Coeliac disease: new clinical aspects

Screening for coeliac disease

Celikey® in publications
Coeliac disease (CD) is an autoimmune condition characterized by an immune mediated enteropathy of the small intestine. The condition occurs in genetically predisposed individuals who ingest prolamins that are found in wheat, rye and barley. Carmen Ribes-Koninckx from Valencia, Spain, has a long experience in diagnosing coeliac disease and is member of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPHGAN). Together with her colleague Ester Donat Aliaga, she reviews the newest knowledge about coeliac disease in her article on page 3.

Only two years ago an Elias Journal with the same topic – coeliac disease – was published (Nr. 1 / 2001). At that time, the reason for this focus was the launch of Celikey®, our new ELISA for the detection of anti-tTG-Antibodies. In 1997 Dieterich et al. brought antibodies to tTG up for discussion as marker for coeliac disease. Today, most of the assays on the market are based on guinea pig liver tTG as antigen. Human recombinant antigen is still very rare and Celikey® is probably the only assay worldwide, which uses human recombinant antigen produced in a eukaryotic system (baculovirus/insect cell system). Our first data when developing the assay were very promising. The antigen showed a high purity and the assay-performance was correspondingly good.

In the meantime, we could gain experience with Celikey® in practice – we could follow how it came off in comparative studies and how it is regarded by experienced clinicians. It was a pleasure to see the outstanding performance of the assay in all of these studies. On page 9 you will find a summary of all publications of the year 2002, in which Celikey® was compared to other methods and/or to other assays.

Ger T. Rijkers, Rik A. Brooimans and Roderick Houwen from Utrecht, the Netherlands, were coauthors of a publication in the European Journal of Pediatrics (Wolters et al., 2002, summarized on page 9), and they were so kind to write an article for the Elias Journal (see page 7). They found a superior performance of Celikey® and concluded, that the assay is an excellent tool to screen for CD in patients with gastrointestinal complaints in the absence of IgA deficiency. It may also prove useful for the screening of populations at low or medium risk for CD.

It was stated in the report of the working group 2000 on coeliac disease, that “the flat intestinal mucosa is no longer the only diagnostic paradigm of CD. But rather, clinical characteristics along with antibody testing have become the new paradigm for diagnosing CD accurately and for monitoring the compliance of a gluten-free diet.” For this task, a serological test should have the highest sensitivity and specificity possible. With Celikey® we are able to support these new directions in diagnostics.

Enjoy reading,
Your Elias Journal editorial team.

Nina Olschowka
Coeliac disease: new clinical aspects

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1. DEFINITION – PREVALENCE
Coeliac disease (CD) has been defined as a permanent intolerance to gliadins (wheat prolamins) and other related prolamins which induce characteristic enteropathy in genetically susceptible individuals.

It was not until the 2nd World War, in the 1940s, that Dicke, a Dutch pediatrician, established an association between wheat flour intake and the disease. Since then knowledge about the disease’s clinical aspects as well as its epidemiology, pathogenesis and diagnosis has widened.

Incidence of CD is much lower in the US than in Europe, 1:1000-2000 versus 1:6000, considering cases diagnosed on clinical grounds. Recent surveys with serological screenings, however, showed a similar prevalence, which points to atypical / silent CD being more prevalent in the US. Speculations about the cause of this difference suggest either environmental factors, or differences in the non HLA-restricted genes, because the frequency of DQ2 DQ8 is similar across the US and Europe. Screening studies conducted all over the world (Europe, America, North Africa, Australia, the Middle East) indicate that the true prevalence of CD is about 1:200. As shown by earlier studies of Catassi et al., screening programs detect as much as 7 previously undetected CD cases for each previously identified CD patient. Thus symptomatic CD represents the top of the “Coeliac Iceberg”, the base of which includes not only silent cases but also other clinical forms of the so-called Coeliac Condition, such as Latent and Potential CD. Latent CD relates to patients who have a normal mucosa, but have had a mucosal atrophy before or since, from which they recovered on a gluten-free diet (GFD). The term of potential CD has been proposed for symptomatic or asymptomatic patients with a high intraepithelial lymphocyte count and a characteristic antibody production pattern at the intestinal level.

2. GENETICS – PATHOGENESIS
Multiple genetic and environmental factors – which have not yet been completely identified – contribute to susceptibility for CD. Besides gliadin, which acts as a trigger, other factors may act as precipitating factors or as protectors. This would explain the discordance for the disease in monozygotic twins. A recent Swedish report on the epidemics of CD demonstrates a protective pattern of infant feeding, namely prolonged breast-feeding and lower gluten intake. The importance of genetic factors is, however, supported by the high prevalence of the disease among relatives – about 10% in first degree relatives, and 30% in HLA identical siblings – as well as by the up to 75% concordance rate in monozygotic twins.

Although a number of genes have been suggested to be implicated in CD, up to now only the susceptibility determined by factors mapping in the HLA region on chromosome 6 has been established. The HLA system (class I and II genes) encodes proteins involved in the presentation of peptides to T-cell receptors. It was shown that the primary CD susceptibility is due to particular HLA-DQ heterodimers encoded by DQA1 & DQB1 genes: DQ2 encoded by DQA1*05 / DQB1*02 and DQ8 encoded by DQA1*03/DQB1*03. Several studies have shown that approximately 95% of coeliac patients express the DQ2 heterodimer (compared to 20-30% of the general population), and that only 2-5% are DQ2 DQ8 negative. However, these numbers also show, that DQ2 and DQ8 are not the only genetic factor for CD in the HLA region.

Genes outside the HLA region may also play a predisposing role in CD. Genes which encode proteins involved in the T-cell response are candidate genes under investigation. Recent studies carried out by the European Genetic Cluster confirm the presence of a genetic risk factor in the 5q31-33 region.

The mechanisms involved in CD pathogenesis have not been completely defined. It is known that an inappropriate immune response, triggered by gliadin and with involvement of intestinal mucosa T cells, results in intestinal lesions. T-cells recognize gliadin peptides only if they are presented by class II HLA-restricted antigen presenting cells. Tissue transglutaminase (t-TG) binds to gliadin and deaminates it. This modification enhances the gliadin-specific immune response through T-cells expressing the α/β T-cell receptor. Other lymphocytes (T and B) are then activated to generate cytokines, which leads to mucosal damage. Another consequence is the parallel activation of plasma cells to produce antibodies, especially anti-gliadin, anti-endomysial, anti-reticulin and anti-tissue transglutaminase antibodies, whose role in the pathogenesis of mucosal damage is still unclear. No strict correlation exists between the presence of these antibodies and mucosal injury.

3. CLINICAL MANIFESTATIONS
Classic CD presents as a gastrointestinal disease. In the pediatric population symptoms may start at an early age, with infants aged 4-24 months showing chronic diarrhea, abdominal distension and weight loss or impaired growth. Vomiting, anorexia, pallor and irritability are also commonly observed. Less frequently, parents report constipation or recurrent abdominal pain. When diagnosis is delayed older children may present with symptoms suggesting dyspepsia or irritable bowel syndrome which are mostly due to vitamin K deficiency, short stature or pubertal delay. Coeliac adults sometimes presenting with clinical symptoms in childhood, but in most cases there is no history suggesting the onset of CD at a young age. Similar to pediatric patients, adults with classic CD present with severe diarrhea and weight loss, but milder symptoms suggesting dyspepsia or irritable bowel syndrome are also frequently found. Atypical forms of the disease are observed in 50% of adults; in pediatric patients these are reported in the older age groups and in adolescents. A wide variety of extraintestinal manifestations, as shown in table I (see next page), have been reported in children as well as in adults. Noteworthy the most recent observations concerning neurological symptoms are associated to gluten sensitization. The widespread use of serological markers for CD has shown that patients with other disorders – especially autoimmune dis-
cases – have a higher risk of contracting CD. They mostly have atypical or even silent forms of the disease, i.e. individuals with enteropathies of diverse severity show no clinical symptoms. The groups that are most at risk are listed in table II. For these patients, serological screening programs are mandatory. Dermatitis herpetiformis is one such condition where, partially due to the importance of the skin manifestations, intestinal symptoms are only rarely reported, although the majority are mucosal abnormalities ranging from mild inflammatory infiltration to severe atrophy. First-degree relatives should be screened for CD although the best screening policy (serological or genetic markers, or a combination of both) remains to be defined and decided. A higher than expected prevalence of CD has also been reported in patients with recurrent perircarditis, sarcoidosis, cystic fibrosis, inflammatory bowel disease, autoimmune hepatitis, primary biliary cirrhosis and schizophrenia, among others, although the association between CD and these conditions is still awaiting confirmation. This raises the question whether GFD will change the natural course of asymptomatic patients of these risk groups.

Some authors have suggested routine CD screening of the general population, however this approach is controversial as there is no data about the optimal screening age and as screening once in a lifetime would not be sufficient to exclude CD.

### 4. Diagnosis

The biochemical and hematological testing of the levels of iron, folate, calcium, vitamins, and of clotting values of transaminases shows different degrees of deficiencies, but is in no way characteristic for CD. Only patients with severe enteropathy will show abnormal intestinal absorption in tests. Imaging studies such as small-bowel barium are of little use because they deliver unspecific results. Abdominal computed tomography or magnetic resonance imaging might, however, be a valuable tool in cases of lymphoma.

Untreated CD patients have circulating antigliadin antibodies and antibodies against reticulin, endomysium and – as has recently been discovered – against tissue transglutaminase (t-TG). The availability of different methods for the serological detection of these antibodies has contributed to increasing the number of diagnoses by raising the suspicion even in asymptomatic or mild symptomatic patients. These so-called serological CD markers display a high sensitivity and specificity, especially t-TG, which makes them useful tools for disease screening but also for monitoring adherence and response to the GFD, or response to challenge. Especially t-TG assays, and more specifically those based on human recombinant t-TG, show an extremely high sensitivity and specificity.

Although serological tests are very efficient at detecting the presence of antibodies involved in the immune response in CD, the diagnosis of CD relies on the study of the intestinal mucosa. Performing at least one small intestinal biopsy is, therefore, mandatory.

The specimen should be obtained from the 2nd or 3rd part of the duodenum, the characteristic lesion observed in patients with untreated CD being a flat mucosa with absence of villi and hyperplastic elongated crypts, together with abnormalities and pseudosecretion of the epithelial cells and an increased number of intraepithelial lymphocytes (IEL). At the lamina propria the number of plasma cells, lymphocytes, mast cells and eosinophils is also elevated. However, these lesions are not exclusive for CD, and not all untreated patients do show the same extent or severity of mucosal lesion. According to the evolution of the mucosal lesion described by Marsh et al., mild abnormalities such as a high IEL count without destruction of villi are early phases in the evolution of mucosal damage; hence gluten-dependent enteropathy is not restricted to a flat mucosa.

As histological changes are not pathognomonic of CD, the gluten dependence of the intestinal lesions has to be unquestionably established. To meet this requirement, in 1969 the European Society for Pediatric Gastroenterology and Nutrition established the ‘3 biopsies rule’, which meant performing one small intestinal biopsy before starting on a gluten-free diet (GFD), followed by a 2nd biopsy to show complete mucosal recovery after a GFD period of at least two years, followed by a gluten challenge and a 3rd small intestinal biopsy to ascertain histological relapse. In 1989 these criteria were reconsidered on the basis of clinical experience and the diagnostic criteria reduced to two requirements: characteristic histological abnormalities of the mucosa while the patient is on a normal gluten containing diet and a complete clinical recovery on a GFD.

Gluten challenge is still recommended in patients with a first small intestinal biopsy performed before the age of two as in this age group other disorders, such as cow’s milk protein intolerance or transient gluten hypersensitivity can be responsible for the intestinal lesion. This is also true, when there is some doubt about the diagnosis, such as negativity of serological markers or ill-defined histological changes of the mucosa, or in patients who started a GFD without the evidence of mucosal injury. In these cases, response to gluten intake should be carefully monitored. Serological tests are especially helpful in determining the optimal timing for post challenge biopsy. This must be indicated after 3-6 months or when clinical relapse occurs. Lack of mucosal injury obliges to strict follow-up and a further biopsy after two years even if there is no clinical response. If histological changes are still not evident, clinical and serological follow-up is recommended and biopsies should be repeated if a recurrence of the disease is suspected.

### Table I. Less common manifestations in coeliac disease.

- Short stature
- Delayed puberty
- Recurrent aphthous stomatitis
- Recurrent abdominal pain
- Steatorrhea
- Folate-deficient anemia
- Osteopenia or osteoporosis
- Dental-enameal hypoplasia
- Vitamin K deficiency
- Thrombocytosis (hyposplenism)
- Primary biliary cirrhosis
- Autoimmune thyroid disease
- Sjögren’s Syndrome
- Microscopic colitis
- Rheumatoid arthritis
- Down syndrome
- IgA nephropathy
- Dermatitis herpetiformis
- Type I diabetes
- IgA deficiency
- Autoimmune thyroid disease
- Microscopic colitis
- Rheumatoid arthritis
- Down syndrome
- IgA nephropathy

### Table II. Disorders associated with coeliac disease.

- Short stature
- Delayed puberty
- Recurrent aphthous stomatitis
- Recurrent abdominal pain
- Steatorrhea
- Folate-deficient anemia
- Osteopenia or osteoporosis
- Dental-enameal hypoplasia
- Vitamin K deficiency
- Thrombocytosis (hyposplenism)
- Primary biliary cirrhosis
- Autoimmune thyroid disease
- Sjögren’s Syndrome
- Microscopic colitis
- Rheumatoid arthritis
- Down syndrome
- IgA nephropathy
- Dermatitis herpetiformis
- Type I diabetes
- Autoimmune thyroid disease
- Microscopic colitis
- Rheumatoid arthritis
- Down syndrome
- IgA nephropathy
suspected. In adult patients the risk for refractory CD must be kept in mind and challenge be restricted to carefully selected cases. Because of the potential nutritional implications, challenges should not be performed in children younger than six, and not during puberty.

5. TREATMENT

The only effective treatment for CD is a strict and permanent gluten-free diet, which means excluding not only wheat but also rye and barley. Cereals considered not harmful for CD patients include rice, maize, corn, buckwheat and tapioca flours. The toxicity of oat prolamins (avenins) is still controversial. Although it has been reported that the intake of oats does not prevent mucosal recovery or produces a relapse of the intestinal lesion, in vitro experiments showed that avenin were able to induce mucosal T-cell activity. In a more recent study Feighery et al. showed that avenin fails to induce a Th1 response in coeliac tissue after in vitro culture. Although a daily consumption of 50-70g of oats for 6-12 months has proven to be not toxic, the low prolamin content of oats as compared to the toxic cereals raises the question of long-term safety especially in children or in dermatitis herpetiformis patients. The risk of contamination with wheat speaks for avoiding oats in newly diagnosed patients, at least until clinical and/or histological recovery. In fact it is extremely difficult to carry out a strict GFD, as wheat flour is widely used in the food industry and even inadvertent contamination with gluten represents a real danger. Moreover, trace amounts of gluten in elaborated foods are not always detected by conventional commercial methods. New strategies combining both immunological and non-immunological methods have been developed by Mendez and coworkers over the last few years, allowing workers over the last few years, allowing for the possibility of a pharmacological approach to the spectrum of gluten sensitivity (“coeliac sprue”).

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A lifelong strict GFD prevents long-term complications of CD and disease-associated malignancies. However, although patients with osteopathy associated to CD improve on a GFD, complete recovery of the bone mineral density is not always achieved. Patients who do not respond after six months of GFD and with no overt lymphoma, are considered as having refractory sprue and are at a higher risk of developing collagenous sprue or an enteropathy-associated T-cell lymphoma. This is because of the presence of an aberrant clonal intraepithelial T-cell population in about 75% of these patients. Results from the Biomed Working Group on Coeliac Disease and Malignancy show that prevalence of CD in patients with Non Hodgkin’s lymphoma was 1.2%, compared with 0.5% CD in the control group. CD patients developed malignancy at a mean of eight years after diagnosis and the majority had never been on a GFD.
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Gastroenterology 115, 1322–1328
Celiac disease (CD) is an autoimmune enteropathy triggered by the ingestion of gluten in genetically-susceptible individuals (1). On intestinal biopsy a flattened jejunal mucosa is seen. This typical intestinal damage resolves completely upon elimination of gluten-containing grains from the diet. The prevalence of CD in the general population might be as high as 0.5% (2). However, not all patients present with the classical triad of growth failure, chronic diarrhoea and abdominal distention. The remainder will either manifest less specific symptoms such as constipation, abdominal pain or anaemia or will have no symptoms at all. Serological markers for CD were developed primarily to detect CD in the group of patients presenting with these atypical symptoms. Initially IgG and IgA antibodies against gliadin were used. However, IgG antibodies to gliadin are not very specific because elevated levels can be found in other diseases and in healthy individuals (3). IgA antibodies against gliadin are more specific, but in most studies more than 10% of the patients with CD are missed (3). Nevertheless, those tests are still widely used because of low costs. Until recently the presence of anti-endomysium antibodies EMA was the most reliable in predicting CD. Although this test is more sensitive and specific than the anti-gliadin antibody (AGA) assays, nevertheless 5%-10% of patients with CD are missed, while some children without CD are falsely labeled as patients (3,4). Therefore a more reliable serological test for CD is needed.

A breakthrough in understanding the pathophysiology of celiac disease has been the discovery and identification of tissue transglutaminase (tTG) as the self-antigen for endomysial antibodies (5). As guinea pig tTG, but not human tTG, was directly available, the first enzyme linked immunosorbent assays (ELISAs) developed used this substrate. However, the sensitivity of this test did not exceed that of the EMA (6,7). These results might have been caused by the only partial homology between guinea pig tTG and human tTG. We have evaluated the diagnostic potential of a newly developed ELISA based on human tTG (Celikey®), as compared to the other celiac screening tests mentioned above in a biopsy-controlled study.

We studied 101 serum samples from patients with suspected CD which were seen in the Department of Paediatric Gastroenterology. Patients had various clinical symptoms such as chronic diarrhea, growth failure, abdominal distention, constipation, abdominal pain or anaemia. In all patients several duodenal samples were taken during endoscopy from the second to third part of the duodenum. In 32 patients, histology was considered to be diagnostic for CD as the small intestinal mucosa revealed (sub)total villus atrophy in combination with crypt hyperplasia, an inflammatory infiltrate in the lamina propria and an increased intraepithelial lymphocyte count. In 49 patients, histological examination revealed no abnormalities or only non-specific changes.

A total of 52 samples from children with biopsy-verified CD were collected (14 males, 38 females, mean age 4.0 years, range 1.1-14.4 years). Clinical and serological improvement was shown in all patients after termination of gluten ingestion. Human IgA tTG was elevated in all but two patients, giving a sensitivity of 96%. Also fifty patients had elevated guinea pig IgA-tTG (sensitivity 96%) and in 48 IgA EMA was positive (sensitivity 92%). IgA anti-gliadin was elevated in 43 patients (sensitivity 83%) and IgG anti-gliadin was elevated in 43 patients (sensitivity 83%).

A total of 49 samples from children with biopsy-verified non CD were collected as a disease control group (29 males, 20 females, mean age 5.1 years, range 0.8-19.2 years). These control patients had symptoms suggestive of CD, but had a normal intestinal biopsy. None of the patients had elevated human IgA tTG, thus giving a specificity of 100%. 45 patients had no elevation

![Fig. 1: Histology of normal (left panel) and celiac disease duodenal biopsy (right panel)]
Clinical Study

![Fig. 2: Correlation between IgA anti-tTG ELISA using either guinea pig liver (X-axis) or recombinant human tTG (Y-axis) as substrate. Cut-off values are indicated by dashed lines. Red symbols are sera from biopsy proven CD negative patients which scored false positive on guinea pig tTG. The green symbol indicates a CD patient false negative on guinea pig tTG. Further explanation see text.](image)

Table: Serological screening for coeliac disease

<table>
<thead>
<tr>
<th>Serological test</th>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG anti-gliadin</td>
<td>80%</td>
<td>83%</td>
</tr>
<tr>
<td>IgA anti-gliadin</td>
<td>86%</td>
<td>83%</td>
</tr>
<tr>
<td>IgA anti-endomysium</td>
<td>90%</td>
<td>92%</td>
</tr>
<tr>
<td>IgA anti-tissue transglutaminase (guinea pig liver)</td>
<td>92%</td>
<td>96%</td>
</tr>
<tr>
<td>IgA anti-tissue transglutaminase (recombinant human)</td>
<td>100%</td>
<td>96%</td>
</tr>
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of guinea pig IgA tTG (specificity 92%) and 44 were IgA EMA negative (specificity 90%). IgA anti-gliadin was normal in 42 patients (specificity 86%) and IgG anti-gliadin was not elevated in 39 patients (specificity 80%).

In our hands the Celikey® test correlated very well with CD with a sensitivity of 96% and a specificity of 100%. Although in a larger series, a false-positive result might be obtained with this test, as is also indicated by the confidence intervals for the specificity (93-100%), the human IgA tTG ELISA seems to be the best test for identifying patients with CD and its implementation might result in less unnecessary intestinal biopsies.

Patients included in this study had complaints suggestive of CD. It can be expected that a lower reliability will be obtained when using the human IgA tTG ELISA for population screening, as was the case for the well validated EMA test in a mass screening project in the Netherlands (2). In that study almost 50% of the subjects positive for EMA had a normal small intestinal biopsy.

In the follow-up of patients with CD, IgA anti-gliadin is widely used as an indicator of dietary compliance, although the EMA seems to be more sensitive to detect long-standing periods of gluten intake (3). As human IgA tTG ELISA gives quantitative values, like IgA AGA, and has proven to be a more sensitive and specific test to identify CD, this test might be used in the future in the follow-up of patients with established CD. However, further investigations are required to evaluate the role of human IgA tTG in monitoring the compliance with a gluten-free diet.

We conclude that the Celikey® test for CD, human IgA tTG ELISA, is an excellent tool to screen for CD in patients with gastrointestinal complaints in the absence of IgA deficiency (8). This assay may also prove useful for the screening of populations at low or medium risk for CD.

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Human tissue transglutaminase enzyme linked immunosorbent assay outperforms both the guinea pig based tissue transglutaminase assay and anti-endomysium antibodies when screening for coeliac disease.
Eur J Pediatr 161, 284-287
Celiac disease (CD) is an autoimmune disease triggered by ingestion of gluten in genetically susceptible individuals.

The identification of tissue transglutaminase (tTG) as the major autoantigen against which the endomyosal antibody is directed has led to a greater understanding of the pathogenesis of the disorder and to the development of assays to detect autoantibodies to tTG. First generation tests for IgA class anti-tissue transglutaminase antibodies used guinea pig liver tTG as antigen. But despite initial optimism that the test might replace the immunofluorescent assays for endomysial antibody (EmA), most clinical studies have reported figures for diagnostic sensitivity and specificity that are inferior to those for the current EmA method.

Second generation commercial kits for IgA against tTG use either purified human tTG or human recombinant tTG as antigen. In July 2000 Pharmacia Diagnostics provided the market with an anti-tTG IgA ELISA based on human recombinant tTG produced in the baculovirus system and not in a prokaryotic system, like some competitors later on did. Still, Celikey® represents the only commercially available ELISA with human tTG expressed in the eukaryotic baculovirus system. In 2002, some studies were published which compared Celikey® to other methods for the diagnosis of celiac disease. These papers shall be summarized in the following.

### Antitissue transglutaminase antibodies outside coeliac disease (Clemente et al.)

Maria G Clemente et al. from Cagliari, Italy investigated the presence of IgA-antibodies against tTG (IgA tTG) in sera from 111 patients with untreated Coeliac Disease (CD), 96 patients with other autoimmune conditions and from 100 healthy controls using guinea pig tTG-ELISA and Celikey®. Western blotting with guinea pig tTG was also performed.

94 patients with CD who tested positive for anti-endomysial antibodies (EmA) and one who tested negative for EmA showed antibodies against the guinea pig tTG. Among controls, 50% of patients with autoimmune liver diseases and 6.5% of patients with insulin-dependent diabetes mellitus tested positive with this test. Clemente et al. showed by Western-blotting analysis that none of the EmA-negative sera that reacted positively in guinea pig tTG-ELISA recognized the tTG protein band at 78 kD. Especially when examining sera from patients with other autoimmune diseases, impurities may cause a high rate of non-specific reactivity.

Celikey® was not only positive in all patients who tested positive for EmA but also in two patients from the autoimmune controls, one of whom tested negative for EmA. Both of these patients had the histologic features of CD at intestinal biopsy and successfully began a gluten-free diet.

The authors concluded that human tTG ELISAs could be used in large screening programs for CD, because it is both, easy to handle and fast.

### Human tissue transglutaminase enzyme linked immunosorbent assay outperforms both the guinea pig based tissue transglutaminase assay and anti-endomyosial antibodies when screening for coeliac disease (Wolters et al.)

Victorien Wolters et al. from The Netherlands compared the sensitivity and specificity of Celikey® versus guinea pig IgA tTG, anti-gliadin antibodies (AGA) and EmA.

A total of 52 samples from children with biopsy-verified CD were investigated. Results are shown in table 1.

The authors concluded, that Celikey® is an excellent tool to screen for CD in patients with gastrointestinal complaints in the absence of IgA deficiency. It may also prove useful for the screening of populations at low or medium risk for CD.

### Recombinant human tissue transglutaminase for diagnosis and follow-up of childhood coeliac disease (Hansson et al.)

Tony Hansson from Stockholm, Sweden, and his group used Celikey® to measure the levels of IgA anti-tTG antibodies in 226 serum samples from 57 children with biopsy-verified coeliac disease, 29 diseases control subjects, and 24 healthy control subjects. All samples were also analyzed for IgG anti-tTG antibodies and EmA. The levels of IgA and IgG anti-tTG antibodies correlated with the condition of the small intestinal villous structure and the serum levels of IgA EmA. All of the 25 serum samples obtained from untreated patients contained IgA anti-tTG antibodies, and 24 of 25 also had IgA EmA. Of the serum samples from 53 control children, two had IgA anti-tTG antibodies and two had IgA EmA. Sensitivity and specificity are listed in table 2.

The authors concluded that although the criteria-based diagnosis of childhood coeliac disease still depends on histologic evaluation of intestinal biopsies, detection of anti-tTG antibodies provides useful complementary diagnostic information. The human recombinant tTG-based ELISA can be used as a sensitive and specific test to support the diagnosis and may also be used in the follow-up of treatment in childhood coeliac disease.

### Antibodies against human tissue transglutaminase and endomysium in diagnosing and monitoring coeliac disease (Bürgin-Wolff et al.)

Annemarie Bürgin-Wolff et al. from Switzerland tested sera from 208 CD patients and 157 disease controls, both groups with

### Table 1. Sensitivity and specificity of different screening tests for CD. The 95% confidence intervals are given in parentheses. gp = guinea pig.

<table>
<thead>
<tr>
<th></th>
<th>AGA IgA</th>
<th>AGA IgG</th>
<th>EmA IgA</th>
<th>gp tTG IgA</th>
<th>Celikey®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>83 (70-92)</td>
<td>83 (70-92)</td>
<td>92 (81-98)</td>
<td>96 (87-100)</td>
<td>96 (87-100)</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>86 (73-94)</td>
<td>80 (66-90)</td>
<td>90 (78-97)</td>
<td>92 (80-98)</td>
<td>100 (93-100)</td>
</tr>
</tbody>
</table>

### Table 2. Comparison of sensitivity and specificity of the serum IgA and IgG AGA assay, the serum IgA EmA test, the human recombinant IgG tTG assay and Celikey®.

<table>
<thead>
<tr>
<th></th>
<th>IgA AGA</th>
<th>IgG AGA</th>
<th>IgA EmA</th>
<th>h tTG IgG</th>
<th>Celikey®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>96</td>
<td>84</td>
<td>96</td>
<td>96</td>
<td>100</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>89</td>
<td>92</td>
<td>96</td>
<td>91</td>
<td>96</td>
</tr>
</tbody>
</table>
jejunal biopsy on a gluten-containing diet and from 41 patients on a gluten-free diet. All patients were tested for EmA and for IgA anti-human tTG antibodies with Celikey®.

200/208 patients with CD had positive IgA tTG, while only 1/157 of the control patients were positive. Sensitivity and specificity of the test were 96% and 99% for the study population. Only 4/365 patients (1%) presented discordant IgA tTG and EmA results, 2 of them had only IgA tTG and 2 only EmA. The IgA tTG levels and the EmA titres were closely correlated to the duration of gluten-free diet and gluten challenge, respectively. The authors concluded, that IgA tTG can be used as an accurate observer-independent alternative to EmA in diagnosing or monitoring CD.

**Comparative evaluation of serologic tests for coeliac disease diagnosis and follow-up (Martini et al.)**

Martini et al. from Torino, Italy, compared various serologic tests by investigating 101 untreated patients with CD, 101 disease controls and 89 healthy controls. Serum EmA were detected by immunofluorescence. Antibodies to tTG were measured by using four commercially available ELISAs that use human recombinant antigen and with an ELISA that uses guinea pig tTG antigen. Sensitivities and specificities of the assays are shown in table 3.

The authors concluded, that, at present, ELISAs using human recombinant antigen cannot replace EmA evaluation, but could be used as a first-level investigation for noninvasive testing in the diagnostic panel for CD.

**Sero logical testing for coeliac disease (Basso et al.)**

Dania Basso, Graziella Guariso and Mario Plebani referred directly to the publication of Martini et al. They studied 285 children, of whom 134 had CD and had been on a gluten-free diet. 151 children underwent upper gastrointestinal endoscopy: CD was histologically diagnosed in 81 and excluded in 70 control cases. IgA or IgG tTG were measured with seven different quantitative ELISAs, 6 of whom use human antigen, one uses murine antigen (results see table 4).

Although Basso et al. found comparable results in children to the data from Martini et al. in adults, Basso et al. did not agree with Martini et al. concerning the superiority of the EmA test over ELISAs. They suggest that ELISAs that include human recombinant antigens can be used as first-level investigatory tool in the diagnostic panel for CD. Basso et al. found that EmA testing appears to have limited clinical value in dubious cases. The quantitative ELISAs also seem to be useful indices for monitoring compliance with a gluten-free diet, especially in children.

**A comparison of 13 guinea pig and human anti-tissue transglutaminase antibody ELISA kits (Wong et al.)**

In July 2002 a study from Richard CW Wong et al. was published, where 13 commercial IgA tTG ELISA kits were compared. Seven ELISAs were coated with guinea pig tTG, two with purified human tTG and four with recombinant tTG. 49 IgA EMA positive adult patients with CD and 64 adult disease controls were measured to establish the sensitivity and specificity of each kit (see table 5 and 6).

The human tTG based kits generally demonstrated superior performance (especially specificity) to the guinea pig tTG based kits. However, the use of human tTG alone was not sufficient to confer performance equal to the IgA EmA IIF assay; only two kits produced closely comparable results (h TG4 and Celikey®). Two of the guinea pig tTG based kits had AUC estimations that were not significantly different from the human tTG based kits. This demonstrates that factors other than antigen source are important in determining kit performance.

**Autoantibodies to human tissue transglutaminase: superior predictors of coeliac disease (Blackwell et al.)**

Penny Blackwell et al. from Derby, UK, also compared sensitivity and specificity of five different IgA tTG ELISAs, four commercial IgA tTG kits which use human tTG as antigen and one guinea pig tTG based ELISA (see table 7). Serum samples from 32 adult patients with untreated CD were investigated. They were from a series of 130 cases and chosen to bias the group towards subjects with negative autoantibodies when measured with guinea pig tTG as antigen. In all cases of CD the diagnosis was confirmed by small-bowel biopsy. All were EMA-positive. Samples from 38 controls subjects (biased towards false-positive levels with guinea pig antigen) were used to compare specificity.

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**Table 3. Sensitivity and specificity of different assays with different cutoffs. All assays with human tTG use a recombinant protein, produced in prokaryotic cells (h TG1-3) or in eukaryotic cells (Celikey®). For comparison, the anti-EmA had a sensitivity of 94%. Its specificity was not determined because a negative result was a selection criterion for the control group.**

<table>
<thead>
<tr>
<th></th>
<th>h tTG1 IgA</th>
<th>h tTG2 IgA</th>
<th>h tTG3 IgA</th>
<th>Celikey®</th>
<th>gp tTG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity (%)</strong></td>
<td>86</td>
<td>96</td>
<td>95</td>
<td>54</td>
<td>89</td>
</tr>
<tr>
<td><strong>Specificity (%)</strong></td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>95</td>
</tr>
</tbody>
</table>

**Table 4. Sensitivity of 7 different kits, 6 with human antigen, 1 with murine antigen. The cutoffs correspond to a 95% specificity for control cases.**

<table>
<thead>
<tr>
<th></th>
<th>h tTG1 IgA</th>
<th>h tTG2 IgA</th>
<th>h tTG3 IgA</th>
<th>h tTG4 IgG</th>
<th>m tTG6 IgA</th>
<th>m tTG7 IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity (%)</strong></td>
<td>86</td>
<td>96</td>
<td>95</td>
<td>54</td>
<td>89</td>
<td>86</td>
</tr>
<tr>
<td><strong>Specificity (%)</strong></td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>95</td>
</tr>
</tbody>
</table>
The comparative data for the control group show wide variation in the proportion of false-positive results (3%-16%) for the four human tTG based assays. Only one sample was positive by Celikey® and this gave consistently ‘false’-positive results with all five assays. This was an EmA-positive sample from a patient whose small-bowel biopsy was normal, however a potential CD cannot be excluded. If this subject is excluded, then there were no false-positives with Celikey®.

The authors anticipate specificities of 99% for Celikey® and 95%-97% for the other kits. They conclude, that with an appropriate antigen, IgA tTG is an effective alternative to EmA when screening a population under suspicion of CD.

**Conclusion**

In the last year, eight studies were published which investigated the performance of Celikey®, compared with other methods and other tests. All studies found Celikey® to be a highly specific and sensitive method to measure anti-tTG antibodies, close to EmA testing, sometimes even superior to EmA.

Most of the authors do not question biopsy as the last and not replaceable method for diagnosing CD. However, measurement of antibodies to tTG, gliadin and endomy- sium effectively diminish the necessity of a small-bowel biopsy for the histological assessment of coeliac disease. If the antibodies are present, there is 99-100% likelihood of a flat mucosa. Conversely, if the antibodies are absent, the chance of a flat mucosa is under 1% in children and 2% in adults (Hadziselimovic and Bürgin-Wolff, 1998). Therefore, the necessity of a small-bowel biopsy could be abrogated by non-invasive immunological investigations. The antibody assay is a simple and powerful tool to estimate the compliance of patients on a strict gluten-free diet.

**References**


**Table 5.** IgA tTG results using the manufacturers’ cutoff and ROC plot analysis derived decision thresholds for the seven guinea pig liver tTG based ELISA kits.

<table>
<thead>
<tr>
<th>Sensitivity (%)</th>
<th>gp tTG1</th>
<th>gp tTG2</th>
<th>gp tTG3</th>
<th>gp tTG4</th>
<th>gp tTG5</th>
<th>gp tTG6</th>
<th>pg tTG7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer’s cutoff</td>
<td>88</td>
<td>98</td>
<td>96</td>
<td>92</td>
<td>100</td>
<td>86</td>
<td>98</td>
</tr>
<tr>
<td>ROC plot analysis</td>
<td>88</td>
<td>94</td>
<td>90</td>
<td>90</td>
<td>88</td>
<td>92</td>
<td>88</td>
</tr>
</tbody>
</table>

**Table 6.** IgA tTG results using the manufacturers’ cutoff and ROC plot analysis derived decision thresholds for the seven human tTG based ELISA kits.

<table>
<thead>
<tr>
<th>Sensitivity (%)</th>
<th>h tTG1</th>
<th>h tTG2</th>
<th>h tTG3</th>
<th>h tTG4</th>
<th>h tTG5</th>
<th>Celikey®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer’s cutoff</td>
<td>71</td>
<td>98</td>
<td>96</td>
<td>98</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>ROC plot analysis</td>
<td>96</td>
<td>96</td>
<td>96</td>
<td>98</td>
<td>98</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 7.** IgA tTG sensitivity and specificity using the manufacturers’ cutoff for four human tTG based ELISA kits and one guinea pig tTG based ELISA kit. Borderline results are considered as positive. Precision profiles were by comparing the coefficient of variance at normal, weak positive and positive concentrations.

<table>
<thead>
<tr>
<th>Sensitivity (%)</th>
<th>h tTG1</th>
<th>h tTG2</th>
<th>h tTG3</th>
<th>Celikey®</th>
<th>gp tTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer’s cutoff</td>
<td>100</td>
<td>88</td>
<td>94</td>
<td>91</td>
<td>50</td>
</tr>
<tr>
<td>ROC plot analysis</td>
<td>84</td>
<td>87</td>
<td>92</td>
<td>97</td>
<td>74</td>
</tr>
<tr>
<td>CV %</td>
<td>11-17</td>
<td>3-7</td>
<td>12-20</td>
<td>7-9</td>
<td>17-30</td>
</tr>
</tbody>
</table>