Antibody tests in the management and follow-up of coeliac disease

by Dr Jacqueline Gosink

In 2022, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) published a position paper on the management and follow-up of children and adolescents with coeliac disease (CD), following publication of new guidelines for the diagnosis of CD in 2020. This article discusses the different markers that can be used for the diagnosis and monitoring of CD as well as the ESPGHAN recommendations.

Overview

Coeliac disease (CD) is a common autoimmune disease which requires life-long adherence to a gluten-free diet (GFD). A new position paper published in 2022 by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) provides recommendations for follow-up of children and adolescents diagnosed with CD and supplements the diagnostic guidelines published in 2020. Antibodies of class IgA against tissue transglutaminase (TGA-IgA) are the most important diagnostic marker for CD and in the new paper are recommended as the first-line parameter for monitoring patients. In patients with IgA deficiency, CD-specific antibodies of class IgG serve as an alternative. The recommended time interval for follow-up is 3–6 months after start of a GFD and every 6 months thereafter until the antibody level has normalized. The antibody measurements serve as a proxy for mucosal healing and aid assessment of patient compliance with the prescribed diet.

Coeliac disease (CD)

CD is an immunologically mediated systemic disease with a pronounced genetic disposition. Its prevalence is estimated at 0.5–1%, although a large number of undiagnosed cases due to atypical or mild symptoms is suspected. CD is triggered by consumption of gluten, which accounts for around 90% of the protein content of many grain seeds such as wheat, barley or rye. The classic manifestation of CD is severe inflammation of the small-intestinal mucosa characterized by villous atrophy and cryptic hyperplasia. Due to reduced nutrient absorption, a broad spectrum of clinical gastrointestinal and non-gastrointestinal symptoms can develop, including chronic diarrhoea, vomiting, abdominal pain, growth disorders, weight loss, anemia, delayed puberty and osteoporosis. The chronic skin condition dermatitis herpetiformis (Duhring's disease) can also occur. Risk groups for CD include, for example, first-degree relatives of CD patients and individuals with certain diseases such as type I diabetes mellitus, Down's syndrome, or selective IgA deficiency. Potential CD refers to cases with CD autoimmunity but no villous atrophy, which may evolve into classic CD. The only effective treatment for CD is a strict GFD to enable regeneration of the small-intestinal mucosa and regression of symptoms.

Key serological markers

TGA-IgA (antibodies of class IgA against tissue transglutaminase) are the most important serological marker in CD. They occur in CD at a prevalence of 95–100% and are virtually never found in healthy individuals or patients with other intestinal diseases. Endomysial antibodies (EMA) directed against endomysium (a connective tissue layer), represent another well-established marker for CD in the context of the indirect immunofluorescence detection method. The main target antigen of these antibodies is transglutaminase (TG).

Components of gluten, especially gliadin, are the exogenous trigger of the inflammatory reactions in CD. Antibodies against deamidated gliadin peptides (DGP) comprise a further sensitive and specific serological marker for CD. Detection of DGP-IgG antibodies is especially useful in patients with an IgA deficiency.

HLA-DQ2 and -DQ8 are the principal determinants of genetic susceptibility for CD and possess a very high negative predictive value for CD. If HLA-DQ2/DQ8 are negative, CD can be as good as excluded. As these alleles occur in around 30% of the healthy population, their determination is used primarily for exclusion diagnostics.

ESPGHAN diagnostic algorithm

The current diagnostic guidelines from ESPGHAN centre on the determination of CD-specific antibodies and in some situations HLA-DQ2/DQ8 alleles [1]. The diagnostic algorithm is applied in children and adolescents with symptoms suggestive of CD as well as in asymptomatic persons with a high risk of CD. The antibody determination must be performed under a gluten-containing diet, as the antibodies steadily decline with a GFD.

TGA-IgA is the recommended first-line test for CD, due to its accuracy and cost-effectiveness. Total IgA is determined in parallel to exclude an IgA deficiency. If the TGA-IgA titer is more than 10 times the upper limit of normal (≥10× ULN) and this result is reinforced by positive EMA-IgA in a second serum sample, an intestinal biopsy is not required. Biopsy remains necessary in children with a TGA-IgA titer of <10× ULN, in cases of suspected CD but negative serology, and for the diagnosis of CD in adults. If total IgA is low, a test for CD-specific IgG (DGP, TGA or EMA) is undertaken along with a biopsy.

HLA-DQ2/DQ8 determination is not obligatory for CD diagnostics but may be useful in some circumstances, such as in patients without high TGA-IgA or patients from risk groups. Gluten challenge is only performed in exceptional circumstances, for example, in children with suspected CD who started a GFD before the diagnosis. HLA-DQ2/DQ8 typing should be performed beforehand to potentially exclude CD. Gluten challenge should be avoided in children and adolescents during periods of accelerated growth.

ESPGHAN follow-up recommendations

According to the new position paper from ESPGHAN, follow-up of children and adolescents with CD after diagnosis is necessary to assess growth and development, resolution of symptoms, possible complications, as well as compliance with the GFD [2].

Initial follow-up should take place 3–6 months after diagnosis. TGA-IgA measured by ELISA or other enzyme immunoassays is recommended for monitoring CD patients. The TGA-IgA titer usually falls significantly after 3 months of a strict GFD (Fig. 1) and serves as a surrogate marker for mucosal healing. Further tests should be performed every 6 months until the TGA-IgA value has normalized to less than 1 ULN. After this, the TGA-IgA level should be checked every 12–24 months. If no reduction in the TGA-IgA titer is observed after 6–12 months or if slightly elevated levels persistent over a long time, this usually indicates non-compliance with the GFD.

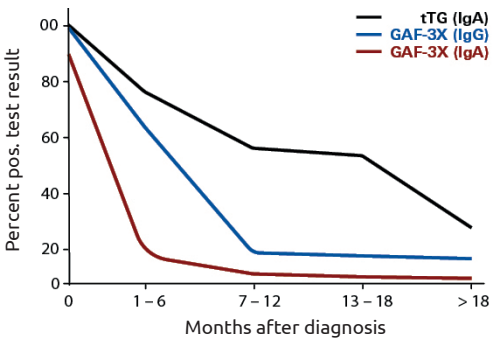

Figure 1. Reduction in antibody titers in patients on a gluten-free diet

For monitoring IgA-insufficient patients, an IgG test for CD-specific antibodies such as DGP, TGA, or EMA should be performed at the same time intervals.

More frequent follow-ups may be required if the patient has difficulties following the diet, if symptoms persist or worsen despite strict adherence to the GFD, or if clinical presentation such as malnutrition or laboratory abnormalities require earlier follow-up. Use of biopsy to routinely assess mucosal healing is not recommended in children with CD following a GFD. Biopsy should only be considered in selected cases based on specific clinical grounds, such as doubts about original diagnosis or suspicion of an additional condition.

ESPGHAN emphasizes the importance of using the same manufacturer's assay for diagnosis and monitoring to circumvent differences between tests and allow meaningful comparison of antibody levels.

TGA detection

TGA are measured monospecifically using enzyme immunoassays such as ELISA, chemiluminescence immunoassay (ChLIA) or immunoblot based on recombinant human TG antigen, which are available for the immunoglobulin classes A and G. ELISA and ChLIA are ideal for high-throughput quantitative analysis of large sample numbers. They provide high sensitivity and specificity and are, moreover, cost-effective, simple to perform and fully automatable.

In scientific studies the EUROIMMUN Anti-Tissue Transglutaminase ELISA (IgA) contributed to a clear CD diagnosis without biopsy [See 3–6] and, in comparison with tests from other manufacturers, reacted most sensitively to increasing titers when the GFD was not adhered to [6].

EMA detection

EMA are detected by the indirect immunofluorescence test (IIFT). IIFT is considered a very specific and sensitive method for detection of CD-specific antibodies. However, since the microscopic evaluation is demanding, the method is used mainly in a confirmatory capacity.

The tissue substrates liver, esophagus and intestine are equally suitable for EMA detection, since the target antigen TG is expressed strongly in these tissues (Fig. 2). The liver substrate offers the advantage that it is easier to interpret due to less interference from other autoantibodies [7]. For example, the interpretation of EMA on esophagus is complicated if anti-smooth muscle autoantibodies (ASMA) are also present. EUROIMMUN IIFT BIOCHIP Mosaics comprising different miniaturized substrates in one test field enable investigation of further antibodies in parallel to EMA, for example antibodies against DGP using antigen dots.

Anti-DGP detection

Anti-DGP antibodies can be determined by ELISA, ChLIA, immunoblot or monospecific IIFT. The specificity and sensitivity of anti-DGP detection is greatly increased through use of a specially designed antigen comprising a deamidated gliadin-analogous fusion peptide containing only the diagnostically relevant epitopes which is expressed in trimeric form (GAF-3X, Fig. 3) [8]. Conventional full-length native gliadin is no longer recommended as a detection antigen due to its low specificity.

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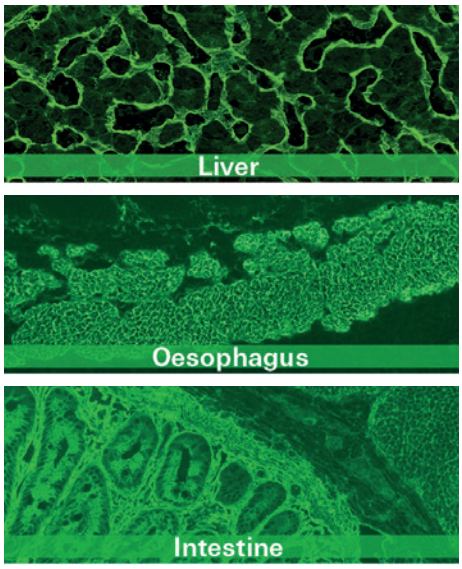

Figure 2. Detection of endomysial antibodies (EMA) on the tissue substrates liver, esophagus and intestine

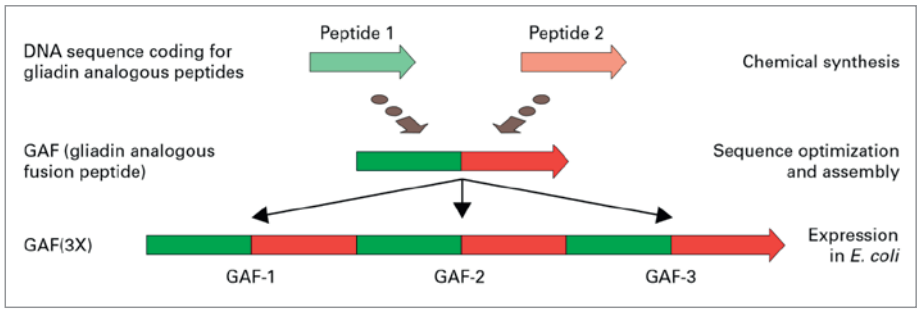

Figure 3. Schematic illustration of trimeric gliadin-analogous fusion peptide (GAF-3X) for detection of anti-deamidated gliadin peptide (DGP) antibodies

>> The performance of the GAF-3X antigen has been proven in many studies. For example, a detection combination of TGA-IgA and GAF-3X-IgG yielded the best performance for diagnosis and exclusion of CD [4]. In patients with IgA deficiency, detection of GAF-3X-IgG demonstrated higher accuracy than detection of TGA-IgG [9,10].

Multiparameter antibody detection

Line blots are useful for multiparameter antibody determination. For example, the EUROLINE Coeliac Disease Profile provides parallel detection of TGA and anti-DGP and also includes an IgA control band to support exclusion of an IgA deficiency. The EUROLINE Autoimmune Gastrointestinal Diseases Profile combines TG and DGP with additional markers for differentiation of CD from Crohn's disease, autoimmune gastritis and pernicious anemia.

Outlook

CD is nowadays recognized as a commonly occurring multi-organ disease with a wide range of signs and symptoms which is under-diagnosed due to its heterogeneous presentation. Serological testing forms the backbone of CD diagnostics in children and adolescents, with biopsy used mainly to clarify difficult cases. The new ESPGHAN position paper on management of CD patients cements the use of serological parameters for follow-up examinations. The recommendations aid assessment of GFD compliance, catch-up growth potential, anemia treatment and necessity of biopsy or gluten challenge. Additional recommendations are provided for monitoring patients with associated type 1 diabetes, IgA deficiency or potential CD. Further aspects of the guideline are useful for assessing the impact of CD and GFD on the patient's quality of life, improving communication with children, parents and caregivers, and easing the transition from pediatric to adult healthcare.

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