研究用

EUROLINE Autoimmune Gastrointestinal Diseases (IgG/IgA)

Sensitivity and specificity:

tTG and GAF-3X:

44 serum samples (pre-characterised with a CE-labelled reference test) were investigated for autoantibodies against tTG (borderline results were not included in the calculation).

n = 44		ELISA Anti-tTG IgG		
		positive	borderline	negative
EUROLINE Autoimmune Gastrointestinal Diseases Anti-tTG IgG	positive	10	0	1
	borderline	0	0	2
	negative	0	0	31

In the investigated panel, a sensitivity of 100% at a specificity of 96.9% was determined with respect to the reference system.

46 serum samples (pre-characterised with a CE-labelled reference test) were investigated for autoantibodies against gliadin (GAF-3X) (borderline results were not included in the calculation).

n = 46		ELISA Anti-Gliadin (GAF-3X) IgG		
		positive	borderline	negative
EUROLINE Autoimmune Gastrointestinal Diseases Anti-GAF-3X IgG	positive	8	0	1
	borderline	3	0	1
	negative	1	0	32

In the investigated panel, a sensitivity of 88.9% at a specificity of 97.0% was determined with respect to the reference system.

Other studies: The prevalence of coeliac disease specific autoantibodies was investigated in patients with SLE, RA, diabetes or Crohn's disease.

Patient panel	n (73)	Anti-GAF-3X IgG positive	Anti-tTG IgG positive
SLE	15	0%	0%
RA	20	0%	0%
Diabetes	18	5.6%	0%
Crohn's disease	20	0%	0%

Reference range: The reference range was determined in a sample panel of healthy blood donors (n = 150). All blood donors reacted negative.

Parietal cells antigen (PCA)

30 serum samples (pre-characterised with a CE-labelled reference test) were investigated for antibodies against PCA (borderline results were not included in the calculation).

n = 30		Gastro-LIA (Human, Imtec): Anti-PCA IgG		
		positive	borderline	negative
EUROLINE Autoimmune Gastrointestinal Diseases: Anti-PCA IgG	positive	13	2	1
	borderline	0	0	2
	negative	0	3	9

In the investigated panel, a sensitivity of 100% at a specificity of 90.0% was determined with respect to the reference system.

In a study, 29 sera from patients with chronic autoimmune gastritis (University, Italy) and 152 healthy blood donor sera were investigated for antibodies against PCA. The prevalence was as follows:

Disease	**Prevalence of antibodies against PCA**	
	Number of samples	**Anti-PCA IgG positive (%)**
Chronic autoimmune gastritis	29	96.6
Healthy blood donors	152	13.2

Intrinsic factor:

30 serum samples (pre-characterised with a CE-labelled reference test) were investigated for autoantibodies against IF (borderline results were not included in the calculation).

n = 30		Gastro-LIA (Human, Imtec): Anti-IF IgG		
		positive	borderline	negative
EUROLINE Autoimmune Gastrointestinal Diseases: Anti-IF IgG	positive	4	0	0
	borderline	0	0	0
	negative	0	1	25

In the investigated panel, a sensitivity of 100% at a specificity of 100% was determined with respect to the reference system.

In a study, 29 sera from patients with chronic autoimmune gastritis (University, Italy) and 150 healthy blood donor sera were investigated for antibodies against IF. The prevalence was as follows:

Disease	**Prevalence of antibodies against Intrinsic factor**	
	Number of samples	**Anti-IF IgG positive (%)**
Chronic autoimmune gastritis	29	31.0
Healthy blood donors	150	0.0

ASCA (Mannan from Saccharomyces cerevisiae):

30 serum samples (pre-characterised with a CE-labelled reference test) were investigated for antibodies against Mannan (ASCA) (borderline results were not included in the calculation).

n = 30		Gastro-LIA (Human, Imtec): ASCA IgG		
		positive	borderline	negative
EUROLINE Autoimmune Gastrointestinal Diseases: ASCA IgG	positive	4	0	0
	borderline	0	0	1
	negative	0	0	25

In the investigated panel, a sensitivity of 100% at a specificity of 100% was determined with respect to the reference system.

In a study, 31 sera from patients with Crohn´s disease, 15 from Ulcerative colitis patients and 150 healthy blood donor sera were investigated for antibodies against Mannan (ASCA). The prevalence was as follows:

Disease	**Prevalence of antibodies against Mannan (ASCA)**	
	Number of samples	**ASCA IgG positive (%)**
Crohn´s disease	31	67.7
Ulcerative colitis	15	20.0
Healthy blood donors	150	13.3

Sensitivity and specificity:

tTG and GAF-3X:

44 serum samples (pre-characterised with a CE-labelled reference test) were investigated for autoantibodies against tTG (borderline results were not included in the calculation).

n = 44		ELISA Anti-tTG IgA		
		positive	borderline	negative
EUROLINE Autoimmune Gastrointestinal Diseases Anti-tTG IgA	positive	24	0	0
	borderline	1	0	0
	negative	0	0	19

In the investigated panel, a sensitivity of 100% at a specificity of 100% was determined with respect to the reference system.

45 serum samples (pre-characterised with a CE-labelled reference test) were investigated for autoantibodies against gliadin (GAF-3X) (borderline results were not included in the calculation).

n = 45		ELISA Anti-Gliadin (GAF-3X) IgA		
		positive	borderline	negative
EUROLINE Autoimmune Gastrointestinal Diseases Anti-GAF-3X IgA	positive	16	0	0
	borderline	3	0	1
	negative	2	0	23

In the investigated panel, a sensitivity of 88.9% at a specificity of 100% was determined with respect to the reference system.

Other studies: The prevalence of coeliac disease specific autoantibodies was investigated in patients with SLE, RA, diabetes or Crohn's disease.

Patient panel	n (80)	Anti-GAF-3X IgA positive	Anti-tTG IgA positive
SLE	20	0%	0%
RA	20	0%	0%
Diabetes	20	20.0%	15.0%
Crohn's disease	20	0%	0%

Reference range: The reference range was determined in a sample panel of healthy blood donors (n = 150). All blood donors reacted negative, except for one positive reaction with GAF-3X.

ASCA (Mannan from Saccharomyces cerevisiae):

49 serum samples (pre-characterised with a CE-labelled reference test) were investigated for antibodies against Mannan (ASCA) (borderline results were not included in the calculation).

n = 49		ELISA Anti-Saccharomyces cerevisiae IgA	
		positive	negative
EUROLINE Autoimmune Gastrointestinal Diseases Mannan IgA	positive	20	2
	borderline	2	2
	negative	2	21

In the investigated panel, a sensitivity of 90.9% at a specificity of 91.3% was determined with respect to the reference system.

In one study 30 sera from patients with Crohn´s disease, 15 from ulcerative colitis and 150 healthy blood donor sera were investigated for antibodies against Mannan (ASCA). The prevalence was as follows:

Disease	Prevalence of antibodies against Mannan (ASCA)	
	Number of samples	ASCA IgA positive (%)
Crohn´s disease	30	60.0
Ulcerative colitis	15	20.0
Healthy blood donors	150	8.7

Clinical significance

Coeliac disease is a chronic disease of the small intestine which develops due to a hypersensitivity to the gluten present in many grains (wheat, rye, barley, etc.) [1-4].

During the physiological digestive process, gliadin, a subfraction of gluten, is split proteolytically. Subsequently, the glutamin-containing fragments are deamidated by the enzyme tissue trans-glutaminase (tTG), transforming glutamine into glutamic acid. The deamidated gliadin fragments cause a T-cell mediated immune response which leads to inflammation of the intestinal epithelium, intraepithelial

lymphocytosis and villous atrophy [1, 2, 5].

The clinical picture is characterised by diarrhoea and the consequences of malabsorption, such as weight loss, fatigue, bad temper, and, in infants, failure to thrive [2-5]. In most cases, the disease appears in early childhood, soon after first contact with flour products [5]. One manifestation of coeliac disease in the skin is dermatitis herpetiformis Duhring (DH), a chronic dermatosis which is accompanied by blisters and characterised by strong pruritus. Coeliac disease can be detected in all cases of DH [3, 4].

Several antibodies are associated with coeliac disease, mainly antibodies against gliadin and **autoantibodies against tTG** [1-3, 5, 6]. Anti-tTG antibodies are virtually absent in healthy persons and patients with other gastrointestinal diseases; their prevalence in untreated coeliac disease in contrast, is almost 100%. Antibodies against gliadin are present in 92% of patients. The determination of IgG antibodies against whole gliadin with conventional tests is useless for the diagnosis of coeliac disease, since a quarter of the normal population reacts positively [4]. In patients with coeliac disease, however, very specific antibodies against deamidated gliadin fragments are formed [7].

Coeliac disease affects up to 1% of the population [2]. In suspected cases of coeliac disease, serological investigations are especially indicated. The standard method is the detection of antibodies against endomysium (EMA, IgA) by indirect immunofluorescence, or of IgA against tTG by enzyme immunoassays [1-3, 6]. The target antigen of EMA is tTG [4]. 2 to 3% of patients with coeliac disease have a selective IgA deficiency. Therefore, the most effective combination is the determination of tTG IgA and IgG against deamidated gliadin fragments [1-4, 6]. For the detection of antibodies against deamidated gliadin fragments, EUROIMMUN uses the antigen **GAF-3X**, a **gliadin-analogue fusion peptide**, expressed as a **trimer** [8].

In adults, the diagnosis of coeliac disease can be made by means of positive serological results, a positive histology and serological improvement under gluten-free diet. In children, the diagnosis can be made **without histology**, if the results for anti-tTG IgA are 10 times higher than the cut-off of the test system, a positive anti-EMA IgA result is obtained from a follow-up sample, the patient is HLA-DQ2 or -DQ8 positive, and the symptoms disappear with a gluten-free diet [3, 9, 10].

Baker’s and brewer's yeast *Saccharomyces cerevisiae* is taken up with food and persists in the intestine. There, it is amongst the most frequent fungi [11]. Antibodies against *S. cerevisiae* (ASCA) are directed against mannan in the cell wall of *S. cerevisiae*. ASCA enrich the serological diagnosis of chronic inflammatory bowel disease (CIBD) by a further parameter, alongside autoantibodies against exocrine pancreas (specific for Crohn’s disease), against intestinal goblet cells (pathognomonic of ulcerative colitis), and against granulocytes (pANCA, mainly associated with ulcerative colitis). **ASCA are a specific serological marker of Crohn's disease** [4, 12-16].

Patients with Crohn’s disease (children and adults) with high ASCA titers (also high anti-I2, anti-OmpC, anti-CBir1 antibody titers) present a complicated disease course with strictures and intestinal perforation, requiring surgery [12, 17]. Retrospective studies have shown that ASCA (also pANCA, Anti-CBir1 and Anti-OmpC) occur in around one third of patients with Crohn's disease and ulcerative colitis, on average four years before onset of the disease [13, 17]. ASCA titers do not correlate with the disease activity and in most cases remain unchanged in Crohn's disease and ulcerative colitis [18].

ASCA (IgG and IgA) [13, 16] appear in up to 70% of patients of Crohn's disease, in around 15% of patients with ulcerative colitis, in 11% of control patients with other bowel diseases, and in up to 10% of healthy control persons [14, 16-18]. ASCA can be detected in 20 to 25% of healthy relatives of patients with Crohn's disease. High ASCA titers are found in Caucasian patients with Crohn’s disease, low titers in patients from Japan and South Korea and none in patients from Hong Kong (Han Chinese) [16].

The determination of ASCA plus pANCA provides a diagnostic specificity of around 90% for chronic inflammatory bowel diseases [16]. Diagnostic specificity of the serological tests for the detection of ASCA for Crohn's disease amount to up to 95% at a sensitivity of up to 79% [12, 13, 15, 16].

Autoimmune gastritis (AIG) is a chronic inflammation of the stomach mucosa which leads to atrophic gastritis with malabsorption – iron and vitamin B_{12} uptake are hindered. Young patients develop iron-deficiency anaemia. Due to the vitamin B_{12} deficiency, **pernicious anaemia (PA)** develops over several years [19-24].

The **autoantibodies** in AIG are directed against **parietal cells (APCA)** and against **intrinsic factor**:

- The parietal cells are destroyed. The enzyme H^+/K^+-ATPase is the target antigen of APCA, the proton-potassium pump is responsible for the production of gastric acid [20-24].
- The intrinsic factor is a glycoprotein which is secreted by the parietal cells. Antibody against intrinsic factor interfere with the absorption of the intrinsic factor/vitamin B_{12} complex in the ileum [21-23].

In most patients, AIG remains asymptomatic for many years, until reaching advanced stages of atrophy [19, 20]. Symptoms of PA encompass anaemia, fatigue, lightheadedness and tachycardia. The lack of vitamin B_{12} hinders DNA synthesis and megaloblasts form, e.g. in the bone marrow and in the gastrointestinal epithelium. As a result, malabsorption and diarrhoea with weight loss, anorexia, glossitis, icterus and neurological complaints occur [21-23]. PA is the most frequent reason for vitamin B_{12} deficiency [22, 24].

It usually occurs in people of 30 years of age, men and women are equally affected. PA is especially frequent in northern Europe, mainly in Scandinavia. Its prevalence amounts from 2.5 to 12%, elderly are affected more frequently [21, 24]. The incidence of AIG is estimated to be 2%, its prevalence amounts to up to 19.5% [22, 24].

AIG/PA is often associated with other autoimmune diseases such as diabetes mellitus type 1, auto-immune thyropathy (mainly Hashimoto's), vitiligo, Sjögren's syndrome, coeliac disease and Addison's disease. Patients with AIG/PA have a significantly higher risk of developing a stomach carcinoma [19-21, 23, 24].

APCA are the most sensitive biomarkers for AIG. They occur in 80 to 90% of PA patients, especially in the early stage of the disease, and may appear several years before the clinical symptoms [4, 20, 21, 24]. With progression of the gastritis and the loss in parietal cells, APCA are less frequently found (in 55% of PA patients in the advanced stages) [20, 21, 23]. In the serum, IgG, IgA and IgM, in the gastric juice, mainly IgA and IgG against parietal cells are present [21, 24].

APCA are not specific for AIG and appear also in other autoimmune diseases, such as Hashimoto's thyroiditis, vitiligo or type 1 diabetes [19, 21, 24]. Moreover, APCA are detectable in around 20% of patients with *Helicobacter pylori* infections. APCA are found in up to 9% of the healthy population, the frequency increases with the age [21, 22, 24].

Antibodies against intrinsic factor are highly specific for AIG. However, they only occur in 40 to 60% of patients with PA, with longer disease, in 60 to 80% [20, 21, 23, 24]. Two types (both IgG) are found in serum: Type 1 reacts with the vitamin B_{12} binding site, type 2 hinders the binding of intrinisc factor/vitamin B_{12} complex to the receptors in the ileum [21, 24]. Type 1 antibodies are detected in 70% and type 2 antibodies in 30 to 40% of PA patients [21, 24]. Type 2 antibodies occur mainly together with type 1 antibodies [4, 21, 23, 24]. In approximately 80% of PA patients, antibodies against intrinsic factor (IgA) are found in the gastric acid, secreted by plasma cells which have migrated into the mucosa [21].

PA diagnostics are based on the detection of megaloblastic anaemia, low vitamin B_{12} levels in serum, atrophy of the stomach mucosa (affecting corpus and fundus) and antibodies against parietal cells and intrinsic factor [19-21, 23]. The diagnostic sensitivity of the APCA tests for PA amounts to around 90% [4, 19, 21]. Asymptomatic AIG is diagnosed based on the presence of APCA [22]. Antibodies against intrinsic factor indicate developing or established PA [19].

The following differential diagnoses must be taken into account: microcytic anaemia, alcohol abuse, liver diseases, myelodysplastic syndrome and other factors which cause a vitamin B_{12} deficiency such as a strictly vegetarian diet, particular drugs and infections, as well as stomach surgery [21]. Chronic-atrophic gastritis is not only caused by AIG, but primarily by infection with *H. pylori* [20].

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