Journal

Scientific news, opinions and reports

*Immuno*Diagnostics

12th Dresden Symposium on Autoantibodies: EASI (European Autoimmunity Standardization Initiative) Session on Celiac Disease

The EASI session at the 12th Dresden Symposium on Autoantibodies took place on September 25, 2015. More than 200 delegates attended the two hour symposium, chaired by Professor Markku Mäki and Dr Eckart Mummert, to listen to experts presenting around the identification and management of celiac disease, and the role of a gluten-free diet on IgA mediated disease. A summary of each presentation is included in this volume of the ImmunoDiagnostics Journal.

Serology and the diagnosis of celiac disease

Early diet and the development of celiac disease

The influence of a gluten-free diet on IgA mediated disease

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New Insights in the Identification of Celiac Disease

On September 25, 2015, in the EASI session at the 12th Dresden Symposium on Autoantibodies, experts from across Europe presented thought provoking lectures around a central theme of celiac disease. Over 200 delegates attended and, through insightful questions, engaged in the session. One of the Chairmen, Prof Mäki from the University of Tampere, Finland, started the session with an overview of celiac disease and the challenges of diagnostics. He challenged thoughts regarding the use of endoscopy as the gold standard test, considering not every user is a gold standard user, and predicted a move towards serology, particularly tissue transglutaminase (tTG/TG2) IgA, becoming the gold standard. Prof Mäki's presentation was followed by a riveting presentation from Ilma Korponay-Szabó from the University of Debrecen, Hungary. Dr Korponay-Szabó was presenting on behalf of the PreventCD Research Group and presented evidence that highlighted that early gluten intake does not prevent celiac disease, and additional evidence to support the use of tTG IgA. Xavier Bossuyt, from University Hospitals Leuven, described how the approach to diagnosing celiac disease (combining antibody tests and taking into account antibody levels) in Belgium improves serologic diagnosis of celiac disease. The final talk of the day was from Dr Renato Monteiro, from France, who discussed the influence of a gluten-free diet on IgA mediated diseases.

Overall, the session was interesting and insightful, and we hope you find the summary of these presentations useful.

Enjoy reading,
Jason Cunningham and Nina Olschowka

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Overview of celiac disease and the special challenges in diagnostics

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An overview of celiac disease

Celiac disease is a systemic disorder in genetically susceptible persons; perpetuated by the daily ingested gluten cereals wheat, rye, and barley; with manifestations in the intestine and in organs outside the gut. Today it is understood that the nature of celiac disease is much more than simply intestinal malabsorption, which as such, is, in fact, no longer essential for the diagnosis. Furthermore, celiac disease is a good model for autoimmunity:

- The environmental trigger and driving force is known – the cereal gluten

- The disease requires a unique genetic background for antigen presentation – expression of the *HLA class II molecules DQ2* or *DQ8*

- The ingested gluten mediates both intestinal adaptive and innate immune responses where transglutaminase 2 (tTG), the autoantigen, is also targeted by specific autoantibody

- The disease remission is highly dependent on a strict gluten-free diet

- Before knowing what the driving force was and thus not excluding the daily food gluten, the disease was self-perpetuating similarly to other autoimmune diseases

In many other autoimmune diseases the environmental insult, trigger and driving force are not known and cannot thus be excluded. Early suggestions and evidence of gluten-induced operative autoimmune mechanisms in celiac disease and existing gluten-triggered reticulin/endomysial autoantibodies against the self[1-4] were heavily criticized.[5] Today the autoimmune nature of celiac disease is widely accepted[6] and a range of other autoimmune disorders could benefit from the lessons from celiac disease.[7]

In Finland, the prevalence of biopsy-proven celiac disease is 0.8% of the total population. The true prevalence in large population-representative materials is 1.5% in children, 2% in adults and 2.7% in the elderly population; it is apparent that new seroconversions and small-intestinal deteriorations increase by age.[8-10] A twenty-fold increase in the number of adults receiving a diagnosis of celiac disease since the 1970s has been observed in Finland.[9] This increase can be attributed to awareness programs, autoantibody screening tools and open access endoscopy (endoscopies performed in the primary care to get biopsy-proven diagnosis). Of all biopsy-proven diagnosed cases, only 40% have gastrointestinal symptoms. The disease is heavily underdiagnosed worldwide but true prevalence differences occur, even within Europe.[11,12] It has also become evident the true prevalence of celiac disease over
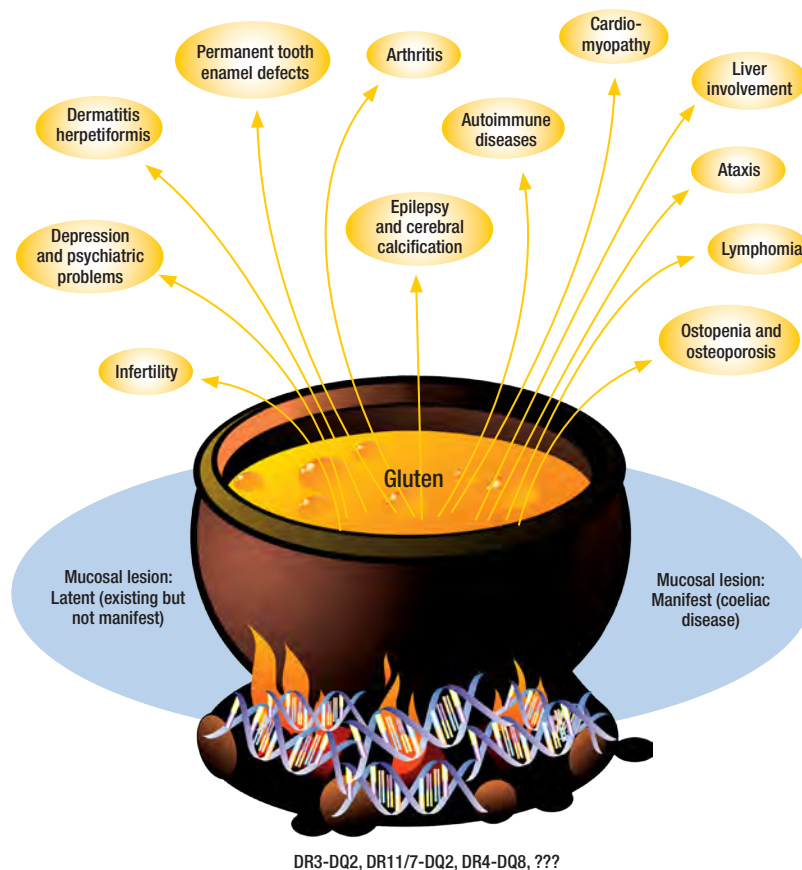

DR3-DQ2, DR11/7-DQ2, DR4-DQ8, ???

Figure 1. Extra-intestinal disease manifestations that can present with celiac disease. Adapted from Mäki M, 2012.[16]

time in the population is on the increase, in Finland from 1% to 2%.[9] Many patients are clinically silent but have manifested a gluten-dependent small-intestinal mucosal injury. This is especially the case among first-degree relatives of celiac disease patients[13] and in patients at increased risk of celiac disease, e.g. patients with type 1 diabetes.[14] Extra-intestinal disease manifestations are common (Figure 1).[15,16]

Serum autoantibody tests in celiac disease and diagnostic challenges

The classical serological test for celiac disease is the measurement of endomysium antibodies (EMA, earlier called R1-reticulin) using rodent and primate tissues. This immunofluorescence test is highly sensitive and specific for untreated celiac disease.[3,8,13-15] With the identification of tissue transglutaminase 2 (tTG) as the actual antigen of EMA, more and more commercial tests have been developed.[17,18,19] Today, anti-tTG tests with human recombinant antigen have a performance which is very similar to the more elaborate EMA test and, therefore, are most widely used today.[20] They are highly sensitive and specific for untreated celiac disease.[3,8,13-15] The IgA-class tTG antibody test is very efficient in celiac disease case finding at the population-based level and a positive test goes hand in hand with celiac-type HLA.[8] A point-of-care EMA test is also highly predictive of untreated celiac disease.[14,21] In selective IgA deficiency, which is relatively common if you consider that approximately 10% of IgA deficient blood donors do have a clinically silent untreated celiac disease, an IgG-class test must be used.[22]

There are several diagnostic challenges that can present in regards to diagnosing a patient with celiac disease. One of the foremost issues is to ensure test requestors are aware that when using tTG antibody (anti-tTG) ELISA tests, positivity does not equal to celiac disease; positive tests may be unspecific, especially in low serum titers[20] and should be interpreted in the context of the clinical history. This is similar in other autoimmune diseases.[23] Another major diagnostic challenge is the reading of the small-intestinal biopsy specimen, the gold-standard test. Inter-observer variations between pathologists is not good when injury is evaluated using a grouped classification, the Marsh classes.[24] Perhaps the main issue, in regards to variation, is that only villi are looked at and orientation of the biopsy is thought to be good when villi are seen. To ensure interpretation is correct, the pathologist must first look at crypts, and ensure the crypts have been cut longitudinally. The landmark of poor orientation and tangential cutting is when cross-sections of crypts are observed. Such biopsies should not be evaluated; the sample should be re-oriented and a fresh section cut. Quantitative, reliable and reproducible morphometric results can be obtained in celiac disease histopathology.[24] In-fact, when produced correctly, small-intestinal biopsy showing a gluten-induced manifest mucosal lesion (villous atrophy combined with crypt hyperplasia) is highly representative of untreated celiac disease and is still the gold standard. However, one thing to bear in mind is when serology is widely used in case finding in clinics, and especially when taking

the autoantibody tests to the primary care, biopsy outcome may be normal (Marsh 0) or may just show inflammatory changes with increased density of intraepithelial lymphocytes (IELs) (Marsh 1). The Marsh 1 lesion is very unspecific for celiac disease (sensitivity and specificity of 60% to predict forthcoming mucosal deterioration and celiac disease).[25] True latent celiac disease patients (existing disease but not yet manifest at the mucosal level) are also picked up by serology. Anti-tTG positivity is highly predictive for celiac disease at either the time of testing or later development.[26] The spectrum of gluten-induced disease entities and the extra intestinal manifestations shown in Figure 1 have been shown to occur irrespective of the degree of mucosal injury, even when according to today's diagnostic criteria the disease was excluded on biopsy. The autoimmune insult to the morphologically normal intestinal mucosa is, in fact, present if searched for. In the small intestine, the disease-pathognomonic autoantibodies target extracellular tTG and might be detected as IgA deposits in biopsy tissues before intestinal injury is seen with conventional methods and also before anti-tTG is detected in serum.[23,25,27-29]

In conclusion, celiac disease remains an under-diagnosed condition; however, with a good clinical history, the use of appropriate serology followed by a well-orientated biopsy, and critical interpretation of results, coeliac disease patients can be accurately diagnosed and managed.

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Influence of diet in the first year of life on the development of celiac disease. Major results of the PreventCD study

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PreventCD is a multinational effort to understand early immunological features leading to gluten-induced celiac autoimmunity and celiac disease. While the exact pathomechanism of this disorder is not fully understood, it is well documented that cereal gluten proteins in wheat, rye and barley act as triggers to induce autoantibodies against type-2 (tissue) transglutaminase (anti-tTG), inflammatory changes in the gut and villous flattening. Normal tolerance to gluten is achieved by the complex interplay of the immune system with host and environmental factors like genetic polymorphism, epithelial integrity, food components and microbiota.

HLA-DQ2 and *DQ8* alleles, which occur in 25-30% of caucasian populations, are responsible for the presentation of gluten-derived gliadin peptides to specific T-lymphocytes. Antigen presentation is augmented by tTG which is able to convert native gliadin peptides into more potent and more immunogenic deamidated forms fitting better into the groove of DQ2 or DQ8 molecules, having preference for negative charges, (as occur in deamidated peptides) at certain key positions. Yet not all *DQ2* and *DQ8* positive persons develop celiac disease and thus further genetic or environmental triggers are necessary for the pathologic reaction to occur.

Celiac autoimmunity is a peculiar human disorder without sufficiently similar animal models, thus these contributing factors cannot be easily investigated in experimental ways. As established celiac disease is not curable, current research is focused on possible prevention strategies by modifying gluten intake in early life.

Observational studies indicated that breast feeding, especially at the time of gluten introduction, as well as the time, amount and how gluten is introduced in the diet of infants may influence the development of celiac disease symptoms and its prevalence later in life.[1] Further, it also has been suggested that a window of opportunity exists between the age of 4-6 months to achieve the lowest rate of adverse response while the immune system is still immature.

With the aim to establish oral tolerance by administering small doses of gluten to young infants, an EU-financed large prospective intervention study was initiated in 2007. 944 *HLA-DQ2* and/or *DQ8* positive new-borns with at least one already affected celiac first-degree family member in 7 European countries (Croatia, Germany, Hungary, Italy, The Netherlands, Poland, Spain) and in Israel were enrolled. Children aged 0-3 months were randomized to receive 100 mg of gluten daily or placebo (double-blind) for 8 weeks from age of 17 weeks. Gluten-containing foods were gradually introduced in identical fashion in both groups after the age of 6 months. The participants were then carefully monitored with clinical, dietary and serologic investigations for anti-gliadin antibodies (EliA Gliadin test and EliA Gliadin*DP* in positive cases, both from Phadia) and anti-tTG antibodies (VarelisA Celikey™, Phadia) 7 times until the age of 3 years and annually thereafter. The primary endpoint was biopsy-proven celiac disease at the age of 3 years (published in 2014[2]). Follow up is still continuing.

Major results

Early gluten intake in low doses does not prevent celiac disease

Children exposed to 100 mg of gluten at 4-6 months of age (n=475) and those assigned to placebo (n=469) and starting gluten with only 6 months had similar cumulative incidence of celiac disease at 3, 4 and 5 years (5.9% versus 4.5%, 10.3% vs. 7.3%, and 13.5% versus 10.6%, p=0.047).[2]

Early gluten intake may be associated with higher frequency of celiac disease and autoantibody positivity in girls (8.9% in the gluten group vs. 5.5% in the placebo group, HR 1.99) (non-significant)

This subgroup analysis did not reach statistical significance due to other confounding factors. The analysis contained data until September 2013 and a new analysis is planned when all children will reach 6 years of age. There were no significant changes in the amount of gluten intake between the two intervention groups after 6 months of age, moreover, children developing celiac disease did not consume larger amounts of gluten than those who remained healthy.

Breast feeding does not protect against celiac disease

Children never breastfed or breastfed for various times had no different frequencies of anti-tTG antibody positivity or biopsy-proven celiac disease.[2] Neither exclusive nor partial breast-feeding seemed protective. However, children were only randomized to gluten and placebo; breastfeeding was the choice of parents. Similar results were obtained in other recent cohort studies.

HLA-DQ2 homozygosity confers elevated risks for developing celiac disease in young age

Country of origin, number of and type of affected family members (parent or sibling), gastrointestinal infections or rotavirus vaccination were not associated with higher risk of celiac disease, whereas genetic background was. Children with two copies of HLA-DQ2.5 or having DQ2.5/DQ2.2 had cumulative incidence of celiac disease at 3, 4, and 5 years 14.9%, 23.9% and 26.9%, respectively, thus much higher than the whole cohort.[2]

Primary immune response to gluten in healthy children results in antibody production against native and deamidated gliadin

The administered amounts of gluten (100 mg) were highly immunogenic at this age. Children randomized to gluten regularly produced both IgA and IgG anti-gliadin antibodies measurable in serum at 6, 9, and 12 months. 35% displayed high serum values (many even >100 U/ml) at 6 months with a gradual decrease after 1 year of age.

Antibodies reacted with deamidated gliadin peptides (DGP) using established clinical diagnostic assays,[3] but this reaction was not predictive of celiac disease and disappeared spontaneously, and the children remained symptom-free. 7 children had undergone small intestinal biopsy because of anti-DGP positivity (negative anti-tTG antibodies), but none of these displayed villous damage and were not diagnosed with celiac disease. Interestingly, homozygous DQ2.5 children had lower antibody gliadin response than children carrying one or two copies of DQ2.2, yet had higher frequency of celiac disease.[3] In a further extended study with food antigen arrays, IgG and IgA reactivity against wheat proteins other than gliadin also appeared when the children started to eat gluten from other sources revealing that this systemic antibody response simply indicated food intake and no pathologic response. These results heavily challenge the diagnostic role of anti-DGP antibody positivity not accompanied by anti-tTG reactivity. It seems that elaboration of normal tolerance to gluten requires an active presentation of gluten to the immune system and that DGP antibodies are not always linked to celiac disease and autoimmunity to tTG.

Antibody production to tTG is the best indicator of celiac disease even in young age

Reactivity to tTG appeared at later age than gliadin antibodies. In most cases, early gliadin antibodies recessed to seronegativity before this happened. When celiac disease developed, anti-tTG antibodies and anti-DGP antibodies appeared jointly at a median age of 2.8 years[2] and all prospectively diagnosed celiac cases in the intervention cohort displayed serum anti-tTG antibodies as measured by the Celikey test (Phadia GmbH, Freiburg, Germany). Thus monitoring serum anti-tTG reactivity is the best tool to monitor high risk cohorts. In earlier studies, anti-DGP seemed to precede anti-tTG reactivity, but in our study, most celiac cases turned to positive for both types of antibodies nearly at the same time and quite abrupt rise of serum levels was observed.

Anti-tTG antibodies in serum above 10 x ULN indicate celiac disease with villous atrophy

The PreventCD study prospectively evaluated the diagnostic guidelines for celiac disease proposed in 2012 by ESPGHAN. According to these new ESPGHAN criteria symptomatic children with serum anti-tTG values exceeding 10 times the upper limit of normal (10 x ULN) and also positive for endomysial antibodies and HLA DQ2 or DQ8 may be diagnosed without biopsy. During follow up, 87 children developed high anti-tTG values (>30 U/ml) and parents of these 81 children agreed to diagnostic biopsies according to the predefined study protocol. All 81 biopsies showed Marsh 3 grade villous atrophy and thus confirmed the diagnosis of celiac disease in the traditional way. Neither symptoms nor endomysial antibody positivity were necessary to predict celiac disease in this cohort when the Celikey assay indicated high antibody positivity.

>10 x ULN anti-tTG results have good predictive values for villous atrophy in a symptom-free cohort

Another arm of the PreventCD project, closely related to the ETICS study, investigated the screen-detected prevalence of celiac disease in two birth cohorts of Swedish children.[4,5] The first cohort was born during the years when Sweden experienced an increase in the early clinical diagnoses of celiac disease due to high gluten consumption at weaning. Asymptomatic children in this cohort were screened at the age of 11 years for anti-tTG. The results were compared with screen-detected celiac disease at 11 years of age in a cohort born after infant feeding practices in Sweden changed to lower gluten intake. The first cohort still showed higher frequency of celiac disease (2.9% versus 2.2%), indicating that high gluten intake in infancy may be a disease inducing factor.[4] The screening study also confirmed the good predictive values of >10 x ULN anti-tTG values for villous atrophy again in a symptom-free cohort,[5] but this study applied 50 U/ml cut-off value also taking into account the grey zone of the ELISA test.

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Laboratory diagnosis of celiac disease

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The clinical presentation of celiac disease

Symptoms suggestive of celiac disease include: chronic or intermittent diarrhoea, failure to thrive, weight loss, stunted growth, delayed puberty, amenorrhoea, iron-deficiency anaemia, nausea or vomiting, chronic abdominal pain, cramping or distension, chronic constipation, chronic fatigue, recurrent aphthous stomatitis (mouth ulcers), dermatitis herpetiformis-like rash, fracture with inadequate traumas/ osteopenia/ osteoporosis, and abnormal liver biochemistry. Celiac disease may be associated with IgA deficiency, Down syndrome and autoimmune disorders, such as type 1 diabetes and autoimmune thyroid disorders.[1-4]

Diagnosing celiac disease

Celiac specific antibodies comprise anti-endomysium antibodies (EMA), anti-tissue transglutaminase antibodies (anti-tTG) and antibodies to deamidated gliadin peptide (DGP). In recent years, serology is credited a more prominent role in the diagnostic work-up of celiac disease, which is reflected in the latest diagnostic guidelines from the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN).[4] In this overview we focus on the ESPGHAN diagnostic criteria for celiac disease (see figure on page 8).

An overview of ESPGHAN diagnostic criteria

The first diagnostic criteria for celiac disease were issued by ESPGHAN in 1970. Three consecutive duodenal biopsy specimens were required, showing (1) initial mucosal villous atrophy, (2) histologic remission under a gluten-free diet, and (3) histologic relapse upon re-challenge with gluten.[5] The revised ESPGHAN criteria (from 1990) abolished the need for follow-up biopsies if the initial biopsy showed villous atrophy and the patient showed clinical remission after withdrawal of gluten from the diet.[6] The presence of disease-specific IgA antibodies at the time of diagnosis and their disappearance in parallel to a clinical response to a gluten-free diet supports the diagnosis.[6] In 2012, ESPGHAN developed new guidelines with different diagnostic approaches for two groups of patients: (1) children with symptoms suggestive of celiac disease and (2) asymptomatic children at increased risk for celiac disease.[4] In these newly proposed guidelines, it is stated that symptomatic patients should be tested for anti-tTG IgA and total IgA (to exclude IgA deficiency).[1] If IgA anti-tTG exceeds 10 x upper limit of normal (ULN), there is the possibility to diagnose

celiac disease without duodenal biopsy, if supported by EMA testing (on a separate blood sample) and *HLA* typing (positive *HLA-DQ2*).[4] The authors of the ESPGHAN guidelines based their recommendations on studies by Vivas *et al.*,[7] Hill and Homes,[8] and Dahlbom *et al.*[9]

Duodenal biopsy has been the gold standard for diagnosis of celiac disease diagnosis for many years. However, it has downsides and requires an invasive procedure. In the ESPGHAN diagnostic guidelines, small bowel biopsy is no longer regarded as mandatory in children who meet specific criteria. Several studies have been published evaluating these guidelines:

> **Alessio, *et al.*[10]** A retrospective analysis of 412 consecutively referred patients (age range 10 months-72 years) who underwent small-bowel biopsy for suspicion of celiac disease. Three hundred ninety-six patients (96.1%) had histological findings consistent with celiac disease (Marsh ≥2). An anti-tTG ratio ≥7 fold cut-off (with three different assays) had a positive predictive value of 100% for significant mucosal damage (Marsh ≥2).[23]

> **Vermeersch, *et al.*[11]** A well-characterized population using 4 different commercial assays. The levels of IgA anti-tTG were evaluated in serum samples from 104 consecutive pediatric and adult patients who were not deficient in IgA and diagnosed with celiac disease, and in 537 consecutive patients without celiac disease (controls) who underwent intestinal biopsy analysis. The likelihood ratio (LR) for celiac disease increased with levels of IgA anti-tTG in all assays. Depending on the assay, the LR for levels ≥10 x ULN ranged from 111 to 294. The percentage of patients with celiac disease with levels of IgA anti-tTG ≥10 x ULN ranged from 41% to 61%, depending on the assay. Patients with a high pre-test probability and levels of anti-tTG ≥10 x ULN have a high probability for having celiac disease, aiding clinical decision making. The authors suggested that it might be best to define thresholds for levels of IgA anti-tTG based on a predefined LR or post-test probability, instead of a multiple of a cut-off value.[11]

> **Klapp, *et al.*[12]** A retrospective review of 150 symptomatic patients suspected of having celiac disease who had undergone a small bowel biopsy, testing for anti-tTG IgA and EMA, and *HLA* genotype. The triple test was positive if anti-tTG IgA was ≥10 x ULN, plus positive EMA plus
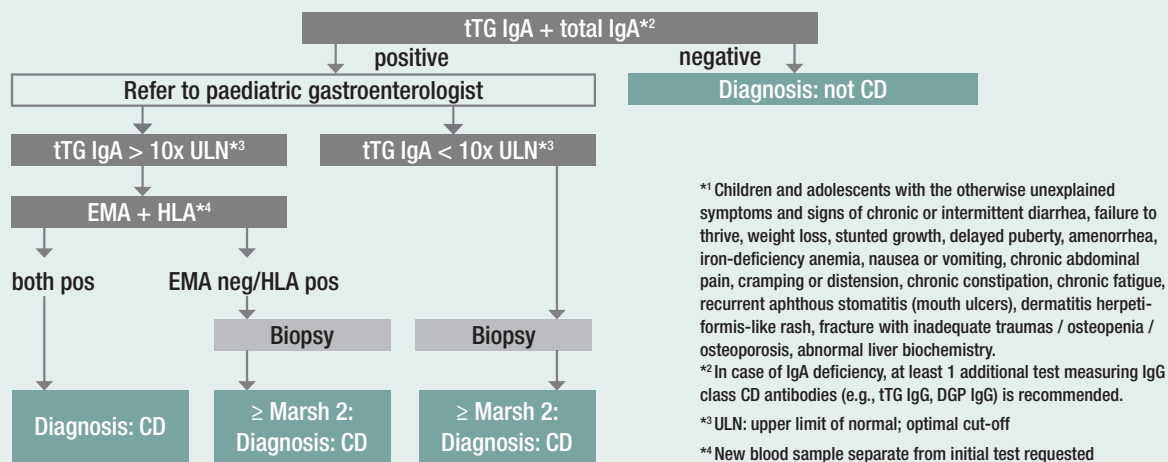
HLA DQ2/DQ8. 116 were positive for the triple test; 113/116 (97.4%) had a Marsh 2/3 histological lesion and had been considered to have celiac disease. The other 3 cases (2.6%) had Marsh 0/1 lesion. However, on follow-up, all 3 children developed histological damage after a gluten challenge. Thus, the positive predictive value of the triple test was 100%. This study supports the view that in selected children who are symptomatic and positive for the triple test, celiac disease diagnosis could be established independent of histological findings. One patient without celiac disease had anti-tTG ≥10 x ULN, but was negative for EMA. He was diagnosed as having cow's-milk protein allergy. Another patient had anti-tTG ≥10 x ULN, was positive for EMA and negative for *HLA DQ2/8*. He was also diagnosed as having cow's-milk protein allergy.

Kruppa, *et al.*[13] Evaluation of human red blood cell tTG antibody test (RBC-tTG) (Quanta Lite, Inova) to a large cohort of child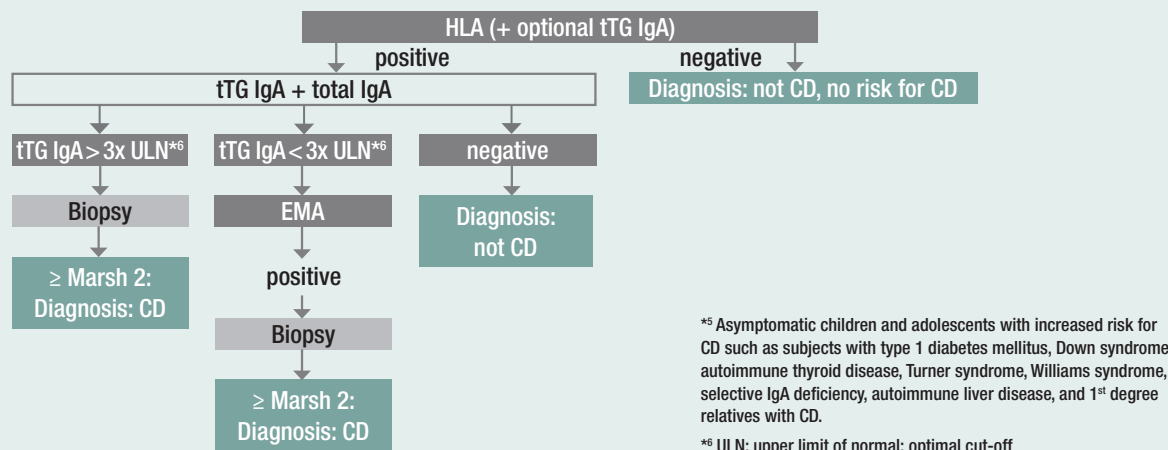ren and adults belonging to at-risk groups. They found that strong positive RBC-tTG (>5 x ULN) showed good accuracy (PPV 94%) and excellent correlation with the other antibodies (Celikey Phadia, EMA) and celiac-type *HLA*. In contrast, low or moderately positive RBC-tTG values were of unsatisfactory prognostic value for a subsequent diagnosis.

Nevoral, *et al.*[14] Children and adolescents (n=345; age 16 months to 19 years) who were examined for suspicion of celiac disease were retrospectively assessed. Evaluation of clinical symptoms and the presence of anti-tTG IgA and EMA-IgA as well as intestinal biopsies were performed in all patients. In patients who were asymptomatic, but positive for EMA and anti-tTG antibodies ≥10 x ULN, the specificity of tests for Marsh 2-3 was only 85%. In symptomatic patients with the same antibody level the specificity was 99%. The authors concluded that because of the high accuracy of serological tests in combination with clinical symptoms for diagnosis of celiac disease, the new guideline seems to be applicable even without the use of *HLA* testing. In this study, intestinal biopsies could be omitted in 28% of patients.

Algorithm 1: For children or adolescents with symptoms or signs suggestive of CD (including atypical symptoms).*[1]

tTG IgA + total IgA*[2]

positive → Refer to paediatric gastroenterologist

negative → Diagnosis: not CD

tTG IgA > 10x ULN*[3]

tTG IgA < 10x ULN*[3]

EMA + HLA*[4]

both pos → Diagnosis: CD

EMA neg/HLA pos → Biopsy → ≥ Marsh 2: Diagnosis: CD

Biopsy → ≥ Marsh 2: Diagnosis: CD

*[1] Children and adolescents with the otherwise unexplained symptoms and signs of chronic or intermittent diarrhea, failure to thrive, weight loss, stunted growth, delayed puberty, amenorrhea, iron-deficiency anemia, nausea or vomiting, chronic abdominal pain, cramping or distension, chronic constipation, chronic fatigue, recurrent aphthous stomatitis (mouth ulcers), dermatitis herpeti-formis-like rash, fracture with inadequate traumas / osteopenia / osteoporosis, abnormal liver biochemistry.

*[2] In case of IgA deficiency, at least 1 additional test measuring IgG class CD antibodies (e.g., tTG IgG, DGP IgG) is recommended.

*[3] ULN: upper limit of normal; optimal cut-off

*[4] New blood sample separate from initial test requested

Algorithm 2: For asymptomatic children or adolescents with CD associated conditions.*[5]

HLA (+ optional tTG IgA)

positive → tTG IgA + total IgA

negative → Diagnosis: not CD, no risk for CD

tTG IgA > 3x ULN*[6] → Biopsy → ≥ Marsh 2: Diagnosis: CD

tTG IgA < 3x ULN*[6] → EMA → positive → Biopsy → ≥ Marsh 2: Diagnosis: CD

negative → Diagnosis: not CD

*[5] Asymptomatic children and adolescents with increased risk for CD such as subjects with type 1 diabetes mellitus, Down syndrome, autoimmune thyroid disease, Turner syndrome, Williams syndrome, selective IgA deficiency, autoimmune liver disease, and 1st degree relatives with CD.

*[6] ULN: upper limit of normal; optimal cut-off

Egner and colleagues (organizers of the UK NEQUAS external quality control program)[15] expressed concerns about the use of common multiples of ULN.[15] They warned for errors of generalization, both between methods and between laboratories. They illustrated the variability between assays in data obtained from external quality assessment distributions. They argue that the variability is such that one cannot reliably distinguish 6 x ULN from 14 x ULN with a high degree of certainty in multiple laboratories using the same assay kit and sample. Egner, *et al.* concluded that the updated guidance is too generalized for use with all the commercial anti-tTG kits and is therefore not translatable for use in all centres. They also criticized the fact that in the ESPGHAN recommendations for screening asymptomatic individuals, the least specific and most expensive test (*HLA DQ*) has been placed at the front of the algorithm. They advise clinicians to consider whether using a cheaper, equally sensitive, and more specific test first (anti-tTG or EMA) would be more cost-effective.

Beltran, et al.[16] Analysis of retrospective laboratory data to investigate the relationship between tTG antibody levels and Marsh 3 histology in the seropositive population of adults and children at a single centre. Amongst 202 seropositive patients with corresponding biopsies, they were able to define a tTG antibody cut-off with 100% specificity for Marsh 3 histology, at just over 10 x ULN. This confirms that high-titre tTG antibody levels have strong predictive value for villous atrophy in adults and children. These authors also reported data from UK NEQAS that show a wide dispersion of results and poor consensus, both between methods and between laboratories using the same method. The authors concluded that decision cut-offs to guide biopsy requirement require local validation and that tTG antibody assay harmonization is a priority.

Spanish retrospective study.[17] Anti-tTG IgA antibody levels, EMA, and *HLA* were evaluated in 751 patients, 640 symptomatic and 111 asymptomatic. Anti-tTG (>10 x ULN), EMA and *HLA DQ2/DQ8* were found in 336 symptomatic patients, all of them with a final celiac disease diagnosis, and in 69 asymptomatic patients. Of the 69 asymptomatic patients, 4 did not have celiac disease.

North American retrospective study.[18] Efficacy of the ESPGHAN criteria was evaluated in 17,505 patients with celiac serology. An anti-tTG >10 x ULN was observed in 336 patients, including 270 who underwent biopsy. Of the 270 patients with biopsy, 263 had positive EMA and 7 negative EMA. Celiac disease was diagnosed on biopsy in 256 EMA-positive patients. Eight symptomatic patients with anti-tTG >10 x ULN had biopsies not diagnostic for celiac disease (4 were EMA positive and all were *HLA DQ2* or *DQ8* positive). Of the 4 EMA positive patients, one patient developed type 1 diabetes and celiac disease, another patient had a biopsy two years later that was negative for celiac disease, and two other patients improved on gluten-free diet. Of the 4 EMA negative patients, three had a normalization of the anti-tTG (two of

them had a recent gastrointestinal infection at the time of initial screening) and one was lost for follow-up. The authors conclude that clinicians must understand the performance of their celiac serologic tests and that false positives may occur using the non-biopsy criteria.

Anti-DGP

Antibodies against deamidated gliadin peptide (anti-DGP) are increasingly considered a candidate to improve serologic diagnosis of celiac disease.

Wolff, et al.[19] Multicentre study (including 376 celiac disease patients and 695 disease-controls). The high specificity of anti-DGP IgG >10 x ULN was demonstrated. The combination of anti-DGP IgG and anti-tTG IgA eliminates the need to measure total IgA to detect IgA deficiency and may improve the accuracy of serologic testing.

Bürgin-Wolff, et al.[20] A retrospective study including sera from 149 celiac disease patients and 119 controls, all with intestinal biopsy. A combination of four tests (anti-DGP IgA, anti-DPG IgG, anti- tTG IgA, and anti-EMA IgA) yielded a positive LR of 86 with a negative LR of 0.00. Omitting EMA still yielded a positive LR of 87 with a negative LR of 0.01. The authors concluded that a combination of three or four antibody tests including anti-tTG IgA and/or EMA IgA permitted diagnosis or exclusion of celiac disease without intestinal biopsy in a high proportion of patients (78%). Jejunal biopsy would be necessary in patients with discordant antibody results (22%). With this two-step procedure, only patients with no celiac disease-specific antibodies would be missed.

Vermeersch, et al.[21] Investigation of anti-tTG IgA and anti-DGP IgG (using assays from 2 manufacturers) in 107 consecutive adult celiac disease and 542 consecutive disease controls (with biopsy). Double positive test results had a higher LR for celiac disease than single positive test results, whereas double negative test results had a lower LR than single negative test results.

Oyaert, et al.[22] Investigation of whether combining anti-tTG IgA and anti-DGP IgG, and taking into account the antibody levels, further improves clinical interpretation. All patients (n=153) and controls (n=974) included underwent duodenal biopsy. Likelihood ratios for celiac disease markedly increased with double positivity and increasing antibody levels of anti-tTG IgA and anti-DGP IgG. Patients with double positivity and high antibody levels (≥3 x ULN, ≥10 x ULN) had a high probability for having celiac disease (LR ≥649 for ≥3 x ULN and ∞ for ≥10 x ULN). The fraction of celiac disease patients with double positivity and high antibody levels was 59%-67% (depending on the assay) for ≥3 x ULN and 33%-36% (depending on the assay) for ≥10 x ULN, respectively. Thus, combining anti-DGP IgG with anti-tTG IgA and defining thresholds for antibody levels improves the serologic diagnosis of celiac disease.

The 2012 ESPGHAN guideline proposes follow-up testing with EMA in case of an increased anti-tTG antibody result. Given the high specificity of high levels (>10 x ULN) of anti-DGP IgG, anti-DGP IgG might be a substitute for EMA. Anti-DGP antibodies can be automated and, therefore, are an appealing alternative for the manual (and more subjective) EMA. Determining anti-DGP IgG also could overcome the need for determining serum IgA level in all patients to avoid false-negative results in patients with a selective IgA deficiency. Moreover, anti-DGP IgG may detect celiac disease patients that are negative for EMA or anti-tTG (the target antigen of EMA). Future studies are needed to evaluate the added value of anti-DGP (compared to manual EMA).

In conclusion, ESPGHAN addressed the question whether duodenal biopsies could be omitted in some clinical circumstances in the diagnosis of celiac disease. According to the 2012 ESPGHAN guidelines, small bowel biopsy is no longer regarded as mandatory for the diagnosis of celiac disease in patients that meet certain specific criteria (i.e. highly elevated anti-tTG levels). Several studies have confirmed that the higher the antibody level, the higher the likelihood for celiac disease. However, concerns have been raised about the lack of standardization between assays, which makes it difficult to harmonize results across assays. Prospective data that further validate the ESPGHAN recommendations are needed. Additional studies should be conducted to evaluate whether combining antibody tests that can be automated (e.g. anti-tTG IgA and anti-DGP IgG) and taking into account antibody levels further improves serologic diagnosis of celiac disease.

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The influence of a gluten-free diet on IgA mediated diseases

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IgA is the most abundant immunoglobulin in many mammals because of its mucosal secretion. In humans, serum IgA would appear to have special significance because of the unique presence of monomeric IgA1, which contains high amounts of O-liked sugar in its long hinge region representing about 6% of total molecular mass. Monomeric IgA1 plays a non-dispensable anti-inflammatory role in immunity and homeostasis. Several inflammatory diseases are however characterized by an increase in serum IgA levels, often paralleled by IgA tissue deposition. These disorders include IgA nephropathy (IgAN), Henoch-Schönlein purpura, ankylosing spondylitis, Sjögren's syndrome, alcoholic liver cirrhosis, celiac disease, inflammatory bowel disease, and dermatitis herpetiform. Increased IgA levels are also observed in patients with AIDS.

IgAN is the most common IgA-associated disease. The cause of IgAN, which is variable in the severity of its presentation, remains unknown. It occurs as a primary disease but it can be associated with Henoch-Schönlein purpura, celiac disease or inflammatory bowel disease. In IgAN, the IgA deposited in the kidney is primarily polymeric IgA1. There are many reports indicating that circulating IgA in patients with the disease are abnormal in terms of levels, molecular size or aggregation state. The O-linked carbohydrate chains present in the hinge region of IgA1 in IgAN patients appear to be different from normal individuals. This lack of specific galactose residues could have marked effects on the properties of the molecule and create new epitopes that in turn could generate immune response with IgG antibodies against degalactosylated IgA1. These complexes induce increased binding and activation of the IgA Fc receptor (CD89) on myeloid cells leading to its cleavage and the release of a soluble form. Soluble CD89 complexed with IgA are nephrotoxic and associated with disease progression.[1]

Evidence supporting impaired gut mucosal immunity in IgA-mediated diseases including IgAN is well established. Among environmental and infectious antigens, dietary components (especially gliadin) have been proposed to play a role in the onset of IgAN. It has been shown by others that gliadin induces mesangial IgA deposits in BALB/c mice and that IgA anti-gliadin antibodies (AGA), associated with elevated IgA levels, are increased in the serum of IgAN patients.[2] Furthermore, the prevalence of celiac disease increases from 0.5-1% in the general population to 4% in IgAN patients. Gliadin was also described to favor polymeric IgA interactions with the mesangial cells through "lectinic" receptors. Other food-derived antigens (bovine serum albumin, soy, casein and ß-lactoglobulin) have also been identified in serum and/or in the mesangium of a subgroup of IgAN patients.[3] The role of food antigens in IgAN development remains however unknown.

To determine whether a gluten-free diet could influence IgA-mediated disease such as IgA nephropathy in mice, a double transgenic mouse model, reported previously, that expresses both human IgA1 and the human myeloid Fcα receptor was used. α1KI-CD89Tg mice develop a spontaneous model of IgA nephropathy on this diet.[4] In this model, both components are necessary to produce the disease phenotype. IgA1-CD89 complexes are formed and deposit in the mesangium, resulting in hematuria, proteinuria and renal impairment. The renal phenotype requires interaction between the IgA1-CD89 complexes; another IgA1 receptor, transferrin receptor 1 (TfR1/CD71); and transglutaminase 2, a cross-linking enzyme that may amplify complex deposition and is implicated in other models of renal fibrosis. Mice were rendered gluten sensitive by being submitted to a gluten-free diet for three generations.[5] This led to a significant reduction in IgA1 mesangial deposition, a reduction in mesangial transferrin 1 and transglutaminase 2, reduced glomerular inflammatory-cell infiltration (CD11b+ and CD3+ cells), a reduction in hematuria, and reduced IgA1-sCD89 complexes in the serum and kidney eluates. Exposure to gluten in these sensitized mice led to intestinal injury, demonstrated by inflammation and villous atrophy; increased IgA1-sCD89 complexes, mesangial IgA1 deposition and elevated serum IgA1 anti-gliadin antibodies that correlated with the level of proteinuria. Interestingly, a correlation was also found between anti-gliadin antibody levels in IgA nephropathy patients and proteinuria. Pertinently, early introduction of a gluten-free diet in α1KI-CD89Tg mice at 3 weeks of age prevented the typical development of mesangial IgA1 deposition and hematuria.[5]

This work provides novel mechanistic insights into the interactions required for IgA deposition in IgAN. Screening IgAN patients with unexplained gastrointestinal symptoms for undiagnosed celiac disease by simple serological tests would seem appropriate; especially given that gluten restriction, in specific cases in which patients are proven to have both conditions, can have beneficial effects on glomerular disease. Additional clinical data using large cohorts are required, however, before gluten restriction can be more generally recommended for patients.

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▪ Celiac disease remains an under-diagnosed condition; however, with a good clinical history, the use of appropriate serology followed by a well-orientated biopsy, and critical interpretation of results, coeliac disease patients can be accurately diagnosed and managed

▪ Early gluten intake in low doses does not prevent celiac disease; breast feeding does not protect against celiac disease; high gluten intake in infancy may be a disease inducing factor

▪ Antibody production to tissue transglutaminase (anti-tTG) is the best indicator of celiac disease even in young age

▪ Anti-tTG antibodies in serum above 10 times upper limit of normal (>10 x ULN) indicate celiac disease with villous atrophy

▪ DGP IgG antibodies are frequently produced in healthy children aged less than 2 years

▪ The suggestion to replace the need for EMA testing when anti-DGP IgG levels are high (>10 x ULN) is under further evaluation

▪ Determining anti-DGP IgG also could overcome the need for determining serum IgA level in all patients to avoid false-negative results in patients with a selective IgA deficiency. Moreover, anti-DGP IgG may detect celiac disease patients that are negative for EMA or anti-tTG (the target antigen of EMA)

▪ Screening IgA nephropathy patients with unexplained gastrointestinal symptoms for undiagnosed celiac disease by simple serological tests would seem appropriate; especially given that gluten restriction, in specific cases in which patients are proven to have both conditions, can have beneficial effects on glomerular disease

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