
Hp infection	736	100.4±36.4	13.6±9.5	9.0±3.7	7.7±10.0	94.4±51.6
AGA	5	59.5±22.5	6.2±2.6	10.8±6.4	0.5±0.4	103.0±26.2
AGC	43	25.8±28.9	13.3±10.7	1.7±1.0	34.6±11.3	63.4±43.2
AGP	3	26.1±2.7	6.8±4.0	5.7±5.0	0.9±0.3	87.7±43.1

AGA: Atrophic gastritis of the antrum; AGC: atrophic gastritis of the corpus; AGP: atrophic gastritis of both antrum and corpus; G-17: gastrin-17; Hp: *Helicobacter pylori*; HpA: *Helicobacter pylori* IgG antibody by enzyme-linked immunosorbent assay; PGI: pepsinogen I; PGII: pepsinogen II. Data are the means±standard deviation.

Table II. *Results of the GastroPanel® test in the 21 study subjects who completed the second round of testing.*

Biomarker profile	No. of cases	PGI, μg/l	PGII, μg/l	PGI:PGII	Gastrin-17, pmol/l	HpA, EIU
AGC	20	11.4±9.4	7.4±3.9	1.3±0.9	31.9±14.0	48.1±46.8
AGP	1	26.5	2.3	11.5	1.2	38.7

AGA: Atrophic gastritis of the antrum; AGC: atrophic gastritis of the corpus; AGP: atrophic gastritis of both antrum and corpus; G-17: gastrin-17; HpA: *Helicobacter pylori* IgG antibody by enzyme-linked immunosorbent assay; PGI: pepsinogen I; PGII: pepsinogen II. Data are the means±standard deviation.

severe in 13. Not unexpectedly, chromogranin expression in the ECL cells was almost invariably intense (19/21 cases).

The laboratory tests designed for finding evidence of AAG disclosed iron deficiency in four patients (Table III). Vitamin B12 deficiency was revealed in 6/21 (28.6%) cases. Similarly, PCA or IFA were detected in six (28.6%) of the patients, whereas eight (38%) patients had high titers of TPA.

Table IV summarizes the case profiles of all 21 second-round study subjects, listing the results of the clinical and laboratory tests used to reach the final diagnosis. For each of these 21 subjects, at least one of the tests was abnormal, in addition to biopsy-confirmed AGC/AGP. There was one patient (no. 5; a female aged 46 years), however, in whom the biopsy failed to confirm AG and all the AAG tests were normal. However, the clinical finding in gastroscopy was definite AG, and the GastroPanel® biomarker profile was typical for AGC. Based on the laboratory and clinical findings listed in Table IV, the final diagnosis of the 21 cases were as follows: a) normal stomach in one; b) AG with no evidence on autoimmune origin in five; c) AAG in 15.

Discussion

The prevalence of AAG is dependent on the technique used for the diagnosis, *i.e.*, whether based on detection of vitamin B12 deficiency, IFA or PCA (1, 3, 6-9, 14-16). According to the global data, the prevalence of AAG in the general population varies between 0.1% and 1-2% (1-9). In studies based on esophagogastroduodenoscopy, the prevalence of AAG is higher, from 0.3% to 2.7% (1, 3, 30, 32). AAG is significantly more common, however, among particular groups of people, particularly those with other autoimmune disorders (11-24). Accordingly, 36-44% of patients with AAG have AITD (20-24), while DM1 increases the risk of AAG by up to 3-5-fold as compared with the general population (15, 17, 19). There is at least a 2:1 female predominance among patients with AAG (1, 3).

Studies on AAG prevalence in the Russian Federation are scant (55, 56). In two recent surveys based on serological biomarker screening for *H. pylori* infection and AG in the Russian Federation using GastroPanel® (45, 47), the prevalence of *H. pylori* infection in a population with mean age of 50+ years was high (64-76%), and AG (any topography) was diagnosed in 10.8% (99 out of the 918 subjects screened) (45). Both surveys used the GastroPanel® test, which does not include a specific biomarker for AAG (40-42, 51), precluding an accurate assessment of the proportion of AAG. However, indirect evidence can be obtained by using the assumption that all AGC and AGP cases that are *H. pylori*-negative are potentially an autoimmune type of AG. Using this approach in the recent survey data (45), where out of 78 AGC/AGP cases, 28 proved to be *H. pylori*-negative, results in an AAG prevalence estimate of 3.05% (28/918) (45). Undoubtedly, this estimate might be inaccurate because of the known fact that Helicobacter frequently disappears during the protracted

clinical course of AGC/AGP, and is no longer detectable in severe AG (42-49, 57). As discussed later, however, this estimate seems to match fairly with the results of the present study (2.6% AAG prevalence), where specific tests were used for diagnosis of AAG, and when the AAG prevalence (15/1,283) is being corrected for the non-attendees (33/1,283) in the second study round.

The present study is the first ever conducted screening of asymptomatic healthy adults for AAG by a non-invasive serological test in the Russian Federation (45, 47, 55, 56). In this study, the serological biomarker test (GastroPanel®), which accurately detects AG and its topography (AGA, AGC, AGP) as well as *H. pylori* infection as the causative agent, was supplemented with other diagnostic tests that are used for diagnosis of AAG-associated co-morbidity (iron deficiency and vitamin B12 deficiency), detecting autoantibodies to gastric H^+/K^+ ATPase in parietal cells (PCA) and their secretory product, IF, as well as measuring TPA as an indicator of AITD (which frequently accompanies AAG) (20-24).

The GastroPanel® test was performed for all 1,283 subjects who consented to participate in the screening trial (Table I). As anticipated in an asymptomatic cohort of subject aged 38±11.2 years, the expected rate of gastric pathologies was rather low. Consistent with published GastroPanel® screening studies in the Russian Federation, *H. pylori* infection was a frequent finding, detected in 57.4% of the subjects (45, 47). In our cohort, the prevalence of *H. pylori* infection was slightly lower than that (64-76%) in the two previous studies. This is explained by the fact that the mean age of the subjects in the present cohort was 12 years younger than that of the two previous studies (around 50 years), indicating that in this country, the prevalence of *H. pylori* infection also seems to be declining in a birth cohort-related manner as shown elsewhere (58).

GastroPanel® biomarker profiles were consistent with AG in 51 cases, *i.e.*, in 3.97% of the screened individuals (Table I). Because of the fact that AAG (targeting the PCs in the corpus) does not affect the antrum (1-5), the five patients with the GastroPanel® profile of AGA were not of interest in this study. Given that AGC has a dual etiology: namely chronic *H. pylori* infection, and an autoimmune mechanism, one could argue that the correct prevalence of AAG could be simply reached by assigning all AGC and AGP cases that are *H. pylori*-negative in the GastroPanel® test to the AAG group. This would be in line with the original thinking when the GastroPanel® test was designed, and indeed, re-iterated in many studies and reviews addressing this biomarker test (40-51, 57). In the light of the accumulated new evidence, however, that would be an oversimplification of the issue, and lead to underestimates of the AAG prevalence, as discussed below.

The reasons for this discordance are two-fold: i) in chronic AGC, Helicobacter frequently disappears during the protracted course of the disease spanning over decades (59-61), and ii) recent evidence implied that *H. pylori* can be a trigger of the autoimmune process leading to AAG, *i.e.*, be involved in pathogenesis of AAG (62-65). Several prospective follow-up studies have confirmed that in chronic AG (both AGA and AGC), *H. pylori* itself can disappear from an atrophic mucosa, and in the absence of antigen stimulus, a normal decay of the IgG antibody molecules will gradually lead to *H. pylori* antibody levels below the 30 EIU cut-off (59-61). Thus, finding AGC/AGP with no evidence of *H. pylori* infection in the GastroPanel® test can denote two things: i) *H. pylori*-related AGC/AGP, where the pathogenic bacteria have disappeared, or ii) true AAG, with *H. pylori* never having been involved in the disease process.

As to the later point, the classical pathogenetic mechanism of AAG is well established. The target autoantigens in AAG are the 100-kDa catalytic α-subunit, and the 60- to 90-kDa glycoprotein β-subunit of gastric H^+/K^+ ATPase (66, 67). PCA and IFA are present in the serum and in gastric juice.

Table III. *Clinical and laboratory findings of the 21 participants who completed the study.*

Finding	Subgroup	Value
Gender, n (%)	Women	15 (71.4)
	Men	6 (28.6)
Age, years	Mean±SD	50.8±13.6
	Range	25-76
Gastroscopy, n (%)	Atrophic gastritis	14 (66.7)
	AG and IM	7 (33.3)
OLGA Stage, n (%)	0	1 (4.7)
	1	3 (14.3)
	2	7 (33.3)
	3	10 (47.7)
USS Classification, n (%)	Normal	1 (4.7)
	AGA	1 (4.7)
	AGC	18 (85.7)
	AGP	1 (4.7)
ECL hyperplasia, n (%)	None	4 (19.0)
	Mild	4 (19.0)
	Moderate	9 (43.0)
	Severe	4 (19.0)
Chromogranin expression*, n (%)	Normal	2 (9.4)
	Intense	19 (90.6)
Deficiency, n (%)	Iron (anemia)	4 (19.0)
	Vitamin B12	6 (28.6)
Antibody-positive, n (%)	Parietal cell	5 (23.8)
	Intrinsic factor	1 (4.7)
	Thyroid peroxidase	8 (38.0)

AG Atrophic gastritis; AGA: atrophic gastritis of the antrum; AGC: atrophic gastritis of the corpus; AGP: atrophic gastritis of both antrum and corpus; ECL: enterochromaffin-like cell; IM: intestinal metaplasia; USS. Updated Sydney System for classification of gastritis. *Immunohistochemical staining of gastric biopsies.

Table IV. *Case profiles of the 21 subjects who participated in the second round of the study.*

ID (gender; age in years)	Fe (9-30.4 μmol/l)	Ferritin (10-120 μg/l)	Total B12 (127-883 pmol/l)	Holo-TC (21-123 pmol/l)	PCA (<1:40)	IFA (1.21-1.52 AU/ml)	TPA (<5.6 IU/ml)	OLGA Stage	USS Grade	ECL hyp/ Chr	ELISA HpA (<30 EIU)	Gastrin-17 (1-7) pmol/l	Final diagnosis
1 (F; 43)	**8.77**	30	272.00	26.15	–	–	**1,000.0**	3	AGC	2/3	9.2	40.00	AAG
2 (M; 62)	–	–	–	–	–	–	–	2	AGC	0/3	8.2	5.50	AAG
3 (M; 33)	14.14	18	526.00	35.52	<1:40	1.70	**46.3**	3	AGC	2/3	**32.9**	40.00	AAG
4 (F; 53)	29.84	177	232.00	35.82	–	–	**88.7**	3	AGC	2/3	3.5	40.00	AAG
5 (F; 46)	20.01	100	359.00	44.61	–	–	3.0	0	N	0/0	**45.2**	40.00	N
6 (F; 56)	10.48	16	**21.50**	**15.77**	–	–	**144.6**	3	AGC	3/3	14.8	40.00	AAG
7 (F; 42)	**6.80**	**1.0**	285.00	25.09	–	–	3.0	2	AGC	2/3	27.5	40.00	AAG
8 (F; 62)	12.33	44	294.00	72.87	–	–	3.6	1	AGC	1/3	**38.7**	1.20	AGC
9 (F; 69)	29.22	60	**126.00**	33.85	<1:40	0.70	**836.0**	3	AGC	2/3	14.9	24.10	AAG
10 (F; 48)	11.88	11	398.00	63.69	**1:1,280**	1.00	3.0	2	AGC	2/3	**79.9**	40.00	AAG
11 (F; 38)	**4.72**	**3**	**4.43**	–	<1:40	1.10	**394.8**	1	AGA	0/3	17.3	2.90	AAG
12 (F; 40)	13.63	33	162.00	21.19	**1:320**	0.90	3.0	3	AGC	2/3	7.0	40.00	AAG
13 (M; 51)	12.22	74	379.00	86.73	<1:40	0.70	3.0	1	AGC	0/0	**112.1**	40.00	AGC
14 (M; 53)	24.92	49	246.00	45.42	<1:40	0.70	3.0	2	AGC	1/3	**112.1**	40.00	AGC
15 (F; 69)	14.89	15	136.00	**17.81**	**1:1,280**	1.00	**1,000.0**	3	AGC	2/3	12.5	6.18	AAG
16 (F; 62)	26.32	60	**125.00**	27.46	<1:40	0.90	3.0	2	AGC	3/3	19.9	40.00	AAG
17 (M; 25)	**5.30**	**6**	329.00	77.05	<1:40	1.40	3.0	2	AGC	1/3	**91.3**	40.00	AGC
18 (F; 65)	20.48	20	153.00	**20.80**	–	–	**61.5**	3	AGC	2/3	**102.2**	32.90	AAG
19 (M; 37)	19.98	49	279.00	64.62	**1:2,560**	1.10	3.0	3	AGC	3/3	7.1	40.00	AAG
20 (F; 38)	10.91	21	278.00	–	**1:5,120**	1.00	3.2	2	AGC	3/3	**93.0**	40.00	AAG
21 (F; 76)	–	–	–	–	–	–	–	3	AGP	1/3	**156.1**	8.30	AGP

AAG: Autoimmune atrophic gastritis; AGC: Atrophic gastritis of the corpus; AGP: atrophic gastritis of both antrum and corpus; N, normal mucosa; ECL hyp: enterochromaffin-like cell hyperplasia; Fe: serum iron; G-17: gastrin-17; Holo-TC: holotranscobalamine (active vitamin B12); HpA: *Helicobacter pylori* IgG antibody; IFA: intrinsic factor antibody; OLGA: operative link to gastric atrophy; PCA: parietal cell antibody; Total B12: total vitamin B12; TPO Ab: thyroid peroxidase antibody; USS: Updated Sydney System. All abnormal test results are shown in bold. Reference values for parameters are shown in parentheses.

The titer of PCA is closely correlated with the severity of AGC and is inversely proportional to the density of PCs. During normal cell turnover, PCs release H^+/K^+ ATPase, which may result in its selective uptake and processing by antigen-presenting cells (68). It is the $CD4^+$ T-cells that recognize PC H^+/K^+ ATPase and facilitate AAG (64-68).

Alternatively, *H. pylori* infection may play an initiating role in the pathogenesis of AAG (62-65) by inducing autoreactive T-cells through gastric H^+/K^+ ATPase – *H. pylori* molecular mimicry at the T-cell level (64, 65). B-Cells produce autoantibodies to gastric H^+/K^+ ATPase and to their secretory product, IF, with help from activated $CD4^+$ T-cells (67). Finally, PC loss from the gastric mucosa may result from $CD4^+$ T-cell-initiated perforin-mediated cytotoxicity or Fas-FasL-induced apoptosis (65). Although the role of *H. pylori* as a trigger of AAG still needs additional confirmation (17), the role of this bacterium in the pathogenesis of AAG cannot be ruled out (62-68). The *H. pylori* -status in the GastroPanel® test is therefore not a reliable indicator for confirming or excluding the diagnosis of AAG. Of note, of the 15 confirmed AAG cases in the present cohort, four tested positive for *H. pylori* in the GastroPanel® test.

Thus, if in the present study, all AGC/AGP cases that remained *H. pylori*-negative in the GastroPanel® test had been defined as AAG without applying any of the other diagnostic tests for AAG, one would end up with a markedly biased underestimate of the AAG prevalence. Accordingly, of the 1,283 tested subjects, AGC and AGP comprised 46 cases (Table I). Of those 46 cases, 31 were classified as *H. pylori*-positive (using the 30 EIU cut-off), while 15 were *H. pylori*-negative. This would translate to an AAG prevalence of 15/1,283 (1.2%) (data not shown). The truth, however, is that of the 46 AG-positive subjects, 25 missed the invitation or refused further examinations, *i.e.*, were non-attenders in the second study round, causing substantial verification bias (*i.e.*, subjects with no confirmatory tests applied).

To estimate the true prevalence of AAG in the present cohort necessitates that the established numbers (15 AAG cases out of 21 subjects with complete verification) must be corrected for this verification bias. When this is done, by assuming that the same proportion of AGC/AGP cases in reality represent AAG among i) the 21 cases with full verification and ii) the 25 non-attendees, the true

prevalence of AAG would be 2.6% (33/1,283) for this cohort. As compared with the figures reported in the world literature, with AAG prevalence in the general population ranging between 0.1% and 1-2% (1-9), the prevalence in the present cohort is among the highest ever reported for asymptomatic, population-based cohorts. AAG prevalence rates of a similar scale (0.3-2.7%) have been reported only in studies where esophagogastroduodenoscopy was used as the screening tool for AAG (1, 3, 30, 32). This is a clear indication to support the use of the present strategy, where asymptomatic subjects are first screened by a non-invasive biomarker test (GastroPanel®) to reveal those potentially affected by AAG (*i.e.* all cases with on AGC/AGP biomarker profile), who should be further analyzed by gastroscopy and biopsies, as well as by the laboratory tests for AAG-specific biomarkers (PCA and IFA), AAG-related co-morbidities (iron deficiency and PA)(30-33), and other related autoimmune conditions (TPA) frequently accompanying AAG (1, 3, 20-24).

In the present series, a carefully selected panel of tests (in addition to gastroscopy) was used to confirm or exclude the presence of AAG among those who were diagnosed with AGC/AGP in the initial GastroPanel® screening (Table IV). As anticipated from recent studies (46-48), there was an extremely close correlation between the GastroPanel® results and gastroscopic examination, all AGC/AGP cases being confirmed by the macroscopic observations in gastroscopy. The same was true for the gastroscopic biopsies, except for one case where the biopsy did not find atrophy diagnosed in the GastroPanel® test and confirmed in gastroscopy (case 5, Table IV). The only feasible reason to explain this obvious discrepancy is the failure to correctly target the biopsy at the site of atrophy. As to the detection of high-titer PCA and IFA, these were present in 5/21 and 1/21 subjects. These results are in alignment with studies that examined PCA and IFA in different settings of patients with AA to the (21-24, 28, 29). Similar to the disappearance of Helicobacter in end-stage AGC (59-61), PCA and IFA can also disappear (or undergo decay) when their autoantigen targets (PCs) disappear in the severely atrophic mucosa. Indeed, this was the situation in the majority of the present cases, who represented advanced OLGA stages of gastric atrophy (Table IV).

In this series, the tests measuring AAG-associated co-morbidities as well as the coexistent autoimmune disorders proved to be highly informative. Altogether, 10 out of 21 patients were diagnosed with iron deficiency or PA, and in eight of these patients, evidence for AITD was provided by high-titer TPA. Given that all these 1,283 individuals represented otherwise healthy, asymptomatic adults, this whole cohort is an outstanding example of this disease; in the vast majority of cases, AAG is asymptomatic and diagnosed as an incidental finding among patients examined for other conditions, such as iron-deficiency anemia, PA or other autoimmune conditions (36). The present study also underlines the important role of serological biomarker screening by GastroPanel®, as this non-invasive diagnostic tool provides first-line evidence for potential AAG; having revealed the characteristic biomarker profile of AGC/AGP, the final diagnosis of AAG can easily be reached using specific laboratory tests.

Conclusion

According to the present screening of asymptomatic healthy adults by the non-invasive serological biomarker assay GastroPanel®, the unadjusted prevalence of AAG in the Russian Federation is 1.2% (15/1,283), representing a higher than average rate in the global statistics. Because of the verification bias due to 25 non-attendees for the reference test, the adjusted prevalence of AAG was 2.6% (33/1,283), which is among the highest reported figures globally, usually found in studies using endoscopic screening. Given that in the vast majority of cases, this condition is discovered as an incidental finding, this study confirms that a non-invasive serological biomarker test (GastroPanel®) is an optimal screening tool in the diagnostic protocol that leads to the final diagnosis of AG. On the basis of the present discovery of *H. pylori* in almost 30% of AAG cases, one cannot rule out the role of this bacterium as a trigger of AAG.

Conflicts of Interest

None declared.

Authors' Contributions

All Authors have met all the following four criteria: i) Substantial contributions to the conception or design of the work or the acquisition, analysis, or interpretation of data for the work. ii) Drafting of the work or revising it critically for important intellectual content. iii) Final approval of the version to be published. iv) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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