The role of systemic autoimmune disorders in the symptoms, course and prognosis of chronic gastritis and their incidence in gastric cancer

Doctoral (Ph.D.) thesis

2022.

Noémi Zádori M.D.
Institute for Translational Medicine
Medical School
University of Pécs

Leader of the Doctoral School:
Erika Pintér M.D. Ph.D. D.Sc.
Program Leader:
Péter Hegyi M.D. Ph.D. D.Sc.
Supervisor:
József Czimmer M.D. Ph.D.
Pécs

1 PUBLICATIONS AND CITATIONS

1.1 Scientific metrics

Summarized IF from all the publications: **78.183**

Summarized IF of all first authorship: **38.765**

Citations: 237 (independent citations: 211)

Hirsch index: 6 (MTMT) / 7 (Google Scholar)

1.2 Publications related to the thesis (without abstracts) - Summarized IF: 13.073

1. **Zádori N**, Szakó L, Váncsa S, et. al. Six Autoimmune Disorders Are Associated With Increased Incidence of Gastric Cancer: A Systematic Review and Meta-Analysis of Half a Million Patients. Front Immunol. 2021 Nov 23;12:750533. **IF: 8.786; Q1/D1**
2. **Zádori N**, Németh D, Szakó L et al. Prevalence of Autoimmune-phenomena behind Chronic Gastritis of Unknown Origin, and their Role in the Poor Histological Outcome of the Stomach: A Single-centre, Retrospective Cross-sectional Study. J Gastrointestin Liver Dis. 2022 Jun 12;31(2):168-175. **IF: 2.142; Q3**
3. **Zádori N**, Németh D, Frim L, et al J. Dyspepsia-Like Symptoms in Helicobacter pylori-Negative Chronic Gastritis are Associated with ASCA-, ANCA-, and Celiac Seropositivity but Not with Other Autoimmune Parameters: A Single-Centre, Retrospective Cross-Sectional Study. Int J Gen Med. 2022;15:7789-7796. **IF:2.145; Q2**

1.3 Publications not related to the thesis (without abstracts)

1.3.1 First author - Summarized IF: 25.692

1. **Zádori N,** Váncsa S, Farkas N, et al. The negative impact of comorbidities on the disease course of COVID-19. Intensive Care Med. 2020 Sep;46(9):1784-1786. **IF: 17.440; Q1/D1**

2. **Zádori N**, Párniczky A, Szentesi A, Hegyi P. Insufficient implementation of the IAP/APA guidelines on aetiology in acute pancreatitis: Is there a need for implementation managers in pancreatology? United European Gastroenterol J. 2020 Apr;8(3):246-248. **IF: 4.623; Q1**
3. **Zádori N**, Gede N, Antal J, et al. EarLy Elimination of Fatty Acids iN hypertriglyceridemia-induced acuTe pancreatitis (ELEFANT trial): Protocol of an open-label, multicenter, adaptive randomized clinical trial. Pancreatology. 2020 Apr;20(3):369-376. **IF: 3.629; Q1**
4. **Zádori N**, Molnár Zs. Hyperinflammatio és modulációja kritikus állapotú Covid-betegekben. LEGE ARTIS MEDICINAE 31 : 10 pp. 423-431. , 9 p. (2021)

1.3.2 Co-author - Summarized IF: 39.418

1. Juhász MF, Vereczkei Zs, Ocskay K, …**Zádori N**,...et al. The EFFect of dietary fat content on the recurrence of pancreaTitis (EFFORT): Protocol of a multicenter randomized controlled trial. PANCREATOLOGY 22 : 1 pp. 51-57. , 7 p. (2022)
2. Ocskay K, Juhász MF, Farkas N, **Zádori N** et al. Recurrent acute pancreatitis prevention by the elimination of alcohol and cigarette smoking (REAPPEAR): protocol of a randomised controlled trial and a cohort study. BMJ OPEN 12 : 1 Paper: e050821 , 9 p. (2022)
3. Kanjo A, Molnar Zs, **Zádori N** et al. Dosing of Extracorporeal Cytokine Removal In Septic Shock (DECRISS): protocol of a prospective, randomised, adaptive, multicentre clinical trial. BMJ OPEN 11: 8 Paper: e050464 (2021)
4. Kiss Sz, Gede N, Hegyi P, … **Zádori N** et al. Early changes in laboratory parameters are predictors of mortality and ICU admission in patients with COVID-19: a systematic review and meta-analysis. MEDICAL MICROBIOLOGY AND IMMUNOLOGY 210 pp. 33-47. , 15 p. (2021)
5. Molnár G, Gyarmathy V.A, **Zádori N** et al. Severe Hypertriglyceridemia-Induced Acute Pancreatitis. CASE REPORTS IN GASTROENTEROLOGY 15 : 1 pp. 218-224. , 7 p. (2021)
6. Szakó L, Farkas N, Kiss Sz,… **Zádori N** et al. Convalescent plasma therapy for COVID-19 patients : a protocol of a prospective meta-analysis of randomized controlled trials. TRIALS 22 : 1 Paper: 112 , 8 p. (2021)
7. Erős A, Dohos D, Veres G,… **Zádori N** et al. Effect of joint transition visits on quality of life in adolescents with inflammatory bowel diseases: a protocol for a prospective, randomised, multicentre, controlled trial (TRANS-IBD). BMJ OPEN 10 : 10 Paper: e038410 , 9 p. (2020)
8. Erőss B, Molnár Zs, Szakács Zs, **Zádori N** et al. Personalised health education against health damage of COVID-19 epidemic in the elderly Hungarian population (PROACTIVE-19) : protocol of an adaptive randomised controlled clinical trial. TRIALS 21 : 1 Paper: 809 , 9 p. (2020)

9. Földi M, Farkas K, Kiss Sz, **Zádori N** et al. Obesity is a Risk Factor for Developing Critical Condition in COVID-19 Patients : a Systematic Review and Meta-analysis. OBESITY REVIEWS 21 : 10 Paper: e13095 , 9 p. (2020)
10. Illés D, Ivány E, Holzinger G, … **Zádori N** et al. New Onset of DiabetEs in aSsociation with pancreatic ductal adenocarcinoma (NODES Trial): protocol of a prospective, multicentre observational trial. BMJ OPEN 10 : 11 Paper: e037267 , 7 p. (2020)
11. Koncz B, Darvasi E, Erdősi D, … **Zádori N** et al. LIFEStyle, Prevention and Risk of Acute PaNcreatitis (LIFESPAN) : protocol of a multicentre and multinational observational case-control study. BMJ OPEN 10 : 1 Paper: e029660 , 9 p. (2020)
12. Váncsa Sz, Hegyi PJ, **Zádori N** et al. Pre-existing liver diseases and on-admission liver-related laboratory tests in COVID-19: a prognostic accuracy meta-analysis with systematic review. FRONTIERS IN MEDICINE 7 Paper: 572115 , 11 p. (2020)
13. Kucserik L.P, Márta K, Vincze Á, … **Zádori N.** et al. Endoscopic sphincterotoMy for delayIng choLecystectomy in mild acute biliarY pancreatitis (EMILY study): Protocol of a multicentre randomised clinical trial. BMJ OPEN 9 : 7 Paper: 025551 , 8 p. (2019)

2 INTRODUCTION

Chronic gastritis (CG) is a multistep, progressive, life-long inflammatory condition of the gastric mucosa. It is one of the most common findings during upper gastrointestinal (GI) endoscopy in Eastern countries. Literature suggests that more than half of the world population may live with CG to some extent.

The two most frequently recognised causes of CG are: (1.) *H. pylori* infection, (2.) autoimmune gastritis (AIG). *H. pylori* infection is the primary etiological factor of CG: 50% of the world's population is infected with *H. pylori*, which is behind one-third of the CG cases. However, the prevalence of *H. pylori* has markedly declined over the past few decades, which resulted in the decline of CG prevalence in parallel. The superficial inflammation caused by *H. pylori* may progress into chronic inflammation, gastric atrophy, intestinal metaplasia (IM), dysplasia, and cancer. A decrease in the infection rate resulted in a decline in the prevalence of CG caused by *H. pylori* and its sequelae.

The second most common, well-known etiological factor besides *H. pylori* infection behind CG is AIG: it occurs in 7.8–19.5% of the CG cases. Autoantibodies are directed against IF and parietal cells (cytoplasmic and plasma membrane antigens). Patients with AIG have a 3-5 times higher risk of co-existing other autoimmune (AI) disorders than the general population, in particular, AI-thyroid disease and diabetes mellitus type I (T1DM). In terms of atrophy, IM and dysplasia, AIG may be more rapid and progressive than *H. pylori* gastritis. Many systemic and glandular autoimmune diseases can also be associated with gastritis; however, their prognostic value is controversial.

2.1 Symptoms

Despite the frequent occurrence during upper GI endoscopy, CG does not cause mostly typical signs and symptoms. Studies have shown that acute *H. pylori* gastritis is associated with

dyspeptic symptoms (e.g., epigastric burning, distention or bloating, belching, episodic nausea, flatulence, and halitosis). However, this association is not justified in chronic infection.
The prevalence of dyspeptic symptoms in the general population is 20-40%, and it is one of the most common indications for upper GI endoscopy, with a controversial diagnostic value. Therefore, performing endoscopy should be considered due to its invasiveness and low-cost effectiveness.

Two types can be distinguished: organic and functional. The current standard for diagnosis of functional dyspepsia (FD) is the Rome IV criteria: the presence of one or more of the four following symptoms: bothersome postprandial fullness, early satiety, epigastric pain, and epigastric burning sensation, and no evidence of the existence of any organic disease that may explain the symptoms. The only exception is *H. pylori* infection. Extensive, population-based studies have shown that *H. pylori* might play a crucial role in the pathogenesis of FD, and it also was included in the definition of FD according to the ROME III criteria.

The exact pathophysiological features behind FD are unknown; however, anxiety and visceral hypersensitivity, such as gastric hypersensitivity to distension and acids, abnormal central pain processing, slow gastric motility, and gastric accommodation failure might play an essential role. Data regarding the connection between *H. pylori-negative* chronic gastritis and dyspeptic symptoms are lacking. Clarifying the aetiology behind *H. pylori-negative* CG and FD and the cause of their possible relationship might be beneficial in establishing the optimal treatment strategy. Immune activation might play a role in the pathogenesis of FD. It has been shown that there is innate immune activation in the mucosa in the case of FD, but the prevalence of AI disorders due to immune activation is uncertain.

2.2 Clinical significance

The significance of CG in clinical practice is largely underrated; however, its role in gastric carcinogenesis is well known: gastric cancer is the final result of the progressive changes in the gastric mucosa, starting with CG, followed by atrophy and IM. The estimated annual cancer

risk is 0.1% within five years after diagnosis. Patients with CG are at increased risk for developing both gastric neuroendocrine tumours and adenocarcinomas. Neuroendocrine tumours are characteristic of AIG, and in patients with *H. pylori* infection is believed to be rare but has been described. There are limited data available about carcinoid tumours in patients with CG, but literature data suggest an annual incidence of 0.68 per cent per person-years.

Gastric adenocarcinoma is the most common histological type among gastric cancers (about 90 to 95%). It arises from the gland cells of the mucosa and develops in the milieu of mucosal atrophy and IM. It is one of the most common cancers worldwide, affecting over 20,000 patients yearly in the USA. At the time of presentations, 50% of the patients already have advanced staged cancer, which extends beyond locoregional confines, and only half of those can undergo curative resection. Tumours with early stages are usually asymptomatic and can be detected by screening programs. The average 5-year survival rate is less than 20%. The prognosis can be improved by early diagnosis and therapy: detecting the tumour before reaching the muscular layer of the stomach is important. **In that case, the 5-year survival rate can be up to 90%.**

A significant decrease in gastric cancer incidence and mortality has been observed recently. It is possible due to the identification and elimination of the underlying causes, globally decreased incidence of *H. pylori* infection and reduced use of tobacco and dietary salt.
Despite this reduction and the effective *H. pylori* eradication strategy, gastric cancer is still the fifth most common cancer worldwide, which raises the possibility of further causative factors. Besides AIG and *H. pylori* infection, several studies suggest a potential association between systemic AI disorders and gastric cancer; however, the relevant data have been controversial.

Although there is no specific treatment for CG, identifying and treating the underlying cause would be crucial in preventing gastric cancer: it can result in the normalisation of the gastric mucosa in cases where the gastritis is not developed to the atrophic end stages. Therefore, a specific follow-up strategy should be set up; endoscopic surveillance has not been fully established in terms of follow-up intervals and duration.

3 AIMS AND OBJECTIVES

A) Autoimmune disorders and gastric cancer

Given the controversial data regarding the connection between systemic AI disorders and gastric cancer,

1) we aimed to provide a comprehensive summary of the potential association and the incidence of gastric cancer in AI disorders in a meta-analysis and systematic review.

B) Chronic gastritis and autoimmunity

Although chronic gastritis is one of the most common GI disorders, the aetiology often remains unknown. However, clarifying the underlying aetiology might be beneficial for preventing the development of gastric neoplasms**.** In a retrospective study, we aimed to

2) discover the possible etiologic factors of CG;
3) to investigate the possible relationship between these factors and IM and atrophy;
4) to determine the prevalence of systemic AI disorder-related autoantibody positivity in CG in southwestern Hungary;
5) to investigate the role of autoantibody positivity in the course and progression of CG;
6) to revise the current clinical practice in diagnosing and managing CG.

C. Dyspepsia and autoimmunity in *H. pylori*-negative chronic gastritis

Immune activation might play a role in the pathogenesis of FD, but the correlation between systemic AI disorders and FD is uncertain. Regarding the uncertainty in this topic, in a retrospective study, we aimed

7) to determine the prevalence and investigate the possible role of dyspeptic symptoms in patients with *H. pylori*-negative CG;
8) to assess the occurrence and pattern of other GI symptoms in CG.

4 METHODS

4.1 Methods of the meta-analysis

Our work was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 Statement and the study protocol was registered on PROSPERO International Prospective Register of Systematic Reviews under registration number CRD42021262875 (see https://www.crd.york.ac.uk/prospero). We did not deviate from the protocol defined in advance.

The systematic search was performed in four scientific databases—MEDLINE via PubMed; Cochrane Central Register of Controlled Trials (CENTRAL); Embase; and Web of Science, Latin American and Caribbean Health Sciences Literature (LILACS)—up to May 17, 2021.

We included studies reporting the standardised incidence ratio (SIR) of gastric cancer in an AI disorder in the general population. We included only full texts and excluded studies with no event rate of SIR. We pooled SIRs of gastric cancer in each autoimmune disorder. After the extraction of SIRs, pooling was carried out by the inverse variance method and random-effects model with the restricted maximum likelihood (REML) estimation. These results were displayed on forest plots. Summary SIR estimation, *p*-value, and 95% CI were also calculated.

Statistical heterogeneity was assessed using χ^2 and the I^2 statistics to acquire probability values, and I^2 represents the percentage of effect size heterogeneity that cannot be explained by random chance. Significant heterogeneity is considered when $p < 0.1$. To check for publication bias, the visual inspection of funnel plots and Eggers' tests were performed (alpha = 0·1). In the case of more than ten studies included, the Eggers test was carried out for each AI disorder.
Subgroup analyses were performed for gender and high-and low-incidence countries in gastric cancer. Quantitative synthesis was performed when more than three articles reported eligible data for analysis. Otherwise, the findings were summarised in the qualitative synthesis. All

analyses were performed using R statistical software (R Foundation, Vienna, Austria) with the meta package (Guido Schwarzer, v4.18-2).

The quality of the included studies was analysed with the Quality in Prognostic Studies (QUIPS) tool, focusing on the definition of prognostic factors and outcomes.

4.2 Methods of the studies

To answer questions 2-8, our studies were conducted from January 2016 to January 2020 with the enrolment of all patients with histologically proved CG who underwent immune serological testing. The diagnosis of CG was set up according to the Updated Sydney system. To avoid performance bias, all included the same single-unit medical team managed patients (single examining endoscopic specialist, one pathologist specialised in GI pathology reviewed all the histological findings). Patients were enrolled on the studies from regular patient care.

We excluded all patients from the studies having any of the followings: (1) acute gastritis; (2) reactive gastropathy; (4) subjects without any serology testing results; (5) *H. pylori* positivity; (6) gastro-oesophageal reflux disease (GERD); (7) ulcer; (8) cancer.

Eligible patients were identified from an electronic database. We categorised patients into two groups according to their AI-serology results: autoantibody seropositive (AISP) and autoantibody seronegative (AISN). The data collection and research were approved by the director of the Clinical Centre and the director of the First Department of Medicine of the University of Pécs (Institutional Review Board; case number: KK/999-1/2020). To ensure personal data protection and privacy, all included patients received a numeric code.

4.2.1 Endpoints for AIMs 2-6

We assigned a composite endpoint for the primary endpoint, which included gastric atrophy and IM. In addition, the following secondary endpoints were assessed: the prevalence of each antibody positivity and the stage of the atrophy based on the Operative Link On Gastritis

Assessment (OLGA) score. All parameters were analysed at the level of AI disease-, AISP-, and AISN groups. If the sample size reached at least eight patients, additional analysis was performed at the individual level of each autoantibody. The role of simultaneous AI positivity (2 or more AI diseases are present) in elevated risk of precancerous lesions was also examined.

4.2.2 Endpoints for AIMs 7-8

The primary endpoint was the association between AI positivity and dyspepsia-like symptoms (according to the Rome IV criteria). Secondary endpoints were the frequency of symptoms in CG, the association between AISP and the most frequently occurring symptoms, the location of the inflammation in the stomach, and the association between AISP and the affected region of the inflammation. All endpoints were analysed on the level of AI disease, AISP-, and AISN groups.

SPSS 25.0 software was used for analyses. Descriptive statistics (mean, standard deviation (SD), and minimum and maximum values) and univariate analyses were performed for both studies. 2-sided Pearson Chi-square was carried out to compare dichotomous variables for patient frequencies. In the case of significant differences, standardised residuals were also observed to arrive at the exact results. Regarding continuous variables, an independent sample t-test was used. We followed the distribution on Q-Q-plot. A p-value of less than 0.05 was considered statistically significant.

Furthermore, to answer questions 2-6, multivariate analyses (adjusted for gender and age) were performed, and odds ratios (OR)s with a 95% CI were calculated. Multinominal logistic regression was performed when co-factors were also considered.

5 RESULTS

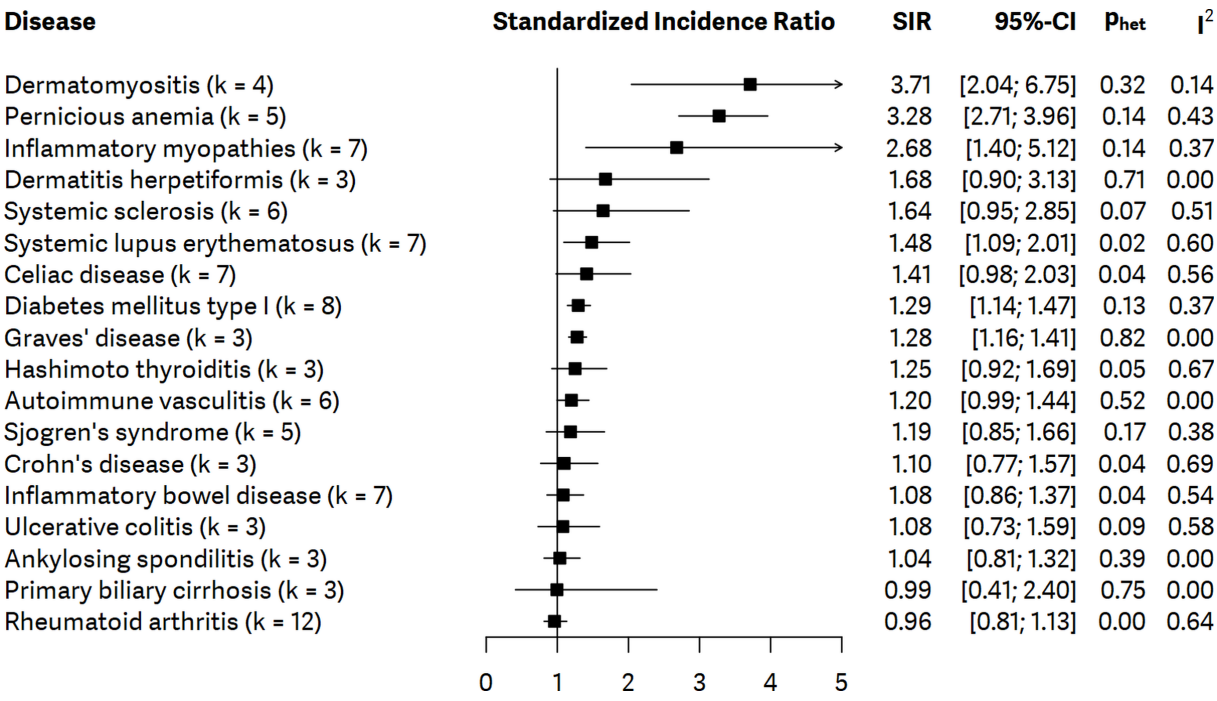
5.1 Results of the meta-analysis

Our systematic search identified 8,206 records, of which 12,420 remained after duplicate removal, of which 43 studies were included in the final analysis. Forty studies were included in the meta-analysis, and nine additional studies were in the systematic review. The included 43 studies describe 36 AI diseases, with 499,427 patients from four continents (America, Europe, Asia, and Australia) and 15 countries. Of the included articles, four studies were retrospective, and 39 were prospective.

5.1.1 Analytical results of associations between autoimmune diseases and gastric cancer

The statistical analysis of the included studies found significantly increased incidence of gastric cancer in the cases **dermatomyositis** (SIR= 3.71; 95%CI: 2.04, 6.75; p< 0.0001) based on four studies, **pernicious anemia (PA)** (SIR= 3.28; 95%CI: 2.71, 3.96; p< 0.0001) based on five studies, **inflammatory myopathies** (SIR= 2.68; 95%CI:1.40; 5.12; p=0.0029) based on seven studies, **systemic lupus erythematosus (SLE)** (SIR= 1.48; 95%CI: 1.09, 2.01; p=0.0116) based on seven studies, **diabetes mellitus type 1 (T1DM)** (SIR= 1.29; 95%CI:1.14, 1.47; p< 0.0001) according to eight studies, and **Graves' disease** (SIR= 1.28; 95%CI: 1.16, 1.41; p< 0.0001) based on three studies. The analysis could not prove increased gastric cancer incidence in AI vasculitis, celiac disease, systemic sclerosis (SSc), dermatitis herpetiformis, Hashimoto thyroiditis, Sjogren's syndrome, inflammatory bowel disease (IBD), Crohn's disease, rheumatoid arthritis (RA), ulcerative colitis, ankylosing spondylitis, and primary biliary cirrhosis *(Fig.1.)*.

Fig. 1. Summarizing forest plot with pooled standardised incidence ratios (SIRs)



the number of studies – k.

5.1.2 Subgroup analysis based on gender

Regarding the subgroup analysis based on gender, an increased incidence of gastric cancer was observed in female patients with T1DM (SIR= 1.62; 95% CI: 1.20, 2.18) but not in male patients. Rheumatoid arthritis did not increase the incidence of gastric cancer, neither in male nor female patients. Subgroup analysis could not be carried out with other autoimmune diseases due to the lack of data on gender.

5.1.3 Subgroup analysis based on the incidence of gastric cancer

In low-incidence countries, increased incidence of gastric cancer was observed in case of PA (SIR= 3.28; 95% CI: 2.71, 3.96), T1DM (SIR= 1.41; 95% CI: 1.02, 1.95), Graves' disease (SIR= 1.28; 95% CI: 1.61, 1.41), and autoimmune vasculitis (SIR= 1.21; 95% CI: 1.01, 1.44). In the case of dermatomyositis (SIR= 5.10; 95% CI: 1.90, 13.67), a higher incidence of gastric cancer was also shown based on two studies; therefore, subgroup analysis could not be carried

out. In high-incidence countries, only SLE (SIR= 1.69; 95% CI: 1.21, 2.36) was associated with an increased incidence of gastric cancer. Regarding other AI disorders, the statistical analysis could not prove any association with gastric cancer.

5.1.4 Qualitative synthesis

Besides the quantitative synthesis, eighteen other AI disorders were included in the qualitative synthesis. In the individual articles, increased incidence of gastric cancer was described in the cases of immune thrombocytopenic purpura, membranous nephropathy, Addison's disease, discoid lupus, Bechet's disease, sarcoidosis, myasthenia gravis, Takayasu arteritis, polymyalgia rheumatica, localised scleroderma, psoriasis. Chronic rheumatic heart disease, IgG4-related disease, ANCA-vasculitis multiple sclerosis, and granulomatosis with polyangiitis seem not to be associated with elevated incidence of gastric cancer according to the individual studies.

5.2 Results of the studies

A total of 285 CG patients were assessed between January 2016 to January 2020, with the final enrolment of 175 patients (52 men and 123 women). There were no significant differences regarding baseline characteristics between AISP and AISN groups. Ten patients (out of 167 patients) had anaemia (5.99%), of which eight patients were in the AISP group. In these patients, the antibody positivity was distributed as follows: three patients had AIG-related, one had celiac disease-, one had IBD-, and three had SLE-related antibody positivity. Of 175 included patients with CG, 53 had atrophy with fibrosis (30.29%), and 49 had atrophy with IM (28%). Detailed results of baseline characteristics of the analysed population can be seen in *Table 1.* in detail.

Table 1. Baseline characteristics of the population

Parameter	Overall (n=175)	AISP (n=97)	AISN (n=78)	p-value
Age (mean, SD)	61.66; 15.13	62.68; 15.03	60.40; 15.13	0.321
Female N^0 (%)	123 (70.29)	64 (65.98)	59 (75.64)	0.641
BMI (mean, SD)	25.89, 5.42	25.81, 5.44	25.81, 5.51	1.000
Alcohol consumption N^0 (%) *	29/74 (39.19)	16/41 (39.02)	13/33 (39.39)	0.946
Smoking N^0 (%) *	20/115 (17.39)	11/58 (18.97)	9/57 (15.79)	0.238
GERD N^0 (%)	81 (46.29)	40 (41.24)	41 (52.56)	0.888
Anaemia N^0 (%) *	10/167 (5.99)	8 (8.25)	2 (2.56)	0.188
Precancerous lesion				
Atrophy with intestinal metaplasia N^0 (%)	49 (28.00)	33 (34.02)	16 (20.51)	<0.001
Atrophy with fibrosis without intestinal metaplasia N^0 (%)	53 (30.29)	37 (38.14)	16 (20.51)	<0.001

**Indicates missing data. The total number of patients with information on smoking status is 115, of whom 20 are smokers; in the case of alcohol consumption, the total number is 74, of whom 29 are regular alcohol consumers (daily).*

5.2.1 Prevalence of autoantibody positivity

Out of 175 CG patients, 97 had positive AI serology results (55.43%). The prevalence of AIG was 21.71% (38/175); out of 38 patients with AIG, 35 (20.00%) had anti-parietal cell antibody positivity, and three patients (1.71%) had both anti-parietal cell and anti-intrinsic factor antibody. Celiac disease-related antibody positivity was present in 8% of the patients (14/175), anti-gliadin antibody positivity was found in all 14 patients (100.00%), anti-endomysium antibody positivity in two patients (1.14%), and tissue transglutaminase antibody IgA and/or IgG in six patients (3.43%). AI thyroiditis-related antibody positivity was found in 17.54% (20/114) of the patients, while 11.90% of the subjects (15/126) had ASCA positivity. The most found antibody was ANA in 19.13% of the patients (22/115). Antibodies against nucleosome (8.70% of CG patients), RF (7.34% of the analysed population) and ds-DNA (6.07% of the patients) were also observed. AI hepatitis-related serology was positive in 9.52% (6/63) of the subjects. In 3.48% of the cases (4/115), anti-b2 glycoprotein positivity was found. Three patients out of 126 (2.38%) had positive serology regarding ANCA, and three out of 111 (2.70%) showed positive SSA. Anti-cardiolipin, anti-centromere, anti-C1q, SSB and myositis-specific antibodies were also found in fewer cases (<1%). Full details of antibody positivity are provided in *Table 2*. Regarding the prevalence of each antibody positivity, no significant difference was observed between females and males ($p>0.05$). There was no significant relationship between gender and AISP ($p>0.05$).

Table 2. Prevalence of autoantibody positivity in patients with chronic gastritis.

Autoimmune disease (attributed antibodies)	Positive (n)	Total number of patients tested	%
AI gastritis (AIG)	**38**	175	**21.71**
Anti-parietal cell antibodies	35	175	20.00
Anti-intrinsic factor antibodies	3	175	1.71
Celiac disease	14	175	8.00
Anti-gliadin	14	175	8.00
Anti-endomysium	2	175	1.14
Tissue transglutaminase antibodies IgA	3	175	1.71
Tissue transglutaminase antibodies IgG	3	175	1.71
Sjögren's syndrome	3	111	2.70
Anti-Sjögren's syndrome-related antigen A (SSA)	3	111	2.70
Anti-Sjögren's syndrome-related antigen B (SSB)	0	111	0.00
Systemic lupus erythematosus (SLE)	**31**	**115**	**26.96**
Anti-nuclear antibodies (ANA)	22	115	19.13
Anti-nucleosome antibodies	10	115	8.70
Anti-cardiolipin	2	115	1.74
Anti-centromere	1	115	0.87
Anti-C1q	1	115	0.87
Anti-b2 glycoprotein	4	115	3.48
Anti-double-stranded DNA (ds-DNA)	7	115	6.07
Autoimmune hepatitis	**6**	**63**	**9.52**
Anti-smooth muscle antibodies (SMA)	1	63	1.59
Anti-liver/kidney microsomal antibodies (LKM-1, LKM-2, LKM-3)	0	63	0.00
Anti-soluble liver antigens (SLA)	0	63	0.00
Liver–pancreas antigens (LP)	0	63	0.00
Anti-mitochondrial antibodies (AMA)	3	63	4.76
Anti-filamentous actin 1 antibodies (F1 actin)	2	63	3.17
Rheumatoid arthritis	**8**	**109**	**7.34**
Anti-cyclic citrullinated peptide antibodies (CCP)	0	109	0.00
Anti-rheumatoid factor (RF) antibodies	8	109	7.34
Systemic sclerosis (Ssc)	**1**	**96**	**1.04**
Anti-Scl-70 antibodies	0	96	0.00
Anti-centromere antibodies	1	96	1.04
Polymyositis/dermatomyositis	**0**	**99**	**0.00**
Anti-Jo-1 antibody	0	99	0.00
Inflammatory bowel disease (IBD)	**18**	**126**	**14.29**
Anti-yeast *Saccharomyces cerevisiae* (ASCA)	15	126	11.9
Anti-neutrophil cytoplasmic antibodies (ANCA)	3	126	2.38
AI thyroiditis	**20**	**114**	**17.54**
Anti-thyroid peroxidase (TPO)	13	114	11.40
Anti-TSH receptor antibodies (TRAb)	2	114	1.75
Anti-thyroglobulin antibodies (Tg)	5	114	4.39

5.2.2 Poor histological outcomes and autoimmune positivity

Concerning precancerous lesions, the AISP group was associated more with atrophy alone (37 vs 16 patients, p<0.001). Atrophy with IM was observed in 33 (34.02%) and 16 (20.51%) patients in the AISP and AISN groups, respectively (p<0.001).

5.2.2.1 Univariate analyses

Based on the results of the univariate analyses, a significant association was observed between AI positivity and precancerous lesions of the stomach. Atrophy was found more frequently in the AISP group (p=0.015). The co-occurrence of atrophy and IM was also correlated with AISP (p=0.039).

AIG-related antibody positivity, especially anti-parietal cell antibody positivity associated with atrophy with IM (p=0.033). No significant correlation was found between any other AI disease-related antibodies and precancerous lesions. No difference was observed concerning worse OLGA score (OLGA 3–4) and AI positivity. Comparisons on individual AI bodies were not carried out due to the low number of cases.

5.2.2.2 Bivariate analyses

Results of bivariate analyses adjusted for age found significant associations in the following relations: AIG-related antibodies with atrophy (OR 2.250; 95% CI 1.945 to 5.357; p<0.001) and atrophy with IM (OR 2.229; 95% CI 1.019 to 4.877; p<0.001); SLE-related antibodies and atrophy (OR 2.288; 95% CI 1.523 to 3.176; p=0.002) and atrophy with IM (OR 2.340; 95% CI 1.375 to 5.841; p=0.006); IBD-related antibody (ASCA and ANCA) positivity with atrophy with IM (OR 2.760; 95% CI 1.218 to 2.645; p=0.017) and atrophy without IM (OR 5.308; 95% CI 1.480 to 19.036; p=0.001); anti-parietal cell antibody with atrophy with IM (OR 2.229; 95% CI 1.019 to 4.877 p=0.006).

Concerning the results of bivariate analyses adjusted for gender, the following associations was found: AIG-related antibodies with atrophy (OR 2.732; 95% CI 1.350 to 2.349; p<0.001) and atrophy with IM (OR 2.222; 95% CI 1.040 to 4.749; p<0.001); SLE-related antibody positivity with atrophy (OR 2.766; 95% CI 1.755 to 4.132; p<0.001) and atrophy with IM (OR 4.294; 95% CI 1.313 to 14.043; p=0.001); ASCA and ANCA positivity and atrophy without IM (OR 2.352; 95% CI 1.032 to 6.645; p=0.007); ANA positivity (OR 2.044; 95% CI 1.097 to 5.242; p=0.029) and AI thyroiditis-related antibody positivity (OR 2.566; 95% CI 1.574 to 4.274; p=0.048). Anti-parietal cell antibody positivity was also associated with worse histological outcomes (OR 2.222; 95% CI 1.040 to 4.749; p=0.038). Sjögren's syndrome, AI hepatitis, RA, SSc and polymyositis/dermatomyositis-related antibody positivity did not significantly affect precancerous lesions.

The analysis regarding simultaneous AI positivity showed a higher risk for precancerous lesions in some cases: SLE-related antibodies (OR 4.778; 95% CI 1.945 to 2.089; p=0.058); AIG-related antibodies (OR 3.182; 95% CI 1.708 to 8.142).

5.2.3 Location and extent of the inflammation

In the AISP group, 57 patients out of 97 had pangastritis (58.76%), while 47 out of 78 (60.28%) were in the AISN group. The inflammation affected only the antrum in 33 subjects in the AISP group (34%) and 23 in AISN. Antrum gastritis was associated more with AI positivity (p=0.042). Isolated corpus gastritis was associated with AI positivity also (p=0.023); affection of the corpus was found in 9 (9.28%) AISP and 6 (7.70%) AISN patients, respectively.

5.2.4 Clinical symptoms

The most frequent symptoms in patients with *H. pylori*-negative CG were as follows: retrosternal burning sensation in 17.14% (30/175 patients); bloating and/or diarrhoea in 9.14% (16/175); diffuse abdominal discomfort/pain not relating to meals in 8.57% (15/175); globus sensation in 4% (7/175); nausea in 4.57% (8/175) and vomitus in 2.29% (4/175).

Diffuse abdominal pain/discomfort was more common in the AISP group than in the AISN (9 vs six patients, respectively, p=0.023). Globus pharyngeus was associated with AISP (p<0.001): 6 patients experienced globus sensation in the AISP group, while one was in the AISN group. Regarding other symptoms, significant differences between AISP and AISN groups could not be observed. Twelve patients experienced a retrosternal burning sensation in the AISP group and 18 in the AISN group (p=0.0713). Less common symptoms were nausea (4 AISP and 4 AISN patients, p=1.000), vomiting (1 AISP and 3 AISN patients, p=0.325), and bloating and/or diarrhoea (9 AISP and 7 AISN patients, p= 0.152). *(Table 3.)*

Table 3. Distribution of frequently occurring symptoms and location of the inflammation between autoimmune positive and negative groups.

	Overall (n=175)	**AISP (n=97)**	**AISN (n=78)**	**p-value**
Key symptom				
Dyspepsia-like symptoms N^0 (%)	95 (54.29)	58 (58.76)	37 (48.72)	**0.012**
Retrosternal burning N^0 (%)	30 (17.14)	12 (12.37)	18 (23.08)	0.0713
Globus pharyngeus N^0 (%)	7 (4.00)	6 (6.19)	1 (1.28)	**<0.001**
Nausea N^0 (%)	8 (4.57)	4 (4.12)	4 (5.13)	1.000
Vomiting N^0 (%)	4 (2.29)	1 (1.03)	3 (3.85)	0.325
Bloating, Diarrhoea N^0 (%)	16 (9.14)	9 (9.28)	7 (8.97)	0.152
Abdominal discomfort/pain N^0 (%)	15 (8.57)	9 (9.28)	6 (7.70)	**0.023**
Location of the gastritis				
Antrum N^0 (%)	56 (32.00)	33 (34.02)	23 (29.49)	**0.042**
Corpus N^0 (%)	15 (8.57)	9 (9.28)	6 (7.70)	**0.023**
Pangastritis N^0 (%)	104 (59.43)	57 (58.76)	47 (60.26)	0.269

5.2.5 Dyspepsia-like symptoms in autoimmune seropositivity

The prevalence of dyspepsia-like symptoms was 54.29% and correlated with AISP (p=0.012). Celiac-disease antibody positivity (p=0.045), ANCA and ASCA positivity (p=0.043) were also associated with dyspepsia. However, the analysis could not prove any relation between dyspepsia-like symptoms and other AI-related antibody positivity, like Sjögren's syndrome, SLE, AI hepatitis, RA, SSc, polymyositis/dermatomyositis, AI thyroiditis (p>0,05). No association was observed between AIG-related antibody positivity and dyspepsia either (p=0.677).

6 DISCUSSION

6.1 Autoimmunity and poor histological outcomes in the stomach: from precancerous lesions to gastric cancer

To summarise the results of our study, one of our major findings was that 55% of the patients with CG had positive AI serology. Our results align with previous findings from other countries: the prevalence of AIG was measured at about 20%, and anti-parietal cell antibodies were found to be more common than anti-intrinsic factor antibodies. Regarding AI disorders besides AIG, SLE-related antibodies, AI thyroiditis, IBD-, celiac disease-, and RA- related antibodies were also commonly positive in our patients. Autoimmunity was associated with precancerous lesions in the stomach: atrophy and atrophy combined with IM. AIG-, SLE- and IBD-related (ASCA and ANCA) positivity were also associated with atrophy and atrophy with IM, seeming to be significant risk factors for poor prognosis. AI thyroiditis-related antibodies and ANA positivity by itself correlated with atrophy alone. Our analysis could not prove any role of other examined antibodies in gastric carcinogenesis. Higher OLGA score and AI positivity showed no significant difference.
Concerning the incidence of gastric cancer in AI disorders, our meta-analysis, including data from 499,427 patients collected from 43 studies, showed that PA, Graves' disease, dermatomyositis, T1DM, inflammatory myopathies and SLE were associated with gastric cancer.

Although this kind of correlation between CG and autoimmunity has not been investigated before, it has been shown that AI diseases often have GI manifestations. The literature shows CG is common in patients with IBD and celiac disease. SLE can affect the GI tract as well; however, according to previous results, histologically proven gastritis is rare in these patients. Regarding the GI tract involvement of RA, it can affect both the GI tract and the liver. Marcolongo et al. found chronic superficial and chronic atrophic gastritis in 30 and 62.5% of patients with RA. A study by Lecouffe-Desprets et al. described SLE, RA, SSc, inflammatory myopathies, Sjögren's syndrome and scleromyositis or other overlapping connective tissue

diseases (5% each) are associated with eosinophilic gastrointestinal disorders. As regards the connection between poor histological prognosis of CG and systemic AI disorders, the context of precancerous lesions was not examined before. However, results of our meta-analysis reassert our study results: AIG, SLE and AI thyroiditis were associated with higher risk for precancerous lesions as well as higher incidence of gastric cancer.

An elevated incidence of various GI tumours has been described in patients with RA, SLE, Sjögren's syndrome, celiac disease, idiopathic inflammatory myositis, and SSc. Studies have shown an increased risk of gastric cancer in patients with dermatomyositis, RA, scleroderma, SLE, or T1DM. In line with our results, Song et al. concluded that patients with dermatomyositis, PA, Addison's disease, dermatitis herpetiformis, IgG4-related disease, primary biliary cirrhosis, T1DM, SLE, and Graves' disease had increased risk for gastric cancer.

The connection between AIG and precancerous lesions of the stomach and/or gastric cancer is well known. A previous prospective cohort study described an annual incidence of 0.25% per person-year for gastric cancer (95% CI 0.07–0.6%), 0.43% per person-year for gastric dysplasia (95% CI 0.2–0.9%) and 0.68% per person-year for type 1 gastric neuroendocrine tumour (95% CI 0.3–1.2%) in patients with AIG. Patients with AIG have 3–7-fold increased risk for gastric adenocarcinoma.

Hsing et al. described that PA was associated with gastric cancer since it is in relation to AIG and results from gastric mucosal damage. The mice model of this pathomechanism suggests correlations between carcinogenesis and autoimmunity. The frequent co-occurrence of AI thyroiditis, T1DM, vitiligo, and Addison disease with PA has been shown, which raises the possibility of a direct mechanistic interpretation through its pathological correlate, AIG.

Epidemiologic evidence for an AI contribution to gastric carcinogenesis shows an elevated incidence of AI disorders in patients with neoplasms. Inversely, an increased incidence of tumours has been demonstrated in patients with AI disorders. Recently an increment in the incidence of both AI disorders and parallelly cancers could be observed. The specific AI

inflammation is often associated with the tumorous disorder of the affected organ. This phenomenon is most conspicuous in people below 50 years of age, and regarding gastric cancer, it affects females more.

Although autoimmunity may significantly affect the development of different neoplasms, the exact pathomechanism remains unclear. Chronic inflammation precedes tumour formation in time. Immune dysregulations, which play a pivotal role in autoimmunity, are also thought to be important in carcinogenesis: AI disorders may lead to antigen specificity-driven tissue damage causing chronic inflammation. Moreover, several other factors can be identified, such as immunosuppression, infections, dietary habits, and environmental factors. These can induce chronic cell damage and trigger AI conditions or cancer.

6.2 Autoimmunity and dyspepsia-like symptoms

Our dyspepsia and autoimmunity-related study investigated the relationship between autoimmunity and dyspeptic symptoms in patients with *H. pylori-negative* chronic gastritis. To summarise our results, the prevalence of dyspepsia-like symptoms was 54.29%. Regarding the connection with autoimmunity, dyspeptic symptoms, diffuse abdominal pain/discomfort, and globus pharyngeus correlated with the presence of autoimmunity. Based on disease-specific analysis celiac disease-related antibody positivity, ASCA and ANCA positivity were associated with dyspeptic symptoms. However, the analysis could not prove correlation between any other investigated AI disease-related antibody positivity and dyspepsia of unknown origin in CG patients.

It is well known that *H. pylori* infection might be associated with FD: the prevalence of *H. pylori* infection is more frequent in dyspeptic patients than in healthy controls. *H. pylori* infection may alter gastric functions, causing increased gastrin-, pepsinogen-, and acid secretion, which might play an important role in the pathogenesis of FD.

However, an increased incidence of dyspepsia-like symptoms was described in *H. pylori*-negative CG as well. As mentioned previously, CG is one of the most common findings during upper GI endoscopy; however, the underlying cause often remains undetectable. In most cases CG is discovered accidentally without causing any symptom. Therefore, we investigated possible etiological factors behind CG that could be associated with dyspepsia-like symptoms.

In our study, 55% of the patients with CG had AI positivity, which was associated with dyspepsia-like symptoms. Several studies suggest a possible connection between AI disorders and dyspepsia in line with our hypothesis and results. These studies described that more than half of the patients with AI disorders have dyspepsia-like symptoms, which may be the consequence of gastroparesis and antral distension. Since immune dysregulation might play a pivotal role in developing both AI- and functional GI disorders, autoimmunity could be a risk factor for functional diseases such as FD. It is confirmed by Koloski et al., describing that AI diseases are associated with FD and irritable bowel syndrome, independent of psychological distress.

In line with our results, Jocelyn A Silvester et al. described that FD occurs in 27% of patients with coeliac disease, which is relieved by a gluten-free diet. A case report by A. Maertens et al. reported how dyspepsia led to a diagnosis of Crohn's disease. Furthermore, our study confirms the work of Lebwohl et al. about the association between *H. pylori*-negative CG with celiac disease. An elevated rate of dyspepsia has also been shown in patients with Sjögren's syndrome, SLE, RA, and AI thyroiditis; however, our study could not prove these correlations.

As mentioned previously, AI disorders often have GI manifestations, and the symptoms might be subclinical and non-specific, with considerable overlap among different conditions. Sometimes it can be the only presented sign of an underlying AI disease. Serologic testing for immune-mediated GI disorders (e.g., celiac disease, IBD) allows broader screening, helping differentiate organic disease from functional GI disorders.

7 CONCLUSIONS

7.1 Summary of novel findings

1. Our meta-analysis confirmed the relationship between AI disorders and gastric cancer. It concludes that PA, Graves' disease, dermatomyositis, T1DM, inflammatory myopathies, and SLE are associated with higher incidence rates of gastric cancer.
2. Our meta-analysis did not confirm any association between gastric cancer and AI vasculitis, celiac disease, SSC, dermatitis herpetiformis, Hashimoto thyroiditis, Sjogren's syndrome, IBD, Crohn's disease, RA, ulcerative colitis, ankylosing spondylitis or primary biliary cirrhosis.
3. Our study confirmed that AI positivity often underlies gastritis of unknown aetiology and predisposes to precancerous lesions in the stomach. It concludes that in the southwestern Hungarian population, anti-parietal cell antibody, ANA, ANCA and ASCA positivity correlated with a worse histological outcome, such as atrophy with or without IM.
4. Our study did not confirm any association between Sjögren's syndrome, AI hepatitis, RA, SSc and polymyositis/dermatomyositis-related antibody positivity and precancerous lesions of the stomach.
5. Our study confirmed that AI positivity in histologically established *H. pylori*-negative CG may predispose to dyspeptic symptoms and may be the causative factor behind uninvestigated FD. It concludes that celiac disease-related antibody positivity, ASCA and ANCA positivity were associated with dyspeptic symptoms.
6. Our study did not confirm any association between dyspepsia-like symptoms and Sjögren's syndrome, SLE, AI hepatitis, RA, SSc, polymyositis/dermatomyositis, AI thyroiditis, or even AIG.

7.2 Clinical practice and future perspectives

Based on the results of our meta-analysis, close gastroenterological follow-up or symptom-oriented routinely performed upper GI endoscopy based on the results of serological screening, may be cost-effective and clinically helpful to prevent subsequent malignancy for patients diagnosed with the above-mentioned six AI disorders.

Based on the data of our studies, it is suggested to look for autoimmunity in patients with CG when a clear etiological factor cannot be identified. Antibodies can serve as non-invasive markers to aid in the identification of the optimal timing of an endoscopic follow-up strategy based on the risk of atrophy/IM development. Given that our study population did not suffer from diagnosed manifest AI disorder, it also raises the possibility that gastritis may predict the development of a later AI disease. Thus, if there is no clear explanation for the aetiology of CG or if the symptoms persist after eradication of *H. pylori*, it is advisable to assess these patients for systemic AI-related antibodies and, if positive, to involve an immunologist for a close follow-up; therefore, AI disorders can be recognisable in the early stages of the disease.

Screening for celiac disease or ASCA and ANCA-related AI disorders (IBD, vasculitis) in case of dyspeptic symptoms might be worthwhile as well. Furthermore, it can be helpful in the earlier diagnosis of these AI disorders.

To establish a higher quality of evidence, further prospective studies are required to prove these associations.

8 ACKNOWLEDGMENTS

I would like to express my gratitude to all those who have made it possible for me to complete this thesis.

First and foremost, I would like to thank my supervisor Dr József Czimmer, for leading my PhD topic and for all his help and cooperation during my work.

I would also like to thank Professor Dr Péter Hegyi, head of the Institute for Translational Medicine, for allowing me to participate in the PhD program and contributing to my scientific and professional development. Furthermore, I am grateful to him for helping my scientific carrier and for teaching me to always reach higher and set the highest standards.

I would also like to thank Professor Dr Kálmán Tóth, head of the First Department of Internal Medicine, for providing the clinical background necessary for my work.

I am very grateful to the multidisciplinary staff of the Institute for Translational Medicine, who assisted me during the planning, writing, and publishing of my research. I would like to thank the site of recruitment of the Division of Gastroenterology and student researcher Levente Frim, whose efforts deserve to be highlighted for assisting with the project.

Special thanks go to my dear friends and colleagues, Dr Nóra Vörhendi, Dr Lajos Szakó and Dr Szilárd Váncsa, for their continuous professional and personal support and help.
Finally, I would also like to express my emphasised gratitude to my parents, my sister, and friends and especially for my love for their trust, support, and motivation, without which this thesis could not have been completed.