

-CONFIDENTIAL-

-GastroPanel test and Autoimmune Thyroid Disease-

**Biohit \*GastroPanel® test in Screening of the Patients with  
Autoimmune Thyroid Disease (AITD) for Autoimmune Atrophic Gastritis (AAG)**

**Jointly Executed by:**

Hospital X (City Y);  
Biohit Oyj (Helsinki)

**Research Team:**

A B, B C, C D, Lea Paloheimo, Panu Hendolin, Carita Eklund, Kari Syrjänen

---

\*The new **Unified GastroPanel®** test: ELISA biomarker test for pepsinogen I (P-PGI), pepsinogen II (P-PGII), gastrin-17 (P-G-17), and *H.pylori* IgG antibodies (P-HpAb).

## Summary

**Background:** Autoimmune atrophic gastritis (AAG) and pernicious anemia (PA) are common autoimmune disorders, being present in up to 2% of the general population. However, in patients with **autoimmune thyroid disease (AITD)** (Hashimoto thyroiditis and Grave's disease), the prevalence is 3- to 5-fold. AAG is characterized by: 1) atrophy of the corpus and fundus; 2) autoantibodies to the parietal cell (PC) and to the intrinsic factor (IF); 3) achlorhydria; 4) iron deficiency anemia; 5) hypergastrinemia; 6) vitamin-B12 deficiency leading to PA; 7) in up to 10% of the patients, AAG may predispose to gastric cancer (GC) or carcinoid tumour. In addition to autoimmune disease, *Helicobacter pylori* (HP)-infection is the other important causative agent of atrophic gastritis (AG), suspected to be **a trigger of AAG** as well.

The high prevalence of AAG in AITD patients, with all its potentially serious consequences provide **a strong rationale for i) screening** of these patients, ii) early diagnosis of AAG, as well as iii) their regular monitoring by gastroscopy. However, whether regular **gastroscopic** surveillance is needed for all patients with AAG/PA is still **controversial**. To obviate the need for this invasive diagnostic tool (gastroscopy) for monitoring these patients, Biohit Oyj (Helsinki, Finland) launched several years ago an ELISA-based assay designed to measure the concentrations of four stomach-specific biomarkers (Pepsinogen I, Pepsinogen II, Gastrin-17, *H. pylori* IgG antibodies) from a single blood sample. This GastroPanel® test is **the first non-invasive** diagnostic tool for stomach health (testing both the structure and function). The new-generation **Unified GastroPanel®** is a technically advanced version of this test, where the processing conditions of all biomarkers are uniform, making the test more versatile.

**Objective:** To conduct a systematic GastroPanel® screening of AITD patients to establish the **prevalence of AAG** and its associated risks (PA included). Another objective is to assess the utility of this non-invasive serum biomarker test as **a substitute to gastroscopy** in the **systematic monitoring** of the AITD patients for AAG and its severe clinical sequels.

**Study Design:** This study is a targeted screening trial for asymptomatic and symptomatic AITD patients. A cohort of patients with prevalent AITD (newly diagnosed cases excluded) are subjected to screening by the Unified GastroPanel® test. The result is classified as one of the optional diagnostic categories: 1) healthy stomach, 2) superficial HP-gastritis, 3) AG of the antrum; 4) AG of the corpus, and 5) AG of the antrum and corpus (pan-gastritis). AG patients testing HP-negative are likely to represent AAG. All patients with GastroPanel® result suggesting AG/AAG will be referred for gastroscopy and biopsy confirmation used as gold standard.

**Methods:** Study subjects (above 18 years of age, both genders) are enrolled among the AITD patients controlled in the outpatient department for endocrinology at Hospital X. All eligible patients who consent to participate will be invited for blood sampling for GastroPanel® testing, instructed to be compliant with the patient preparation. All blood samples will be stored at -70C until delivered for analysis in the laboratory of Biohit Oyj (Helsinki). On the occasion of blood sampling, all patients fill in questions from **validated questionnaires** exploring possible dyspeptic symptoms and potential sequels of AAG. Patients with suggested AG/AAG in the GastroPanel® test will be referred to gastroscopic examination, with directed gastric biopsies, following the protocol of the Updated Sydney System (USS). Statistical analyses include calculation of the performance indicators of the GastroPanel® test for individual study endpoints, including ROC analysis for the optimal sensitivity/specificity balance, using

moderate/severe AG/AAG as the endpoint.

**Specific Aims:** The main goal of this screening trial is to establish **the prevalence of AGG** among the patients with prevalent AITD. Related to the assessment of the validity of GastroPanel in systematic surveillance of AITD patients, three clinically relevant issues need to be addressed: 1) the **rate of unnecessary referrals** for gastroscopy (false positive rate; 1-PPV) following a positive GastroPanel® test; 2) the **rate of gastroscopies to be avoided** after a negative GastroPanel® examination (true negative rate; NPV), and 3) the rate of clinically **significant diseases** (conditions) that are **missed** by the GastroPanel® examination (i.e., false negative rate; 1-SE).

**Study execution and time table:** The study plan necessitates a review by the regional review board (Hospital X, Ethical Committee) before start. Given that the subjects in the study will be enrolled among AITD patients attending the outpatient department of Hospital X, it is estimated that the screening of a cohort of 500 patients will take approximately ? months. It is estimated that a cohort of 100-150 subjects (enriched by all grades of AG/AAG) should be enough to give this GastroPanel® study the necessary statistical power.

**Impact of the study:** The known high prevalence of AAG in AITD and its potentially severe clinical sequels advocate 1) screening, 2) early diagnosis, 3) regular surveillance, and 4) treatment. However, it is still **controversial**, whether **regular gastroscopic surveillance** is needed for AITD patients with AAG/PA. The present study will provide new insights i) in the true prevalence of AAG among the AITD patients, as well as ii) on the utility of the **non-invasive biomarker** test (GastroPanel®) as a substitute of gastroscopy in the systematic **surveillance** of AITD patients for AAG.

## 1. BACKGROUND

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, increasing with age (1,2,3). In patients with autoimmune thyroid disease (4, 5) and those with type 1 diabetes (DM1)(6, 7) or, the prevalence is 3- to 5-fold increased. AAG is characterized by atrophy of the corpus and fundus, and the presence of circulating autoantibodies to the parietal cell (PCA) and to their secretory product, intrinsic factor (IF) (8). Chronic auto-aggression to the gastric proton pump, H<sup>+</sup>/K<sup>+</sup>ATPase, may result in decreased gastric acid secretion, hypergastrinemia, and iron deficiency anemia (9, 10). In a later stage of the disease, PA may result from vitamin-B12 deficiency, which is significantly more common among AITD patients than in non-AITD patients (11). Finally, in up to 10% of these patients, AAG may predispose to gastric carcinoid tumors or adenocarcinomas (12-18). Determining the demographic, immunological, and genetic risk factors, as well as an early diagnosis of AAG are all very important to prevent and treat iron deficiency anemia, PA, and (pre)malignant gastric lesions: intestinal metaplasia (IM), enterochromaffin-like cell (ECL) hyperplasia, and dysplasia (7).

### 1.1. Definition of autoimmune thyroid disease (AITD)

There are two major clinical diseases associated with thyroid autoimmunity, **hypothyroidism** (Hashimoto thyroiditis) and **hyperthyroidism** (Grave's disease)(19). Two of the principal thyroid autoantigens in the former are **thyroid peroxidase** and **thyroglobulin**. In the latter, the principal autoantigen is **thyroid stimulating hormone receptor (TSHR)**. Thyroid autoantibodies can also reflect disease activity and progression and be valuable in disease prediction and classification (20). Accordingly, autoantibodies to these antigens rarely develop before 20 years of age, but they may presage subsequent clinical disease (primary hypothyroidism)(21). The probability of developing overt hypothyroidism within the next 20 years in women who are thyroid peroxidase antibody–negative with a thyroid stimulating hormone (TSH) less than 2 mU/l is less than 5% (22). That probability increases logarithmically to 55% when the TSH is greater than 6 mU/l in thyroid peroxidase antibody–positive subjects, an annual rate of progression of 4.3%, as compared with just 2.6% for those with only elevated TSH and 2.1% for those with only peroxidase autoantibodies (22).

Higher rates of progression have been found in men, who are 5 times more likely than women to progress to overt disease; in women aged 45 years or older; in patients with TSH levels

greater than 20 mU/l; and in patients with thyroid antibody titers greater than 1:100,000 (22, 23). About 10% of thyroid antibody-positive subjects in these studies subsequently became antibody-negative, and in some subjects mildly elevated TSH returned to normal. These observations in AITD mimic some of the features associated with DM1 autoimmunity (5,6,7), including the dual parameter model in which evidence of both autoimmunity and target organ failure are predictive of progression to clinical disease (20) and the risk of progression to disease is related to antibody titer and persistence. These findings are consistent with the broad spectrum of clinical consequences of AITDs.

### **1.2. Autoimmune thyroid disease (AITD) and autoimmune atrophic gastritis (AAG)**

The association of AITD and other autoimmune diseases was first suggested in the 1960's (5,24,25,26,27). More recently, the association between AITD and PA has been included in type IIIb polyglandular autoimmune disease (PGA)(28). A more systematic survey of the association of AITD with AAG was started more recently by Centanni et al. (4). They studied 62 patients with AITD for the presence of AAG by assaying serum gastrin levels. Patients with hypergastrinemia underwent gastroscopy followed by the histological examination of multiple biopsy specimens. Twenty-two (35%) of 62 patients had hypergastrinemia and the diagnosis of AG was histologically confirmed in all 22 patients (4). PCA were detected in 68% (15/22) of the patients, but anemia was observed in 82% (18/22) of the patients with AITD and AAG, but only in 22% (9/40) of the patients without AAG. The authors confirmed that in the patients with AITD, **one third had AAG**, which was diagnosed also in young patients (4).

Subsequently, this close association has been confirmed in several large clinical studies (29,30,31,32). In the 5-year follow-up study of Tozzoli et al. (29), at baseline, 51/208 (24.5%) AITD patients were positive for PCA and 10/208 (4.8%) for IFA. After 5 years, 6 (24%) of the 25 patients (consented to follow-up) showed a histologically proven AAG. During the follow-up, the PCA autoantibody levels rise progressively over time, reach a peak and then fall, in parallel with the progressive destruction of the gastric mucosa and disappearance of the target autoantigen (PP)(29). The presence of PCA predicts the development of AAG in AITD patients. Recently, Venerito et al. (30) made a case-control study in AITD patients to evaluate the usefulness of serum pepsinogens in the identification of AGC, and to determine the relationship of *Helicobacter pylori* with AAG, including 34 AITD patients and 30 controls. Serological (Gastro Panel+) AAG was present only in AITD patients (8/34, **23.5%**, OR 8.3, 95% CI=1.9-36.2),

confirmed by biopsies in all 8 cases (30). The authors made several important conclusions: 1) sero-prevalence of oxyntic (=corpus) gastric atrophy is high in patients with AITD; 2) **testing of serum pepsinogens (GastroPanel) should be included in the clinical assessment of these patients**; 3) *H. pylori* infection is unlikely to be a principal factor in the pathogenesis of AAG in AITD patients; and 4) in AAG, mucosal atrophy can spread from the corpus towards the antral mucosa (30).

In their comprehensive analysis, Castoro et al. (31) evaluated, in a consecutive series of patients followed-up for AITD, the prevalence of AAG during a 10-year period in one clinic. Altogether, 242 AITD patients underwent a screening including PCA, vitamin-B12, ferritin, iron, and hemoglobin testing, with gastroscopy confirmation of all PCA-positive cases. Altogether, 57/242 (**23.5 %**) patients tested PCA-positive. Of these, 33/57 (57.8 %), were affected by GD and 24/57 (42.1 %) by HT. These data also confirm the common association of AAG with AITD, not infrequently as a part of the PGA type III syndrome, which emphasizes the importance of regular monitoring of these patients (31). Even more impressive figures on AITD-AAG association were reported recently by Lahner et al. (32) in their cross-sectional study on 319 consecutive outpatients with atrophic corpus gastritis (AGC). Of these 319 AGC patients, 169 (**53%**) had an associated thyroid disorder, confirmed to be of AITD type in 128 patients (75.7%). AITD and AAG occur in a closely linked fashion (128/319, 40.1%), suggesting that AGC patients should be investigated for an occult AITD, in particular the women with positive PCA antibodies (32).

At present, this frequent **association of AITD with AAG is a generally accepted fact**, and presented as such also in recent textbooks (33).

### **1.3. Subsets of autoimmune thyroid disease (AITD)**

#### 1.3.1. Hashimoto's thyroiditis (HT)

Autoimmune thyroid diseases (AITDs) include many thyroid gland disorders, with different histological and clinical pictures ranging from the hypothyroidism of chronic lymphocytic thyroiditis to the hyperthyroidism of Graves' disease. Like other autoimmune diseases, also the chronic lymphocytic thyroiditis, also known as Hashimoto's thyroiditis (HT) according to the physician who first described this condition, derives from a combination of genetic susceptibility and some environmental trigger factors (33,34).

HT is more frequent in females (3.5/1000) than in males (0.8/1000) and global prevalence is increasing with age. HT is the most common cause of acquired hypothyroidism in children and adolescents and usually presents itself during early adolescence or among schoolchildren, with or without gout, with a prevalence of 1% among schoolchildren (35). Susceptibility to HT is determined by individual genetic background, including both major histocompatibility complex (MHC) and non-MHC genes. Associations have been reported between HT and HLA- DR3, HLADR4, or HLA-DR5. Furthermore, in children and adolescents paternal alleles and antibodies status have been shown to influence susceptibility to AITD. The expression of HLA-DR antigens on thyroid cells have a potential role in perpetuating the immune response, related to certain HLA-DR subtypes. As regards non-HLA susceptibility genes, several studies demonstrated the association between a polymorphism of the CTLA-4 gene and autoimmune thyroid disease (36).

HT is an organ-specific autoimmune disease, characterized histologically by a lymphocytic infiltration of the thyroid gland, initially characterized by hyperplasia and subsequently by infiltration of lymphocytes and plasma cells between follicles, then resulting in a follicle atrophy (33). Lymphocytic infiltration is composed of B lymphocytes, about 30%, and T-lymphocytes, about 60%, including CD4+ helper and CD 8+ suppressor. The process is exacerbated by the action of auto-antibodies directed against several thyroid antigens, like thyroid peroxidase antibodies (TPO-Abs), detectable in 90% of patients with HT. They inhibit enzyme activity and stimulate cytotoxicity by natural killer. Anti-thyroglobulin antibodies (TgA) are detectable in a small percentage of patients, while high levels of thyrotropin receptor-blocking antibodies are often present, particularly in patients who develop autoimmune hypothyroidism (33,34,35).

### 1.3.2. Graves disease (GD)

Graves' disease (GD) is the most important cause of **thyrotoxicosis** in pediatrics and affects about 0.02% in children and adolescents. Its frequency increases with age: it is rare before the four years, gradually rises, reaching a peak during adolescence, with a preponderance for female gender (37). In GD, the immune system develops antibodies that cause the thyroid to grow and make more thyroid hormone than the body needs. These thyroid-stimulating immunoglobulins (TSIs) bind to thyroid cell receptors and trick the thyroid into growing and producing too much thyroid hormone, leading to **hyperthyroidism**.

In GD, the main **autoantigen** is the thyroid stimulating hormone receptor (TSHR), which is expressed primarily in the thyroid but also in adipocytes, fibroblasts, bone cells, and a variety of additional sites including the heart (38). The TSHR is a G-protein coupled receptor with 7 transmembrane-spanning domains. TSH, acting via the TSHR, regulates thyroid growth and thyroid hormone production and secretion. The TSHR undergoes complex post-translational processing involving dimerization and intramolecular cleavage; the latter modification leaves a 2-subunit structural form of the receptor which eventually undergoes degradation or shedding of the ecto-domain (39). Each of these post-translational events may influence the antigenicity of the receptor and, furthermore, this complex processing may contribute to a break in self-tolerance. For example, the affinity of TSHR antibodies for the TSHR ecto-domain is greater than for the holo-receptor itself.

One of the unique characteristics of GD, not found in normal individuals or in the rest of the animal kingdom, is the **presence of TSHR antibodies** (TSHR-Abs) easily detectable in the vast majority of patients (39,40). In patients with GD, as for other antigens in other autoimmune diseases, TSHR-reactive B-cells survive deletion and can potentially present thyroid autoantigen to T cells inducing pro-inflammatory cytokines (41). Hence both B-cells and T-cells play a central role not only in producing TSHR-antibodies but also in mediating chronic inflammatory changes of the disease seen in the thyroid gland, in the retro-orbit and in the skin (39,40,41). A common view of GD is that TSHR-Abs promote the disease by enhancing thyroid antigen expression. Stimulating TSHR-Abs are certainly capable of this role and may interact directly with the immune system including stimulation of maturing thymocytes (42). However, T-cell activation and subsequent thyroid infiltration in GD patients are not just the result of direct autoantibody-induced mechanisms. In fact, GD appears to develop on a background of concurrent autoimmune thyroiditis. Therefore, the observations supporting the induction of apoptosis by cleavage TSHR-Abs suggests that such antibodies may be active very early in the disease and may serve to perpetuate the disorder once established. Indeed TSHR-Abs can even be detected in a small number of patients with HT as well (41).

## **2. DEFINITION OF AUTOIMMUNE ATROPHIC GASTRITIS (AAG)**

AAG affects the parietal cell-containing gastric corpus and fundus with sparing of the antrum (8,43). PCA, targeted against gastric H<sup>+</sup>/K<sup>+</sup> ATPase, are detected in 60–85% and IF antibodies in 30–50% of patients with AAG (7,44). Besides PA, iron deficiency anemia is frequently

observed (9,10). Furthermore, AAG is characterized by hypo- or achlorhydria, high serum gastrin, and low pepsinogen I concentrations (45,46). Chronic hypergastrinemia causes the ECL cells in the oxyntic mucosa to undergo hyperplasia (47), which may progress toward dysplasia and gastric carcinoid tumors (12,46).

PCA are detected by immunofluorescence staining of the cytoplasm of gastric parietal cells (48). However, Karlsson et al. (49) showed that the ELISA to detect gastric H<sup>+</sup>/K<sup>+</sup> ATPase antibodies is 10-fold more sensitive than the indirect immunofluorescence technique and has a high specificity. Current ELISAs have a sensitivity and specificity of respectively 85–93% and 80–85%. PCA are detected 60–90% of the patients with AAG and/or PA (1,8,48).

The recognition of antibodies to IF (AIF) derives from the work of Taylor et al. (50) and Schwartz (16). Two types of autoantibodies bind to IF. Type I AIFs block the binding of vitamin-B12 to IF, thereby preventing the transport of vitamin-B12 from the stomach to its absorption site in the terminal ileum. Type I AIFs are demonstrable in 70% of the patients with PA (49). Type II AIFs do not interfere with vitamin-B12 transport. They can be found in 30–40% of patients with PA.

The destruction of H<sup>+</sup>/K<sup>+</sup>ATPase-containing parietal cells results in hypo- or achlorhydria. This can be measured using 24-h gastric pH-metry or after stimulation with pentagastrin. Hypochlorhydria is defined as a maximal acid output less than 15 mmol H<sup>+</sup>/h after injection of pentagastrin. A progressive decrease in acid secretion in the case of AAG with a decreased parietal cell mass has been found (46,51,52). Total achlorhydria is diagnostic of PA. Achlorhydria interrupts the negative feedback of somatostatin on antral gastrin-producing G cells causing hypergastrinemia (53). Fasting serum gastrin (G-17) levels correlate negatively with peak acid output, and positively with the degree of corpus atrophy (46,54) and with PCA levels (26). Low serum pepsinogen I levels, resulting from destruction of chief cells or zymogenic cells, are also characteristic to AAG (45,55,56).

## **2.1. Endoscopy and pathology of AAG**

On endoscopy, the affected corpus and fundus mucosa appears shiny and red because of the visibility of submucosal blood vessels. The stomach wall is thinned, and the rugal folds flatten or disappear. In biopsy specimens, lymphocytic infiltrates are present in the submucosa and

lamina propria (44,46). In the next stage, there is a marked reduction in the number of oxyntic glands, parietal and zymogenic cells, followed by replacement of normal glands by glandular structures lined with mucus-containing cells resembling those of the small bowel mucosa (intestinal metaplasia, IM). A proliferation of ECL cells in the oxyntic mucosa (47), due to sustained hypergastrinemia, can be seen, which may progress in a small proportion of patients toward carcinoid tumors (12,57,58,59).

## 2.2. Epidemiology of AAG

In the general population, there is an age-related increase in the prevalence of parietal cell antibodies (PCA), from 2.5% in the third decade to 12% in the eighth decade (1,2). The prevalence is even higher in subjects affected by another autoimmune disorder. In DM1, PCAs are found in 10–15% of the children and in 15–25% of the adults (4,5,60,61,62). The respective prevalence of AAG and PA in the general population are 2% and 0.15–1% (2,3,63,64), compared with 5–10% and 2.6–4%, respectively, in DM1 patients (7,26,63,65,66). In **patients with AITD**, AAG is concomitantly detected up to one third of the patients (29,30,31,32,33). Iron deficiency anemia is present in 20–40% of patients with AAG (10, 67), whereas PA can be diagnosed in up to 15–25% of the patients (68). The progression of AAG to PA is likely to span 20–30 years (69).

Finally, gastric carcinoid tumors are observed in 4–9% of the patients with AAG/PA, which is 13 times more frequent than in controls (12,57,58,59,70). Patients with AAG/PA also have a 3- to 6-fold increased risk of GC, ranging from 0.9–9% (12,57,59,71,72,73).

## 2.3. Pathogenesis of AAG

The target autoantigens in AAG are the 100-kd catalytic  $\alpha$ -subunit and the 60- to 90-kd glycoprotein  $\beta$ -subunit of the gastric H<sup>+</sup>/K<sup>+</sup>ATPase (74,75). **Autoantibodies to parietal cells (PCA)** and to their secretory product, IF, are present in the serum and in gastric juice. The titer of PCAs correlates with the severity of corpus atrophy and is inversely proportional to the concentration of parietal cells (26,54). CD4<sup>+</sup>T-cells recognizing parietal cell H<sup>+</sup>/K<sup>+</sup> ATPase mediate autoimmune gastritis. During normal cell turnover, PCs release H<sup>+</sup>/K<sup>+</sup> ATPase, which may result in its selective uptake and processing by antigen-presenting cells (76).

Alternatively, *Helicobacter pylori* (HP)-infection may play **an initiating role** in the pathogenesis of AAG and PA (77,78,79,80) by inducing autoreactive T-cells through gastric

H<sup>+</sup>/K<sup>+</sup> ATPase-HP molecular mimicry at the T-cell level (78,79), epitope spreading, and bystander activation. B-cells produce autoantibodies to gastric H<sup>+</sup>/K<sup>+</sup> ATPase and to their secretory product, IF, with help from activated CD4<sup>+</sup>T-cells (75). Finally, PC loss from the gastric mucosa may result from CD4<sup>+</sup> T-cells initiated perforin-mediated cytotoxicity or Fas-FasL apoptosis (80).

Regardless of whether PCAs are pathogenic or not, their presence provides a convenient diagnostic probe for AAG. A precise understanding of the pathogenesis of autoimmunity may lead to rational therapeutic strategies directed toward restoration of tolerance or impeding the progression of autoimmunity. Whether HP could trigger AAG or not remains controversial (7). However, should this be the case, HP eradication might be able to prevent AAG. Currently, it is recommended that HP-infection should be tested and treated in patients with gastric atrophy, intestinal metaplasia/dysplasia, and hypo- or achlorhydria (7).

#### **2.4. Demographic factors**

Advancing age is a risk factor that has been associated with PCA positivity (7). In the general population, PCA positivity increases from 2.5% in the third decade to 12% in the eighth decade (1,2). In AITD patients, PCA are present in 10–15% of the children and 15–25% of the adults (66). Some authors (6,60) report a female preponderance for PCA positivity, although this has not been consistently confirmed in other studies (62,66).

#### **2.5. Endocrine factors**

AAG is frequently accompanied by other autoimmune diseases, including DM1 (7) and autoimmune thyroid disease (Hashimoto's thyroiditis and Graves' disease) (4,63,81). AAG is also part of the **autoimmune polyglandular syndrome** type III (PGAII)(28,82). PA occurs in up to 2–12% of patients with AITD (4,83), 6% of those with Addison's disease, 9% of those with primary hypoparathyroidism, and 3–8% of those with vitiligo (1,7).

In patients with DM1, immunological risk factors that have been associated with PCA positivity include persistent islet cell antibody positivity (60,61), glutamic acid decarboxylase-65 antibody positivity (66,84), and thyroid peroxidase autoantibody positivity (66,84). The association with glutamic acid decarboxylase-65 antibodies might be explained by the fact that glutamate decarboxylase-65 is not only present in the pancreas and brain but can also be found

**in the thyroid gland** and stomach. PCA are more frequent in DM1 patients than in their first-degree relatives, even after HLA matching, suggesting that the diabetic condition itself plays an important role (85).

## 2.6. Environmental factors

*Helicobacter pylori* (HP) might be implicated in the induction of AAG (77,78,80). This hypothesis is supported by studies reporting a high prevalence of HP-seropositivity and a low prevalence of positive HP-staining in subjects with atrophic corpus gastritis (86,87,88,89). Furthermore, the finding of gastric autoantibodies in 20–50% of HP-infected patients and reports of a positive correlation between gastric autoantibodies and antibodies to HP in patients with AAG/PA (88,90,91,92,93) suggest that chronic HP-infection is linked with gastric autoimmunity. However, a correlation between HP and PCAs has not been reported in all studies (64,94,95). Moreover, others found no or a negative link between HP and atrophic corpus gastritis (96). On the other hand, HP eradication in patients who have antigastric antibodies leads to the loss of those antibodies in some subjects (97). These data revisit the concept of possible reversibility of gastric mucosa atrophy (7).

## 2.7. Monitoring the patients at risk for AAG and its sequels

AG/AAG is the single most powerful independent risk factor for distal (non-cardia) GC (98,99,100,101). It is estimated that 50% of all GC cases develop through the “Correa cascade” (99,102,103,104), leading from HP-associated- or autoimmune gastritis to mucosal atrophy, intestinal metaplasia, dysplasia, and to invasive adenocarcinoma. There are some implications that early eradication of HP-infection can slow down or even revert this cascade (105,106). Because this process takes several decades, there should be good prospects for early detection of precancerous lesions (107), but the problem is lack of a suitable test for GC screening (108). Furthermore, most of the patients report only a short period of symptoms before the diagnosis of GC, and up to 40% report no dyspeptic symptoms at all (109).

It is **controversial** whether patients with AAG/PA should be placed under a surveillance program with **regular gastroscopies**, including multiple gastric biopsies (7). The gastric carcinoids that occur in these patients generally do not pose a great threat to life, whereas the risk of developing GC is real. Nevertheless, in many clinics, endoscopy with biopsies remains the gold standard diagnostic tool, disclosing HP-infection, mucosal atrophy, intestinal

metaplasia or dysplasia, and their topography (98,110) However, this invasive method is uncomfortable, distressing and quite costly, emphasizing the need for rapid, reliable and inexpensive non-invasive tests for screening and monitoring these patients(108,109,111).

### 2.7.1. Non-invasive diagnostic tools for monitoring

Such non-invasive methods providing information on the structure and function of gastric mucosa were introduced in the early 1980's, when Miki et al. (112) and Samloff et al. (113) developed assays measuring pepsinogen (PG) concentrations in the blood. The latest development in this field represents the panel combining serum pepsinogen I (PGI) and II (PGII), gastrin-17 (G-17) and HP IgG antibodies (IgG-HP) using ELISA technique (GastroPanel® test, Biohit Oyj, Helsinki), proposed as the first-line diagnostic test for dyspeptic symptoms (114,115,116). According to a recent meta-analysis, serum PGs are not suitable for GC screening, however, but they proved to be useful for detecting the patients at risk for GC (117). Recently, these stomach-specific biomarkers were recommended by an authoritative international group of experts for diagnosis and screening of AG (118).

The GastroPanel test is based on combined analysis of PG-I, PG-II, amidated G-17 and HP-antibodies, designed to give information on both the structure and function of the stomach mucosa. Most importantly, this panel gives accurate estimates of the capacity of the corpus and antrum mucosa to secrete acid and G-17, respectively, as well as of important gastric pathologies like inflammation, grade and topography of AG (116,119,120,121). Normal plasma levels of these biomarkers indicate that the stomach mucosa has normal structure and function, whereas the abnormal levels are signs of a non-healthy stomach, reflecting the disturbances in the feedback mechanisms between the acid output, PGs and G-17 (122).

GastroPanel test has been on the market for several years by now, and during this time, it has been validated in clinical studies in Finland and elsewhere (123,124,125,126). Due to the inherent characteristics of the natural history of AG/AAG, the PGI values (and PGI/PGII ratio) remain within normal range as long as AG of the corpus is graded only as mild by the USS. However, mild AG/AAG is a poorly reproducible diagnostic category even among experienced pathologists, and because of this, mild AG of the corpus should never be used as the study endpoint in calculating the performance indicators of the PGI, PGI/PGII, as repeatedly emphasized (125,126,127). The only appropriate way of calculating the predictive indicators

of PGI and PGI/PGII ratio for AG of the corpus is to use the combined moderate/severe AG as the study endpoint. Using this approach in an adequately powered study with validated USS classification gives ROC (AUC) values above 0.970 for PGI and >0.950 for the PGI/PGII ratio as predictors of moderate/severe AG of the corpus (125,127).

### **3. THE GASTROPANEL® TEST**

The GastroPanel® is a user-friendly ELISA technique, intended both for research purposes and for clinical practice. The GastroPanel® test contains four biomarkers specific for the stomach mucosa: 1) Pepsinogen I (P-PGI), 2) Pepsinogen II (P-PGII), 3) Gastrin-17 (P-G-17) and 4) H. pylori antibody (P-HpAb) (114,115,116)([www.gastropanel.com](http://www.gastropanel.com)).

#### **3.1. ELISA test for Pepsinogen I and Pepsinogen II**

P-PGI is secreted solely by the chief cells (chief cell/mucous neck cells) of the corpus mucosa. AG of the corpus leads to loss of these cells and, as a result, the P-PGI level in circulation decreases. P-PGII is produced by the chief cells and mucous neck cells of the gastric mucosa, by pyloric glands in the gastric antrum and by Brunner's glands in the proximal duodenum. The ratio of PGI to PGII concentration in the plasma of normal subjects is above 3.0.

#### **3.2. ELISA test for Gastrin-17**

The P-G-17 ELISA method in the GastroPanel® is specific to amidated G-17 molecule. G-17 peptide is the most important member of the gastrin/cholecystokinin-family which regulates the physiology of the upper gastrointestinal tract. This peptide is the biologically most active gastrin peptide, stimulating gastric acid secretion with 6-times higher potency than the biologically next most active gastrin, G-34. The G-17 ELISA in GastroPanel® assay allows estimation of the number and function of antral G-cells, without background noise and cross-reactivity with other gastrin fragments. G-17 is secreted exclusively by the gastrin-cells (G-cells) in the antrum, representing a fraction of the total gastrin concentration in the circulation. When dormant, the G-cells in antrum secrete only small amounts of G-17 hormone. The maximal secretion is achieved after physiological protein stimulation, or when the acid secretion in the stomach decreases, is low or absent ([www.gastropanel.com](http://www.gastropanel.com)/GastroPanel Sample Collection Instructions: Stimulated Gastrin-17s). As a result of antral atrophy (i.e., loss of glands), the amount of G-cells decreases and, consequently, both the basal and post-prandial secretion of G-17 will decrease.

### 3.3. ELISA test for *Helicobacter pylori* (HpAb ELISA)

The *H. pylori* infection is the most important cause of chronic gastritis. Another well-known cause for severe AG is the autoimmune mechanism, which can also be activated by *H. pylori* infection (77,78,79,80). GastroPanel® test for *H. pylori* is performed from the plasma samples. The test is based on an ELISA technique, with purified *H. pylori* bacterial antigen, adsorbed on a microplate, and a detection antibody labeled with horseradish peroxidase (HRP).

### 3.4. The new unified GastroPanel® test

Prompted by the design of the original GastroPanel itself, where all four biomarkers are being processed under different laboratory (incubation) conditions, Biohit R&D Department started a project towards unified GastroPanel® test in 2013. The concept was to develop a new assay, where all four markers are being processed under the same conditions using an automatic ELISA instrument or manual processing. The new Unified GastroPanel® test contains the same four markers as the current version (1.1), maintaining its basic design as an ELISA test. Because of the crucial modifications in the key test components, the unified test will be treated as a novel test by the registration authorities. Because of this fact, the new version must undergo all the necessary steps needed for CE registration, including clinical validation in a cohort of study subjects with relevant gastric pathologies.

## 4. STUDY DESIGN

The present study is designed as a targeted screening of patients with prevalent AITD, with or without signs of other autoimmune disease (e.g., PGAIII), enrolled from the outpatient clinic of the Hospital X. The cohort is screened by the Unified GastroPanel® test distinguishing 5 diagnostic categories: 1) healthy stomach, 2) HP-gastritis, 3) AG of the antrum, 4) AG of the corpus and 5) AG of both antrum and corpus. The test performance indicators will be calculated using moderate/severe AG/AAG as the endpoint, separately for markers of the antrum (G-17) and corpus (PGI, PGI/PGII ratio), respectively. As an additional endpoint, down-stream in the path to AG, is the detection of *H. pylori* infection in the antrum or in corpus.

### 4.1. Aims of the study

With the aid of a target screening, to establish the **true prevalence of AAG** among the patients with prevalent AITD, irrespective whether symptomatic or asymptomatic. Among AITD

patients, AAG can develop as a part of PGAIII syndrome or as a solitary manifestation of their autoimmune disease. Disclosing AAG as early as possible is important to be able to adopt adequate surveillance measures to prevent the serious clinical sequels of AAG in these patients.

Another aim is to demonstrate whether the currently **validated cut-off values** for the four biomarkers of GastroPanel (123,124,125,126,127) are applicable also for the atrophic gastritis developed through an autoimmune mechanism (AAG), apart from HP-associated AG. Given that GastroPanel® is a quantitative test, ROC analysis can be used to estimate the best SE/SP balance for each single marker against the relevant endpoint to adjust the optimal clinical performance of the new GastroPanel® test.

Related to the applicability of GastroPanel test in the **systematic surveillance** of AITD patients, three clinically relevant issues will be addressed in this **biopsy-confirmed** study: 1) the rate of unnecessary referrals for gastroscopy (false positive rate; 1-PPV) following a positive GastroPanel® test; 2) the rate of gastroscopies to be avoided after a negative GastroPanel® examination (true negative rate; NPV), and 3) the proportion of clinically significant diseases (conditions) that are missed by the GastroPanel® examination (i.e., false negative rate; 1-SE).

#### **4.2. Patients**

This targeted trial is conducted in collaboration between Hospital X (City Y) and the Clinical Research Department of Biohit Oyj (Helsinki). The clinical arm is performed exclusively by the Hospital, while the laboratory analyses will be done in the service laboratory of Biohit Oyj.

Enrolment of the patients in the study will take place exclusively at Hospital X, including consecutive patients over 18 years of age, attending their regular monitoring visit for AITD at the outpatient department (ambulatory). The eligible patients can be asymptomatic or symptomatic (=dyspepsia), all having prevalent AITD diagnosed years before. The estimated cohort to be screened for GastroPanel is at least 500 subjects (both genders), to reach a cohort of 100-150 patents enriched with roughly **equal numbers of the relevant study endpoints** (different grades of AAG).

Patient enrollment is taking place in a single step. In brief, the potentially eligible patients are identified among the AITD outpatients by the members of the research team. At this stage, every

patient will be asked to consent the study and sign a written consent to participate. All consented patients will be interviewed using previously validated questionnaires (by the nurse administrator). They will be scheduled for an appointment to GastroPanel testing at the laboratory, to ensure compliance with the preparatory steps needed before the GastroPanel® sampling. Most importantly, apart from the recommended 10h fasting (overnight), the use of PPI-medication should be discontinued preferably one week before GastroPanel® sampling. If not possible, a notice of that must be included in the test request form.

Eligible patients are all adults aging 18 years and above, irrespective whether symptomatic or asymptomatic as to their upper gastrointestinal tract. However, the following patients should be considered **non-eligible**: 1) the patients whose treatment requires surgery, or immediate follow-up treatment for major symptoms, as well as 2) those who refuse to sign the written consent.

#### 4.2.1. Patient preparation

Proper conduction of and reliable results from the GastroPanel® examination necessitate some preparatory measures of the patient. Detailed instructions are usually given to each test subject at the time of his/her consenting to participate, but this does not apply here, because all subjects already complete the preparation for gastroscopy. Their compliance with the taking of medicines listed below will be controlled before taking the blood sample.

The patient should not drink, eat or smoke for at least 4 hours before the sample collection, e.g., 10-hour fasting overnight is perfect. The patients are allowed to take their prescribed, regular medication. However, it is necessary to report any use of proton pump inhibitors (PPIs, such as Esomeprazole, Lanzoprazole, Omeprazole and Rabeprazole), and the time of discontinuation in PPI use) on the Request Form, because these medicines interfere with the output of GastroPanel® biomarkers ([www.gastropanel.com](http://www.gastropanel.com)/GastroPanel® Sample collection Instructions).

### **4.3. Methods**

#### 4.3.1. Questionnaire of symptoms

GI-symptom questionnaire on i) functional dyspepsia and IBS according to the Rome III criteria,

and on ii) reflux disease symptoms according to the Montreal classification will be completed prior to blood sampling (128).

#### 4.3.2. Sample collection for GastroPanel® test

The person taking the blood sample shall fill the TEST REQUIST FORM as complete as possible. For each patient, 1 EDTA tube (4ml tube) for ancestry determination and 2 plasma tubes for GastroPanel®, will be taken. Plasma sample for GastroPanel® examination is taken after fasting (see above). Additionally, Gastrin-17 can be determined also after stimulation (see below). A minimum of 2 ml EDTA plasma from a fasting blood sample is taken into an EDTA tube (e.g. Biohit Cat. no. 454235 Vacuette 4ml tube containing K2EDTA). Use of Gastrin-17 stabilizer 100µl/2ml plasma (Biohit Cat. No. 601 050 or 601 051) allows the sample transfer at room temperature (20-25°C), and permits the ELISA tests within 4 days from the sample collection.

#### 4.3.3. Sample processing

The blood sample needs to be centrifuged within 30 minutes, at 1800-2000 g for 10 minutes (e.g. Vacuette, Biohit Cat. no. 454235) or as prescribed by tube manufacturer or centrifuge manufacturer (e.g. StatsSpin Express 2, at 4000 g for 2 minutes). Unless immediately used for testing, the EDTA plasma needs to be frozen instantly (-70°C). Preferable storage temperature of the sample with the Gastrin-17 stabilizer is in the refrigerator at 2-8°C, for up to 4 days. If the sample cannot be analysed within 4 days, it should be stored frozen at -15 to -20°C, but for any storage of over 2 weeks, the temperature should be -70°C.

The samples should be mixed thoroughly after thawing. Multiple freezing and thawing cycles should be avoided. Lipemic or cloudy specimens must not be used. If a postprandial blood sample is needed, it should be taken into an EDTA tube after 20 minutes upon the intake of the protein drink. For further details, refer to the section describing Gastrin-17 stimulation (see [http://www.biohithealthcare.com/GastroPanel Sample Collection Instructions](http://www.biohithealthcare.com/GastroPanel%20Sample%20Collection%20Instructions) and below).

#### 4.3.4. Stimulated G-17

If basal Gastrin-17b concentration is low (below 1.0 pmol/l) and the patient has no H.pylori infection (antibodies below 30 EIU, with no eradication history), the result suggests high acid

output (with no AG in the antrum). In this case, there is no need for testing of Gastrin-17s.

If GastroPanel reveals *H. pylori* infection (antibodies over 30 EIU) and low Gastrin-17b (below 1.0 pmol/l), the result can indicate either high acid output of the corpus or AG in the antrum. Distinction between these two conditions is clinically relevant by measuring protein drink-stimulated Gastrin-17s. If stimulated Gastrin-17s concentration is over 3.0 pmol/l, AG in the antrum is excluded. If, however, Gastrin-17s remains below 3.0 pmol/l after protein stimulation, AG in the antrum is likely, advocating further examinations, e.g., gastroscopy.

The secretion of G-17 can be stimulated by the intake of a protein drink having average protein content of 77% [Biohit Cat. No. 601038 (50x20 g), Cat. No. 601037 (5x20 g)]. This stimulation should not be performed for patients who are sensitive to lactose (i.e., lactose intolerance or hypolactasia). To prepare the protein juice, 20 g of protein (one foil bag of protein powder) is mixed to 150 ml of water. The stimulated (post-prandial) blood sample must be taken 20 minutes after the intake of the protein juice ([www.gastropanel.com](http://www.gastropanel.com)/GastroPanel Sample Collection Instructions: Stimulated Gastrin-17s).

#### 4.3.5. Evaluation of GastroPanel® results

The results of the GastroPanel® test are evaluated using the GastroSoft® interpretation software. GastroPanel test is optimized for the Updated Sydney System (USS) for classification of gastritis, both including 5 diagnostic categories (see above for study endpoints). The test results will be delivered online to Hospital X, where the results are linked with the other clinical data of the patients, including the results of (upcoming) gastroscopy and biopsy histology.

#### **4.4. Gastroscopy and biopsy procedures**

In this screening study, all patients are examined with the GastroPanel® test first. Only those who test GastroPanel-positive, i.e., **the result is classified as AG/AAG**, will be subjected to gastroscopy. This provides the histological confirmation to be used as the gold standard in calculating the performance indicators for the test. Gastroscopy referrals will be made to the outpatient department of Gastroenterology, Hospital X (City Y). It is important that also the taking of gastric biopsies follows the same USS system, including biopsy sampling from the antrum and corpus as specified by USS (120,125,129). In endoscopy, all observed abnormal mucosal lesions are noted and photographed, and if necessary (e.g. suspicion of malignancy) subjected to additional biopsy. Endoscopic findings from the esophagus, stomach, duodenal

bulb and the second part of the duodenum will be recorded according to a predefined protocol to improve consistency between the endoscopists. At the time of endoscopy, the endoscopists will be blinded to the questionnaires. The future management and surveillance of the patients will be arranged according to the normal practices of the clinic.

#### 4.4.1. Biopsy protocols

The optimal use of the USS system necessitates that the biopsy protocol follows an agreed systematic. In each patient, routine biopsy specimens are taken from the antrum and corpus, at least two biopsies from each. These biopsies are taken from the large and small curvature of the middle antrum (biopsies 1 and 4) and from the large curvature of the corpus (biopsies 5 and 6). In addition, two extra biopsies are recommended to be taken from the incisura angularis (biopsies 2 and 3). Importantly, to facilitate the pathology reading, the biopsies from the antrum and incisura (Biopsies 1, 2, 3 and 4) must be immersed into one and the same formalin bottle, and embedded into the same paraffin block (Block No. 1; labeled **ANTRUM**). The two biopsies from the corpus are set into one and the same formalin tube, and embedded into the same paraffin block (Block No. 2; labeled **CORPUS**). In addition, biopsies from the bulb and the second part of the duodenum, distal and mid esophagus will be taken.

#### 4.4.2. Interpretation of the biopsies

All gastroscopy biopsies are examined by the expert pathologists as part of their daily routine at the Department of Pathology, Hospital X. The diagnoses are reported using the USS for classification of gastritis, and diagnosed into different “phenotypes” of gastritis (120,121,122,125,129).

### **4.5. Statistical analyses**

All statistical analyses will be performed using the SPSS 25.0.0.1 for Windows (IBM, NY, USA) and STATA/SE 15.1 software (STATA Corp., Texas, USA). The descriptive statistics will be done according to routine procedures. Performance indicators (sensitivity, specificity, positive predictive value, PPV, negative predictive value, NPV and their 95%CI) of individual markers and whole GastroPanel® test will be calculated separately for each study endpoint, using the STATA/SE software and the *diagti* algorithm introduced by Seed et al. (2001)(130). This algorithm also calculates the area under ROC (Receiver Operating Characteristics) called AUC, for each biomarker at each endpoint. Because GastroPanel® is a quantitative ELISA test, these

ROC curves can be used to identify the optimal sensitivity/specificity balance that gives each biomarker an optimal threshold for detection of each study endpoint. Significance of the difference between AUC values can be estimated using STATA's roccomb test with 95%CI.

## 5. ETHICAL ISSUES

The study design and its execution does not involve any significant ethical issues except those in other clinical studies of similar type. The study protocol will be submitted for approval to the Regional Ethical Committee (Hospital X), and the study is conducted in accordance with the Declaration of Helsinki.

Patients are enrolled among consecutive AITD patients attending the outpatient department of Hospital X for the scheduled appointment to control their disease. Thus, they represent regular AITD outpatients controlled in the hospital as part of their routine clinical surveillance for AITD. The only additional procedures carried out to the patients include filling of questionnaires and the blood sampling. The maximum amount of venous blood taken is 10 ml. All patients must sign the informed consent for their participation. When the result of the GastroPanel® test is available, clinical judgement is used to make the referral for gastroscopy. Based on existing clinical practice, however, any patient with suspected AG/AAG needs gastroscopic confirmation of the disease severity (7,129). In this respect, the study protocol does not include any diagnostic procedure additional to the existing clinical practices. Once all results are available, the patients will be informed about the results, following the usual clinical practices, including an explanation of the test results and the appropriate measures for further conduct.

## 6. TIME FRAME

Given that the subjects in the study will be enrolled among consecutive AITD patients with estimated 10-15% prevalence of AAG/PA, attending the ambulatory of Hospital X, (with attendance rate of **X? patients/week**), and a cohort of patients enriched with sufficient numbers of all study endpoints will be needed, it is estimated that GastroPanel screening of a minimum of 500 subjects will take **approximately ? months** of clinical work. The laboratory arm of this study is expected to proceed online with the progress of patient enrollment and gastroscopies. Despite a minor delay (of days) due to the biopsy examination by the pathologists, the full database of the patients will be ready for statistical analysis practically on

real-time after completion of the enrollment of the cohort and examination of their blood and biopsy samples.

#### **7. PROJECTED COSTS TO BE COVERED by Biohit Oyj**

The company will compensate the extra effort put in the project by Hospital X. The details are subject to discussions and contractual agreement.

## REFERENCES

1. Whittingham S, Mackay IR. Pernicious anemia and gastric atrophy. In: Rose NR, Mackay IR, eds. *The autoimmune diseases*. New York: Academic Press; 1985; 243–266.
2. Jacobson DL, Gange SJ, Rose NR, Graham NM. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin Immunol Immunopathol* 1997; 84:223–243.
3. Carmel R. Prevalence of undiagnosed pernicious anemia in the elderly. *Arch Intern Med* 1996;156:1097–1100.
4. Centanni M, Marignani M, Gargano L, Corleto VD, Casini A, Delle Fave G, Andreoli M, Annibale B. Atrophic body gastritis in patients with autoimmune thyroid disease. An underdiagnosed association. *Arch Intern Med* 1999;159:1726–1730.
5. Irvine WJ, Scarth L, Clarke BF, Cullen R, Duncan LJP. Thyroid and gastric autoimmunity in patients with diabetes mellitus. *Lancet* 1970;2:163–168.
6. Riley WJ, Toskes PP, Maclaren NK, Silverstein J. Predictive value of gastric parietal cell autoantibodies as a marker for gastric and hematologic abnormalities associated with insulin dependent diabetes. *Diabetes* 1982;31:1051–1055.
7. De Block CEM, De Leeuw IH, Van Gaal LF. Autoimmune Gastritis in Type 1 Diabetes: A Clinically Oriented Review. *J Clin Endocrinol Metab* 2008;93:363–371.
8. Strickland RG, Mackay I. A reappraisal of the nature and significance of chronic atrophic gastritis. *Am J Dig Dis* 1973;18:426–440.
9. Marignani M, Delle Fave G, Mecarocci S, Bordi C, Angeletti S, D'Ambra G, Aprile MMR, Corleto VD, Monarca B, Annibale B. High prevalence of atrophic body gastritis in patients with unexplained microcytic and macrocytic anemia. *Am J Gastroenterol* 1999;94:766–772.
10. De Block CE, Van Campenhout CM, De Leeuw IH, Keenoy BM, Martin M, Van Hoof V, Van Gaal LF. Soluble transferrin receptor level: a new marker of iron deficiency anemia, a common manifestation of gastric autoimmunity in type 1 diabetes. *Diabetes Care* 2000;23:1384–1388.
11. Collins AB, Pawlak R. Prevalence of vitamin B-12 deficiency among patients with thyroid dysfunction. *Asia Pac J Clin Nutr* 2016;25:221–226. doi: 10.6133/apjcn.2016.25.2.22.
12. Kokkola A, Sjöblom SM, Haapiainen R, Sipponen P, Puolakainen P, Järvinen H. The risk of gastric carcinoma and carcinoid tumours in patients with pernicious anaemia: a prospective follow-up study. *Scand J Gastroenterol* 1998;33:88–92.
13. Addison T. Anaemia: disease of the suprarenal capsules. *London Med Gaz* 1849; 8:517–518.
14. Flint A. A clinical lecture on anaemia, delivered at the Long Island College Hospital. *American Medical Times* 1860;1:181–186.
15. Castle WB. Development of knowledge concerning the gastric intrinsic factor and its relation to pernicious anemia. *N Engl J Med* 1953;249:603–614.
16. Schwartz M. Intrinsic factor antibody in serum from patients with pernicious anaemia. *Lancet* 1960;2:1263–1267.
17. Irvine WJ, Davies SH, Delamore IW, Williams AW. Immunological relationship between pernicious anemia and thyroid disease. *Br J Med* 1962;2:454–456.
18. Irvine W. Immunologic aspects of pernicious anemia. *N Engl J Med* 1965;273:432–438.
19. Leslie D, Lipsky P, Notkins AL. Autoantibodies as predictors of disease. *J Clin Invest* 2001;108:1417–1422. DOI:10.1172/JCI200114452.
20. Rapoport B, McLachlan SM. Thyroid autoimmunity. *J Clin Invest* 2001;108:1253–1259.
21. Dayan CM, Daniels GH. Chronic autoimmune thyroiditis. *N Engl J Med* 1996;335:99–107.
22. Vanderpump MP, Tunbridge WM, French JM, Appleton D, Bates D, Clark F, Grimley Evans J, Hasan DM, Rodgers H, Tunbridge F. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. *Clin Endocrinol (Oxf)* 1995;43:55–68.

23. Rosenthal MJ, Hunt WC, Garry PJ, Goodwin JS. Thyroid failure in the elderly. Microsomal antibodies as discriminant for therapy. *JAMA* 1987;258:209–213.
24. Doniah D, Roit IM, Taylor KB. Autoimmune phenomena in pernicious anaemia. Serological overlap with thyroiditis, thyrotoxicosis, and systemic lupus erythematosus. *Br Med J* 1963;25;1(5342):1374-1379.
25. Singer W, Sahay BM. Myasthenia gravis, Hashimoto's thyroiditis, and pernicious anaemia. *Br Med J*. 1966; 9;1(5492):904.
26. Cruchoad A, Juditz E. An analysis of gastric parietal cell antibodies and thyroid cell antibodies in patients with pernicious anaemia and thyroid disorders. *Clin Exp Immunol*. 1968;3(8):771-781.
27. Irvine WJ. The association of atrophic gastritis and autoimmune thyroid disease. *Clin Endocrinol Metab* 1975;4:351-377.
28. Neufelt M, Blizzard RM. Polyglandular autoimmune disease. In: Pinchera A (Ed). *Autoimmune Aspects of Endocrine Disorders*. New York, Academic Press. 1980;357-365.
29. Tozzoli R, Kodermaz G, Perosa AR, Tampoia M, Zucano A, Antico A, Bizzaro N. Autoantibodies to parietal cells as predictors of atrophic body gastritis: a five-year prospective study in patients with autoimmune thyroid diseases. *Autoimmun Rev* 2010;10(2):80-83. doi: 10.1016/j.autrev.2010.08.006.
30. Venerito M, Radünz M, Reschke K, Reinhold D, Frauenschläger K, Jechorek D, Di Mario F, Malfertheiner P. Autoimmune gastritis in autoimmune thyroid disease. *Aliment Pharmacol Ther* 2015;41(7):686-693. doi: 10.1111/apt.13097.
31. Castoro C, Le Moli R, Arpi ML, Tavarelli M, Sapuppo G, Frittitta L, Squatrito S, Pellegriti G. Association of autoimmune thyroid diseases, chronic atrophic gastritis and gastric carcinoid: experience from a single institution. *J Endocrinol Invest* 2016;39:779. doi:10.1007/s40618-016-0445-5
32. Lahner E, Centanni M, Agnello G, Gargano L, Vannella L, Iannoni C, Delle Fave G, Annibale B. Occurrence and risk factors for autoimmune thyroid disease in patients with atrophic body gastritis. *Am J Med* 2008;121:136-141.
33. d'Annunzio G, Russo C, Tallone R, Lorini R. Autoimmune disorders associated to type 1 diabetes mellitus in children and adolescents, Chapter 1. In: *Autoimmune Disorders – Current Concepts and Advances from Bedside to Mechanistic Insights*, Fang-Ping Huang (Ed.), 2011;1-26.
34. Pearce, EN, Farwell AP, Braverman L. Thyroiditis. *N Engl J Med* 2003;348: 2646-2655.
35. Lorini R, Gastaldi R, Traggiai C, Polo Perucchin P. (2003). Hashimoto's thyroiditis. *Ped Endocrinol Rev* 2003;1: Supplement 2, pp. 205-211.
36. Barker JM. Type 1 diabetes-associated autoimmunity: natural history, genetic associations, and screening. *J Clin Endocrinol Metab*, 2006;91:1210-1217.
37. Kaguelidou F, Carel JC, Léger J. Graves' disease in childhood: advances in management with antithyroid drug therapy. *Horm Res*, 2009;71:310-317.
38. Bahn RS, Dutton CM, Natt N, Joba W, Spitzweg C, Heufelder AE. Thyrotropin receptor expression in Graves' orbital adipose/connective tissues: potential autoantigen in Graves' ophthalmopathy. *J Clin Endocrinol Metab* 1998;83:998–1002.
39. Morshed SA, Davies TF. Graves' disease mechanisms: The Role of stimulating, blocking, and cleavage region TSH Receptor antibodies. *Horm Metab Res* 2015;47:727–734.
40. Vlase H, Davies TF. Insights into the molecular mechanisms of the autoimmune thyroid diseases. In: *Endocrine and Organ Specific Autoimmunity*. Eisenbarth GS (ed.). CA: R. G. Landes Co; 1999: 98–132.
41. Bagriacik EU, Klein JR. The thyrotropin (thyroid-stimulating hormone) receptor is expressed on murine dendritic cells and on a subset of CD45RB<sup>high</sup> lymph node T cells: functional role for thyroid-stimulating hormone during immune activation. *J Immunol* 2000;164:6158–6165.
42. Gimenez-Barcons M, Colobran R, Gomez-Pau A, Marin-Sanchez A, Casteras A, Obiols G, Abella R, Fernandez-Doblas J, Tonacchera M, Lucas-Martin A, Pujol-Borrell R. Graves' Disease TSHR-Stimulating Antibodies (TSAbs) Induce the activation of immature thymocytes: A Clue to the riddle of TSAbs generation? *J Immunol* 2015;194: 4199–4206.

43. Kaye MD. Immunological aspects of gastritis and pernicious anaemia. *Baillieres Clin Gastroenterol* 1987;1:487–506.
44. Toh BH, Van Driel IR, Gleeson PA. Mechanisms of disease: pernicious anemia. *N Engl J Med* 1997;337:1441–1448.
45. Varis K, Kekki M, Härkönen M, Sipponen P, Samloff IM. Serum pepsinogen I and serum gastrin in the screening of atrophic pangastritis with high risk of gastric cancer. *Scand J Gastroenterol Suppl* 1991;186:117–123.
46. De Block C, De Leeuw I, Bogers J, Pelckmans P, Ieven M, Van Marck E, Van Acker K, Van Gaal L. Autoimmune gastropathy in type 1 diabetic patients with parietal cell antibodies: histological and clinical findings. *Diabetes Care* 2003;26:82–88.
47. Solcia E, Fiocca T, Villani L, Gianatti A, Cornaggia M, Chiaravalli A, Curzio M, Capella C. Morphology and pathogenesis of endocrine hyperplasias, precarcinoid lesions, and carcinoids arising in chronic atrophic gastritis. *Scand J Gastroenterol Suppl* 1991;180:146–159.
48. Fisher JM, Taylor KB. A comparison of autoimmune phenomena in pernicious anemia and chronic atrophic gastritis. *N Engl J Med* 1965;272:499–503.
49. Karlsson FA, Burman P, Lööf L, Olsson M, Scheynius A, Mardh S. Enzyme linked immunosorbent assay of H+/K+ ATPase, the parietal cell antigen. *Clin Exp Immunol* 1987;70:604–610.
50. Taylor KB, Roitt IM, Doniach D, Couchman KG, Shapland C. Autoimmune phenomena in pernicious anemia: gastric antibodies. *Br Med J* 1962;2:1347–1352.
51. Perasso A, Testino G, de'Angelis P, Augeri C, de Grandi R. Gastric chief cell mass in chronic gastritis. Count and relationships to parietal cell mass and functional indices. *Hepatogastroenterology* 1990;38 (Suppl 1):63–66.
52. Burman P, Mardh S, Norberg L, Karlsson FA. Parietal cell antibodies in pernicious anemia inhibit H+, K+ adenosine triphosphatase, the proton pump of the stomach. *Gastroenterology* 1989;96:1434–1438.
53. Trudeau WL, McGuigan JE. Relations between serum gastrin levels and rates of gastric hydrochloric acid secretion. *N Engl J Med* 1971;284:408–412.
54. Sipponen P, Valle J, Varis K, Kekki M, Ihamäki T, Siurala M. Fasting levels of serum gastrin in different functional and morphologic states of the antrofundal mucosa. An analysis of 860 subjects. *Scand J Gastroenterol* 1990;25:513–519.
55. Samloff IM, Varis K, Ihamaki T, Siurala M, Rotter JJ. Relationships among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology. A study in relatives of patients with pernicious anemia. *Gastroenterol* 1982;83:204–209.
56. Alonso N, Granada ML, Salinas I, Lucas AM, Reverter JL, Junca J, Oriol A, Sanmarti A. Serum pepsinogen I an early marker of pernicious anemia in patients with type 1 diabetes. *J Clin Endocrinol Metab* 2005;90:5254–5258.
57. Armbrecht U, Stockbrügger RW, Rode J, Menon GG, Cotton PB 1990 Development of gastric dysplasia in pernicious anaemia: a clinical and endoscopic follow-up study of 80 patients. *Gut* 31:1105–1109.
58. Borch K, Renvall H, Liedberg G. Gastric endocrine cell hyperplasia and carcinoid tumors in pernicious anemia. *Gastroenterology* 1985;88:638–648.
59. Sjöblom SM, Sipponen P, Järvinen H. Gastroscopic follow-up of pernicious anaemia patients. *Gut* 1993;34:28–32.
60. Maclaren NK, Riley WJ. Thyroid, gastric, and adrenal autoimmunities associated with insulin-dependent diabetes mellitus. *Diabetes Care* 1985; 8 (Suppl1):34–38.
61. Betterle C, Zanette F, Pedini B, Presoto F, Rapp LB, Monciotto CM, Rigon F. Clinical and subclinical organ-specific autoimmune manifestations in type 1 (insulin-dependent) diabetic patients and their first-degree relatives. *Diabetologia* 1984;26:431–436.
62. Landin-Olsson M, Karlsson FA, Lernmark A, Sundkvist G. Islet cell and thyrogastic antibodies in 633 consecutive 15- to 34-yr-old patients in the diabetes incidence study in Sweden. *Diabetes* 1992;41:1022–1027.
63. Betterle C, Mazzi PA, Pedini B, Accordi F, Cecchetto A, Presotto F. Complement-fixing gastric parietal cell autoantibodies. A good marker for the identification of type A chronic atrophic gastritis. *Autoimmunity* 1988;1:267–274.

- 64.Oksanen A, Sipponen P, Karttunen R, Miettinen A, Veijola L, Sarna S, Rautelin H. Atrophic gastritis and *Helicobacter pylori* infection in outpatients referred for gastroscopy. *Gut* 2000;46:460–463.
- 65.Ungar B, Stocks AE, Whittingham S, Martin FIR, Mackay IR. Intrinsic factor antibody, parietal-cell antibody, and latent pernicious anaemia in diabetes mellitus. *Lancet* 1968;2:415–417.
- 66.De Block CE, De Leeuw IH, Vertommen JJ, Rooman RP, Du Caju MV, Van Campenhout CM, Weyler JJ, Winnock F, Van Autreve J, Gorus FK, Belgian Diabetes Registry.  $\beta$ -cell, thyroid, gastric, adrenal and coeliac autoimmunity and HLA-DQ types in type 1 diabetes. *Clin Exp Immunol* 2001;126:236–241.
- 67.Annibale B, Capurso G, Delle Fave G. The stomach and iron deficiency anaemia: a forgotten link. *Dig Liver Dis* 2003;35:288–295.
- 68.Toh BH, Alderuccio F. Pernicious anaemia. *Autoimmunity* 2004;37:357–361.
- 69.Irvine WJ, Cullen DR, Mawhinney H. Natural history of autoimmune achlorhydric atrophic gastritis. *Lancet* 1974;2:482–485.
- 70.Kaplan LM, Graeme-Cook FM. Case records of the Massachusetts General Hospital (Case 9–1997). A 39 year-old woman with pernicious anemia and a gastric mass. *N Engl J Med* 1997;336:861–867.
- 71.Hsing A, Hansson L, McLaughlin J, Nyren O, Blot W, Ekblom A, Fraumeni Jr JF. Pernicious anemia and subsequent cancer: a population-based cohort study. *Cancer* 1993;71:745–750.
- 72.Brinton L, Gridley G, Hrubec Z, Hoover R, Fraumeni Jr JF. Cancer risk following pernicious anaemia. *Br J Cancer* 1989;59:810–813.
- 73.Carpenter C, Patalas E. Case records of the Massachusetts General Hospital (Case 40–2000): a 38-year-old woman with gastric adenocarcinoma. *N Engl J Med* 2000;343:1951–1958.
- 74.Karlsson FA, Burman P, Löf L, Mardh S. Major parietal cell antigen in autoimmune gastritis with pernicious anemia is the acid-producing H<sup>+</sup>, K<sup>+</sup>- adenosine triphosphatase of the stomach. *J Clin Invest* 1988;81:475–479.
- 75.Toh BH, Sentry JW, Alderuccio F. The causative H<sup>+</sup>/K<sup>+</sup> ATPase antigen in the pathogenesis of autoimmune gastritis. *Immunol Today* 2000;21:348–354.
- 76.van Driel IR, Baxter AG, Laurie KL, Zwar TD, La Gruta NL, Judd LM, Scarff KL, Silveira PA, Gleeson PA. Immunopathogenesis, loss of T cell tolerance and genetics of autoimmune gastritis. *Autoimmun Rev* 2002;1:290–297.
- 77.Appelmelk B, Faller G, Claeys D, Kirchner T, Van den Broucke-Grauls C. Bugs on trial: the case of *Helicobacter pylori* and autoimmunity. *Immunol Today* 1998;19:296–299.
- 78.Amedei A, Bergman MP, Appelmelk BJ, Azzurri A, Benagiano M, Tamburini C, van der Zee R, Telford JL, Vandenbroucke-Grauls CM, D’Elios MM, Del Prete G. Molecular mimicry between *Helicobacter pylori* antigens and H<sup>+</sup>, K<sup>+</sup>-adenosine triphosphatase in human gastric autoimmunity. *J Exp Med* 2003;198:1147–1156.
- 79.van Driel IR, Read S, Zwar T, Gleeson PA. Shaping the T cell repertoire to a bona fide autoantigen: lessons from autoimmune gastritis. *Curr Opin Immunol* 2005;17:570–576.
- 80.D’Elios MM, Appelmelk BJ, Amedei A, Bergman MP, Del Prete GF. Gastric autoimmunity: the role of *Helicobacter pylori* and molecular mimicry. *Trends Mol Med* 2004;10:316–323.
- 81.Lam-Tse WK, Batstra MR, Koeleman BP, Roep BO, Bruining MG, Aanstoot HJ, Drexhage HA. The association between autoimmune thyroiditis, autoimmune gastritis and type 1 diabetes. *Pediatr Endocrinol Rev* 2003; 1:22–37.
- 82.Betterle C, Dal Pra C, Mantero F, Zanchetta R. Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. *Endocr Rev* 2002;23:327–364.
- 83.Doniach D, Roitt IM, Taylor KB. Autoimmune phenomena in pernicious anaemia: serological overlap with thyroiditis, thyrotoxicosis and systemic lupus erythematosus. *Br Med J* 1963;1:1374–1379.
- 84.De Block CE, De Leeuw IH, Rooman RP, Winnock F, Du Caju MV, Van Gaal LF. Gastric parietal cell antibodies are associated with glutamic acid decarboxylase-65 antibodies and the HLA DQA1\*0501-DQB1\*0301 haplotype in Type 1 diabetes mellitus. *Belgian Diabetes Registry. Diabet Med* 2000;17:618–622.
- 85.De Block CE, De Leeuw IH, Decochez K, Winnock F, Van Autreve J, Van Campenhout CM, Martin M, Gorus FK,

- Belgian Diabetes Registry. The presence of thyrogastric antibodies in first-degree relatives of type 1 diabetic patients is associated with age and proband antibody status. *J Clin Endocrinol Metab* 2001;86:4358–4363.
- 86.Karnes Jr WE, Samloff IM, Siurala M, Kekki M, Sipponen P, Kim SW, Walsh JH. Positive serum antibody and negative tissue staining for *Helicobacter pylori* in subject with atrophic body gastritis. *Gastroenterol* 1991;101:167–174.
- 87.Fong TL, Dooley CP, Dehesa M, Cohen H, Carmel R, Fitzgibbons PL, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection in pernicious anemia: a prospective controlled study. *Gastroenterol* 1991;100:328–332.
- 88.Ma JY, Borch K, Sjöstrand SE, Janzon L, Mardh S. Positive correlation between H,K-adenosine triphosphatase autoantibodies and *Helicobacter pylori* antibodies in patients with pernicious anemia. *Scand J Gastroenterol* 1994;29:961–965.
- 89.Annibale B, Aprile MR, D'ambra G, Caruana P, Bordi C, Delle Fave G. Cure of *Helicobacter pylori* infection in atrophic body gastritis patients does not improve mucosal atrophy but reduces hypergastrinemia and its related effects on body ECL-cell hyperplasia. *Aliment Pharmacol Ther* 2000;14:625–634.
- 90.Uibo R, Vorobjova T, Metsküla K, Kisand K, Wadström T, Kivik T. Association of *Helicobacter pylori* and gastric autoimmunity: a populationbased study. *FEMS Immunol Med Microbiol* 1995;11:65–68.
- 91.Negrini R, Savio A, Poiesi C, Appelmelk B, Buffoli F, Paterlini A, Cesari P, Graffeo M, Vaira D, Franzin G. Antigenic mimicry between *Helicobacter pylori* and gastric mucosa in the pathogenesis of body atrophic gastritis. *Gastroenterol* 1996;111:655–665.
- 92.Faller G, Steiniger H, Kränzlein J, Maul H, Kerkau T, Hensen J, Hahn EG, Kirchner T. Antigastric autoantibodies in *Helicobacter pylori* infection: implications of histological and clinical parameters of gastritis. *Gut* 1997;41:619–623.
- 93.Claeys D, Faller G, Appelmelk BJ, Negrini R, Kirchner T. The gastric H/K ATPase is a major autoantigen in chronic *Helicobacter pylori* gastritis with body mucosa atrophy. *Gastroenterology* 1998; 115:340–347.
- 94.Kohlstadt IC, Antunez De Mayolo EA. Parietal cell antibodies among Peruvians with gastric pathology changes. *Scand J Gastroenterol* 1993;28:973–977.
- 95.De Block CEM, De Leeuw IH, Bogers JJPM, Pelckmans PA, Ieven M, Van Marck EAE, Van Hoof V, Ma'day E, Van Acker KL, Van Gaal LF. *Helicobacter pylori*, parietal cell antibodies and autoimmune gastropathy in type 1 diabetes mellitus. *Aliment Pharmacol Ther* 2002;16:281–289.
- 96.Villako K, Kekki M, Maaros HI, Sipponen P, Tammur R, Tamm A, Keevallik R. A 12-year follow-up study of chronic gastritis and *Helicobacter pylori* in a population-based random sample. *Scand J Gastroenterol* 1995;30:964–967.
- 97.Faller G, Winter M, Steininger H, Lehn N, Meining A, Bayerdorffer E, Kirchner T. Decrease of antigastric autoantibodies in *Helicobacter pylori* gastritis after cure of infection. *Pathol Res Pract* 1999;195:243–246.
- 98.Sipponen P, Kekki M, Haapakoski J, Ihamäki T, Siurala M. Gastric cancer risk in chronic atrophic gastritis: statistical calculations of cross-sectional data. *Int J Cancer* 1985;35:173–177.
- 99.Correa P, Haenszel W, Cuello C, Zavala D, Fontham E, Zarama G. Gastric precancerous process in a high risk population: cohort follow-up. *Cancer Res* 1990;50:4737–4740.
- 100.Filipe MI, Munoz N, Matko I, Kato I, Pompe-Kirn V, Jutersek A. Intestinal metaplasia types and the risk of gastric cancer: a cohort study in Slovenia. *Int J Cancer* 1994;57:324–329.
- 101.Ohata H, Kitauchi S, Yoshimura N, Mugitani K, Iwane M, Nakamura H. Progression of chronic atrophic gastritis associated with *Helicobacter pylori* infection increases risk of gastric cancer. *Int J Cancer* 2004;109: 138–143.
- 102.Correa P. A human model of gastric carcinogenesis. *Cancer Res* 1988;48:3554–3560.
- 103.Correa P. The epidemiology of gastric cancer. *World J Surg* 1991;15:228–234.
- 104.Correa P. Human gastric carcinogenesis:a multistep and multifactorial process –First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992;52:6735–6740.
- 105.Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ. European *Helicobacter* Study Group. Management of *Helicobacter pylori* infection--the Maastricht IV/ Florence Consensus Report. *Gut* 2012;61: 646–664.

106. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001;345:829-832.
107. Weck MN, Stegmaier C, Rothenbacher D, Brenner H. Epidemiology of chronic atrophic gastritis: population-based study among 9444 older adults from Germany. *Aliment Pharmacol Ther* 2007;26: 879-887.
108. Lomba-Viana R, Dinis-Ribeiro M, Fonseca F, Vieira AS, Bento MJ, Lomba-Viana H. Serum pepsinogen test for early detection of gastric cancer in a European country. *Eur J Gastroenterol Hepatol* 2012;24:37-41.
109. Bornschein J, Selgrad M, Wex T, Kuester D, Malfertheiner P. Serological assessment of gastric mucosal atrophy in gastric cancer. *BMC Gastroenterol* 2012;12:10. doi: 10.1186/1471-230X-12-10.
110. Valle J, Kekki M, Sipponen P, Ihamäki T, Siurala M. Longterm course and consequences of Helicobacter pylori gastritis. Results of a 32-year follow-up study. *Scand J Gastroenterol* 1996;31:546-550.
111. Germaná B, Di Mario F, Cavallaro LG, Moussa AM, Lecis P, Liatoupolou S, Comparato G, Carloni C, Bertiato G, Battistel M, Papa N, Aragona G, Cavestro GM, Iori V, Merli R, Bertolini S, Caruana P, Franzé A. Clinical usefulness of serum pepsinogens I and II, gastrin-17 and anti-Helicobacterpylori antibodies in the management of dyspeptic patients in primary care. *Dig Liver Dis* 2005;37:501-508.
112. Miki K, Ichinose M, Shimizu A, Huang SC, Oka H, Furihata C, Matsushima T, Takahashi K. Serum pepsinogens as a screening test of extensive chronic gastritis. *Gastroenterol Jpn* 1987;22:133-141.
113. Samloff IM, Varis K, Ihamaki T, Siurala M, Rotter JI. Relationships among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology. A study in relatives of patients with pernicious anemia. *Gastroenterol* 1982;83:204-209.
114. Korstanje A, den Hartog G, Biemond I, Lamers CB. The serological gastric biopsy: a non-endoscopic diagnostic approach in management of the dyspeptic patient: significance for primary care based on a survey of the literature. *Scand J Gastroenterol Suppl* 2002;236:22-26.
115. Oksanen A, Sipponen P, Miettinen A, Sarna S, Rautelin H. Evaluation of blood tests to normal gastric mucosa. *Scand J Gastroenterol* 2000;35:791-795.
116. Varis K, Sipponen P, Laxen F, Samloff IM, Huttunen JK, Taylor PR, The Helsinki Gastritis Study Group. Implications of serum pepsinogen I in early endoscopic diagnosis of gastric cancer and dysplasia. *Scand J Gastroenterol* 2000;35:950-956.
117. Miki K. Gastric cancer screening using the serum pepsinogen test method. *Gastric Cancer* 2006;9:245-253.
118. Agréus L, Kuipers EJ, Kupcinkas L, Malfertheiner P, Di Mario F, Leja M, Mahachai V, Yaron N, van Oijen M, Perez Perez G, Rugge M, Ronkainen J, Salaspuro M, Sipponen P, Sugano K, Sung J. Rationale in diagnosis and screening of atrophic gastritis with stomach-specific plasma biomarkers. *Scand J Gastroenterol* 2012;47:136-147.
119. Varis K, Isokoski M. Screening of type A gastritis. *Ann Clin Res* 1981;13:133-138.
120. Sipponen P, Valle J, Varis K, Kekki M, Ihamäki T, Siurala M. Fasting levels of serum gastrin in different functional and morphologic states of the antrofundal mucosa. An analysis of 860 subjects. *Scand J Gastroenterol* 1990;25:513-519.
121. Varis K, Kekki M, Härkönen M, Sipponen P, Samloff IM. Serum pepsinogen I and serum gastrin in the screening of atrophic gastritis with high risk of gastric cancer. *Scand J Gastroenterol* 1991;186:117-123.
122. Sipponen P, Ranta P, Helske T, Kääriäinen I, Mäki T, Linnala A. Serum levels of amidated gastrin-17 and pepsinogen I in atrophic gastritis: an observational case-control study. *Scand J Gastroenterol* 2002;37:785-791.
123. Storskrubb T, Aro P, Ronkainen J, Sipponen P, Nyhlin H, Talley NJ. Serum biomarkers provide an accurate method for diagnosis of atrophic gastritis in a general population: the Kalixanda study. *Scand J Gastroenterol* 2008;43:1448-1455.
124. Telaranta-Keerie A, Kara R, Paloheimo L, Härkönen M, Sipponen P. Prevalence of undiagnosed advanced atrophic corpus gastritis in Finland: an observational study among 4,256 volunteers without specific complaints. *Scand J Gastroenterol* 2010;45:1036-1041.
125. Väänänen H, Vauhkonen M, Helske T. Non-endoscopic diagnosis of atrophic gastritis with a blood test. Correlation between gastric histology and serum levels of gastrin-17 and pepsinogen I: a multicentre study. *Eur J Gastroenterol Hepatol* 2003;15:885-891.

126. Benberin V, Bektayeva R, Karabayeva R. Prevalence of H.pylori infection and atrophic gastritis among asymptomatic and dyspeptic adults in Kazakhstan. A hospital-based screening with a panel of serum biomarkers. *Anticancer Res* 2013;33:4595-4602.
127. Syrjänen KJ, Sipponen P, Härkönen M, Peetsalu A, Korpela S. Accuracy of GastroPanel testing in detection of atrophic gastritis. *Eur J Gastroenterol Hepatol* 2015;27:102-104.
128. Aro P, Ronkainen J, Storskrubb T, Bolling-Sternevald E, Johansson S-E, Talley NJ. Validation of the translation and cross-cultural adaptation into Finnish of the Abdominal Symptom Questionnaire, the Hospital Anxiety and Depression Scale and the Complaint Score Questionnaire. *Scand J Gastroenterol* 2004;39:1201-1208.
129. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996;20: 1161-1181.
130. Seed PT, Tobias A. Summary statistics for diagnostic tests. *Stata Techn Bull* 2001;59:9-12.