Antibody tests for monitoring coeliac disease and gluten-free-diet compliance

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Overview

Coeliac disease is an autoimmune condition that requires lifelong adherence to a gluten-free diet. A recently published position paper from ESPGHAN (European Society for Paediatric Gastroenterology Hepatology and Nutrition) recommends antibody testing for long-term monitoring of children and adolescents diagnosed with coeliac disease. Autoantibodies of immunoglobulin class A (IgA) against tissue transglutaminase (tTG) play the most important role in diagnostics and monitoring, while coeliac-specific IgG antibodies should be analysed in patients with an IgA deficiency.

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Gluten intolerance

Gluten is a component of many grains such as wheat, rye and spelt. It is made up predominantly of gliadin, which is only partly digested in the intestine. Oligopeptides are formed which can pass through naturally occurring gaps in the intestinal epithelium, reaching the underlying connective tissue. Here they are bound by the enzyme tissue transglutaminase (tTG) and modified (deamidated). In people with a certain genetic predisposition, the resulting complexes of tTG and gliadin oligopeptides can trigger an immune reaction leading to the formation of specific antibodies. The immunological overreaction causes inflammation of the small intestinal wall and corresponding symptoms. With progressive damage, the small intestinal epithelium cannot absorb enough nutrients, leading to deficiencies of important minerals and vitamins. Gluten intolerance, known as coeliac disease, is a chronic autoimmune disease. It cannot be cured, but when a strict gluten-free diet is followed, the immune reaction abates and in most cases the level of antibodies drops.

Clinical chameleon

Coeliac disease has a very diverse clinical picture. The classical form often manifests at a very early age. When infants consume their first

gluten-containing food, typical symptoms such as chronic diarrhoea, vomiting, abdominal pain and cramps occur. The resulting malabsorption leads to weight loss, anaemia, growth disorders and delayed puberty. Coeliac disease can also manifest in adulthood. In these cases, untypical symptoms such as constipation, osteoporosis, joint disorders, depression, miscarriages or the chronic skin condition dermatitis herpetiformis (Duhring's disease) can also occur.

Coeliac disease should therefore not be diagnostically excluded just because typical symptoms such as diarrhoea or weight loss are not present. Notably, overweight and obese persons can also suffer from coeliac disease. The diverse symptomology has led to coeliac disease being called the chameleon of gastroenterology. Moreover, it is not a rare disease, with a prevalence estimated at 1 %. A large number of undiagnosed cases is also suspected, as patients and clinicians often do not think of gluten intolerance as a cause of some complaints. According to recommendations from ESPGHAN, coeliac disease should be considered as a possible cause even in cases of unspecific or non-gastrointestinal symptoms.

Diagnostics

Damage to the intestinal mucosa can be identified by intestinal biopsy, but this method is now relegated to a more minor role behind serology for differential diagnostics of coeliac disease. The investigation of antibodies is recommended in patients with symptoms suggestive of coeliac disease, both children and adults, as well as in asymptomatic persons at high risk of developing the disease. At-risk groups include first-degree relatives of coeliac disease patients as well as persons with certain diseases, for example Down's syndrome or type I diabetes mellitus.

The first-line test is the detection of anti-tTG antibodies of the immunoglobulin class A (IgA). These autoantibodies are specific for coeliac disease and do not occur in healthy individuals or persons with other diseases. Total IgA is determined in parallel to exclude IgA deficiency, which occurs with above average frequency in patients with coeliac disease. If a high value for anti-tTG-IgA is obtained, defined as a titer of at least tenfold the upper limit of normal (≥10x ULN), a second blood sample is taken for a confirmatory indirect immunofluorescence test (IIFT). In IIFT the anti-tTG antibodies are known as anti-endomysium antibodies (EmA) since they react with the connective tissue layer endomysium. If the EmA-IgA detection is also positive, a diagnosis of coeliac disease can be made without biopsy. With negative or low titers of anti-tTG or low total IgA, a biopsy remains necessary for diagnosis, but should be supplemented by further tests such as IgG antibodies against deamidated gliadin peptides (DGP), tTG or EmA.

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Antibody detection methods

Coeliac disease-specific antibodies can be determined using different methods such as ELISA, chemiluminescence immunoassay, immunoblot and IIFT. Anti-Tissue Transglutaminase ELISAs (IgA, IgG) from EUROIMMUN provide high sensitivity and specificity and are ideal for fully automated high-throughput processing of many analyses. The IIFT for detection of EmA-IgA is especially suitable for confirmation of a positive anti-tTG-IgA result due to its outstanding specificity and sensitivity. Liver or oesophagus is generally used as the detection substrate (Figure 1), whereby the two substrates provide equivalent sensitivity and specificity.

An additional important antibody test in coeliac disease diagnostics is the determination of antibodies against deamidated gliadin peptides (DGP). The development of a specially designed antigen containing only the diagnostically relevant epitopes of gliadin (gliadin-analogous fusion peptide in trimeric form, GAF-3X, Figure 2) has greatly increased the sensitivity and specificity of this test. The detection of IgG antibodies against DGP by ELISA plays an important role in patients with IgA deficiency. A test combination of anti-tTG-IgA and anti-DGP-IgG significantly increases the serological detection rate for coeliac disease.

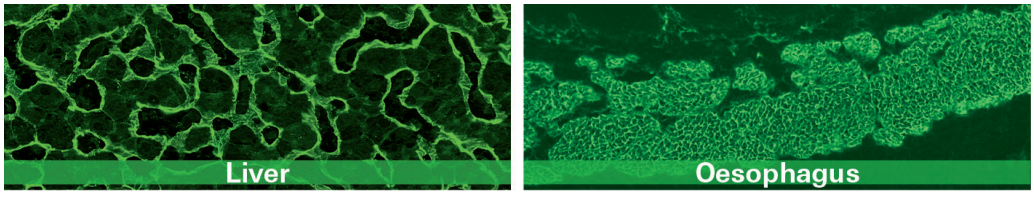

Figure 1. Detection of EmA on the tissue substrates liver and oesophagus

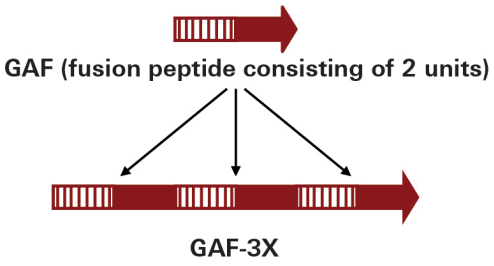

Figure 2. Schematic representation of GAF-3X antigen for detection of anti-DGP antibodies

The immunoblot EUROLINE Coeliac Disease Profile enables multiparameter detection of anti-tTG and anti-DGP antibodies. The IgA blot additionally includes an IgA band to support simultaneous exclusion of an IgA deficiency. The EUROLINE Autoimmune Gastrointestinal Diseases Profile provides these and further markers to aid serological differentiation of coeliac disease from Crohn's disease, autoimmune gastritis and pernicious anaemia.

Genetic analysis

The determination of the genetic parameters HLA-DQ2/HLA-DQ8 is useful, for example, in patients with unclear results or in symptomatic patients who have already started a gluten-free diet and whose antibodies are therefore no longer detectable. Since around a third of the healthy population possesses the HLA-DQ2/HLA-DQ8 alleles, their detection alone is not evidence of coeliac disease. The parameters serve rather for exclusion diagnostics, since without these alleles coeliac disease is very unlikely. The microarray test system EUROArray HLA-DQ2/DQ8-h Direct provides easy determination of all clinically relevant alleles and does not require any previous knowledge of molecular biology. The evaluation, interpretation and documentation of results is fully automated.

Monitoring of gluten-free diet

Once coeliac disease is diagnosed, adherence to a strict gluten-free diet is the only reliable possibility to curb the immune reactions and prevent continued damage to the intestinal epithelium. With a gluten-free diet regeneration of the mucosa can in some cases take several years, although the concentration of the antibodies usually falls within a few months (Figure 3).

A recently published position paper from ESPGHAN provides recommendations for follow-up of children and adolescents with coeliac disease and monitoring of the gluten-free diet. The first follow-up examination should be performed after 3 to 6 months, with measurement of anti-tTG-IgA antibodies by ELISA or other enzyme immunoassays. In patients with IgA deficiency, IgG assays for anti-DGP, anti-tTG or EmA are recommended. With adherence to the diet the antibody titer usually normalises over time (<1x ULN) and further check-ups can be performed at longer time intervals of 12 to 24 months.

It is important to use the same test systems for diagnosis and follow-up in order to circumvent discrepancies between different assays and allow meaningful comparison of antibody levels.

The follow-up of children and adolescents after diagnosis helps to assess growth and development, resolution of symptoms, possible complications, as well as compliance with the gluten-free diet. If no reduction in the tTG-IgA titer is observed after 6 to 12 months or if slightly elevated levels persist over a long time, this usually indicates non-compliance with the gluten-free diet. More frequent follow-ups may be required if the patient has difficulties following the diet or if symptoms persist or worsen despite dietary compliance. Malnutrition or laboratory abnormalities may also indicate the need to assess the patient more regularly. Further aspects of the new position paper cover education of children, parents and caregivers about the disease and improving communication to ensure full adherence to the gluten-free diet.

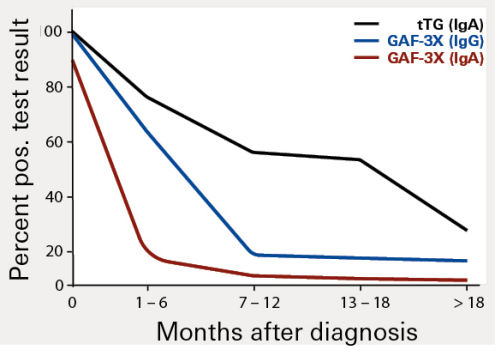

Figure 3. Antibody titer course in patients on a gluten-free diet

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References

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Mearin et al. J Ped Gastroenterol Nutr 1;75(3):369-386 (2022)

Further references available on request