The Significance of IgG Antibodies against Tissue Transglutaminase in Coeliac Disease

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**Abstract**


Coeliac disease (CD) is a multifactorial disease of the small intestine. In genetically predisposed individuals the, ingestion of cereals leads to a remodulation of the mucosal architecture, and the production of autoantibodies against tissue transglutaminase (tTG). The treatment is a lifelong gluten-free diet.

The diagnostic procedure relies on the examination of a small-bowel biopsy that displays villous atrophy. A spectrum of clinical manifestations is associated with CD, ranging from overt enteropathy to atypical and silent symptoms. Approximately 1% of the general population has CD, and the majority is undiagnosed. Although most patients with active CD can be detected by the assessment of elevated IgA-tTG, some patients lack these antibodies. Moreover, individuals with IgA-deficiency cannot be identified by means of IgA serology.

The aim of this thesis was to investigate the clinical utility of IgG-tTG for the detection and follow-up of subjects with active CD. The included studies showed that IgG-tTG was highly prevalent in IgA-deficient and IgA-competent patients with CD, whereas non-CD patients rarely had these antibodies. During a gluten-free diet, IgG-tTG decreased, demonstrating that IgG-tTG can be used to follow the patient’s adherence such a diet. Furthermore, 10% of healthy IgA deficient blood donors had elevated IgG-tTG, indicating that they had silent CD.

In IgA-competent subjects, high IgG-tTG levels correlated with a severe mode of CD and profound mucosal deterioration, suggesting that IgG-tTG might be involved in the disease progression. Moreover, we found that although a considerable percentage of IgA-competent patients lack IgG-tTG, the presence of these antibodies in conjunction with high levels of IgA-tTG was highly predictive of a severe small-intestine villous atrophy. It was also demonstrated that IgG-tTG normalisation coincided with clinical remission in IgA-competent CD patients on a gluten-free diet.

**Keywords:** Coeliac disease, Tissue transglutaminase, Endomysium, IgG antibodies

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Facts are stubborn things, but statistics are more pliant.

*Mark Twain*
This thesis is based on the following papers, which will be referred to in the text by their roman numerals:


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Abbreviations

AGA  Anti-gliadin antibodies
AUC  Area under the curve
CD   Coeliac disease
CTLA4 Cytotoxic T-lymphocyte-associated protein 4
DH   Dermatitis herpetiformis
ELISA Enzyme linked immunosorbent assay
EmA  Endomysium antibodies
ESPGAN European Society for Paediatric Gastroenterology and Nutrition
ESPGHAN European Society for Paediatric Gastroenterology, Hepatology and Nutrition
Fc   Fragment crystalline
FcγRIIa Fc gamma receptor IIa
FITC Fluorescein isothiocyanate
GTP  Guanosine tri-phosphate
HLA  Human leucocyte antigen
Ig  Immunoglobulin
IL-15 Interleukin 15
MIC  Major histocompatibility class I related polypeptide chain
MODY Maturity onset diabetes of the young
MYO9B Myosin IX B
NKG2D Natural killer cell group 2D receptor
RBA  Radiobinding assay
ROC Receiver operator curve
tTG  Tissue transglutaminase
Introduction

Coeliac disease (CD) is a multifactorial disease of the small intestine. In genetically susceptible individuals, ingestion of cereals causes an aberrant cellular immune response, disruption of the mucosal integrity and remodulation of the mucosal architecture. Active CD is also characterised by the production IgA autoantibodies against extracellular matrix proteins. A spectrum of clinical manifestations is associated with CD, ranging from the classical childhood malabsorptive form to atypical and silent symptoms. The disease may also be latent for several years prior to the onset of morbidity and mucosal changes (1, 2).

Several screening studies indicate that the prevalence of CD is close to 1% of the general population (3-5) and recent reports imply that the prevalence is increasing (6). The diagnosis is made by means of histological examination of biopsies from the upper small intestine in conjunction with assessment of autoantibodies. The current treatment is a lifelong gluten-free diet, which in most cases leads to recovery of the mucosal structure and remission of symptoms (7).

Untreated patients are at risk of developing refractory CD, a form that is unresponsive to treatment and associated with a higher risk of intestinal malignancy (8). Other complications such as infertility (9), osteoporosis, osteopenia (10), and autoimmunity (11) have been shown to occur in patients with untreated CD.
Coeliac disease

Symptoms

The typical presentation of CD is related to gastrointestinal symptoms in the presence or absence of malabsorption. Classic childhood CD is characterized by diarrhoea with consequences of malabsorption such as failure to thrive, anorexia, abdominal distension, muscle wasting, poor weight gain, or weight loss. The symptoms appear in children between 6 and 24 months of age, sometimes within a few weeks after gluten has been introduced into the diet. Atypical symptoms of childhood CD are often associated with a delayed onset of morbidity and the symptoms may be vague comprising recurrent abdominal pain, vomiting, constipation, or extraintestinal manifestations such as anaemia or puberty delay (12). Adult patients with CD manifest a variety of symptoms including diarrhoea, nausea, abdominal pain, bloating, weight loss, constipation, folate deficiency, migraine, and unexplained fatigue (2).

During the few past decades, it has become evident that atypical and extraintestinal symptoms are the predominating manifestations of CD in both children and adults (7). Iron deficiency anaemia is the most common primary presentation of CD in adults (13) and increased serum levels of transaminases, indicating liver involvement, may occur despite the absence of other symptoms (14). Dental enamel defects on permanent teeth (15), reduced bone mineral density (16), and an increased rate of miscarriage has also been reported in untreated CD patients (17).

Dermatitis herpetiformis (DH), a blistering skin disease, is an extraintestinal form of CD, which is characterised by granular IgA depositions in the papillary dermis of the unaffected skin (18). DH patients often have silent symptoms and milder enteropathy than CD patients.
Patients with clinically silent disease lack symptoms despite the presence of mucosal damage (19, 20). In contrast, potential or latent CD patients exhibit normal mucosa of the small intestine, whereas autoantibodies associated with active CD are elevated (21). Some of these patients may have enteral complaints whereas others are asymptomatic. Another form of the disease is refractory CD, which is unresponsive to gluten exclusion from the diet (22). In these patients the intestinal mucosa may be atrophic, and they often have severe malabsorption. Refractory CD is a severe condition associated with an increased risk of developing small-intestine lymphoma (23, 24).

Associated diseases

A number of autoimmune and genetic diseases have been demonstrated to coexist with CD, such as type I diabetes (25), Sjögren’s syndrome (26), and selective IgA deficiency (27). An increased prevalence of CD in children with Down’s (28), Turner’s (29), and William’s syndromes (30) has also been reported. Selective IgA deficiency, the most common immune deficiency in humans, is associated with an increased risk of developing CD. The general clinical presentation of CD does not differ between patients with and without IgA-deficiency (31). Among IgA-deficient patients the atypical, vague, and silent symptoms dominate (32).

Mucosal features

As a reaction to gluten ingestion, the mucosa of the small intestine deteriorates gradually in CD patients. Several scoring systems for evaluation of the histopathological changes have been suggested. The Marsh classification (33) include four distinct stages of histopathological findings. In the infiltrative stage the mucosal structure is maintained while there is an increased density of intraepithelial lymphocytes (I). In the next hyperplastic stage, the villi are unaltered whereas the crypts are hyperplastic and there is an increased density of intraepithelial lymphocytes (II). During the following destructive stages gradual flattening of the villi is observed, showing partial villous atrophy (IIIA), subtotal atrophy (IIIB) or total atrophy (IIIC) (34), (figure 1). The final hypoplastic stage (IV) is characterised by the absence of villi and hypoplastic crypts.
Figure 1. Histology of the duodenum. Left, normal villi. Right, crypt hyperplasia and villous atrophy of a patient with coeliac disease.

Diagnosis and Treatment

Mandatory for the diagnosis of CD is histological examination of one to three biopsy specimens taken from the upper small intestine that demonstrate mucosal villous atrophy. The original diagnostic criteria were defined in 1969 by the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) (35). The criteria included a repeated biopsy procedure during the course of disease. The first biopsy was taken from untreated patients, the second follow-up biopsy after a period of gluten-free diet, and the final biopsy after the reintroduction of gluten-containing food.

The need for a biopsy after a challenge period was subsequently excluded from the revised criteria, except for children younger than 2 years of age and in ambiguous cases (36). Current diagnostic routines include serology in conjunction with the histological examination of at least one biopsy that demonstrates mucosal villous atrophy while the patient consumes gluten-containing food. When declination of CD-related autoantibodies and remission in symptoms are observed on a gluten-free diet, the second biopsy may not be needed (37).

The only treatment of CD available today is gluten avoidance, which leads to mucosal restitution and relief of symptoms. Mucosal healing and clinical improvement are not always achieved upon treatment (38), which in the majority of cases is due to dietary lapses (39). However, persistent villous atrophy has been observed in symptom-free patients who are compliant with a strict gluten-free diet (40), and it is possible that these individuals are at risk of developing refractory CD.
Epidemiology

CD was previously believed to affect infants and young children only but during the last few decades it has become evident that the disease occurs in individuals of all ages. The symptoms may appear at any time in life and the incidence of CD seems to vary among different countries (41-46). However, screening studies indicate a global prevalence of CD of 0.5-1% (12, 46-48) and symptomatic subjects constitute a minority of cases. It has been demonstrated that between 4 and 7 subjects are undetected for every diagnosed patient (3, 47). One investigation indicated that as many as 90% of CD children may be undiagnosed (49), and a recent study demonstrated that the prevalence of CD has doubled in the two past decades (6).

Genetic factors

The genetic predisposition associated with CD is mainly determined by the major histocompatibility complex class II antigens – referred to as the human leukocyte antigens, (HLA) in humans, encoded by genes localized on chromosome 6q21.3. The majority of CD patients carry the HLA-DQ2 haplotype (95%) and the remainders usually have the DQ8 haplotype. Although this genetic disposition (CELIAC 1) is essential for the development of CD, additional genes seem to contribute to the onset of CD (50).

Genome scans have mapped genetic risk factors for CD to chromosome 5q31-33 (CELIAC 2), chromosome 2q23-32 (CELIAC 3), chromosome 19p13.1 (CELIAC 4), chromosome 15q11-13 (CELIAC 5), and chromosome 4q27 (CELIAC 6). Cytotoxic T-lymphocyte-associated protein 4 (CTLA4), located within CELIAC 3, seem to be associated with an increased risk for CD (51). The gene for myosin 9B (MYO9B) within CELIAC 4 has been demonstrated to be associated with CD in some populations (52, 53). A functional variant of the FcγRIIa gene was recently reported to be associated with CD (54).
Gluten

The external trigger of CD is ingested wheat gluten or related proteins of other cereals. Gluten is the adhesive mass that remains when water-soluble starch and albumins have been washed away from wheat flour. This portion contains proteins that are rich in proline and glutamine residues, the prolamins. Gluten can be divided into alcohol-soluble (gliadin) and alcohol-insoluble (glutenin) fractions.

The gliadin fraction consists of several proteins that have been classified according to their electrophoretic mobility as α-, β-, γ-, and ω-gliadins. The glutenin fraction is divided into high and low molecular weight glutenins (55, 56). Gliadins and glutenins, as well as the related prolamin from rye (secalin) and barley (hordein), elicit symptoms in CD patients, whereas the equivalents from oats (avenin) in general are well tolerated (57, 58).

Due to their high content of proline, gliadins are resistant to proteolytic degradation by the digestive enzymes (59). Several fragments of gliadin and glutenin are able to bind to HLA-DQ2 (60) and/or DQ8 (61) and stimulate CD4+ T cells from CD patients, in particular after deamidation (62). Other sequences exert toxic effects on the mucosal epithelium leading to an increased permeability of the mucosa (56, 63).

Tissue transglutaminase

Tissue transglutaminase (tTG, EC 2.3.2.13) is an enzyme present in most organs. In the gastrointestinal tract, the enzyme is found intracellularly in enterocytes and myofibroblasts. Extracellularly, tTG is located in the membranes of enterocytes and in the subepithelial connective tissue of the small intestine (64). It has been demonstrated that tTG activity is increased in the mucosal lesions of patients with active CD (65).

tTG can form cross-links between glutamine containing (acceptor) and lysine containing (donor) substrates by a transamidation reaction, which is strictly Ca2+-dependent. In the absence of donor substrates, or at low pH, transglutaminase-bound acceptor molecules may become deamidated. In addition to the transamidation activity, tTG may function as an intracellular GTPase. The two enzymatic functions are mutually exclusive (66, 67).

tTG is organised into four domains. The enzymatic sites for transamidation and GTPase activities are located within the second core domain, which is surrounded by an N-terminal β-sandwich domain and two C-terminal β-barrel domains. The two first domains are linked to the others by a loop, which functions as a hinge region (68).
Intracellularly, in the GTP-bound form, tTG adapts a closed conformation (69) that keeps the transamidation site inaccessible. Extracellularly, in the presence of Ca\(^{2+}\), tTG adapts an open conformation, which discloses the active site (70), (Figure 2).

Figure 2. Tissue transglutaminase (tTG) in the GTP-bound conformation (left) and bound to a gliadin analogue (G within the circle) in the presence of Ca\(^{2+}\) (right). D1-D4 denotes the four domains of tTG. Adapted from (70).

tTG has a strong affinity for fibronectin (71), and it has been demonstrated that 2:1 complexes between tTG and fibronectin are formed upon cellular release of tTG (72-74). The fibronectin-binding region has been localised to residues 1-7 and 88-106 of the N-terminal first domain of tTG (75, 76).

The biological function of tTG has not been clarified. However, it has been suggested that tTG is involved in apoptosis, wound healing, and cell adhesion (66, 67, 77, 78). Stabilisation of newly formed extracellular matrix proteins is dependent on the transamidation activity of tTG (79). Activation and deposition of latent transforming growth factor beta in the extracellular matrix is also mediated by the enzymatic activity of tTG (80, 81). Cell surface tTG that is involved in fibronectin-integrin dependent interactions between cells and the extracellular matrix does not require the enzymatic activity (82).

Although tTG knock out mice are born healthy, lack of tTG in these animals cause an abnormal immune response due to the impaired capacity of macrophages to engulf apoptotic cells (83-85). In humans a mutation within or close to the active site of tTG is associated with either maturity onset diabetes of the young (MODY) or early onset type 2 diabetes (86, 87).

Several proteins may serve as donor substrates for tTG (88). Gliadins that contain a high percentage of glutamine are favoured acceptor substrates and tTG molecules may participate in the transamidation reaction either as acceptor or donor substrates (89-92).
It has been revealed that tTG-gliadin complexes are formed *in vitro* as well as in the small intestine of CD patients (93, 94). Moreover, immunostimulatory peptides of gliadin can be deamidated by tTG, which increases their binding to HLA-DQ2 and DQ8 molecules (95, 96).

**Serology**

Patients with active CD frequently have increased serum levels of IgA and IgG antibodies directed against a variety of food components (97, 98) and autoantigens such as actin, calreticulin and collagen (99-102). Detection of elevated serum antibodies is frequently used in the clinical routine as an aid in identifying patients with CD and in monitoring compliance with a gluten-free diet.

**Anti-gliadin antibodies**

IgA and IgG antibodies directed against gliadin (AGA) are often elevated in untreated CD patients. However, AGA is not disease specific, and some patients with active CD lack these antibodies. In contrast, patients with diseases other than CD and healthy individuals occasionally have elevated levels of IgA-AGA and IgG-AGA. The sensitivity of IgA-AGA has been reported to vary from 52% to 100% and the specificity ranges between 71% and 100%. The sensitivity of IgG-AGA is similar to that of IgA-AGA, whereas the specificity of IgG-AGA can be as low as 50% (103). These antibodies disappear rapidly during a gluten-free diet (104).

Native gliadin preparations comprise a mixture of proteins and contaminating components. It has been demonstrated that detection of AGA with purified α-, β-, γ-, or ω-gliadin is more disease specific for CD than native gliadin (105, 106). Additionally, deamidated nonapeptides of gliadin are more accurate for detection of antibodies from untreated CD patients (107, 108). Recently developed assays for assessment of IgA and IgG antibodies directed against deamidated gliadin peptides have been reported to have a sensitivity and specificity higher than 90% (109, 110). In analogy with antibodies directed against native gliadin, the antibodies against deamidated gliadin disappear rapidly after the introduction of gluten-free diet (111, 112).
Anti-endomysium antibodies

Circulating anti-endomysium autoantibodies (EmA) are directed against an amorphous supportive tissue surrounding smooth muscle cells and myofibroblasts of the gastrointestinal tract (113). IgA-EmA are measured by indirect immunofluorescence, using tissue sections from either monkey oesophagus or human umbilical cord (114).

The reported sensitivity of IgA-EmA ranges from 87% to 100%, and the specificity is between 91% and 100% (103, 115). Although IgA-EmA has proved to be a highly specific marker of CD, this type of autoantibodies are not always present in children younger than 2 years of age (104). The IgA-EmA levels correlate with the mucosal damage and eventually disappear on a gluten-free diet (116).

IgG-EmA can also be detected in untreated patients with CD. However, due to the presence of other IgG autoantibodies directed against various tissue autoantigens, IgG-EmA is more difficult to evaluate. There are few studies that clarify the clinical significance of IgG-EmA for the detection of untreated CD patients (117, 118).

Anti-tissue transglutaminase antibodies

The autoantigen of the EmA has been identified as tTG (119). The amino acid sequence of tTG is generally highly conserved among species, displaying an 80% homology between guinea pig and human tTG (120). Thus, the first generation of anti-tTG assays were based on tTG from guinea pig liver preparations (119, 121). However, these antigens contained several contaminating proteins that decreased the specificity of these assays. Subsequently, when purified native and recombinant human tTG became available, it was demonstrated that human tTG bound IgA-tTG more potently than the guinea pig counterpart (122).

ELISAs using recombinant human tTG for detection of IgA-tTG were demonstrated to have sensitivities and specificities higher than 90% (37, 103). The IgA-tTG levels decrease on a gluten-free diet and reappear after the reintroduction of gluten into the diet (123). The significance of IgG-tTG in CD diagnosis has been questioned because elevated levels of IgG-tTG were observed in patients with connective tissue diseases and disorders other than CD (121, 124, 125). The elevation of IgG-tTG detected in these patients was probably caused by the use of assays based on guinea pig tTG, given that assessment of these antibodies with human recombinant tTG increased the specificity (126).
The gut-associated immune system

The intestine is one of the largest mucosal surfaces of the body covering an area of 400 sq m in adult humans (127). The main function of the small bowel epithelium is to digest food and transport nutrients into the bloodstream. In order to maintain mucosal homeostasis, immunological ignorance of harmless antigens, such as commensal microorganisms and food antigens, is required. Concurrently rapid activation of the immune system is needed to limit the expansion of evading pathogens.

The gut-associated immune system is organised in functional and anatomically separate compartments. The inductive sites are represented by Peyer’s patches and solitary lymphoid follicles scattered along the intestine. These structures are covered with a specialised epithelium, the M cells that permit transport of particulate antigens from the lumen into the underlying tissue. Antigen-presenting dendritic cells, B cells, T cells, and macrophages are in close proximity beneath the M cells. After antigen priming at these sites, B cells and T cells migrate to the mesenteric lymph nodes where they expand and differentiate into effector or memory cells. Thereafter they are seeded back to the lamina propria of the intestine, the effector site, where further expansion and differentiation may take place. In the absence of inflammatory stimuli, dendritic cells are conditioned to induce a tolerogenic T cell response, whereas immune activation occurs in the presence of inflammation (128-130).

Among other functions, the intestinal mucosa regulates the development of IgA-secreting plasma cells. Approximately 80% of all plasma cells are located in the intestinal lamina propria. The antibody profile in the upper small intestine is dominated by an IgA1-production, whereas the majority of antibodies produced in the colon are IgA2 (131).

In the bloodstream approximately 90% of the IgA pool is of IgA1 subclass. According to the present conception these antibodies reflect an antibody production of the bone marrow (132). However, it can not be excluded that a fraction of the IgA antibodies found in the peripheral circulation is derived from an intestinal response.

Less than 5% of the plasma cells in the intestine produce IgG. The proximal part of the intestine is dominated by an IgG1 production whereas IgG2 is more prominent in the colon (128). The regulation and function of the IgG response of the intestine has not been clarified. However, lack of IgA at mucosal sites in IgA-deficient individuals is compensated for by and increased production of intestinal IgM and IgG (133).
Pathomechanisms of CD

Neither the underlying mechanisms of the mucosal deterioration observed in CD patients nor the immune activation upon gluten exposure are well understood (134). CD is considered to be a T cell mediated disease (135, 136), triggered by distinct prolamin peptides. The first mucosal alterations are characterised by increased mucosal permeability and an elevated number of intraepithelial CD8+ T lymphocytes (57).

Recent findings indicate that the early mucosal changes are triggered by toxic sequences of gliadin that cause increased interleukin 15 (IL-15) production. In the intestine of CD patients, IL-15 expression is enhanced. This cytokine promotes abnormal expansion of intraepithelial lymphocytes and upregulation of the natural killer cell group 2 D receptor (NKG2D) on their surface (137). Furthermore, IL-15 triggers lysis of stressed enterocytes by increasing their expression of major histocompatibility class I polypeptide related chain (MIC), the ligand of NKG2D (138, 139). Later stages of mucosal remodelling are associated with activation of CD4+ T cells, infiltration of macrophages and plasma cells in the lamina propria (140-142).

tTG can convert certain glutamine residues of undigested gliadin peptides into negatively charged glutamic acid by the deamidation process mentioned previously. The CD4+ T cell response elicited by deamidated gliadin peptides is stronger than that caused by their native counterparts (50). Additionally, it has been shown that the transamidation reaction mediated by tTG may result in the formation of complexes between gliadin and tTG molecules (93). The production of tTG-specific autoantibodies by plasma cells is most likely T cell dependent. It has been proposed that neoepitopes formed by gliadin-tTG complexes may activate tTG-specific T cells. These activated autoreactive T cells may subsequently provide necessary help to B cells for maturation into antibody producing plasma cells (119). However, no such autoreactive T cells have been demonstrated in the intestine of CD patients.

Alternatively, gliadin-tTG complexes might be picked up by tTG-specific B cells. After intracellular processing and loading of gliadin peptides into HLA-DQ2 or DQ8 molecules followed by cognate interaction with gliadin-specific T cells, the B cells may develop into antibody-secreting plasma cells (143), (Figure 3).

Whether tTG-specific antibodies participate in the development of CD or merely reflect destructive events of the intestinal mucosa has not been clarified. tTG-specific plasma cells reside in the intestinal lesion of patients with active CD. Recombinant single chain antibodies derived from intestinal B cells appear to be directed against a limited number of epitopes located in close vicinity to the enzymatic site of tTG (144-147). The tTG-specific antibodies have been shown to inhibit the enzymatic activity of tTG in vitro and in situ (148, 149). Purified IgA from CD patients prevents natural epithelial
villous differentiation *in vitro* to the same extent as monoclonal anti-tTG (150, 151).

IgA depositions containing tTG-specific antibodies are observed in the mucosal lesion of CD patients (152). Moreover, intestinal IgA-depositions have been noted in latent CD and in patients with minor mucosal changes prior to the detection of circulating IgA-tTG (153). A recent report indicated that anti-tTG antibodies modulated the cytoskeleton of epithelial cell lines and fibroblast, as well as inducing proliferation of the intestinal epithelium from treated CD patients (154).

Most of the studies regarding the autoantibody involvement in CD have focused on the effect of IgA-tTG. However, it cannot be ruled out that IgG-tTG also interferes with the natural function of tTG-mediated processes. Additionally, IgG antibodies generally have proinflammatory properties exerted by their binding to activating Fc receptors that are present on various effector cells (155, 156). A functional variant of the FcγRIIa gene was recently reported to be associated with CD (54), indicating a possible role for involvement of IgG-immune complexes in the precipitation of CD.

**Figure 3.** Proposed mechanisms behind the formation of antibodies directed against tissue transglutaminase (tTG). Partially degraded gliadin (triangles) penetrates the epithelium of the small intestine. tTG present in the subepithelial region deamidates gliadin (half-circles), and/or form complexes between gliadin and tTG molecules. Deamidated or native gliadin is captured and processed by antigen presenting dendritic cells (APC). Gliadin fragments presented to T cells in the context of HLA-DQ2 or DQ8 molecules, leads to a priming of gliadin-specific CD4+ T cells. Then, tTG-specific B cells bind and process tTG-gliadin complexes. After presentation of gliadin sequences in the context of HLA-DQ2 or DQ8, and cognate interaction with gliadin-specific T cells, the B cells differentiate into plasma cells producing tTG-specific antibodies, either IgA-tTG or IgG-tTG.
Present investigation

Aim
The main aim of this investigation was to study the contribution of IgG-tTG in CD diagnosis.

I: To evaluate the clinical relevance of IgG-tTG for the detection of untreated CD in IgA-deficient patients, and to investigate the prevalence of CD among healthy blood donors with IgA deficiency.

II: To evaluate whether IgG-tTG can be used as a substitute for IgG-EmA as a marker of CD in laboratory practice.

III: To investigate whether the IgG-tTG binding-epitopes are altered when tTG is immobilised.

IV: To investigate whether quantitative detection of IgG-tTG can be used to predict mucosal damage and disease severity in patients with gluten sensitivity.
Patients

Included in study I were 151 serum samples from IgA-deficient subjects, comprising control subjects and untreated CD patients. Serum samples collected from apparently healthy blood donors at the Finnish Red Cross Blood Transfusion Service, Helsinki, were also included. These samples were divided in two groups according to the presence or absence of IgG-EmA.

Included in study II were 215 serum samples sent to a reference laboratory for investigation of CD-related serology. The samples, which were collected from IgA-deficient and IgA-competent patients, were divided in subgroups according to the presence or absence of IgA-EmA and IgG-EmA.

Included in study III were serum samples consecutively collected from 123 children with symptoms indicative of CD, who all had a undergone a small-intestine biopsy. Included in the study were also samples from 80 CD children on a gluten-free diet.

Included in study IV were serum samples collected from 301 patients with gastrointestinal symptoms that indicated CD, which was diagnosed in accordance with the revised ESPGHAN criteria (36). Included were also serum samples from 134 DH patients diagnosed by means of skin biopsy. Of these patients 131 also underwent small-intestine biopsy.
Methods

Biopsy evaluation
Study I and IV: small-intestine histology was graded 0-5 according to the description by Fontaine and Navarro (157), which is a more detailed grading of partial villous atrophy than the modified Marsh criteria (34).

Study III: small-intestine histology was graded on a scale of 0 – 5 according to a modification of the Marsh criteria (34).

Enzyme linked immunosorbent assay (ELISA)
IgA-tTG and IgG-tTG were quantified by means of ELISA, Celikey™ for IgA-tTG and Recombi-tTG IgG for IgG-tTG (Phadia, Freiburg, Germany) in accordance with the manufacturer’s instructions. In short, microplate wells coated with human recombinant tTG were incubated with serum samples. Horse radish peroxidase labelled secondary antibodies, either anti-IgA or anti-IgG, were used for the detection of tTG-specific antibodies.

The antibody content in serum samples was expressed as arbitrary units/ml (U/ml) calculated from six-point calibrator curves containing tTG specific antibodies ranging from 0 to 100 U/ml.

Radiobinding assay (RBA)
Full-length human tTG cDNA isolated from endothelial cells was subcloned into the pGEM-T Easy Vector. tTG was synthesised by in vitro transcription and translation in the presence of 35S-methionin utilising the TNT SP6 coupled reticulocyte lysate system. For immunoprecipitation of IgA-tTG, goat anti-human IgA agarose was used.

Sepharose covalently linked with anti-human IgG (of the same quality as that used for the detection of IgG-tTG with ELISA), or protein A Sepharose was used for immunoprecipitation of IgG-tTG. Antibody levels were determined against six-point calibrator curves containing tTG-specific antibodies ranging from 0 to 100 U/ml.
Endomysium antibodies (EmA)

Studies I and IV: for binding of EmA, tissue sections from composite blocks of human jejunum and appendix, or human umbilical cord were used. Rabbit anti-human IgA or rabbit anti-human IgG antibodies labelled with FITC were used to visualise antibody binding to the endomysial fibres in tissue. Serum samples yielding fluorescence in dilution 1:2.5 were considered positive and further diluted to obtain the end titre.

Studies II and III: for binding of EmA tissue sections from monkey oesophagus was used. FITC labelled rabbit anti-human IgA or FITC labelled sheep anti-human IgG antibodies, absorbed over monkey tissue was used to visualise antibody binding to the endomysial fibres. Serum samples yielding fluorescence in dilution 1:10 were considered positive and further diluted to obtain the end titre.

Statistics

Depending on differences in dietary habits and environmental factors, antibody levels may vary between studied populations. Therefore, the optimal cut-off for each study was calculated by means of receiver operator curves (ROC) (158). The area under the ROC curve (AUC) quantifies the over-all ability of an assay to discriminate between individuals with and without the disease.

For evaluation of assay performance, sensitivity and specificity were calculated. The sensitivity of an assay is defined as the proportion of individuals having the disease who test positive. The specificity is defined as the proportion of individuals without the disease who test negative.

Non-parametric statistical methods were used for comparisons among groups. The Kruskal-Wallis test with Dunn’s multiple comparisons or the Mann-Whitney U-test was used to compare antibody levels between groups. The Spearman rank method was used to compare correlations between antibody levels, and the Wilcoxon signed rank test was used for pairwise comparisons.

Logistic regression was carried out to compare antibody levels among groups on the basis of age, gender, and biopsy findings. Kaplan-Meyer survival analyses, employing the log-rank method, were used to analyse the normalisation time of antibody levels.
Results and discussion

IgG-tTG as a marker of CD in IgA-deficient patients (Studies I and II)

Selective IgA deficiency, which is defined in adult subjects as serum IgA concentrations lower than 0.05 g/l, occurs in the general population with a frequency of 0.2 – 0.25% (133, 159). A total of 2.6% of CD patients has been reported to be IgA-deficient (160).

Although IgA-tTG and IgA-EmA are reliable markers of active CD in IgA-competent subjects, patients with IgA deficiency will not be detected by means of conventional IgA serology. Determination of total serum IgA concentrations, followed by biopsy of all IgA-deficient individuals with enteral complaints, leads to a high number of unnecessary biopsies, as most of these individuals do not have CD (161). Assessment of IgG-AGA for pre-biopsy selection of IgA-deficient patients has been suggested. However, due to the low sensitivity of this marker, several IgA-deficient CD patients will remain undiagnosed (162). The detection of IgG-EmA has several technical disadvantages, making the method unavailable to most laboratories on a routine basis (163).

Thus, an accurate and easily accessible marker that facilitates the identification of CD patients with IgA deficiency is needed. Here we investigated the clinical performance of IgG-tTG as a marker of CD in IgA-deficient patients, whose diagnose was confirmed by small-intestine biopsy, in healthy blood donors, and in serum samples sent to a routine laboratory for investigation of CD-related autoantibodies.
Biopsy confirmed IgA-deficient patients (Study I)

Despite that the prevalence of CD among IgA-deficient subjects is increased, as compared to the general population, patients with combined IgA deficiency and CD account for only a few cases in gastrointestinal centres (31). Study I demonstrated the clinical performance of IgG-EmA and IgG-tTG as markers of CD in a larger population of IgA-deficient patients than previously studied (117, 118, 160, 164).

We found that IgG-tTG was prevalent in 98.7% of the untreated IgA-deficient CD patients included in the study. There was a positive correlation between IgG-tTG and IgG-EmA ($r=0.91$, $p<0.0001$). The only CD patient without IgG-tTG and IgG-EmA, was a boy eleven moths of age, who developed both antibodies during later gluten challenge. Two of the control subjects had IgG-tTG levels close to the cut-off, one just above and the other just below. However, none of them had IgG-tTG levels as high as the CD patients (Figure 4). None of the control subjects had IgG-EmA.

The ROC analyses displayed a near-perfect discrimination between CD patients and controls, with an AUC of 0.9992 for IgG-tTG and 0.9936 for IgG-EmA. The high correlation between the two methods also suggests that that tTG is the main, and probably the sole, autoantigen of IgG-EmA. Hence, both IgG-tTG and IgG-EmA appear to be highly sensitive and specific for the detection of untreated IgA-deficient CD patients. However, the assessment of IgG-tTG is better suited for screening purposes.

Furthermore, the dynamics of the IgG-tTG levels monitored during a gluten-free diet demonstrated that the serum levels of these antibodies are, at least in part, gluten-dependent. A several-fold reduction in IgG-tTG levels was observed for all patients, and four of them resumed normal serum levels of IgA after a gluten-free diet. Nonetheless, only a minority of patients reached normal IgG-tTG levels during the study period, whereas the mucosal structure was completely restored. This finding contrasts with the observed behaviour of IgA in IgA-competent patients, for whom the serum levels of IgA-tTG actually disappear on a gluten-free diet (164). Furthermore, the IgA-tTG levels are usually rapidly normalised in IgA-competent patients after gluten exclusion (115), sometimes even before mucosal restitution is observed (40, 165).

The sustained IgG-tTG levels could not be explained by poor compliance with the gluten-free diet, as estimated from interviews of the patients. However, given that a strict gluten-free diet might be difficult to achieve, low doses of gluten intake in combination with the reported higher intrinsic permeability of the intestinal mucosa in IgA-deficient subjects (133), might be sufficient to maintain the production IgG autoantibodies.
The clinical consequences of persistent production of IgG-tTG remain to be investigated. However, IgA deficiency has been demonstrated to be associated with several autoimmune disorders with the presence of a variety of autoantibodies (133). It is possible that patients with sustained IgG-tTG elevation are prone to develop future autoimmune disorders.

IgA-deficient blood donors (Study I)

Although IgA-deficient patients lack protective IgA at mucosal surfaces, most of them do not suffer from any particular ailments. We found that the prevalence of IgG-tTG and IgG-EmA was 9.8% in apparently healthy IgA-deficient blood donors, implying that they had silent CD (Figure 4). Biopsies were not performed on these patients. Nor was it possible to establish whether they had CD or not. However, the HLA conferred risk alleles associated with CD was confirmed in 12 of the IgG-EmA positive blood donors. Given the high diagnostic performance of IgG-tTG among the studied clinical cases, our results support the fact that IgA-deficient subjects have at least a 10-fold increased risk of CD (27).

Detection of IgG-tTG in a laboratory setting (Study II)

This study reflected the laboratory situation, in which samples from patients are analysed at a stage when the final diagnosis is unknown. Nor has IgA deficiency been established in most of the cases.

Among the 120 included IgA-deficient patients, 22 had elevated IgG-tTG, and 20 were positive for IgG EmA. Thus, coexistent IgG-tTG and IgG-EmA was observed in 17.4% of the patients, which indicated a high probability that they had CD. Noteworthy is that the cut-off used was adapted to be suitable also for the IgA-competent patients included in the study. However, the ROC analysis indicated perfect discrimination for IgG-tTG among IgA-deficient patients. Thus, a higher cut-off would have resulted in a 100% sensitivity and specificity (Figure 4). Our results also supported the observed poor outcome of IgG-AGA as predictors of CD in IgA-deficient patients (162). Only 8 of 20 patients with positive levels of both IgG-EmA and IgG-tTG had elevated IgG-AGA, whereas 29 of 113 patients without any of the IgG autoantibodies were positive for IgG-AGA.

Included in the study were also samples from IgA-competent patients, divided into groups according to whether or not they had IgA-EmA. All IgA-EmA positive patients were found to also have elevated IgA-tTG. Considering the high diagnostic significance of these autoantibodies (123) it can be assumed that double positivity regarding IgA autoantibodies indicated that all these patients had CD. Only 72% of these patients had elevated IgG-tTG, whereas none of the IgA-EmA negative patients had either of the IgG autoantibodies (Figure 5). Even if the absence of these antibodies does not
exclude CD, it might be assumed that most patients without IgA-tTG and IgG-tTG had diseases other than CD. Nevertheless, the presence of potential CD patients in this group would not have affected the specificity of the IgG-tTG assessment. Thus, it can be concluded that the specificity of IgG-tTG was 100% among the IgA-competent patients included in this study.

Previous studies have indicated that IgG-tTG may occur in patients with a number of disorders other than CD (124, 125). These studies employed assays based on guinea pig-tTG, which clearly are inferior to their human counterparts (122). The high specificity of IgG-tTG found in our studies has recently been confirmed by others (166, 167), although not all IgA-competent CD patients have these antibodies.

Recent guidelines on serology as an aid in CD diagnosis advocate the use of human tTG for assessment of anti-tTG antibodies (168), which was the antigen of use in our studies. Notable, however, is that patients with end-stage cardiac failure may have elevated IgA-tTG as well as IgG-tTG, without any evidence of CD (169, 170). These patients lack IgA-EmA, which indicate that the epitopes of tTG in the endomysial tissue might be different compared to the ELISA format, where tTG is coated onto microwells by hydrophobic interactions.

**Figure 4.** The distribution of tissue transglutaminase specific IgG antibodies (IgG-tTG) in various groups of IgA-deficient subjects included in studies I and II. Coeliac disease patients (I), control subjects (II), blood donors with (III) and without IgG-EmA (IV), patients with (V) and without IgG-EmA (VI). Group I-IV were included in study I and group V-VI were included in study II. The upper limit of the shaded area indicates the cut-off 10 U/ml used in study I, and the lower limit indicates the cut-off 4 U/ml used in study II. The line indicates optimal cut-off of 7 U/ml for both studies.
Summary

In summary studies I and II showed that autoantibodies directed against tTG is a common immunological feature of CD patients, irrespective of IgA deficiency. The results demonstrated that IgG-tTG and IgG-EmA are highly sensitive and specific markers of CD in IgA-deficient patients. The high concordance and correlation between these antibodies indicate that either of them can be used as criterion to select IgA-deficient patients for biopsy referral. However, assessment of IgG-tTG is better suited than IgG-EmA for screening and monitoring purposes than.

Among apparently healthy IgA-deficient blood donors, 10% might have silent CD. In order to prevent potential complications from prolonged gluten exposure, CD should be considered for all IgA-deficient subjects.

Epitope display of tTG for detection of CD related autoantibodies (Study III)

The importance and use of serologic markers have increased during the last decade. In research settings, IgA-tTG detected with ELISA has been shown to be a highly accurate marker of active CD, whereas a lower performance has been observed in clinical practice.

Although IgG-tTG generally seems to be of marginal clinical importance in screening for untreated CD (121), several studies have reported even higher sensitivity of IgG-tTG than their IgA counterparts for untreated CD (171-173). These observations were based on the assessment of autoantibodies with RBA, a method in which tTG is kept in a soluble form. It can not be ruled out that the availability of CD-related epitopes differ between soluble and immobilised tTG, the latter of which is used for detection of autoantibodies with the ELISA technique.

Study III compared the performance of IgG-tTG and IgA-tTG, assessed with ELISA and RBA. We assumed that potential differences in the epitope presentation of tTG would be reflected in divergent diagnostic performances of the two assay formats.
Autoantibodies in untreated children referred for biopsy

We observed that the ELISA and RBA detected elevated IgA-tTG in the same untreated CD children, and the correlation between the IgA-tTG levels measured with the two techniques was high ($r=0.94$, $p<0.0001$). This indicates that the immobilisation procedure did not induce any major alterations of tTG. However, the sensitivity of 89% for IgA-tTG was lower than the previously observed sensitivity of 96% (123). The discrepancy was possibly due to the large proportion of children younger than 2 years of age among the patients. It has previously been reported that children in this age group may not always have detectable serum levels of IgA autoantibodies (104). None of the CD children without IgA-tTG had elevated IgG-tTG. However, all of them had increased levels of IgA-AGA (data not shown).

The prevalence and levels of IgG-tTG in untreated CD children were positively correlated ($r=0.91$, $p<0.0001$) between the ELISA and RBA when anti-human IgG was used for the RBA method. This indicates that the binding epitopes for IgG-tTG were also essentially unaltered after immobilisation. In line with previous observations (115) and the findings of study II, only a fraction of the untreated CD children appeared to have elevated IgG-tTG levels (Figure 5). However, the RBA results obtained with protein A deviated markedly from those of the other tTG-IgG assays.

When protein A Sepharose was used for detection, 36% of untreated CD children without IgG-tTG, according to the other two assyas, were positive. The antibody levels of these children correlated with the IgA-tTG levels ($r=0.85$, $p<0.0001$), whereas no such correlation was observed when comparing with IgG-tTG. It might be speculated that the protein A did not detect IgG-tTG exclusively, given that protein A can bind IgM and IgA, in addition to the high affinity binding of IgG (174). Regardless of which isotype, autoantibody detection with the RBA method based on protein A was highly sensitive and specific for CD, as IgA-deficient CD children were also identified. Thus the method appears to be well suited as a screening assay for untreated CD children.

Autoantibodies in children on a gluten-free diet

Among the treated children, 34% had increased IgA-tTG and/or IgG-tTG levels at follow-up. Most of these children were only positive with the IgA-tTG RBA, which is in agreement with previous observations that IgA-tTG detected with RBA remains elevated longer than EmA and IgA-tTG (171, 172). However, the IgA-tTG levels detected with the RBA method did not correspond with the mucosal structure observed in the treated children who had a biopsy performed at the time for follow-up. Most of these children had a normal histology, despite elevated IgA-tTG assessed with RBA and three
children with partial villous atrophy had normal levels of autoantibodies, irrespective of which method was used for detection.

The discrepancy of IgA-tTG levels observed between the methods indicated a possibility that certain tTG-epitopes might become hidden when tTG is fixed to the solid phase. However, another explanation might be that the RBA method detects lower quantities of autoantibodies than the ELISA technique. Whether elevated IgA-tTG levels represent minute amounts of IgA-tTG from long-standing inflammation of the small intestine, or an early immune response due to temporary dietary transgressions, remains to be determined.

Summary

Taken together, the results of study III suggest that the binding epitopes of CD related autoantibodies are only slightly different when tTG is immobilised as compared to the soluble conformation. This difference had minor impact on the assessment of autoantibodies from untreated CD children, whereas it had a considerable impact on the detection of IgA-tTG when gluten was retracted from the diet.

Moreover, the RBA assay based on protein A for detection of autoantibodies in CD children did not appear to measure IgG-tTG only. For investigation of the clinical significance of IgG-tTG in CD, it is recommended to use an RBA based on an anti-IgG specific resin, or IgG-tTG ELISA.

IgA-tTG and IgG-tTG as predictors of mucosal damage and disease severity (Study IV)

The various clinical symptoms associated with gluten sensitivity are difficult to quantitate. Neither the IgA-EmA titres nor the grade of mucosal villous atrophy have been demonstrated to reflect disease severity (175, 176). Patients with DH, the extraintestinal manifestation of gluten enteropathy, have mucosal alterations similar to those observed in CD, but the spectrum is wider with preserved villi in some cases (177).

Biopsy is the gold standard in diagnosing CD. However, the mucosal lesion may be patchy (178, 179), and the evaluation is subjective and depends on the quality of the tissue specimen. It has been reported that up to 10% of biopsies may be poorly oriented, which may lead to misclassification of the histopathology (180). Most patients, irrespective of symptoms, can be detected by the demonstration of elevated serum IgA-tTG. A correlation between the grade of mucosal damage and the levels of IgA-tTG has been indi-
cated (181). However, the significance of IgG-tTG in IgA-competent patients with respect to mucosal damage and symptoms is unclear.

In study IV we investigated whether the quantification of IgA-tTG and IgG-tTG, either alone or in combination, could be used as an aid in predicting mucosal destruction and disease severity in patients with various manifestations of gluten sensitivity.

Autoantibodies related to mucosal damage

The highest levels of IgG-tTG were found in patients with subtotal or total villous atrophy, but far from all patients with gluten sensitivity had these antibodies. We also noted a correspondence between the grade of mucosal villous atrophy and the IgG-tTG levels when patients with severe villous atrophy and milder lesions were compared (p<0.0001). There was also a correspondence between the IgA-tTG levels and the grade of mucosal villous atrophy, (p<0.0001), with significantly higher IgA-tTG levels in patients with at least a Marsh IIIb lesion and those with milder mucosal alterations.

It was recently suggested that the biopsy procedure might not be needed for patients with high levels of IgA-tTG, as highly elevated IgA-tTG was associated with mucosal abnormalities compatible with CD (182). These results were further strengthened by the observation that IgA-tTG levels above the measuring range of the used assay were associated with at least a Marsh IIIa histology (183). The results of our study also supported the conception that highly elevated IgA-tTG levels were associated with at least a Marsh IIIa lesion. In fact, more than half (61%) of the CD and DH patients had IgA-tTG levels above the measuring range. Noteworthy is that the majority of these patients had at least a Marsh IIIb lesion.

However, serology without biopsy might be doubtful for screening and case-finding in clinical practice, since the presence of autoantibodies in serum may precede the development of mucosal damage in potential and latent CD patients (184). A recent study of children with a HLA-conferred risk for CD, demonstrated that IgA-tTG levels occasionally can reach very high levels, and subsequently revert to normal without any evidence of the development of CD (185, 186).

As it was noted that the IgG-tTG levels correlated with the grade of mucosal villous atrophy in our study, we investigated whether these antibodies could, in conjunction with elevated IgA-tTG, predict the extent of the mucosal lesion. We found that all but one patient with IgA-tTG levels higher than 100 U/ml and concurrent IgG-tTG levels higher than 20 U/ml had at least a Marsh IIIb lesion. The remaining patient had Marsh IIIa villous atrophy.

Thus, the presence of both IgA-tTG and IgG-tTG predicted Marsh IIIb lesion or more in untreated gluten-sensitive patients with 99% specificity. This indicates that there is no need for biopsy to diagnose CD in individuals with
high levels of both IgA-tTG and IgG-tTG. However, further studies are warranted in order to establish whether elevated IgG-tTG, in analogy with IgA-tTG, occurs prior to the development of mucosal alterations. Nevertheless, the presence of high IgA-tTG and IgG-tTG levels may be used as a complement in cases where dubious histopathology is observed.

### Autoantibodies and the clinical presentation

When the clinical presentation of CD was compared with the quantity of IgG-tTG, we found the highest prevalence and levels among the youngest children with the severe mode of enteropathy. All these children were IgG-tTG positive and had higher IgA-tTG levels than all other patient groups. An inverse relation between IgG1-tTG and age has previously been reported (173). Although our study indicated a similar correspondence between IgG-tTG and age of the CD children (r=-0.4958, p<0.0001), age alone did not account for high or low antibody levels, as no such relation was observed among the adult CD patients or the DH group.

The presence and levels of IgG-tTG were comparable in adult CD patients and the DH group. Consistent with our observations in studies II and III, several of these patients had normal levels of IgG-tTG (Figure 5). In contrast, the IgA-tTG was elevated in most of the adult CD and DH patients. Therefore, IgA-tTG seems to be more suitable for clinical evaluation and screening purposes among these patients.

Conversely, it was observed in some of the CD children that the IgA-tTG levels disappeared rapidly, while the clinical recovery and normalisation of IgG-tTG took much longer. Children with the severe mode of CD displayed a particularly rapid decline pattern of IgA-tTG levels as compared to IgG-tTG. Therefore, it is possible that measuring IgG-tTG levels better reflects the clinical condition of CD children, especially in cases with a poor appetite. Those children might have reduced the gluten intake already before a serum sample has been taken and in such instances, IgA-tTG and IgA-EmA can both be negative that has been observed in earlier studies (185, 187).

Whether elevated IgG-tTG reflects more profound inflammation of the intestine, or if they participate in the disease progression could not be determined by this study. However, it may be speculated that the proinflammatory properties of IgG, exerted by their binding to activating Fc receptors on various effector cells, might enhance ongoing inflammation (156, 188). It was recently reported that homozygosity for the isoform of FcγRIIa (CD32a) that binds IgG with higher affinity was associated with CD (54). That indicates that IgG-tTG might be involved in maintaining the intestinal inflammation, at least in some CD patients. Further studies are needed in order to establish whether patients with elevated IgG-tTG are at risk of developing more severe complications than patients who have elevated IgA-tTG only.
Figure 5. The distribution of tissue transglutaminase specific IgG antibodies (IgG-tTG) in various groups of IgA-competent subjects included in studies II, III and IV. Patients with (I) and without IgA-EmA (II) from study II, coeliac disease patients (III) and control subjects (IV) from study III, coeliac disease patients (V) and control subjects (VI) from study IV. The upper limit of the shaded area indicates cut-off 4 U/ml used in studies II and III and the lower limit indicates cut-off 3 U/ml used in study IV.

Summary

Taken together, the results of study IV indicated that the levels of IgG-tTG and IgA-tTG were positively correlated with the grade of mucosal villous atrophy and that the IgG-tTG levels corresponded with more severe manifestations of gluten enteropathy. Whereas elevated IgG-tTG or IgA-tTG taken alone should be interpreted with caution, the combined assessment of high IgA-tTG and IgG-tTG levels predicted a severe villous atrophy. Hence, the biopsy procedure might not be needed to diagnose CD for patients with very high levels of both IgA and IgG against tTG.

During a gluten-free diet, IgA-tTG mostly disappeared prior to clinical and mucosal recovery. Interestingly, the overall clinical condition in children was better reflected by the IgG-tTG levels. For follow-up of adult IgA-competent patients, assessment of IgG-tTG is also recommended, as their presence and levels might indicate a more severe disease.
Conclusions

IgG-tTG are highly prevalent in IgA-deficient CD patients, and these antibodies can be used as substitutes for IgG-EmA in pre-biopsy testing of patients suspected of having CD.

The high prevalence of IgG-tTG in apparently healthy IgA-deficient blood donors indicates that they may have silent CD. Thus, CD should always be considered for IgA-deficient patients.

IgG-tTG can be used for monitoring adherence to a gluten-free diet in IgA-deficient CD patients. However, mucosal recovery is not reflected by the levels of IgG-tTG in treated patients.

The IgG-tTG levels correspond with the disease severity in untreated IgA-competent patients with CD and DH, indicating that it might be possible to use IgG-tTG as a marker of disease activity.

Elevated levels of IgG-tTG in patients with high IgA-tTG levels predicted at least a Marsh IIIb mucosal lesion, indicating that the combined detection of IgG-tTG and IgA-tTG might reduce the need for biopsy in diagnosing CD.
Celiaki, eller glutenintolerans, är en tunntarmssjukdom som drabbar vissa personer då de äter livsmedel som innehåller vete, korn och råg. Den toxiska komponenten från vete kallas gluten. Sjukdomen är genetiskt betingad och då personer som har celiaki äter gluteninneållande mat uppstår en inflammation tunntarmen, vilken leder till att tarmluddet försvinner, s.k. villusatrofi. Det bildas också antikroppar riktade mot ett enzym som finns i tarmen, vävnadstransglutaminas (tTG). Det är ungefär 1 % av hela befolkningen som har celiaki, och under en 20-årsperiod har förekomsten fördubblats.

Tidigare ansåg man att celiaki endast drabbade barn, men numera har det visat sig att sjukdomen förekommer i alla åldrar. De klassiska symptomen på celiaki hos barn är diarré, tillväxtstörningar, och andra yttringar som orsakas av bristande näringsupptag till följd av avsaknad av tarmludd i tunntarmen. Vuxna har ofta mer diffusa symptomet än barn, där järnbrist kan vara den enda indikationen på celiaki. Personer med celiaki löper en ökad risk för att utveckla benskört, anemi, malignitet i tunntarmen och diverse andra komplikationer.

Celiaki kan också yttra sig som en rad extraintestinala manifestationer, som hudsjukdomen dermatitis herpetiformis, migrän eller infertilitet. Det har också visat sig att olika autoimmuna eller genetiskt betingade sjukdomar såsom diabetes, autoimmun tyroidit, Downs’ syndrom och IgA-brist är kopplade till en högre risk för celiaki. En minoritet av personer med celiaki blir diagnostiserade, och det är så många som 90 % av alla med sjukdomen som förblir upptäckta.

Diagnosen ställs med hjälp av mätning av IgA-antikroppar riktade mot tTG (IgA-tTG) och tunntarmsbiopsi, där man kan konstatera villusatrofi. Behandlingen består i en livslång glutenfri kost, varvid inflammationen i tunntarmen klingar av, tarmluddet återställs och serumnivåerna av IgA-tTG normaliseras.

Förhöjda nivåer av IgA-tTG används ofta i den kliniska rutinen som selektionskriterium för att utföra tunntarmsbiopsi. Dessa antikroppar vid uppföljning av hur väl en person med celiaki lyckats genomföra en glutenfri diet. Det har visat sig att IgA-tTG är sjukdomsspecifika och förhöjda nivåer av dessa antikroppar förekommer sällan vid andra sjukdomar än celiaki. Trots detta har inte alla personer med celiaki förhöjda nivåer av IgA-tTG och de peroner som har IgA-brist och celiaki kan inte upptäckas med hjälp av IgA serologi.
Denna avhandling behandlar möjligheten att mäta IgG-tTG som komplement till IgA-tTG och biopsi i diagnosen av celiaki. I de första två studierna visades att IgG-tTG hade en hög diagnostisk sensitivitet och specificitet som markör för celiaki bland personer med IgA-brist. Vid en jämförelse med tidigare tillgänglig metodik visades att mätning av IgG-tTG är ett säkrare och enklare sätt att identifiera celiaki hos personer med IgA-brist. Dessutom visade resultaten att IgG-tTG kan användas som uppföljning av en glutenfri diet. Även om antikroppsnivåerna av IgG-tTG i samtliga studerade patienter minskade från det att gluten uteslutits ur kosten, kvarstod en förhöjd nivå hos flertalet patienter, trots att tarmslemhinnan läkt ut och symptomen försvunnit. Vidare antydde resultaten att så mycket som 10 % av till synes friska blodgivare med IgA-brist har celiaki, varför alla personer med konstaterad IgA-brist bör undersökas för förekomst av celiaki.

I de följande studierna visades att endast 60-75% av alla celiakipatienter med normal serumkoncentration av IgA har förhöjda nivåer av IgG-tTG. Vidare visade resultaten att serumnivåerna av IgG-tTG spegler graden av tunntarmsskada och framför allt symptomens svårighetsgrad hos personer med olika yttringar av celiaki. Liksom hos personer med IgA-brist, minskade nivåerna av IgG-tTG då gluten uteslutits från kosten, och normaliseringen av IgG-tTG följde den kliniska återhämtningen. Slutligen visades att en kombination av höga serumnivåer av IgG-tTG tillsammans med IgA-tTG kan förutsäga förekomsten av en omfattande villusatrofi. Detta antyder att vissa personer med celiaki inte skulle behöva genomgå en biopsi för att erhålla diagnosen celiaki.

Sammantaget visade resultaten i de ingående studierna att IgG-tTG är ett bra komplement till befintliga diagnostiska markörer, och att mätning av IgG-tTG bör ingå i den kliniska rutinen för alla individer med misstänkt celiaki.
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References


to tissue transglutaminase is related to epithelial cell proliferation in celiac disease. Gastroenterology 2007;132:1245-53.


169. Peracchi M, Trovato C, Longhi M, Gasparin M, Conte D, Tarantino C, Prati D, Bardella MT. Tissue transglutaminase antibodies in pa-


glutaminase autoantibodies is the most sensitive and specific screening method for celiac disease. Am J Gastroenterol 2001;96:1536-40.

172. Bilbao JR, Vitoria JC, Ortiz L, Corralesa A, Hualde I, Preciado E, Castano L. Immunoglobulin G autoantibodies against tissue-

173. Agardh D, Borulf S, Lernmark Å, Ivarsson SA. Tissue transglutami-

174. Ljungberg UK, Jansson B, Niss U, Nilsson R, Sandberg BE, Nilsson B. The interaction between different domains of staphylococcal pro-


talo L, Mäki M. Antiendomysial and antihuman recombinant tissue transglutaminase antibodies in the diagnosis of coeliac disease: a bi-


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)