Algorithm of coeliac disease investigation

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Coeliac disease (CD) is an immune-mediated systemic disorder elicited by gluten and related prolamines. It is characterized by the presence of a variable combination of gluten-dependent clinical manifestations, disease-specific antibodies, a strong genetic predisposition, and enteropathy. CD-specific antibodies comprise autoantibodies against tissue transglutaminase type 2 (tTG) including endomysial antibodies (EmA), and antibodies against deamidated forms of gliadin peptides (gliadin-analogous trimeric fusion peptide, GAF-3X). The genetic risk factors for CD encompass the human leukocyte antigens (HLA) DQ2 and DQ8. Nowadays, the determination of CD-specific antibodies and CD-associated DNA alleles plays a central role in the diagnosis of CD and is in some cases sufficient to circumvent the need for intestinal biopsy.

A clinical chameleon

The hallmark of CD is morphological damage to the proximal small intestinal mucosa with atrophy of villi and hyperplasia of cryptae, which can manifest in many different ways. The disease is subdivided into different forms: the classical form with malabsorption syndrome, the atypical form with unspecific symptoms, the silent form with subclinical course and the potential form, in which CD-specific antibodies are detected but no manifestation is apparent.

Classical intestinal symptoms of CD include diarrhoea, vomiting, abdominal distension, pain, malnutrition and weight loss. Other manifestations occur in the CNS, e.g. ataxia, seizures and depression, in the skin, e.g. dermatitis herpetiformis, stomatitis and hair loss, in the bones, e.g. osteoporosis, fractures, arthritis and dental anomalies, in the heart, e.g. carditis, or in the reproductive tract, e.g. infertility and miscarriage. CD may also cause delayed adolescence, anaemia and malignancies.

CD results in lifelong intolerance to gluten. A strict gluten-free diet (GFD) allows regeneration of the small intestinal mucosa and regression of symptoms. Non-adherence to a GFD increases the risk for long-term consequences such as malignoma, pregnancy complications, osteoporosis and development of other autoimmune diseases. A proper diagnosis even of subclinical CD is critical to avoid long-term health problems.

Prevalence in the MENA region

Until the 1990s, CD was believed to almost exclusively affect individuals of European origin and assumed uncommon in Middle East and North African populations. However, CD has since been shown to be as common in these latter regions as in Western Europe, with low levels of clinical suspicion and availability of healthcare resources accounting for the previous underdiagnosis. Evaluation of the current literature reveals prevalences of 0.1 to 1.2% in low risk and of 2.4 to 44% in high risk populations in the Middle East and North Africa.

ESPGHAN diagnostic strategy

Since the publication of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines on the diagnosis of CD in 1990, the perception of the disease has changed from a rather uncommon enteropathy to a common multi-organ disease strongly associated with the haplotypes (HLA)-DQ2 and -DQ8. Moreover, for the diagnosis of CD, which originally mainly relied on intestinal biopsies, serological test systems have gained more and more importance.

In the revised version of the guidelines from 2012, new diagnostic criteria were defined by two strategies addressing either patients (children/adolescents) presenting with symptoms of CD (algorithm 1) or risk group patients without CD-specific symptoms (algorithm 2).

According to the guidelines, biopsy can be omitted in symptomatic patients if the concentration of CD-specific antibodies exceeds 10 times the upper limit of normal (10 ULN) and further confirmatory tests are positive. The future diagnostic strategy in CD is proposed to mainly rely on CD-specific antibodies with intestinal biopsies being applied only to difficult borderline cases.

tTG autoantibodies

IgA antibodies to endomysium or tTG have a prevalence of 95 to 100% in CD patients, while they are virtually absent from healthy individuals or patients with other intestinal diseases. Detection of EmA using indirect immunofluorescence (IIF) is considered the reference standard for CD-specific antibodies. Since the identification of tTG as the major antigen recognized by EmA, IgA ELISA based on (recombinant) human tTG have been developed, showing a high correlation to IIF-based determination of EmA.

Deamidated gliadin antibodies

Antibodies against native gliadin turned out to have unsatisfying diagnostic sensitivity and specificity and their determination is outdated. Recently, antibodies directed against deamidated gliadin have been described as a new diagnostic tool. Based on the finding that only a portion of the epitopes within the gliadin molecule are diagnostically relevant and that these must be present in deamidated form, a novel recombinant gliadin-analogous fusion peptide (GAF), consisting of an immunodominant tTG-deamidated nonapeptide (derived from digested wheat γ-gliadin) fused to the sequence of an empirically adapted immunocompetent gliadin-analogous octapeptide, was developed in trimeric form (3X) (Figure 1).

The use of this designer peptide as antigenic target considerably increases the sensitivity of the immunoassay (IgA: 84%, IgG: 96%), compared to native gliadin-based ELISA (IgA: 54%, IgG: 31%) at a defined specificity of 95% (Figure 2). The overall increase in sensitivity amounts to 30% for IgA and 65% for IgG. The sensitivity of the Anti-Gliadin (GAF-3X) IgG ELISA (96%) is comparable to that of the Anti-tTG IgA ELISA (97%) at a defined specificity of 95%.

Combined testing for anti-gliadin (GAF-3X) and anti-tTG

The highest diagnostic accuracy for CD is achieved by the parallel determination of anti-gliadin (GAF-3X) IgG and anti-tTG IgA antibodies (Figure 3). High titers of anti-gliadin (GAF-3X) can strengthen diagnosis in cases with grey-zone titers of anti-tTG, and vice versa. The Anti-Gliadin (GAF-3X) IgG ELISA is, moreover, valuable for identifying CD patients with an IgA deficiency, which is frequently associated with CD. The test combination of anti-gliadin (GAF-3X) IgG and anti-tTG IgA is able to identify 78% of CD patients (area A) and exclude 92% of control subjects (area B) with a probability close to 100%.

Diagnosis in all age groups

The parameters anti-gliadin (GAF-3X) and anti-tTG are suitable for diagnosing CD in all age groups. The prevalence of anti-gliadin (GAF-3X) and anti-tTG antibodies (IgA and IgG) was demonstrated to be similar in child and adult CD patients. Moreover, the Anti-GAF-3X IgG and Anti-tTG IgA ELISAs performed equally well in panels of children of different ages, namely 0-2 years, 2-4 years and >4 years (Table 1). ▷

Therapy monitoring

Determination of anti-gliadin (GAF-3X) IgG and anti-tTG IgA is suitable for assessing disease activity and for monitoring a GFD or a gluten-load test. Titers of both antibodies decrease in a comparable manner under a GFD. In patients placed on a GFD, antibody titers should decrease significantly within six months and reach normal values within two years. Persistently high titers or increasing titers indicate non-compliance with a GFD, consciously or not.

HLA-DQ2 and -DQ8

HLA-DQ2 and -DQ8 are the principle determinants of genetic susceptibility for CD and are found in virtually all patients. The familial prevalence in first-degree relatives of patients amounts to 10%. These haplotypes are also found in approximately one third of the healthy population.

Since both haplotypes serve as valuable exclusion parameters for CD diagnostics, HLA-DQ2/-DQ8 analysis is recommended as the first-line test for screening asymptomatic persons at high genetic risk for CD (algorithm 2). With a negative result for both HLA-DQ2 and -DQ8, CD can be virtually excluded. Moreover, HLA-DQ2/DQ8 functions as confirmatory parameter in symptomatic persons (algorithm 1), and is particularly useful for clarifying cases with inconclusive biopsy/serology (especially infants and individuals on a gluten-free diet) and for differential diagnostics. The EUROArray HLA-DQ2/DQ8 detects all relevant disease-associated alleles (HLA-DQA1 and -DQB1) and yielded maximal sensitivity and specificity (100%) in clinical studies with pre-characterized samples.

Summary

Due to major advances in assay capabilities, laboratory testing nowadays forms the backbone of CD diagnostics. The recently revised guidelines from ESPGHAN are based primarily on the determination of CD-specific antibodies, with intestinal biopsy used in a confirmatory capacity. The parallel determination of anti-deamidated gliadin (GAF-3X) IgG and anti-tTG IgA antibodies achieves the highest diagnostic accuracy. If the genetic determinants HLA-DQ2 and -DQ8 are negative, CD can be as good as excluded. It is expected that future studies and refinements to the diagnostic strategy will place even greater emphasis on serology and render biopsy unnecessary in the vast majority of cases. ML

▽**Table 1:** Diagnostic accuracy of CD antibodies in relation to age

| | Antibody | Diagnostic accuracy * | | |
		children 0 - 2 years (19 CD, 28 controls)	children 2 - 4 years (32 CD, 30 controls)	children >4 years (130 CD, 162 controls)
IgA	Anti-Gliadin	85%	76%	81%
	Anti-GAF-3X	89%	87%	89%
	Anti-tTG	91%	89%	96%
IgG	Anti-Gliadin	77%	81%	78%
	Anti-GAF-3X	91%	89%	92%
	Anti-tTG	72%	76%	67%

* % correctly classified patients (true positive + true negative)

▽**Figure 1.** GAF-3X construct
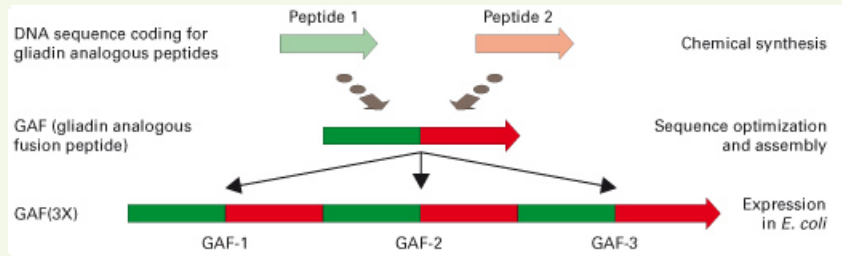
▽**Figure 2.** Sensitivities and specificities of CD assays
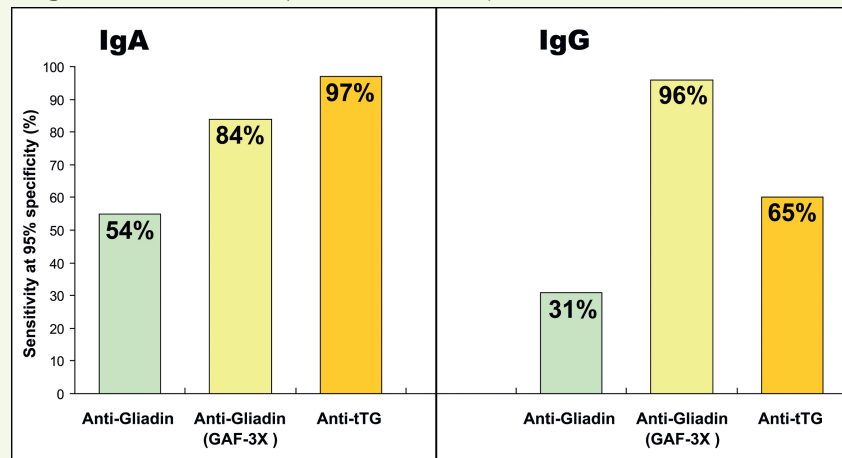
▽**Figure 3.** Combined testing of anti-gliadin (GAF-3X) IgG and anti-tTG IgA