

Identifying True Celiac Disease and Wheat Allergy in the Era of Fashion Driven Gluten-Free Diets

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Keywords

Celiac disease · Specific IgE · Wheat allergy

Abstract

Background: Diagnosing both celiac disease (CD) and wheat allergy (WA) might be challenging due to the increasingly popular gluten-free diets. **Objectives:** This study investigates the value of anti-tissue transglutaminase IgA (tTG IgA) and wheat-specific IgE (WtIgE), and identifies clinical and serological features associated with CD and WA. **Method:** Serological markers of autoimmunity and allergy along with medical charts of patients assessed for tTG IgA and WtIgE between 2010 and 2016 were evaluated. **Results:** During the last years, an increasing number of patients have been tested for tTG IgA, while the number of positive results decreased linearly. Among the 2,965 patients included, 128 patients showed at least once a positive tTG IgA. All patients with tTG IgA levels higher than the 12-fold upper normal limit had CD. The ratio of tTG IgA/total IgA did not perform better as a diagnostic test for CD compared to tTG IgA. tTG IgA and anti-nuclear antibodies were significantly associated. WA was only rarely investigated, particularly in adults. However, positive WtIgE were found in nearly 50% of the cases. WtIgE and

tTG IgA values were negatively correlated. **Conclusions:** tTG IgA were increasingly tested, while the rate of positive results decreased in recent years, possibly reflecting the impact of current alimentary trends on clinical practice. Associated autoimmune disease was frequently found in CD. High levels of tTG IgA accurately predicted CD diagnosis. WA was rarely investigated and deserves more attention, in particular in children with atopic background. WA does not seem to be associated with CD.

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Introduction

In recent years, disorders related to gluten ingestion have become a popular phenomenon with a global estimated prevalence of 5% [1]. Accordingly, the market for gluten-free products is constantly growing, with an annual growth rate of 10% [2]. However, the reasons for this demand is hardly explained by the small but documented increase of the prevalence of celiac disease (CD) [3] or true IgE-mediated wheat allergy (WA), for which epide-

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miological data are sparse. While the rate of CD in the population is estimated to be around 1–2% [4, 5], WA seems rare with an estimated prevalence of 0.3–0.5% in children, and less likely in adults [6]. In otherwise healthy individuals, gluten avoidance has been recently reported to be deleterious [7].

Wheat might be responsible for true IgE-mediated allergy. Possible manifestations are occupational asthma, rhinitis, contact urticaria, rarely anaphylaxis, and wheat-dependent exercise-induced anaphylaxis (WDEIA). Similar to CD, manifestations of WA may include abdominal cramps, dizziness, and bloating. More than 20 different wheat allergens have been described [2]. While gluten is involved in CD, high-molecular-weight glutenin and ω 5-gliadin (Tria19) appear to be the major allergens in WDEIA, and a gluten-free diet is often proposed for prophylaxis in the latter condition [8, 9]. The exact relation between CD, WA, and pollen allergy is not known but several cross-reacting antigens have been described [10].

Recently, another condition termed “non-celiac wheat/gluten sensitivity” has been described to define all those cases in which gluten avoidance leads to an improvement of the subjective health condition, without any objective WA or CD [11, 12]. Interestingly, a recent study reported that both patients with non-celiac wheat/gluten sensitivity and CD develop autoimmune disorders, compared with patients with irritable bowel syndrome [13]. Differentiating between these entities is challenging in clinical practice.

When CD is suspected, the first diagnostic step is most often the analysis of anti-tissue transglutaminase (tTG IgA) [14]. However, this test is also used during follow-up to verify the adherence to a gluten-free diet because it has been shown that more than 90 percent of CD patients on a gluten-free diet will have negative serologies by 5 years [15]. Similarly, when suspecting WA, assessment of anti-wheat IgE (W IgE) is recommended [8]. Using data of patients assessed for tTG IgA or W IgE likely allows to identify most patients who present with symptoms or morbidities (i.e., anemia, osteoporosis) possibly associated with gluten ingestion. Moreover, because CD has been associated with several autoimmune conditions, in particular type 1 diabetes [11], thyroiditis [16, 17], hepatitis [11, 18], primary biliary cholangitis [11, 19], gastritis [11], Sjögren’s syndrome [20], and also IgA deficiency [17, 21], assessing the presence of other auto-antibodies and IgA levels is sometimes performed during work-up of suspected CD, despite the lack of specific recommendations [22]. Eventually, in the presence of IgA deficiency, the assessment of anti-deamidated Gliadin Peptide IgG

(DGP IgG) antibodies has been recently reported to be helpful [23].

This retrospective study longitudinally analyzed the prevalence of positive tTG IgA and W IgE in our tertiary care center. To better define the burden of morbidity associated with gluten and wheat ingestion and to help physicians recognize the clues for true CD or WA, this study investigated the association of tTG IgA and W IgE with other serological and clinical parameters of patients presenting with symptoms associated with gluten and wheat ingestion.

Material and Methods

Patients

We retrospectively analyzed all requests for tTG IgA and W IgE between 2010 and 2016. In patients tested for tTG IgA or W IgE, the presence of associated auto-antibodies during the same period was analyzed. Moreover, in case of positivity, we retrospectively evaluated the medical charts for comorbidities, endoscopy results, associated serological parameters, and physician diagnosis. Initially, because of a too large population, we limited our longitudinal analysis to 2010, 2012, 2014, and 2016 (Fig. 1a, b, 2, 3; Table 1). However, for association analysis among antibodies and for characterizing patients with positive W IgE, all patients assessed between 2010 and 2016 were included (Tables 2, 3).

Criteria for WA and CD were allergist’s diagnosis and gastroenterologist’s diagnosis, respectively. For CD diagnosis, physicians adhered to the European Society for Pediatric Gastroenterology, Hepatology and Nutrition criteria [24], the guidelines from the British Society of Gastroenterology [25] and the American College of Gastroenterology [22]. For WA diagnosis, allergists relied on history, skin tests, and levels of W IgE. According to validated criteria [26], oral food challenge was performed in case of doubt only after obtaining the patient’s consent and when benefits outweighed the risks of a hypersensitivity reaction. The patient’s follow-up in medical charts was used to check whether WA and CD were confirmed, when available.

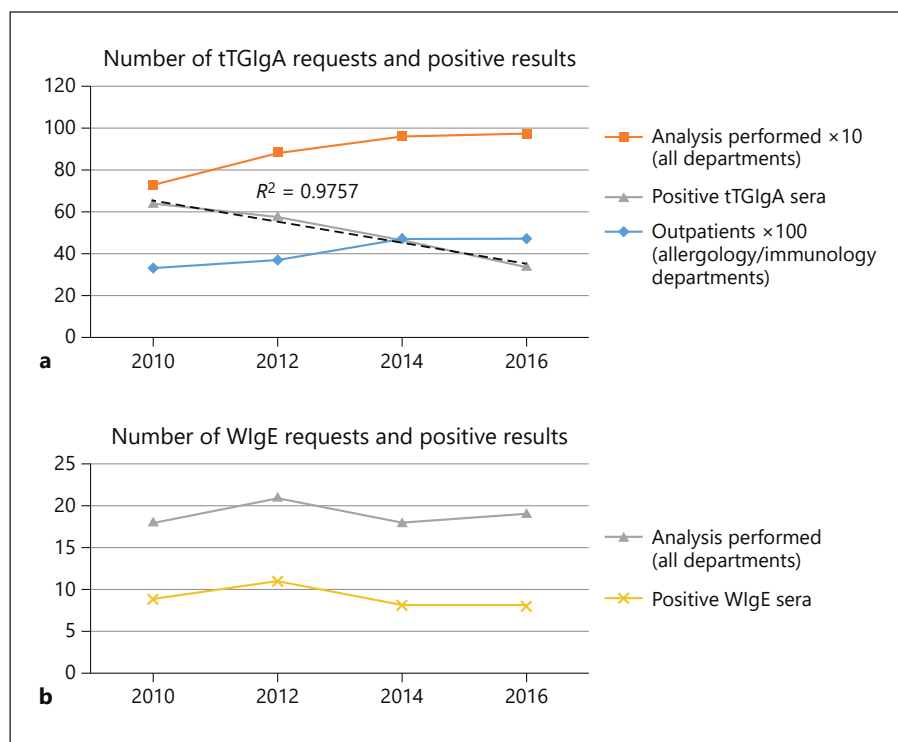
Methods

All the tests were performed in the immunology and allergy clinical laboratory of the University Hospital of Geneva.

tTG IgA (Celikey™) and DGP IgG were determined by fluoro enzymeassays on Unicap 100, according to the manufacturer’s instructions (Thermo Fisher Scientific, Uppsala, Sweden). Normal values are <7 U/mL. Before January 20, 2016, anti-tTG IgA antibodies were detected by a specific semi-quantitative ELISA using native human TG antigen isolated from fresh red blood cells (QUANTA Lite® h-tTG IgA ELISA, Inova Diagnostics, San Diego, CA, USA). Normal values were <20 U. Total IgA were systematically analyzed along with tTG IgA.

W IgE, IgE against rTria19, and grass pollen allergen (rPhlp1, rPhlp5, rPhlp7, rPhlp12) were performed by ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden), according to the manufacturer’s instructions. Results were positive when equal to or greater than 0.35 kUA/L.

Fig. 1. a Decreasing amount of positive tTGlgA despite increasing laboratory requests and increasing number of patients that consulted our adult allergology/immunology outpatient facilities during 2010, 2012, 2014, and 2016. R^2 value close to 1 (dashed line) shows linearity. Y-axis shows raw numbers. **b** Low amounts but high rate of positive results of WlgE analysis during 2010, 2012, 2014, 2016. Y-axis shows raw numbers. tTGlgA, anti-tissue transglutaminase IgA; WlgE, wheat IgE.



A screening of sensitization to aeroallergens (mixture of relevant allergens) was performed by Phadiatop[®] (Thermo Fisher Scientific, Uppsala, Sweden), according to the manufacturer's instructions.

Anti-nuclear antibody (ANA) detection was performed by indirect immunofluorescence using Hep-2 (human epithelial cell line) substrate slides and FITC anti-human IgG conjugate (Nova Lite[™] Inova Diagnostics, San Diego, CA, USA). Serums were titrated from 1/80 to endpoint and results expressed as the last positive dilution. Values above or equal to 1/160 were considered as positive.

Anti-nucleoprotein (Ro [SSA], La [SSB], nRNP, Sm, Jo-1 and Scl-70) detection and quantification were performed by specific ELISAs. A screening ELISA (Quanta Lite[™] ENA 6, Inova Diagnostics, San Diego, CA, USA) was followed in case of positivity by specific ELISAs (Quanta Lite[™] SS-A, Quanta Lite[™] SS-B, Inova Diagnostics, San Diego, CA, USA). For each ELISA, respective purified antigen is bound to the wells under conditions that will preserve the antigen in its native state. Results are expressed in units calculated relatively to a defined positive control. Results above 20 U are considered positive.

Anti-ATPase IgG antibodies (Gastric Parietal Cell Antibodies) were detected by a specific semi-quantitative ELISA using Purified H⁺/K⁺ ATPase isolated from pig gastric mucosa as antigen (Quanta Lite[™] GPA, Inova Diagnostics, San Diego, CA, USA). Results are expressed in units calculated relatively to a defined positive control. Results above 20 U are considered positive. This test was introduced in August 2013.

Anti-intrinsic factor IgG antibodies were detected by a specific semi-quantitative Elisa (Alphadia, Wawre, Belgium). Results are

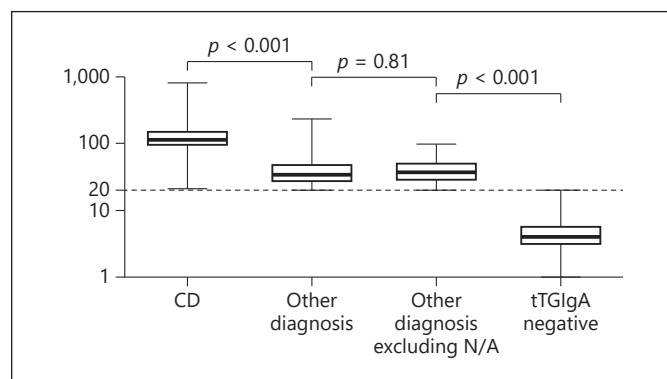


Fig. 2. tTGlgA values of patients with CD diagnosis, with other diagnosis, and with other diagnosis excluding those without information (N/A), compared to patients with negative values. Only analyses prior to January 20, 2016 and of patients analyzed in 2010, 2012, 2014 and 2016 were used (ELISA method). The Y-axis shows tTGlgA values in Units. Whiskers are minimum and maximum values. CD, celiac disease; tTGlgA, anti-tissue transglutaminase IgA; N/A, non-available.

expressed in Binding Index, the ratio between the sample and the cut off's OD and is considered as positive when BI > 1.1.

Total IgA were measured by nephelometry using BN ProSpec System (Siemens, Marburg, Germany). Normal values in adults are: 0.7–4.0 g/L.

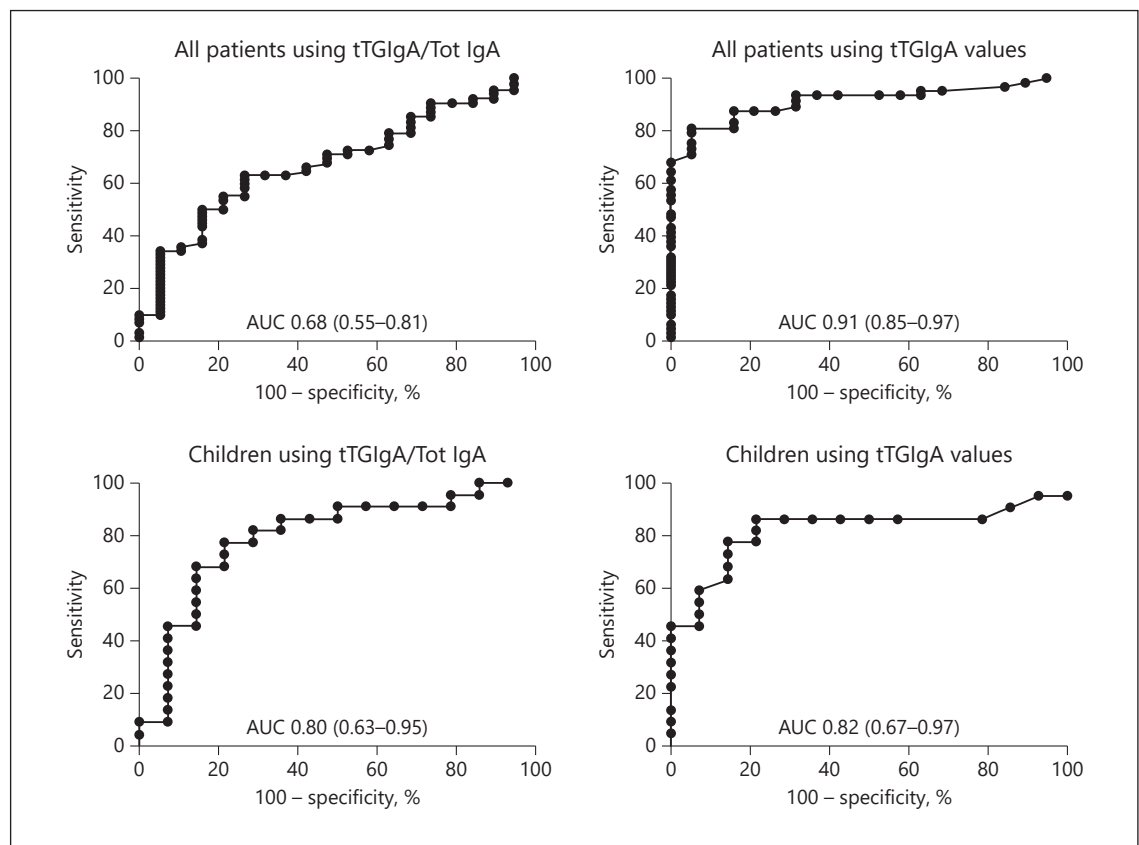


Fig. 3. ROC curve of all patients with positive tTGlgA (ELISA method, 2010, 2012, 2014, and 2016) or only children <6 years old using either normal values or the ratio of tTGlgA/Total IgA ratio. AUC, area under the curve (95% CI); tTGlgA, anti-tissue transglutaminase IgA.

Anti-thyroperoxidase (TPO) antibodies were measured by electro chemiluminescence immunoassay on a Cobas 8000 analyzer (Roche, Basel, Switzerland). Results above 35 units are positive.

Anti-liver-kidney microsome and anti-mitochondria antibodies were detected by indirect immunofluorescence technique using mouse kidney/stomach/liver substrate slides and FITC anti-human IgG conjugate (Nova Lite™ ANA KSL, Inova Diagnostics, San Diego, CA, USA). Serums were titrated from 1/20 to endpoint and results expressed as the last positive dilution. Values above or equal to 1/40 are positive.

All ELISAs are performed on a fully-automated processing system (DSX®, Dynex Technologies, Chantilly, USA). Ethical approval was obtained from Swissethics; study No. 2016-00663.

Statistics

Two-tailed chi-square test without Yates' correction was used for statistical analysis for associations between different antibodies and the presence of tTGlgA. For small sample size, Fisher's exact test was used instead. Mann Whitney U test was used to compare tTGlgA values and Spearman's rank coefficient for correlation of tTGlgA, anti-rTria19, and WlgE values. GraphPad Prism® version 5.00 for Windows, GraphPad Software, San Diego, CA, USA was used for the latter and for ROC curve generation.

Results

Data from Longitudinal Analysis (2010, 2012, 2014, 2016)

tTGlgA Evaluation

A total of 3,548 analysis for tTGlgA have been requested for a total of 2,965 patients during the 4 years analyzed. The number of tTGlgA requests increased by 133% while the number of patients that consulted our adult allergology/immunology outpatient facilities (data from other departments were not available, but likely similar) increased by 141% between 2010 and 2016. The mean age was 29 years (range 0–99) and did not change significantly during the study (26, 28, 31, and 29 for 2010, 2012, 2014, and 2016 respectively), F/M ratio was 1.4/1. The rate of positivity of other antibodies (i.e., IgE towards minor and major grass pollen recombinant allergens, Phadiatop, anti-TPO, ANA) and total IgA did not show a particular trend (data not shown).

Table 1. Characteristics of patients with positive tTGlgA 2010, 2012, 2014, and 2016

Patients, total	128
Gender, F/M	72/56
Leading symptom	
Bloating/cramps	25 (20)
Vomiting/nausea	3 (2)
Stool changes	15 (12)
Non-GI	4 (3)
Anaphylaxis	1 (1)
Other	62 (48)
N/A	18 (14)
tTGlgA value, mean, units	91 (7.1–820)
Age at first positive tTGlgA, mean, years	19 (1–88)
Number of patients with total IgA above normal range	21 (16)
Number of patients with low total IgA	6 (5)
Biopsy	
Consistent with CD	61 (48)
N/A	21 (16)
Not performed	26 (20)
Not consistent	19 (15)
Physician diagnosis	
CD	69 (54)
N/A	39 (30)
Other GI disease	9 (7)
Non GI disease	10 (8)
IBS	1 (1)
Associated autoimmune and other conditions	
Thyroiditis	10 (8)
Diabetes type 1	17 (13)
SLE	2 (2)
Dermatitis herpetiformis	2 (2)
Castleman disease	1 (1)
Eosinophilic esophagitis	1 (1)
Antiphospholipid syndrome	1 (1)
Rheumatoid arthritis	1 (1)

Values are presented as *n* (%) or mean (ranges).

CD, celiac disease; tTGlgA, anti-tissue transglutaminase IgA; GI, gastrointestinal; SLE, systemic lupus erythematosus; N/A, non-available; IBS, irritable bowel syndrome.

Despite an increasing number of tTGlgA requests, the number of positive results decreased during the last years (Fig. 1a) resulting in a substantial decrease in the positivity rate from 8.8% (64/731) in 2010 to 3.5% (34/973) in 2016. A total of 128 patients showed at least once a positive anti-tTGlgA. Among these, 69 patients were eventually diagnosed with CD by the gastroenterologist (2.3% of the initially 2,965 tested; 61 had a consistent histology). Characteristics of tTGlgA-positive patients are shown in Table 1. Type 1 diabetes was the most frequent associated disease and also a major reason for tTGlgA testing.

Table 2. Characteristics of patients with positive WlgE between 2010 and 2016

Patients, total	48
Gender, F/M	15/33
Leading symptom	
Bloating/cramps	2 (4)
Vomiting/Nausea	3 (6)
Stool changes	2 (4)
Non-GI	13 (27)
Anaphylaxis	13 (27)
Other	11 (23)
N/A	4 (8)
WlgE value, mean, units	28 (0.53 to >100)
Number of patients with WlgE >100 kU/L	7 (15)
Age at first positive WlgE, mean, years	9 (0–63)
Age at first positive WlgE, median, years	3
Number of patients with age >18 years old	8 (17)
Number of patients with total IgA above normal range	3 (6)
Number of patients without IgA testing	36 (75)
Number of patients with low total IgA	0 (0)
Biopsy	
Consistent with CD	0 (0)
N/A	2 (4)
Not performed	42 (87)
Not consistent	4 (8)
Physician diagnosis	
WA	23 (48)
N/A	2 (4)
Other GI disease	3 (6)
Non GI disease	20 (42)
IBS	0 (0)
Oral food challenge	
Positive	12 (67)
Doubtful	2 (11)
Associated condition	
Eczema	24 (50)
Food allergy	21 (44)
Eosinophilic esophagitis	2 (4)
Asthma	8 (17)

Values are presented as *n* (%) or mean (ranges).

CD, celiac disease; tTGlgA, anti-tissue transglutaminase IgA; GI, gastrointestinal; N/A, non-available; WA, wheat allergy; WlgE, wheat IgE.

WlgE Evaluation

WlgE was tested far less frequently than tTGlgA, and in younger patients (mean age 20, range 1–79). F/M ratio was 1/1. The number of requests and of positive results (approximately 50%) were stable over the years (Fig. 1b).

Table 3. Amount of *analysis* (*italic*) and number of patients tested for different antibodies alone or in combination with tTGlgA between 2010 and 2016. For IgA, amount of *results* <0.7g/L and patients with values <0.7g/L respectively are given instead of positivity

Analysis	Positive sera/No. sera analyzed, %	Number of patients with tTGlgA assessed concomitantly	Percent of patients with both anti-bodies positive	Number of positive patients among tTGlgA positive patients, %	Number of positive patients among tTGlgA negative patients, %	p value
WlgE	61/123 (49.6)	36	0	0 (N/A)	9 (25)	1
Anti-rTria19	19/121 (15.7)	35	0	0 (N/A)	2 (5.7)	1
Anti-rPhlp1;rPhlp5	498/658 (75.7)	74	1.4	1 (100)	50 (68.5)	1
Anti-rPhlp7;rPhlp12	108/556 (19.4)	60	0	0 (0)	10 (16.9)	1
Anti-ATPase	206/921 (22.3)	92	2.2	2 (100)	29 (32.2)	0.11
Anti-IF	124/2,407 (5.1)	295	0.3	1 (10)	14 (4.9)	0.41
Anti-TPO	762/3,636 (20.1)	703	2.3	16 (23.9)	117 (18.4)	0.28
Anti-LKM	14/2,043 (0.7)	215	0	0 (0)	0 (0)	1
Anti-mitochondrial	143/1,901 (7.5)	182	0	0 (0)	13 (7.3)	1
Phadiatop	636/1,848 (34.4)	334	1.8	6 (42.9)	99 (30.1)	0.35
ANA	5,873/22,185 (26.5)	1,626	1.0	17 (37.8)	367 (23.2)	0.02
Anti-Ro	1,233/2,010 (61.3)	159	1.3	2 (40)	65 (42.2)	1
Anti-La	527/1,970 (26.8)	158	1.3	2 (40)	32 (20.9)	0.29
IgA below 0.7 g/L	8,101/36,854 (22.0)	4,528	0.5	24 (14.4)	548 (12.6)	0.49

Values are presented as *n* (%) or mean (ranges).

Because no patients in this group (zero division).

N/A, non-available; tTGlgA, anti-tissue transglutaminase IgA; WlgE, anti-wheat IgE; ANA, anti-nuclear antibodies; Anti-LKM, anti-liver kidney microsome; Anti-IF, anti-intrinsic factor; Anti-TPO, anti-thyroperoxidase; Anti-ATPase, anti-adenosine triphosphatase (Gastric Parietal Cell Antibodies); Anti-rTria19, anti-triticum aestivum (wheat component); Anti-rPhlp1/5/7/12, anti-phleum pretense (grass pollen component).

Assay and Cut-Off Evaluation

Using data collected from the medical charts for patients with positive tTGlgA, values of tTGlgA of patients with CD were compared to those of patients without CD.

Using data of patients tested by ELISA only, patients with a final diagnosis of CD had significantly higher tTGlgA values (mean 130 U, min 21 U, max 820 U) compared to those with another diagnosis (mean 42 U, min 20 U, max 47 U). Values >240 U, present in 4 patients, were invariably associated with the diagnosis of CD (Fig. 2).

Further, a comparison of the sensitivity and specificity of the tTGlgA/total IgA ratio versus the currently used, simple tTGlgA positivity was performed in positively tested adults or in children under the age of 6 years. ROC curve generated area under the curve (AUC) showed similar test performance, indicating that the tTGlgA/total IgA ratio was not better in identifying patients with CD (Fig. 3).

Non-Longitudinal Analysis of Other Antibodies Using Data from All Available Years (2010–2016)

Wheat IgE

As the number of patients tested for WlgE was low, patients assessed in 2011, 2013, and 2015 were included

in the analysis. Altogether, between January 1 2010 and December 31 2016, 123 WlgE analyses have been performed. Sixty-one of them were positive, relating to 48 patients (Table 2). Non-gastrointestinal symptoms and anaphylaxis were the most frequent reasons for WlgE testing. Only 8 patients were older than 18 years. None had low total IgA defined by values below 0.7 g/L. WA was the most frequent physician's diagnosis, and eczema the most frequent associated condition.

Analysis of the Association between WlgE and Other Antibodies

Forty patients have been tested for both anti-Tria19 and WlgE, while 121 anti-rTria19 assays were performed for 118 patients and 19 were positive. Among these, all pediatric patients had concomitantly positive WlgE and all had a diagnosis of suspected WA, while all adult patients were not tested concomitantly for WlgE and all except one had a diagnosis of suspected WDEIA. Values of anti-Tria19 and WlgE were correlated ($r = 0.79$; $p < 0.001$). None of the patients with positive WlgE has been tested for ANA, this population being predominantly pediatric.

Further, patients tested for both grass pollen (rPhlp1;rPhlp5 or rPhlp7;rPhlp12) and WlgE were analyzed. Only 3 patients were found, one had both antibodies positive, 2 had both antibodies negative. Association analyses of other auto-antibodies with WlgE were not performed because of too small numbers of patients tested for the latter.

Analysis of the Association between tTGlgA and Other Antibodies

Using data from 2010 to 2016, 36 patients have been assessed for both WlgE and tTGlgA, none of them had positive tTGlgA, 9 had single WlgE positivity. WlgE and tTGlgA values were negatively correlated ($r = -0.34$; $p = 0.04$).

Among all the patients tested for both anti-TPO and tTGlgA, more than 2% had both antibodies, indicating a possible association of the 2 conditions when compared to the general population with a prevalence of tTGlgA of approximately 1% (OR 1.39; 95% CI 0.77–2.52), despite the lack of a statistically significant difference.

The association between tTGlgA and different antibodies was tested, and ANA was found to be significantly more frequent in patients with positive tTGlgA (OR 2.01; 95% CI 1.09–3.71; Table 3).

An assay for DGPigG has been introduced in our laboratory in January 2016 for patients with negative tTGlgA and low IgA levels. Fifty-three patients were tested for this antibody and tTGlgA; 3 (5.7%) had single DGPigG positivity. Among these 3, 2 had low IgA (0.13 g/L for 1 patient and 0.08 g/L for the other patient); both were 3 years old at the time of testing.

IgA Analysis

Concerning total IgA levels, 36,854 analyses have been performed (for 17,457 adult and pediatric patients). A value of 0.7 g/L, which is the lower limit of the normal range for adults, was used to define low IgA among all patients because tTGlgA normal values are not age-adjusted. Using the cut-off of 0.7 g/L, among the 36,854 analyses, 8,101 (22%) showed an IgA below 0.7 g/L. Among all patients tested for IgA levels (in average tested 2.1 times), 2,175 patients (12.5%) had IgA levels below 0.7 g/L at least once. A second analysis, using the consensus limit of 0.07 g/L for IgA deficiency, allowed to identify 160 patients among 17,457 investigated (0.9%) that fell into this category.

A total of 4,528 patients have been tested for both IgA and tTGlgA. Low IgA levels were found in 12.6% among patients tested but negative for tTGlgA and 14.4% among

patients with positive tTGlgA (OR 1.17; 95% CI 0.75–1.82), the difference being not significant (Table 3). tTGlgA positivity rate was 4.2% among patients with low IgA levels compared to 3.6% among patients with normal or high IgA levels.

Discussion

Over the last few years, the number of tTGlgA requests increased in our center. However, the number of patients having positive tTGlgA decreased linearly. To our knowledge, despite studies showing a rise in the incidence of CD [3, 12], this is the first study showing a decreasing trend for a marker of CD, possibly reflecting the impact of current alimentary trends on clinical practice. In fact, the vast majority of tTGlgA requests were for individuals tested for the suspicion of CD, not yet known to have CD and as such, having the same diet as the general population. As mentioned earlier, the general population is now consuming increasingly gluten-free food, without an apparent medical reason and with a marked annual growth rate of 10%. One major novelty of our study consists in our data showing a decrease of tTGlgA positivity rate. Assuming a stable CD prevalence during the short study period, lack of analytical bias (the same test was used until January 2016, and internal and external quality controls were regularly performed), unchanged propensity to test for tTGlgA as shown by the fact that the increase in the number of tTGlgA requests paralleled the number of outpatients seen in our clinic, we conclude that a gluten-free diet, as adopted by an increasing larger part of the general population, could possibly explain our longitudinal results. On the long term, the decrease of gluten ingestion could have a bigger impact on the prevalence of CD than of WA (Fig. 4).

In this study, patients with CD had significantly higher levels of tTGlgA than patients with other disorders and levels above 240 U were pathognomonic. This finding is similar to other validation studies proposing to omit biopsy for children with tTGlgA >10-fold the upper normal limit, when associated with other criteria [5, 24, 27, 28].

An analysis of the association of low IgA or IgA deficiency with CD in our study is limited by the fact that we did not have a comprehensive database of all patients with CD. However, we found more patients with tTGlgA positivity among patients with low IgA than among those with normal or high IgA levels, supporting a possible association between the 2 conditions. The rate of analyzed

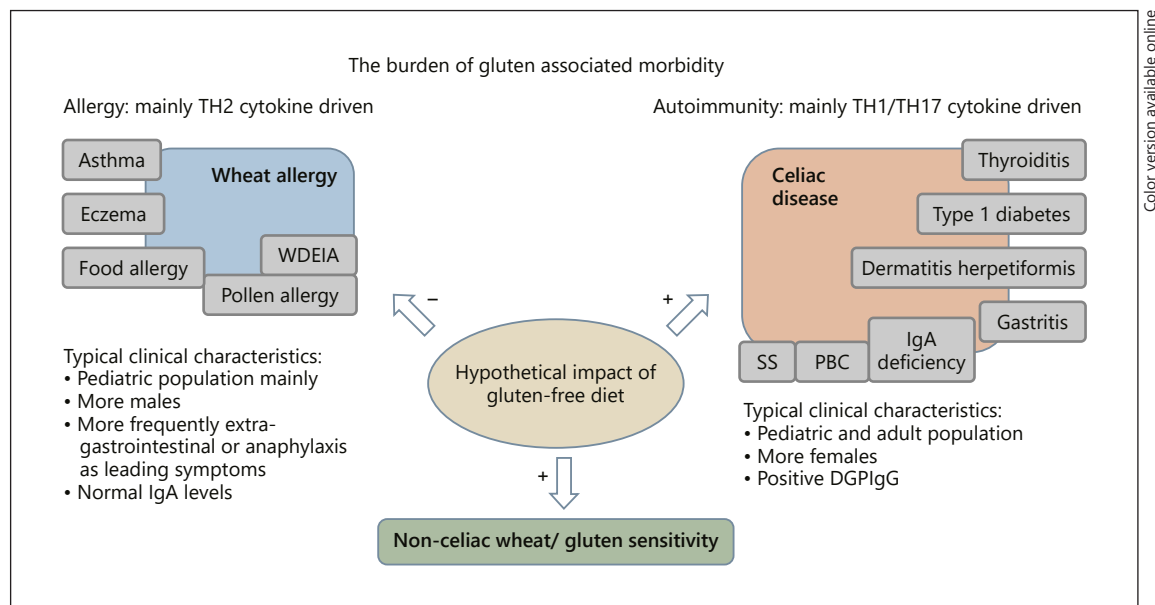


Fig. 4. WA and CD are 2 distinct conditions that have to be evaluated before considering NCGS. Associated conditions and typical clinical characteristics can help in the diagnostic work-up. Reduced gluten intake in the population might reduce the incidence of CD, but will have a negligible impact on WA. SS, Sjögren syndrome; PBC, primary biliary cholangitis; WDEIA, wheat-dependent exercise-induced anaphylaxis; DGPtIgG, anti-deamidated gliadin peptide IgG.

patients with IgA deficiency (below 0.07 g/L) was 0.9%, which is comparable to the one reported in literature [29].

It is worth noting that while IgA cut-off values are age-dependent, that is, infants having lower levels, those for tTGtIgA are not, leading to possibly false-negative results in this population [30, 31]. Accordingly, we analyzed whether a ratio of tTGtIgA/total IgA could perform better as a diagnostic test in this population. However, the AUC in our ROC curves generated with patients having positive tTGtIgA did not show any difference. These results need to be confirmed in prospective studies but for the moment, using age-uncorrected tTGtIgA values in children appears to be appropriate in suspected CD.

Differently than for tTGtIgA, analyses of WtIgE were only rarely performed and mainly in the pediatric population. Up to 50% of the tested patients were found positive for WtIgE, showing that this test was mainly used to confirm rather than to screen for WA. Moreover, 17% of the positive patients were older than 18 years.

WtIgE positivity did not decrease during the study period, in agreement with other studies showing a stable prevalence of food allergies [32]. Of course, there are multiple reasons other than abdominal symptoms linked to gluten ingestion for requesting tTGtIgA (i.e., newly diag-

nosed diabetes type 1, dermatitis herpetiformis, infertility). However, only 1% of patients tested for tTGtIgA have been tested for WtIgE, despite the fact that about 20% of patients tested for tTGtIgA initially presented with bloating or cramps, symptoms compatible with WA. This supports the hypothesis that screening for WA should be implemented, both in children and adults.

Concomitant autoimmune disease was not found in patients with positive WtIgE. Rather, male gender, eczema, other food allergies, and asthma were more frequently seen in association with positive WtIgE, partly confirming previous results [2, 33].

None of the 71 patients tested for WtIgE or anti-rTria19 concomitantly to tTGtIgA had positive tTGtIgA, so that patients with suspected WA or WDEIA do not seem particularly prone to have CD. The negative correlation found between WtIgE and tTGtIgA supports this concept.

Similarly, only 1 patient among 134 tested for anti-rPhlp1;rPhlp5 or rPhlp7;rPhlp12 concomitantly to tTGtIgA had positive tTGtIgA, so that grass pollen allergy does not seem to be associated with CD. Among all patients that were tested for both WtIgE and anti-rTria19, none had anti-rTria19 alone, so that WtIgE testing seems to have a high sensitivity for WDEIA, and testing for an-

ti-rTria19 can be reserved for those patients who had positive WlgE. This result makes sense considering that rTria19 is an allergen of the complete wheat extract. Moreover, no patient was found to have anti-rTria19 without anti-rPhlp1; rPhlp5, so that WDEIA appears to be linked to grass pollen allergy.

Our association analysis showed that the prevalence of tTGlgA-positive antibodies was doubled among patients with anti-TPO antibodies compared to the normal population. Despite the lack of a statistically significant association, this result supports the well-known association of CD with autoimmune thyroiditis [16, 17, 20].

Among the other antibodies, none were found to be significantly associated with the presence of tTGlgA apart from ANA. This confirms that a common pathogenic immune mechanism might be involved in both the development of tTGlgA and ANA [13]. It also underlines a common predisposition to develop autoimmune disease, as showed by the fact that about 10% of patients with positive tTGlgA in our study had either type 1 diabetes or an autoimmune thyroiditis. On the contrary, our study does not support the hypothesis of a link between atopy and CD, in agreement with previous studies [34, 35], nor of a link between food allergy and CD, as reported in recent studies [36].

In summary, wheat and in particular gluten ingestion in our population causes a significant burden of health-related discomfort, as shown by the significant amount of tTGlgA and WlgE requests. Our center performed more than 1 billion outpatient consultation in 2016. Our data shows that in about 0.1% of all consultations, CD was an issue, while WA was suspected almost exclusively in the pediatric population. Moreover, when facing patients with symptoms associated with gluten intake, atopy, male gender and non-gastrointestinal leading symptoms could point to WA compared to CD, which in turn should be

investigated in particular in the presence of an autoimmune disease or IgA deficiency. High levels of tTGlgA might help diagnose CD without endoscopy, while using a ratio of tTGlgA/total IgA does not improve the diagnostic performance, neither in the pediatric nor in the adult population. WA does not seem to be associated with CD and is probably under-diagnosed compared to CD.

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Statement of Ethics

The study protocol has been approved by the research institute's committee on human research. Ethical approval was obtained from Swissethics; study No. 2016-00663.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

D.S. conceived the study, obtained approval from Swissethics, collected and analyzed the data, and drafted the manuscript. C.B. and S.R. helped in collecting data from medical charts. J.C.C. and J.L.F. helped to draft the manuscript. P.R.L. designed the study and helped to draft the manuscript. All authors read and approved the final version.

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