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## Celiac disease - is it just the tip of the iceberg?

### **New strategies for early diagnosis**

Celiac disease: pathogenesis, diagnosis and therapy

Screening - a future trend: useful and economic?

Rapid test for autoimmune diagnostics

5 years of AESKU

Screening - a future trend?

Dr. Torsten Matthias, AESKU

Celiac disease, also known as endemic sprue or gluten-sensitive enteropathy, is an excellent example for the complexity involved in the diagnosis of autoimmune diseases. The availability of reliable serological screening methods also allows the screening of asymptomatic clinical pictures and non-classic courses. It could be clearly demonstrated that in Europe and the US the prevalence of celiac disease may be 15 times higher than previously expected. Cases that have been recognised due to their classic clinical symptoms of early infantile celiac disease, such as bloated abdomen, loss of appetite, vomiting, diarrhea, flatulence and growth retardation, only represent the tip of the iceberg. This is the reason why the second issue of AESKU.SCIENCE primarily focuses on the current opportunities in finding a safe and efficient diagnosis of celiac disease, even in asymptomatic cases.

The first article by Dr. Michael Schultz of the University of Otago at Dunedin in New Zealand offers an extensive overview on the pathophysiology, diagnostics, clinical symptoms and therapy of celiac disease based on the recent literature. The discussion with Prof. Yehuda Shoenfeld from Tel Aviv, Israel, stresses the general importance of screening techniques for the future of autoimmune diagnostics. Current reports take a closer look at a potential correlation between celiac disease and other diseases such as type I diabetes or osteoporosis, reinforce the importance of highly sensitive tests for the diagnosis of celiac disease at the earliest stage possible and introduce highly sensitive test systems which allow economic and reliable screening at the same time.

Enjoy your reading!



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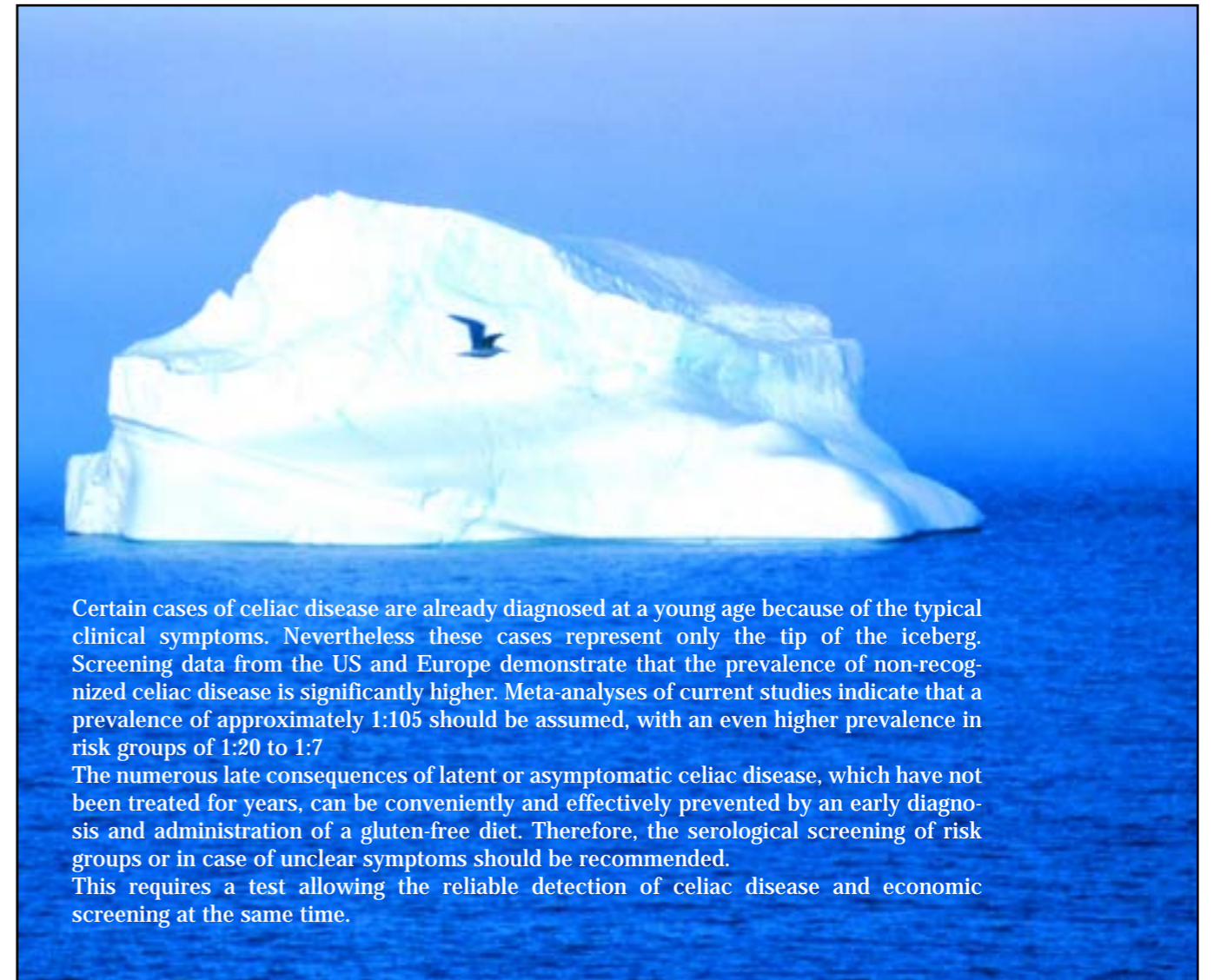
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Celiac disease - more common than previously assumed

Dr. med. Dr. med. habil. Michael Schultz, Department of Medical and Surgical Sciences, University of Otago at Dunedin, New Zealand



Certain cases of celiac disease are already diagnosed at a young age because of the typical clinical symptoms. Nevertheless these cases represent only the tip of the iceberg. Screening data from the US and Europe demonstrate that the prevalence of non-recognized celiac disease is significantly higher. Meta-analyses of current studies indicate that a prevalence of approximately 1:105 should be assumed, with an even higher prevalence in risk groups of 1:20 to 1:7. The numerous late consequences of latent or asymptomatic celiac disease, which have not been treated for years, can be conveniently and effectively prevented by an early diagnosis and administration of a gluten-free diet. Therefore, the serological screening of risk groups or in case of unclear symptoms should be recommended. This requires a test allowing the reliable detection of celiac disease and economic screening at the same time.

Celiac disease, also known as endemic sprue or gluten-sensitive enteropathy, is a genetically determined multi-systemic disease characterized by incompatibility of the wheat gliadin fraction and other alcohol-soluble proteins of rye and barley. This disease was first mentioned already in 1887 by Samuel Gee who also described the typical symptoms diarrhea, faintness and growth retardation <sup>1</sup>. Even at that time, it was postulated that a relationship to food intake may exist. However, it was not before 1954, when Paulley and colleagues described an atrophy of the villi of the small intestine as a typical pathological finding which is accompanied by a chronic inflammation of the mucosa <sup>2</sup>.

A biopsy of the small intestine is the renowned gold standard for the diagnosis of celiac disease <sup>3</sup>. However, more currently, less invasive, serological test methods became established. The introduction of the detection of anti-endomysial and anti-gliadin antibodies together with the combined determination of IgG and IgA made more reliable, however relatively costly screening methods available <sup>4</sup>. In 1997, tissue transglutaminase was identified as the antigen of the specific autoimmune response of celiac disease, shortly followed by commercially available assays <sup>5</sup> which allowed to perform population-wide large-scale screenings and thus to diagnose also asymptomatic pathologies and non-classic courses. It was soon demonstrated that the prevalence of this

disease is considerably higher than previously assumed. The previous assumption was that the maximum prevalence in Western populations would be 1:3000 – 1:10,000<sup>6</sup>, but a currently performed analysis of the available studies demonstrated that a prevalence of approximately 1:105 should be assumed, with an even higher prevalence in risk groups of 1:20 to 1:7<sup>7</sup>. Based on this fact, the US National Institute of Health (NIH) convened a national consensus conference in 2004 to revise the existing diagnostic and management guidelines<sup>8</sup>.

The current paper intends to give a short overview on pathophysiology, diagnostics, clinical symptoms and therapy of celiac disease based on the current literature.

## Prevalence

Celiac disease is a multi-systemic disease which in contrast to previous opinions does not only manifest itself through classic symptoms of the gastrointestinal tract, but it may also involve other organ systems (skin, liver, joints, uterus, brain, heart, etc.)<sup>9</sup>. This represents a severe drawback for clinical, symptom-oriented diagnosis. The partial clarification of the underlying pathophysiological correlation of celiac disease allowed the design of a number of non-invasive serological tests suitable for large-scale population-wide screening tests. Their results demonstrated that the prevalence is considerably higher than previously assumed, with an increasing number of adult patients without indications of the classic gastrointestinal symptoms<sup>7</sup>. This resulted in the definition of the term *potential* or *latent* celiac disease<sup>10</sup>. Although biopsies of the small intestine of patients without any or with only a few symptoms (a- or oligo-symptomatic) does not necessarily demonstrate a clear atrophy of the villi, an increased count of T-cells is detected intraepithelially<sup>11</sup>. The major phenotypic problems of these patients are iron-deficiency anemia or other manifestations of celiac disease. However, detailed interviews demonstrate that retrospectively symptoms often cannot be excluded completely; during the further course of the disease, these patients may develop gastrointestinal or extraintestinal symptoms when left untreated<sup>12,13</sup>.

Prevalence is the rate of patients with the respective disease within a population. According to European studies, the prevalence in children ranges from 1:285 to 1:67, depending on the required diagnostic criteria (i.e. pathologic biopsy of the small intestine, increased IgA transglutaminase titers, etc.)<sup>14-20</sup>. All in all, it can be assumed that the risk of actually contracting celiac disease is approximately 1-3% for asymptomatic European and US populations<sup>21</sup>.

An association with celiac disease has been described for a number of diseases. Particular risk groups are patients with autoimmune diseases like diabetes mellitus type 1 (Dm1) and patients with a positive family history of celiac disease or dermatitis herpetiformis Duhring. Also in cases of unexplained osteoporosis or

iron deficiency anemia, an asymptomatic celiac disease should be taken into consideration.

An association between Dm1 and celiac disease is known since a long time. A number of studies could demonstrate that prevalences of celiac disease between 1.5% and 7% can be assumed for children and adults with Dm1<sup>22</sup>. In addition, it is suspected that celiac disease may not only occur in association with Dm1, but may even precede Dm1 and may eventually influence its later expression. Vice versa, it seems that early diagnosis of celiac disease followed by treatment reduces the risk of developing Dm1. It could be demonstrated that autoantibodies against pancreatic islet cells are preferentially detected when celiac disease is still untreated; nevertheless, in some cases after treatment, those antibodies were no longer detected<sup>23</sup>.

An iron deficiency anemia often leads to diagnostic measures, because iron is absorbed in the proximal small intestine, the classic histological localization of celiac disease<sup>24</sup>. Therefore, in case of a refractory iron deficiency anemia of unclear origin, a deep biopsy of the small intestine should be performed. In such cases, iron deficiency may be the only manifestation of celiac disease<sup>25</sup>. Screening tests detected a prevalence of celiac disease between 3% and 12% in patients with selective iron deficiencies but without other symptoms<sup>26</sup>. A gluten-free diet improves the mucosal atrophy and thus compensates for the iron deficiency by increased resorption<sup>27</sup>.

Celiac disease may also be associated with reduced bone density. The atrophy of the villi related to untreated celiac disease may reduce the resorption of vitamin D and calcium; on the other hand, it may also reduce the calcium supply accompanied by lactose intolerance. The prevalence of celiac disease may reach 5% in patients with reduced bone density<sup>28</sup> in the various studies and it depended on the diagnostic standards used for celiac disease as well as for bone density determination<sup>29</sup>.

A potential genetic predisposition of this disease is suggested by the distinct association with the HLA-DQ2 and HLA-DQ8 genotypes and also the high prevalence in first-degree relatives. Again depending on the applied diagnostic criteria, prevalences up to 40% are assumed<sup>30</sup>. When the diagnosis is based on the small-grade histological changes according to Marsh I classification, the prevalence is even higher than 44%<sup>31</sup>. Monozygotic twins show a concordance of approximately 70-75%<sup>32</sup>.

## Pathogenesis

Celiac disease is induced by the intake of various protein fractions of wheat, rye and barley in food. It is generally named gluten incompatibility; however, more precisely, the name gluten only refers to the inducing protein fraction of wheat (*gliadins* and *glutenins*)<sup>33</sup>, while in rye and barley, the inducing proteins are *hordeins* and *secalins*<sup>34</sup>. Celiac disease is clearly associated with various genotypes of HLA class II genes. Almost all individuals suffering from celiac disease possess alleles coding for a

specific HLA-DQ2 (in 90-95%) or DQ8 (in 5-10%) heterodimer<sup>35,36</sup>. This is a quite frequent constellation in the European population and therefore appears to be necessary but not sufficient for the phenotypic expression of the disease. The search for other genetic associations was not yet successful<sup>37</sup>.

HLA class II molecules are expressed on the cell surface of antigen-presenting cells. There they bind to exogenous peptides (here: gluten) presented from CD4<sup>+</sup> T-cells. This requires the conversion of glutamine to the negatively charged glutamic acid, a reaction catalyzed by the enzyme tissue transglutaminase. The determination of autoantibody titers directed against tissue transglutaminase is used for diagnostic applications<sup>5</sup>. The tissue damages are due to the secretion of  $\gamma$ -interferon<sup>38</sup>. First histological damage can already be observed 1 hour after contact to gluten; therefore, more recent studies have questioned the central role of CD4<sup>+</sup> T-lymphocytes. CD4<sup>+</sup> T-cell activation takes place as a retarded immune response and therefore would require days to be expressed. Just recently, increased IL-15 mucosal levels were measured in active celiac disease. This discovery may be considered as evidence for another potential pathological mechanism<sup>39</sup>.

## Diagnostics

When almost 120 years ago celiac disease was first described, it was only based on the clinical presentation of mostly young patients with the classic symptoms of diarrhea, lethargy and retarded development<sup>1</sup>. Even in 1960, diarrhea prevailed in 100% of the patients. Thanks to the general availability of endoscopy offering the taking of biopsy samples and moreover to non-invasive serological tests for the diagnosis of celiac disease, a pronounced diarrhea is only detected in 50% of the diagnosed patients<sup>40</sup> today.

However, biopsies of the small intestine taken by endoscopy and demonstrating characteristic changes still represent the diagnostic gold standard and should be taken from all patients with a well-founded suspicion of celiac disease (Figs. 1 and 2)<sup>9</sup>.

I	II	III			IV
Infiltration stage	Hyperplasia stage	Destruction stage: atrophy of the villi			Hypoplasia
		IIIA	IIIB	IIIC	
Increase of intraepithelial lymphocytes to 30-40 per 100 enterocytes.	In addition to the infiltration of lymphocytes, a hyperplasia of crypts exists with branching and elongation and a reduced mitosis rate. Normal villi.	Partial atrophy of the villi.	Subtotal atrophy of the villi, individual villi can still be detected.	Complete atrophy of the villi.	Flat atrophic mucosa with irreversible damage.

**Table 1:** Classification of histological mucosal damage in celiac disease according to MARSH<sup>42</sup>

Although zoom endoscopy allows the *in vivo* diagnosis of the pronounced atrophy of the villi, it does not replace biopsy and so far has not become part of clinical routine<sup>41</sup>. As histological changes in the proximal small intestine are often discontinuous, four or more biopsies should be taken. The histological classification is performed according to the MARSH criteria (Table 1). It does not only take the architecture of the villi into account but also the infiltration of inflammatory cells in early stages<sup>42</sup>.

The histological damage of the mucosa of the small intestine is as variable as the phenotypic expression of the disease. In case of doubt and of a well-founded suspicion, another biopsy sample should be taken. For therapy monitoring repeated biopsies are regarded as obsolete, and the symptoms should be used as an orientation. However, a histological stage IV damage with complete atrophy of the villi in celiac disease refractory to therapy should be considered an early stage in the development of an enteropathy-associated T-cell lymphoma<sup>9</sup>.

Three important antigens (i.e. gliadin, endomysium, tissue transglutaminase) are used for the diagnosis of celiac disease. Based on the consensus conference of the NIH, two meta-analyses were performed comprising the data of all studies on sensitivity and specificity of serological tests in the diagnostic field of celiac disease published since 1966<sup>43,44</sup>. The anti-gliadin IgG (AGA IgG) assay is variable with respect to sensitivity (57-100%) and specificity (47-94%). No better results were achieved for the detection of anti-gliadin IgA (AGA IgA; sensitivity 52-100% and specificity 71-100%). There was no significant difference between the results of adult patients and children.

For the detection of anti-endomysium IgA (EMA IgA) antibodies, sensitivities of 86-100% and specificities of 90-100% were demonstrated. As no sufficient data are available on the detection of anti-endomysium IgG (EMA IgG) antibodies, an evaluation is not possible.

Following the identification of tissue transglutaminase (tTg) as the target structure for autoantibodies<sup>5</sup>, assays were made available based on the detection of IgA and IgG against transglutami-

nase. Sensitivity for tTG IgA was 77-100%, specificity ranged from 91% to 100%. It should be noted that today the results would be even better due to the exclusive utilization of the human protein, which is a standard procedure today. tTG IgG was investigated in only a small number of studies. A sensitivity of 85-97% and a specificity of 91-93% were obtained.

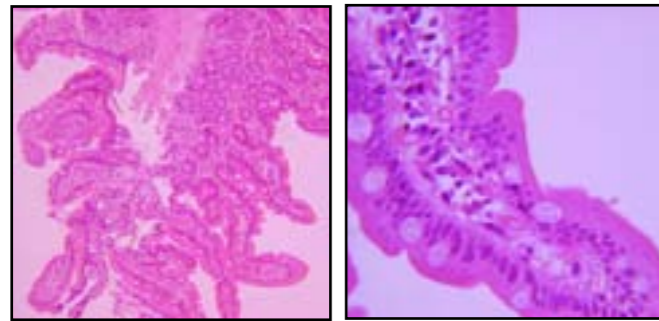


Figure 1A/B: Normal mucosa of the small intestine.

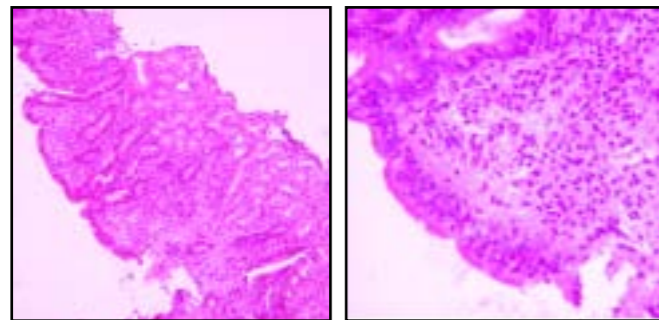


Figure 2A/B: Increase in intraepithelial lymphocyte count with pronounced atrophy of the villi; individual villi can be still detected. This damage refers to MARSH IIIb.

Pictures: Dr. Gail Williams, Department of Pathology, Dunedin Public Hospital

Only few studies investigated the benefits of a test combination compared to the analysis with a single test<sup>44</sup>. When AGA IgA + IgG were used under the condition that at least one test had to be positive, a sensitivity of 83-100% was achieved, while specificity was 71-99%. When the results of both tests had to be identical, sensitivity was only 50%<sup>44</sup>. Only one study was published on the combination of IgA and IgG tTG (human recombinant protein). A sensitivity of 98.5% was obtained under the condition that at least one test had to achieve a positive result (specificity 100%)<sup>45</sup>.

The combination of IgA AGA and IgA EMA resulted in a sensitivity of 100% at a specificity of only 73%, under the condition that at least one test had to achieve a positive result<sup>46</sup>.

Since a short period of time, a test for the detection of HLA-DQ2 and DQ8 is commercially available; however, due to the small number of results available so far, it cannot be recommended for practical clinical use at the moment. On the one hand, more than 95% of all celiac disease patients are positive for this

genotype (high sensitivity), on the other hand, the same applies to 30% of the healthy population (low specificity)<sup>43,44</sup>.

Altogether, EMA IgA and tTG IgA demonstrated the best sensitivity and specificity results to prevent unnecessary endoscopies for biopsies of the small intestine. Antigliadin antibody-detecting tests cannot be recommended for screening purposes, due to their pronouncedly poorer sensitivity and specificity. Significant differences could not be detected in results on children or adults. In patients with selective IgA deficiency, the detection of celiac disease is problematic; only less sensitive IgG-based assays are used. While the combination of various tests only allowed a small improvement of sensitivity, specificity was negatively affected<sup>44</sup>.

## Clinical picture

Due to its many diverse manifestations, celiac disease is considered a chameleon among the diseases covered by internal medicine. A number of studies illustrates that celiac disease is not limited to childhood. A recent retrospective analysis demonstrates that it was diagnosed in 11% of the patients before the 9<sup>th</sup> year of life, but 12% were older than 60<sup>47</sup>. However, while the pathology in children equals more the classic picture of celiac disease, it differs from the presentation in adults. A retrospective study compared the manifestations at diagnosis before and after the availability of serological tests. While before 1993, classic celiac disease with symptomatic diarrhea prevailed (73%), this share was clearly reduced after 1993 (diarrhea 43%)<sup>40</sup>. In addition, today 30% or more of all celiac disease patients have normal weight or are overweight<sup>48</sup>. Other symptoms are iron deficiency anemia, abdominal pain and flatulence.

Another study published in 2001 demonstrated that the majority of the patients were female (ratio males to females 1:2.8) and were diagnosed in their 4<sup>th</sup> – 6<sup>th</sup> decade of life. However, it was surprising that, from the statistical point of view, almost 11 years in average passed until diagnosis and thus treatment; before, 36% of the patients were diagnosed as irritable bowel syndrome patients<sup>49</sup>. The principal symptom in this study was classic diarrhea (85%); all other cases were silent celiac disease with iron deficiency anemia (8%), osteoporosis and osteopenia (7%). Celiac disease is associated with many other diseases including autoimmune diseases. Top-ranking were thyroid diseases (18%), dermatitis herpetiformis Dühring (9.8%)<sup>50</sup>, aphthous oral lesions (9%) and various neurological clinical pictures (ataxia of the cerebellum 7%; peripheral neuropathy 49% and epilepsy 5.5%)<sup>51</sup>. Another study demonstrated diarrhea as the major symptom in only 43% of the patients, 17% were identified based on screening due to other diseases<sup>40</sup>.

In children, celiac disease often manifests itself between the age of 6 to 24 months, following supplementary feeding of wheat products, with the classic symptoms of chronic diarrhea, anorexia and abdominal discomfort. Shortly after that growth retardation,

muscular dystrophy and loss of weight occur, and such children are generally unhappy. In isolated cases, a "celiac disease crisis" may develop with explosive diarrhea, bloated abdomen and water and electrolyte loss, even to the picture of a hypovolemic shock syndrome.

In older children (5-7 years), celiac disease presents itself often less classic with relapsing abdominal complaints, but also with symptoms of chronic malabsorption (growth retardation, dental enamel defects, etc.). This is a silent or atypical (potential) form of celiac disease<sup>7,52</sup> that may not be diagnosed before adult age.

## Complications

In addition to the already discussed secondary diseases resulting from untreated celiac disease as a consequence of the malabsorption of various food ingredients and water and electrolyte loss, early assumptions were made that celiac disease might be associated with malignant diseases.

For the first time, Fairly and Mackie described in 1937 six patients with a lymphoma of the small intestine and steatorrhea<sup>53</sup>, but the term enteropathy-associated lymphoma (EATL) was only created in 1986<sup>54</sup>. The rarely occurring EATL is a T-cell non-Hodgkin lymphoma with an annual incidence of not more than 0.5–1/1,000,000 adults. However, with a share of 35%, it represents one of the most frequent malignant tumors of the small intestine. Normally, 5-10 years pass after celiac disease has been diagnosed before EATL develops, but even periods up to 60 years were described<sup>55</sup>. The tumor develops from a clonal expansion of intraepithelial TCR $\alpha/\beta$  cells and can be partially considered the direct consequence of a celiac disease refractory to therapy<sup>56</sup>. In the majority of the cases, the patients initially responded well to the gluten-free diet but then demonstrated symptoms of new disease activity with loss of appetite, loss of weight and diarrhea. While EATL was mostly reported in the jejunum, it may be also found at other locations within the small intestine and is therefore not suited for "simple" biopsy<sup>55</sup>. However, more recently, the introduction of capsule endoscopy allows the visual examination of the small intestine<sup>56,57</sup>.

## And the winner is ...



Pier Luigi Meroni, Ricard Cervera, Josip Font, Yehuda Shoenfeld (from left to right)

In 2005, the renowned EULAR PRIZE of the European League Against Rheumatism (EULAR), including 30,000 Euro was awarded to Yehuda Shoenfeld and Miri Blank from Israel, Ricard Cervera and Josep Font from Spain and the Italian researchers Pier Luigi Meroni and Elena Raschi.

The scientists were awarded for their research work in the field of the antiphospholipid-syndrome (APS), in particular for their research into the origin of APS and the role of infections as the source and initiator of this autoimmune disease.

The EULAR PRIZE is always awarded to research groups of science or practical work who have made outstanding contributions to the progress in rheumatology. In 2005, Josef Smolen, president of the EULAR, presented the awards during the opening ceremony of the 2005 EULAR Conference on 8<sup>th</sup> of June in Vienna.

In addition, the adenocarcinoma of the small intestine is also associated with celiac disease. It is assumed that the development of the carcinoma follows the adenoma-carcinoma sequence. A British study detected an adenocarcinoma in 13% of the celiac disease patients. However, the demonstration of increased adenoma incidence is still pending.

A number of other studies suggest associations with many other malignant diseases (esophagus, pharynx, mammal, skin, liver carcinoma). However, in most studies the number of examined cases was not sufficient to allow valid conclusions<sup>58-60</sup>.

## Therapy

The only effective therapy for celiac disease is a life-long gluten-free diet. The diet is based on avoiding various proteins inducing celiac disease found in wheat, rye and malt. In earlier times, this was a very restrictive type of diet only allowing corn, rice and potato products as a replacement, but then it was possible to improve compliance significantly by including various types of flour as well as an extended selection of products with an improved nutrient content. Many patient organizations were founded in the late seventies providing affected individuals with support and advice<sup>61</sup>.

However, a strict diet does not guarantee a complete absence of side effects. Non-enriched gluten-free products often lead to a deficiency of vitamins B and D, calcium, iron, zinc, magnesium and fibers. Deficiency syndromes are observed quite often<sup>61</sup>.

Numerous studies were performed to test improved gluten-free products or to establish "how much" gluten is admissible without harming the patient. However, the significance of these studies

is often limited due to low case numbers, ineffective study design and too short follow-up periods<sup>61</sup>. Nevertheless it could be demonstrated that the addition of oatmeal fundamentally improves the absorption of iron, zinc, vitamin B1 and fibers<sup>62</sup>. However, caution is advised, as most commercially available products are contaminated with gluten<sup>63</sup>. Also the addition of wheat starch is controversially discussed. Commercially available wheat starch contains up to 60 mg gluten per 100 g and should therefore be made available as a purified product<sup>61</sup>. The consumption of these products often resulted in an increased number of abdominal complaints. A currently performed study monitored 57 patients receiving either a gluten-free diet or a gluten-free diet supplemented with wheat starch. After one year, differences between the two groups could not be observed with respect to all investigated criteria<sup>64</sup>.

## Summary

Altogether, a significant increase in the incidence of celiac disease was recently observed. Increasingly, patients with so-called silent or potential celiac disease are diagnosed. While in adult patients often malabsorption symptoms such as iron deficiency anemia or osteoporosis prevail, children still more often suffer from classic celiac disease with diarrhea and abdominal discomfort. This shift in symptoms has two reasons, i.e. the current availability of non-invasive screening tests and the increasing knowledge of the multiple manifestations of celiac disease. However, the confirmation of diagnosis still requires a biopsy of the small intestine as the gold standard. From the therapeutic point of view, a gluten-free diet with or without the addition of oatmeal or wheat starch represents the only available alternative.

TEST METHOD	SENSITIVITY (%)	SPECIFICITY (%)
Based on IgA detection		
AGA IgA	52 – 100	71 – 100
EMA IgA	86 – 100	90 – 100
tTG IgA	77 – 100*	91 – 100
Based on IgG detection		
AGA IgG	57 – 100	47 – 94
EMA IgG	-	-
tTG IgG	85 – 97	91 – 93
Combined tests		
IgA+IgG AGA	83 – 100	71 – 99
IgA+IgG tTG	98.5	100
IgA AGA + IgA EMA	100	73

\*93-96% when only studies involving recombinant human antigens were considered.

**Table 2:** Sensitivity and specificity of various serological tests in the diagnostics of celiac disease

## References

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## Screening for celiac disease in patients with type I diabetes - which test is the best?

Type I diabetes is one of the most severe diseases in children and adolescents. The genetic predisposition behind the development of type I diabetes makes the affected individuals also prone to other autoimmune diseases like celiac disease, autoimmune thyroiditis, immunoadrenalitis (Addison's disease), vitiligo, alopecia or gastroenterologic autoimmune diseases.

The fact that all these diseases are related to the occurrence of autoantibodies against the affected organs or cell structures suggests that autoantibody tests might be suitable for the screening of risk groups like type I diabetes patients for autoimmune diseases.

Therefore, the British National Institute for Clinical Excellence (NI-CE) published in the middle of 2004 a current Health Technology Assessment (HTA) Report as part of its NHS R&D Health Technology Assessment (HTA) program with the title "Autoantibody testing in children with newly diagnosed type I diabetes mellitus", with the aim to examine the potential role of autoantibody tests in screening tests.

The NHS R&D HTA program was established to supply comprehensive and high-quality information on efficiency and costs of medical technologies to the decision-makers in the British National Health Service (NHS).

The authors from the Public Health and Epidemiology Department of the Birmingham University had three reasons to primarily focus in their analysis on celiac disease in addition to autoimmune thyroiditis, despite the multitude of autoimmune diseases related to diabetes:

- Autoantibodies are available as suitable markers for the serological diagnostics of celiac disease.
- An asymptomatic or silent celiac disease may harm the patient a long time before it is diagnosed based on clinical symptoms.
- Before the report was published, it was controversially discussed whether type I diabetes patients should be screened for celiac disease, and - if yes - at which time periods a regular monitoring should be performed. Therefore, it was necessary to resolve whether autoantibody tests would be a suitable tool for a medically and economically reasonable screening.

The analysis involved many data sources: scientific papers from

medical databases such as MEDLINE, EMBASE or the Cochrane Library, also references of the identified papers and additional professional and private online information sources. Research groups, laboratories and manufacturers of test systems were contacted, so that even still unfinished studies could be integrated.

The core of the analysis was a detailed comparison of the various test systems currently available on the market for the detection of autoantibodies present in celiac disease. Essential comparison parameters were sensitivities and specificities of the various tests.

The second primary aim of the study was to set up an extensive model for analysis and assessment (decision analytic model) to decide based on cost-benefit considerations whether the screening for celiac disease of individual diabetes patients would be adequate.

### The results

The result of the HTA report was clear: autoantibody testing is a medically and economically suitable tool for the screening of type I diabetes patients for celiac disease.

All evaluated autoantibody tests showed acceptable precision: IgA-EMA, IgA-ARA and IgA-tTg tests demonstrated particularly good suitability, followed by IgG AGA and then by IgG AGA tests. When considering the precision of the individual test systems, two tests clearly stand out due to their specificity and sensitivity, i.e. the IgA EMA immunofluorescence tests and IgA tTg ELISA tests.

In summary, the results of the HTA report attest the IgA EMA immunofluorescence test the best precision, because the number of available studies on only most recently developed tTg tests



was still quite low at the time when the analysis was performed. One of two included studies demonstrated comparable specificities and sensitivities for IgA EMA and IgA tTg, while the second even demonstrated higher sensitivities of the IgA tTg tests.

When focusing on ELISA tests only, that due to their potential for automation are more economic and less labor-intensive and therefore clearly better suited for the screening of large patient populations, the HTA report concludes that the tTg ELISA tests might be preferred for patient screening for celiac disease.

The evaluation of the economic data of the analysis and assessment model demonstrates clearly that the combination of the serological test with the highest precision and biopsy for confirmation of a positive diagnosis represents the most economic screening variant.

Therefore, the lowest cost compared to the abstention from one screening per gained year of life (QALY quality assured life year) is achieved by the combination of IgA EMA with biopsy (12.250 Pounds) and the combination of IgA tTg with biopsy (12.970 Pounds).

The combination of a variety of serological tests in patient screening did not or almost did not result in positive effects.

### Still much to be done ...

The analysis and assessment model developed in the current HTA report points out that screening of type I diabetes patients for celiac disease is not only useful from the medical point of view, but that it is also cost-effective.

However, clinicians and the families of affected children would surely like to know the right time for screening and which consequences the late diagnosis of silent celiac disease might have for the development of the disease to adult age. This information cannot be derived from the model. Furthermore, the question addressing the number of screenings and their time intervals in lifelong affected type I diabetes patients requires further research.

Abbreviation	Test for:	Method
IgA-AGA	IgA antibodies against alpha-gliadin	ELISA
IgG-AGA	IgG antibodies against alpha-gliadin	ELISA
IgA-ARA	IgA antibodies against reticulin	Immunofluorescence
IgA-EMA	IgA antibodies against endomysium	Immunofluorescence
IgA-tTg	IgA antibodies against tissue transglutaminase	ELISA
IgG-tTg	IgG antibodies against tissue transglutaminase	ELISA



Although the current HTA report focuses only on the screening of type I diabetes patients for celiac disease, its systematic analysis of the various available diagnostic test systems and the developed model for cost-benefit considerations also offers a basis for the assessment of screening in other patient groups with an increased risk of celiac disease.

*The extensive HTA report*

*"Autoantibody testing in children with newly diagnosed type I diabetes mellitus" can be downloaded as a PDF file at [www.aesku.com/diagnostics/english/support/](http://www.aesku.com/diagnostics/english/support/)*

References:

Chan AW, Butzner JD, McKenna R, Fritzler MJ. Tissue transglutaminase enzyme-linked immunosorbent assay as a screening test for celiac disease in pediatric patients. *Pediatrics* 2001; 107:E8.  
Kordonouri O, Dietrich W, Schuppan D, Weibert G, Muller C, Sarioglu N, et al. Autoantibodies to tissue transglutaminase are sensitive serological parameters for detecting silent celiac disease in patients with Type I diabetes mellitus. *Diabet Med* 2000; 17: 441-4.

## AESKU.AWARD - More than only fame

From now on, the "AESKU.AWARD for life contribution to autoimmunity" will be awarded with an amount of 30,000 Euro, making it one of the most considerable scientific awards in the entire field of medicine. It will be awarded every two years to three scientists who excelled for many years in the field of autoimmunity, where such a high amount of money represents an exceptional honour.

The award ceremony takes place at the "International Congress on Autoimmunity" every two years; the next meeting will be held

from the 29th of November to the 3rd of December 2006 in Sorrento, Italy.

The AESKU.AWARD has two objectives. It intends to emphasize the importance of research on autoimmune diseases and to attract attention to progress in this field. In addition, it intends to promote interdisciplinary cooperation and to establish autoimmunity as an independent field of research. The prize money will help to improve the financial support of current research projects.

### AESKU.AWARD for life contribution to autoimmunity

The AESKU.AWARD for life contribution to autoimmunity was for the first time awarded at the 4th International Congress on Autoimmunity in November 2004 in Budapest to three pioneers in autoimmune disease research: Donato Alarcon-Segovia of the National Institute of Medical Sciences and Nutrition in Mexico City, who has unfortunately passed away, Ian R. Mackay of the Monash University in Clayton, Australia and Noel R. Rose, Johns Hopkins Medical Institute, Baltimore, USA.

All three scientists have made important contributions to the research on autoimmune diseases since decades.



AESKU.AWARDs



M. E. Gershwin is handing over the AESKU.AWARD to Ian R. Mackay



Congress President Professor M.E. Gershwin, Dr. Torsten Matthias (AESKU), and Congress President Professor Yehuda Shoenfeld (from left to right) are presenting the AESKU.AWARDs



Noel R. Rose receives the AESKU.AWARD

## Only a "Laboratitits"?

Most recent research results, such as the data of the study of William F. Stenson and colleagues of the Washington University demonstrating a clear association between the appearance of osteoporosis and celiac disease, emphasize the importance of large-scale screening tests for autoimmune diseases.

On the one hand, the earliest possible detection of a disease risk helps patients to prevent severe consequences. On the other hand, the diagnosis or prognosis of an autoimmune disease is not good news for obviously healthy individuals.

Therefore, we discussed the benefits of early diagnosis or prognosis with Professor Yehuda Shoenfeld of the Tel Aviv University, and we asked whether screening tests will become a "mega trend" in autoimmune diagnostics.

Professor Shoenfeld, Head of the Department of Internal Medicine, Head of the Center for Autoimmune Diseases of the Tel Aviv University and scientific head of the AESKU.INSTITUTE, has the first chair of autoimmunity worldwide. The chair was established at the Tel Aviv University in March 2003.

Being Congress President of the "International Congress on Autoimmunity" he is always aware of future trends in autoimmunity.

*Professor Shoenfeld, one of your primary main focuses is the development of new opportunities and strategies in the diagnosis of autoimmune diseases. Do you see relevant trends for the near future? Will screening tests for autoimmune diseases become more important?*

Certainly! Screening techniques will have a significant impact on the future of autoimmune disease diagnostics.

"From diagnosis to prognosis," is how I would describe this trend. In addition to the simple diagnosis of an existing disease, the prediction of autoimmune diseases and various specific disease characteristics will increasingly gain in significance in coming years.

This trend has been triggered by a number of retrospective epidemiological studies published in the past two or three years. They demonstrated clearly that autoantibodies associated to autoimmune diseases do not only play a significant role as diagnostic markers but that their occurrence also may have a high predictive value. So what is different now? In the past, when autoantibodies were found in a patient who apparently showed no signs of disease, this was generally assumed to be a false positive result. This was jokingly referred to as "laboratitits".



Prof. Yehuda Shoenfeld

Thanks to some excellent studies performed e.g. with blood samples from recruits stored for many years for documentation purposes, we know today that autoantibodies can occur 10 to 20 years before the outbreak of the respective autoimmune disease, and in some cases even earlier.

The surely most remarkable example is the disease primary biliary cirrhosis (PBC), where the typical anti-mitochondrial antibodies (AMA) may be detected 30 years before the occurrence of the first symptoms. Other studies demonstrate similar results for diabetes, Crohn's disease or ulcerative colitis, where characteristic autoantibodies exist far before the first symptoms appear. Anti-dsDNA antibodies precede the development of lupus erythematosus by 5 to 10 years.

*How is it possible to differentiate truly false positive results?*

Only a consistent follow-up can answer this question. The test result has to be checked after a reasonable period of time. In the case of some diseases, e.g. the antiphospholipid syndrome (APS), the diagnostic criteria already require that tests should be repeated after a couple of weeks, because the antibodies identified may have arisen as a consequence of an infection.

What consequences do these results have for clinicians, patients and laboratories?

We are entering a phase in diagnostics in which the prognosis of the course of a disease will no longer be merely an educated guess. For the first time ever it will be possible to make a concrete prognosis of a disease, right down to a prediction of the individual characteristics of the disease, e.g. which organs will be affected.

All currently available results illustrate the significance and importance of extensive screenings at an early stage. At the same time, however, this development also requires the responsible and ethical use of the new diagnostic possibilities.

Who should be tested? Unquestionably insurance companies and employers would be very interested in the results of screening tests. But should the entire population really be tested or merely those known risk groups such as the relatives of patients with autoimmune diseases or those with a known genetic predisposition? Certainly it makes sense to test those patients who already have a particular autoimmune disease for other related diseases.

What is the benefit of an early prognosis for the affected patient?

Certainly the prediction that an apparently healthy person will eventually suffer from an autoimmune disease is not a positive piece of news. However, many diseases may be efficiently prophylactically treated when an early diagnosis is available. Patients who are diagnosed at an early stage as being at risk of an anti-

phospholipid syndrome may prevent thromboembolic events simply and with virtually no side-effects by taking anticoagulants such as aspirin. With other diseases the treating physician can offer valuable recommendations for future behavior. Even when the development of a disease cannot be prevented, at least an early diagnosis offers the possibility to take therapeutic action at the earliest possible point in time.

#### References

Clinical Significance of anti-dsDNA Antibody Isotypes: IgG/IgM ratio of anti-dsDNA antibodies as a prognostic marker for lupus nephritis. Förger F, Matthias T, Oppermann M, Becker H, Helmke K. *Lupus* 2004, 13: 36-44

Increased prevalence of celiac disease and need for routine screening among patients with osteoporosis. Stenson WF, Newberry R, Lorenz R, Baldus C, Civitelli R. *Archives of Internal Medicine*, 2005, 165: 393-399

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#### Book presentation

### “Autoimmune Diseases - the Enemy from Within”

Autoimmune diseases represent the third most common disease following cardiovascular diseases and cancer. They affect approximately 5% of all adults, and the tendency is increasing. Therefore, also the demand for information is high. Yehuda Shoenfeld and Gisele Zandman-Goddard wrote the book “Autoimmune Diseases - the Enemy from Within” to discuss these issues. It is now also available in the German translation (“Autoimmunerkrankungen - Der Feind in uns”). The book discusses the potential triggers of autoimmune diseases in detail and identifies the principal symptoms of autoimmunity based on typical clinical pictures and also deals with therapeutic options and prevention strategies. As autoimmune diseases are always caused by an alteration in the immune system, the introduction describes the function of the normal immune system in detail. In addition, the authors take a closer look at the exciting past of autoimmune diseases.

For more information, please visit: [www.aesku.com](http://www.aesku.com)

## From research to practical use

### The new generation of transglutaminase tests



The trigger of celiac disease is the incompatibility of a component of most cereals: the protein gliadin representing the alcohol-soluble fraction of gluten. In addition to other antibodies, also antibodies directed against gliadin are detected in the serum of patients suffering from celiac disease.

It has been demonstrated in *in vitro* experiments that peptides of gliadin formed by tryptic or chymotryptic cleavage can bind to the corresponding HLA molecules on the surface of T-cells of

celiac disease patients and can stimulate the cells in culture. These experiments have shown that a 33mer peptide resistant to further proteolytic cleavage is particularly potent. It is capable of stimulating three different patient-specific T-cell epitopes<sup>5</sup>. It is supposed that in celiac disease patients this peptide is the primary trigger of the immune response against gluten. When being modified by tTg, this peptide reacts more specific than all other known natural substrates.

In addition to the cereal compound gliadin, the human enzyme tissue transglutaminase (tTg) plays a principal role in the pathogenetic processes leading to celiac disease (see also Figure 1).

Tissue transglutaminase is an ubiquitous enzyme mainly occurring in the cytoplasm. It can be released by tissue damage and stress related to celiac disease. In 1997, it was identified as the major antigen of the IgA anti-endomysium antibodies.

Tissue transglutaminase can modify gliadin and its proteolytic degradation products by two different reactions: Firstly, glutamine residues in gliadin can be converted to glutamic acid by the action of tTg (deamidation). This reaction requires an acidic environment as occurring in the proximal intestine and to an increased extent due to the inflammatory process at celiac disease<sup>36</sup>. It converts gliadin and its fragments containing almost no negatively charged amino acids to a protein

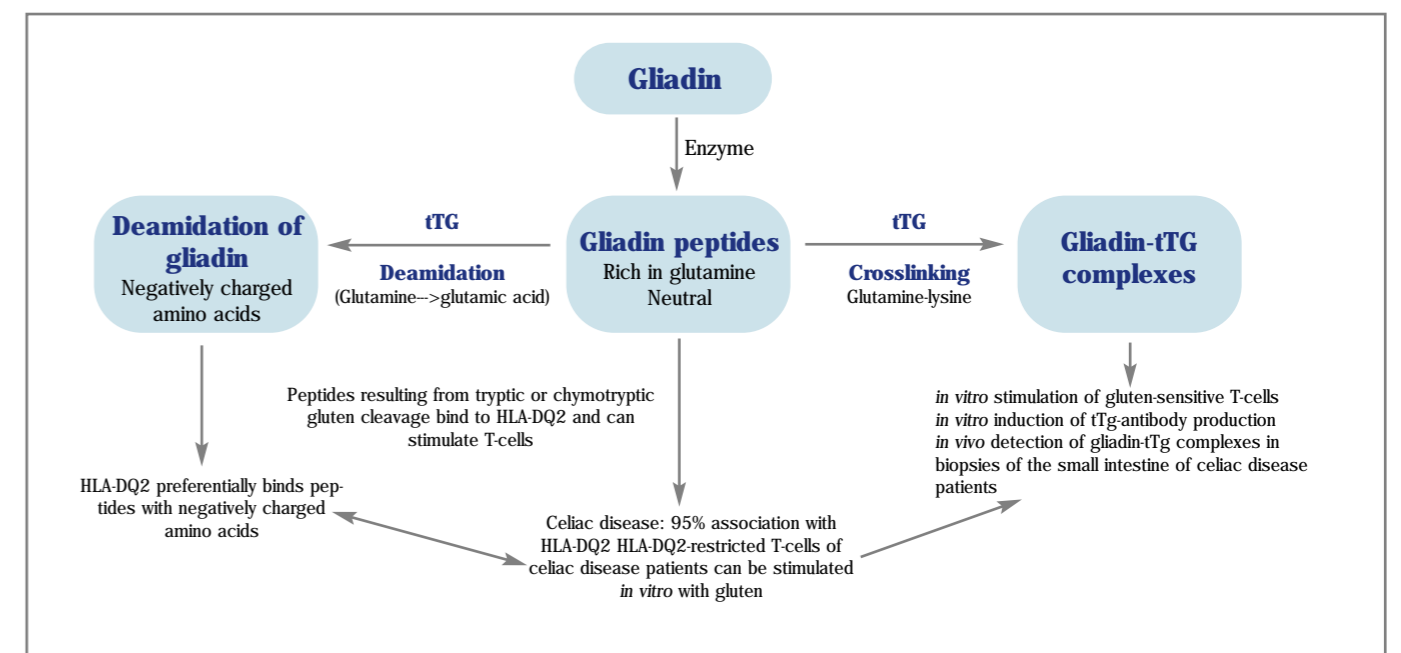


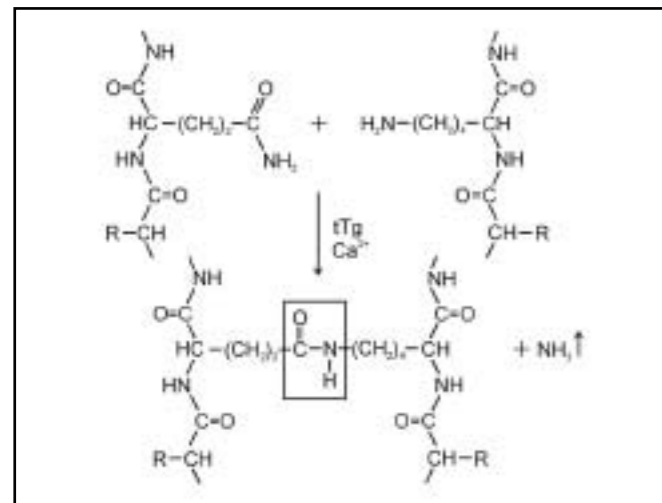
Figure 1

with many negatively charged amino acids. The negatively charged peptides are preferentially bound by HLA-DQ2 and can - as already shown for gluten - stimulate T-cells of celiac disease patients<sup>1,2</sup>. Celiac disease is actually associated with HLA-DQ2 for 95%<sup>6,7</sup>.

From the functional point of view, transglutaminase is a glutamine-glutamyl transferase; therefore, it can also crosslink glutamine and lysine residues (Figure 2). The resulting gliadin-transglutaminase complexes can stimulate gluten-sensitive T-cells *in vitro* and induce the production of anti-tTg antibodies<sup>4,5</sup>. Furthermore, these gliadin-tTg complexes can also be detected *in vivo* in small intestine biopsies of celiac disease patients<sup>8</sup>.

Latest results suggest the hypothesis that the small modified gliadin peptides are recognized by the T-cells, while the tTg complexes are recognized by the antibody-producing B-cells<sup>7</sup>.

ELISA tests for the detection of antibodies against tissue trans-



**Figure 2:** Crosslinking of peptides by tTg

glutaminase (tTg) become increasingly important in the diagnostics of celiac disease; due to their ease of use, reduced training requirements, objective analysis and optional automation they are ideally suited for safe diagnosis and economic screening of celiac disease.

Based on the results and experiments discussed above, the research department of AESKU.Diagnostics has developed a completely new type of tTg test: microtiter plates are coated with human recombinant tissue transglutaminase together with gliadin-specific peptides derived from the described 33mer peptide. The transglutaminase neo-epitopes formed by this crosslinking mimic the physiological tTg epitope.

Therefore, AESKULISA® tTg assays represent a novel generation of assays: due to the detection of tTg neo-epitopes, sensitivity was significantly higher than in conventional tTg assays and endomysium immunofluorescence tests. They are also highly specific and free from cross-reactions with gliadin.

The AESKULISA® tTg IgA test of the new generation achieves a better sensitivity than conventional tTg tests, together with 100% specificity.

Although the detection of IgA tTg antibodies is surely the most important issue in the diagnostics of celiac disease, the AESKULISA® tTg IgG is essential when it comes to the detection of tTg antibodies in patients with IgA deficiency which is more frequent in celiac disease than in the normal population.

The design of the tTg tests of the new generation involved testing of celiac disease patients (with diagnoses confirmed by biopsy) and controls for the presence of IgA and IgG anti-endomysium (IFA) and anti-tTg antibodies. A significantly higher sensitivity for IgG was found with the AESKULISA® tTg ELISA compared to endomysium IFA and the ELISA test of a competitor. These data stress why it is important to assay IgG tTg in all patients and not only in IgA-deficient individuals.

The novel AESKULISA® CeliCheck assay allowing the combined quantitative determination of IgA and IgG tTg represents an ideal screening test system for risk groups and the monitoring of celiac disease patients.

#### References:

1. Investigation of the putative immunodominant T cell epitopes in Coeliac disease. Ellis HJ et al. Gut 2003; 52:212-217
2. Gliadin peptide specific intestinal T cells in coeliac disease. Lundin KEA et al. Gut 2003; 52:162
3. Deamidation and cross-linking of gliadin peptides by transglutaminases and the relation to celiac disease. Skovbjerg H et al. Biochim. Biophys. Act. 2004; 220-230
4. Coeliac disease – a meeting point for genetics, immunology, and protein chemistry. Mowat AM et al. Lancet 2003; 361: 1290-1292
5. Structural basis for gluten intolerance in celiac sprue. Shan L et al. Science 2002; 297: 2275-2279
6. Pathomechanisms in celiac disease. Dietrich et al. Int. Arch. Allergy Immunol 2003; 132: 98-108
7. The pathogenesis of coeliac disease. Dewar et al. Int.J.Biochem.Cell Biol. 2004; 36: 17-24
8. Gliadin and tissue transglutaminase complexes in normal and coeliac duodenal mucosa. Ciccocioppo et al. Clin Exp Immunol 2003; 134: 516-524

## Osteoporosis and celiac disease



“Our results suggest that about 3-4% of all osteoporosis cases are due to an existing celiac disease limiting the absorption of calcium und vitamin D”, says Stenson, who works as a physician at the Barnes-Jewish Hospital of the Washington University.

Within one year, the gluten-free diet did not only improve the gastrointestinal symptoms, but also the bone density of the osteoporosis patients suffering from celiac disease. The positive effect on bone density was even significantly stronger than that of a comparable standard therapy for osteoporosis treatment.

Stenson, who also works as a professor at the Washington University, and his co-workers draw a clear conclusion from their data: the incidence of celiac disease in osteoporosis patients definitely justifies the screening of all osteoporosis patients for this frequent autoimmune disease.

It is a financial matter whether also extended risk groups should be examined. Provided that bone density is highest at the age of 18, it may appear wise to test all individuals with a high risk of osteoporosis – i.e. young Caucasian women – for celiac disease, writes Alan L. Buchman, M.D., M.P.H., of the Feinberg School of Medicine of the Northwestern University in Chicago in his comment to Stenson’s study.

However, the cost-benefit relationship of such an extensive study considering actually detected cases and prevented consequences of the disease may not be very convincing. Buchman calculates that the prevention of a single fracture in a celiac disease patient with osteoporosis would cost about 43,000 dollars.

Therefore, highly efficient serological screening techniques are required to allow the most sensitive, reliable and also economic (e.g. via automation) diagnosis of celiac disease.

#### References:

- Stenson WF, Newberry R, Lorenz R, Baldus C, Civitelli R. Increased prevalence of celiac disease and need for routine screening among patients with osteoporosis. Archives of Internal Medicine; vol. 165, pp. 393-399, Feb. 28, 2005.

Osteoporosis is a disease of the skeleton that due to reduced bone mass and destruction of the microarchitecture of the bone tissue increases the risk of fractures. It may also be caused by longterm deficiency in important minerals and vitamins together with untreated celiac disease.

But does the association between osteoporosis and celiac disease justify the examination of all osteoporosis patients for celiac disease? This question was investigated in a current study performed at the Washington University School of Medicine in St. Louis.

The researcher team of William F. Stenson M.D. proves that osteoporosis patients suffer significantly more often from celiac disease than other individuals, and he strongly recommends to screen osteoporosis patients for celiac disease with suitable serological tests, because a diet for the treatment of celiac disease may also considerably improve the bone density of the affected individuals.

The total study population included more than 800 individuals, i.e. 266 with and 547 without osteoporosis. They were first screened using a serological test for celiac disease. Positive diagnoses for celiac disease were confirmed by endoscopic biopsies. All affected individuals where biopsy confirmed the diagnosis of celiac disease consumed a gluten-free diet. The development of their bone density was monitored.

12 of the 266 subjects with and 6 of the 547 subjects without osteoporosis were detected to be positive for celiac disease in the serological screening. The diagnosis was confirmed by biopsy in 9 of the osteoporosis patients but in only one healthy person.

The relation becomes even clearer when relative values are considered: 3.4% of the osteoporosis patients but only 0.2% of the healthy subjects demonstrated positive results for celiac disease in serological tests.

## NIH publishes recommendations on the diagnosis of celiac disease

Two parallel developments were the reasons why the US National Institute of Health (NIH) summoned the "Consensus Development Conference" on celiac disease in the summer of 2004:

- On the one hand, an increasing number of particularly European but also US studies demonstrated that the prevalence of celiac disease is generally higher than previously assumed. Projected figures suggest that in the US up to 3 million people, i.e. 1% of the total population, might be affected
- The identification of new autoantibodies related to celiac disease allowed the development of novel serological tests that thanks to their sensitivity also identify affected individuals not demonstrating the typical symptoms of celiac disease.

It was the objective of the NIH Consensus Development Conference to strengthen the awareness for the importance of celiac disease and also to design a guideline for action to improve diagnosis and management of this autoimmune disease.

For two and a half days, the discussions of experts from science, hospital and public health covered the following issues:

- How can celiac disease be reliably diagnosed?
- What is the actual prevalence of celiac disease?
- Which clinical manifestations and late consequences does the disease have?
- Who should be tested for celiac disease?
- What should the ideal management of celiac disease look like?
- Which recommendations can be made for future research projects?

The results of the conference are recorded in the NIH Consensus Development Conference Statement; they give clear advice for the diagnostics of celiac disease.

The statement proposes to perform a serological test first, that - if positive - should be confirmed by a biopsy of the small intestine. According to the opinion of the NIH Consensus Development Conference, IgA tTg ELISA tests and IgA EMA immunofluorescence tests are the best test systems due to their high sensitivity and specificity.

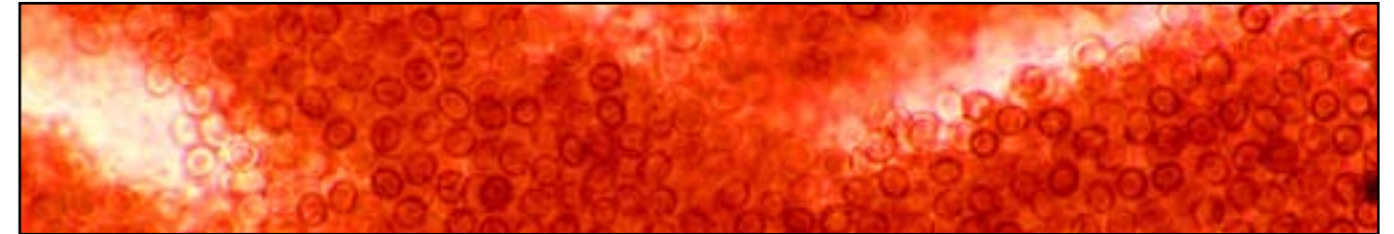
Due to the high portion of atypical or silent courses of the disease, a variety of very different patient groups should be examined either demonstrating potential atypical symptoms or belonging to known risk groups. The statement only rejects the screening of the total population, because insufficient data are available to support such a measure.

The conference proposed that future research projects should investigate for example which factors trigger celiac disease at an existing genetic predisposition, which relationships exist between celiac disease and other autoimmune or neurological diseases or which arguments might support or argue against the screening of the total population. The participants of the conference also wish the development of a serological test that in a non-invasive way can quantify the current activity of the disease.

*The complete "NIH CONSENSUS DEVELOPMENT CONFERENCE STATEMENT" can be downloaded as a PDF file from the AESKU homepage at [www.aesku.com/diagnostics/english/support/](http://www.aesku.com/diagnostics/english/support/)*

## Pernicious anemia

### New tests for the detection of antibodies against parietal cells and intrinsic factor



Pernicious anemia, also called Biermer anemia, is the final stage of autoimmune gastritis (type A gastritis) characterized by the destruction of the gastric mucosa. The typical clinical picture shows the atrophy of the mucosa, a selective loss of parietal cells and chief cells and a lymphocyte infiltration of the submucosa.

10-15% of all patients with autoimmune gastritis develop a pernicious anemia in the course of the disease.

Pernicious anemia is the most common cause of vitamin B12 deficiency in Western populations. It is caused by a deficiency in intrinsic factor, a glycoprotein required for the absorption of vitamin B12 from the gastrointestinal tract. Intrinsic factor is synthesized by the parietal cells of the gastric mucosa. Vitamin B12 plays an important role in hematopoiesis, a deficiency results in anemia.

Although this disease can appear in all ethnic groups, it is most frequent in Scandinavian and Northern European populations. Women are slightly more frequently affected than men.

Pernicious anemia does not appear before the 30<sup>th</sup> year of life, the mean age at diagnosis is 60.

Just recently it could be actually demonstrated that up to 2% of the people over 60 suffer from pernicious anemia.

### **AESKULISA®: maximum sensitivity and specificity at minimum effort**

The target of autoantibodies at pernicious anemia are the parietal cells, in particular the H<sup>+</sup>-transporting enzyme H<sup>+</sup>/K<sup>+</sup> ATPase being responsible for the acidification of the gastric lumen, and the glycoprotein intrinsic factor synthesized by parietal cells. Anti-parietal cell antibodies can be detected by indirect immunofluorescence or ELISA tests.

The handling of ELISA tests offers a number of benefits: convenient use, objective analysis and opportunities for automation provide more user-friendliness and cost-effectiveness.

AESKU.DIAGNOSTICS has therefore developed two ELISA tests offering maximum sensitivity and specificity in the diagnosis of pernicious anemia:

- **7511 AESKULISA® Parietal Cell**  
ELISA assay for the quantitative and qualitative detection of IgG autoantibodies against parietal cells in human serum.  
Coated antigen: Native H<sup>+</sup>/K<sup>+</sup> ATPase from parietal cells.
- **7512 AESKULISA® Intrinsic Factor**  
ELISA assay for the quantitative and qualitative detection of IgG autoantibodies against intrinsic factor in human serum.  
Coated antigen: Human recombinant intrinsic factor

Antibodies against parietal cells demonstrate a sensitivity of 80-90%, but are also detected in up to 5% of the normal population.

Antibodies against intrinsic factor demonstrate a sensitivity of 50-70% and a specificity of 100% in a population of healthy blood donors.

Therefore, the assays **AESKULISA® Parietal Cell** and **Intrinsic Factor** are valuable tools to differentiate between pernicious anemia and other causes of vitamin B12 deficiency.

Both tests share of course all benefits that make the **AESKULISA®** product family the ideal partner for automation: identical protocols, ready-to-use reagents and short incubation times.

**DISCOVER NEW  
POSSIBILITIES  
MEDICA 2005**

**AESKU.DIAGNOSTICS Hall 3 Booth H34**

## Face to face in Wendelsheim

### 1<sup>st</sup> International Meeting of AESKU Distributors



The participants of the 1. International AESKU Distributors Meeting in Wendelsheim had the opportunity to meet each other face to face and to exchange experiences with the team at the Wendelsheim facility.

The primary aim of AESKU.DIAGNOSTICS is the networking of research, development and everyday practice in laboratory diagnostics. By using AESKU.DIAGNOSTICS products, users in the lab have the opportunity to benefit from current research results in autoimmune diagnostics. However, the flow of information is also needed in the opposite direction to keep AESKU.DIAGNOSTICS up to date on the users' requirements that will find their way into product development.

AESKU.DIAGNOSTICS's field product specialists and an extensive network of specialized distributors are the indispensable interface for information exchange between the Wendelsheim facility and users all over the world.

Although current means of communication make the world appear pronouncedly smaller – international exchange of information via telephone and e-mail makes life easier – personal contact is still essential. Therefore, the 1st International Meeting of AESKU Distributors took place in Wendelsheim on 11 and 12 April 2005; 22 participants from 14 countries visited the Wendelsheim facility, most of them from European countries such as Greece, Italy, Great Britain and France but some of them even crossed the Atlantic Ocean and came from the US or Venezuela.

A comprehensive product training was performed by sales and research employees of AESKU, which provided the participants with essential current knowledge.

New members of the distributor circle profited mainly from the comprehensive introduction into the unique AESKU product range and its company and product philosophy. But also

distributors working with AESKU for many years were provided with new information from AESKU including new product developments.

At the same time, the meeting was intended for mutual acquaintance, exchange of experience and intensive discussion about current market trends and customer requirements.

"Tests" were not only an issue at the meeting, but also during the evening, although the type of "samples" had changed. Obviously, a winetasting with various wines from the region Rheinhessen could weaken a number of prejudices against German wine. Even the guests from France were positively surprised about the quality of the served wines.

While the AESKU distributors are dedicated product and application specialists at daytime, they develop completely other talents at night. The meeting was finished with a night boat trip on the Rhine. The highlight of the evening was a karaoke show revealing so far undetected talents. It may even be that some of the participants will change to show business.

AESKU would like to thank again all participants in this meeting for coming and their committed cooperation. The next distributors meeting is scheduled for April 2006.

## AESKU.Seven-Up: when every minute counts in autoimmune diagnostics

Every minute counts when it comes to the diagnosis of life-threatening autoimmune diseases like the Goodpasture syndrome that may lead to massive pulmonary bleedings and a rapidly progressing glomerulonephritis. Only the quickest diagnosis and therapy can improve the patient's prognosis. This is why the corresponding laboratory results have to be available immediately, even outside the normal routine or during overnight emergency service.

A novel test system now offers decisive time advantages for patients, clinicians and laboratory staff. Up to now, even fully automated test methods for autoimmune diagnostics needed at least one hour and up to two and a half hours to provide the result; now the vital data are available after only 21 minutes.



The AESKU.Seven-Up, named after the time-saving 3x7-minute protocol, was developed by AESKU.DIAGNOSTICS, the manufacturer of the largest product portfolio of ELISA tests for autoimmune diagnostics, together with DIESSE Diagnostica Senese SpA, the Italian manufacturer of the laboratory analyzer Chorus, which also serves as the platform.

AESKU.Seven-Up requires only 3x7 minutes for the entire test sequence. Despite its speed, the system provides quantitative and not only qualitative data.

Many parameters from the large AESKULISA® product portfolio from the fields of rheumatology, thyroid diseases, vasculitis, thrombosis, hepatology and gastroenterology were already

adapted to the new platform. Absolute priority was given to the time-critical parameters anti-GBM antibodies (anti-basal membrane antibodies) for the diagnosis of rapidly progressing glomerulonephritis and Goodpasture syndrome and anti-PR3 antibodies (proteinase 3) for the reliable diagnosis of Wegener's Granulomatosis where rapid diagnosis may be life-saving.

From now, AESKU.Seven-Up also allows the rapid, fully automated analysis of anti-phospholipid antibodies (against cardiolipin and  $\beta_2$ -glycoprotein I) – a vital requirement for quick diagnosis when APS is suspected, in young stroke patients and in case of exceptional thrombotic events.

### Rapid, reliable and flexible

Test strips can be individually inserted, therefore allowing the parallel analysis of different parameters. Up to 30 different tests can be individually combined. Thanks to the low costs of the test strips, it even makes economic sense to test the serum of individual patients immediately.

All benefits of automation have been utilized: The user merely has to add the sample and start the program; the complete test will run fully automated.

It is even unnecessary to run a standard curve, because the barcode of a test strip does not only define its lot number and expiry date, but it includes also a 5-point standard curve.

Daily laboratory routine requires easily comprehensible software and convenient handling - in particular under time pressure. AESKU.Seven-Up offers a clearly designed user interface.

Special safety measures guarantee reproducible results: with AESKU.Seven-Up all tests are performed independently from the laboratory temperature at 38.5 °C (101.3 °F). This makes the results much more safe and precise, which is particularly important when readings are close to the cut-off value.



In addition to the analysis of acute parameters, AESKU.Seven-Up is also an economic alternative for the laboratory for the automated immediate routine testing of autoimmune diseases. Also smaller labs with lower sample throughput can benefit from the safety and economy of automation in autoimmune diagnostics.

For more information visit us at:  
www.aesku.com

Benefits
Serum samples can be tested immediately
Rapid analysis of up to 30 parameters in parallel
7+7+7 minutes incubation time
Quantitative and qualitative analysis
Fully-automated system
User-friendly software
Transfer of the results to EXCEL
Constant incubation temperature

Unique specifications ensure benefits to patients, physicians and laboratory staff.

Parameters
The first available parameters:
GBM
MPO
PR3
Cardiolipin IgG
Cardiolipin IgM
$\beta_2$ -GPI
dsDNA
Rheumatoid factor IgM
a-TG
a-TPO

Numerous products from the broad AESKULISA® product range have already been adapted for the new test system.

## Start young ...



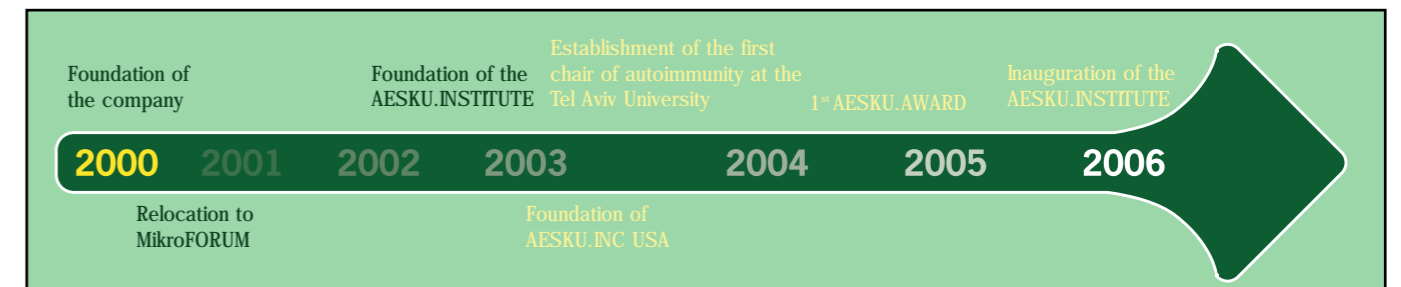
Not only the industrial site Germany but also AESKU as a research-oriented enterprise strongly depends on the skills and enthusiasm of its employees. Therefore, AESKU commits itself not only to interdisciplinary autoimmunity research but also to get young people enthusiastic about research and development.

The German initiative "Wissenschaft in die Schulen! (WIS)" (Science into Schools!) motivated AESKU to offer the opportunity to relate biology more to current research in the special subject L12 of the Stefan-George High School in Bingen.

WIS is an initiative of the German popular scientific journal "Spektrum der Wissenschaft" under the auspices of the Gesellschaft für Biochemie und Molekularbiologie (Society of Biochemistry and Molecular Biology) e.V. and the Max-Planck Institute of Astronomy. WIS does not only provide current expert knowledge to the pupils but also intends to forge links between companies and schools.

However, AESKU starts forging links even between companies and much younger talents. In 2004, AESKU provided the kindergarten in Wendelsheim with a complete computer equipment; now the older kindergarten children could get an idea how a research-oriented enterprise works during a visit at AESKU. It was an exciting day with surprising insights for both parties – or did you know before that a centrifuge is as fast as the motor of a Ferrari participating in Formula 1?

## Looking back at 5 years



1 January 2000 was not only the day when a new millennium started but also the foundation day of AESKU.DIAGNOSTICS under its initial name Aesku.lab Diagnostika. Only three months later, on 1 April 2000, the young company relocated into the Biotechnology Center Mikroforum in Wendelsheim, just the right place for its various activities in research, product development and production.

### Looking back at 5 years of AESKU history

Based on intensive research and development, AESKU.DIAGNOSTICS succeeded in establishing the worldwide largest product range for the diagnostics of autoimmune diseases. Today, AESKU.DIAGNOSTICS is represented in 42 countries worldwide by a network of in total 49 well-selected, highly qualified distributors.

In 2003, a sales organization was founded in the US, i.e. AESKU.INC located in Miami, Florida.

More than 100 large laboratories all over the world trust in AESKU products. Exclusive sales partnerships were closed.

### That's what counts!

But there is more to a company than only sales figure, data and facts. In the last five years, AESKU.DIAGNOSTICS created many new jobs in Wendelsheim.

Innovative tests and technology platforms like AESKU.Seven-Up create innovative diagnostic opportunities – for the benefit of patients and physicians.

Alternative techniques that are safer, simpler, faster, and first of all more efficient than the techniques used so far, support laboratory users.

Exciting research cooperations support new insights into diagnosis and therapy of autoimmune diseases. The AESKU.INSTITUTE, founded in 2003, is just the right place for this.

Supported by AESKU, the first chair of autoimmunity worldwide was established at the Tel Aviv University in 2003.

Since 2004, the AESKU.AWARD donated by AESKU awards outstanding achievements in autoimmunity, thus strengthening the awareness of the importance of this field of research.

We hope that AESKU has contributed new insights to research, diagnosis and prognosis of autoimmune diseases within the last years. We thank our customers, our partners in research and business and our friends who accompanied us on this way.

Five years AESKU – a reason for a short look back without leaning back. New exciting and challenging research and development projects are ahead ...

Your AESKU-Team

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