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Accuracy in Diagnosis of Celiac Disease Without Biopsies in Clinical Practice

Short title: The non-biopsy approach to diagnose celiac disease

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Abbreviations:
CD Celiac Disease**; CI** Confidence Interval**; DGP** antibodies against deamidated gliadin peptides**; EMA** endomysium antibodies; **HLA** human leukocyte antigen**; T1DM** type 1 diabetes mellitus; **TGA** autoantibodies against tissue-transglutaminase; **PPV** positive predictive value**; ULN** upper limit of normal**;**

Authors' contribution:
K.J. Werkstetter (K.W.): study design & management, data acquisition, analyses & interpretation, writing of the manuscript
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Abstract

Background & Aims: The guidelines of the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition allow for diagnosis of celiac disease without biopsies in children with symptoms and levels of immunoglobulin A against tissue-transglutaminase (TGA-IgA) 10-fold or more the upper limit of normal (ULN), confirmed by detection of endomysium antibodies (EMA) and positivity for HLA-DQ2/DQ8. We performed a large, international prospective study to validate this approach.

Methods: We collected data from consecutive pediatric patients (18 years or younger) on a gluten-containing diet who tested positive for TGA-IgA from November 2011 through May 2014, seen at 33 pediatric gastroenterology units in 21 countries. Local centers recorded symptoms; measurements of total IgA, TGA, and EMA; and histopathology findings from duodenal biopsies. Children were considered to have malabsorption if they had chronic diarrhea, weight loss (or insufficient gain), growth failure, or anemia. We directly compared central findings from 16 antibody tests (8 for TGA-IgA, 1 for TGA-IgG, 6 for IgG against deamidated gliadin peptides, and 1 for EMA, from 5 different manufacturers) 2 HLA-DQ2/DQ8 tests from 2 manufacturers, and histopathology findings from the reference pathologist. Final diagnoses were based on local and central results. If all local and central results were concordant for celiac disease, cases were classified as proven celiac disease. Patients with only a low level of TGA-IgA (3-fold or less below the ULN) but no other results indicating celiac disease were classified as no celiac disease. Central histo-morphometry analyses were performed on all other biopsies and cases were carefully reviewed in a blinded manner. Inconclusive cases were regarded as not having celiac disease for calculation of diagnostic accuracy. The primary aim was to determine whether the non-biopsy approach identifies children with celiac disease with a positive predictive value (PPV) above 99% in clinical practice. Secondary aims included comparing performance of different serological tests and to determine whether the suggested criteria can be simplified.

Results: Of 803 children recruited for the study, 96 were excluded due to incomplete data, low level of IgA, or poor-quality biopsies. In the remaining 707 children (65.1% girls; median age, 6.2 years) 645 were diagnosed with celiac disease, 46 were found not to have celiac disease, and 16 had inconclusive results. Findings from local laboratories of TGA-IgA 10-fold or more the ULN, a positive result from the test for EMA, and any symptom identified children with celiac disease (n=399) with a PPV of 99.75 (95% CI, 98.61–99.99); the PPV was 100.00 (95% CI, 98.68–100.00) when only malabsorption symptoms were used instead of any symptom (n=278). Inclusion of HLA analyses did not increase accuracy. Findings from central laboratories differed greatly for patients with lower levels of antibodies, but when levels of TGA-IgA were 10-fold or more the ULN, PPVs ranged from 99.63 (95% CI, 98.67–99.96) to 100.00 (95% CI, 99.23–100.00).

Conclusions: Children can be accurately diagnosed with celiac disease without biopsy analysis. Diagnosis based on level of TGA-IgA 10-fold or more the ULN, a positive result from the EMA tests in a second blood sample, and the presence of at least 1 symptom could avoid risks and costs of endoscopy for more than half the children with celiac disease

worldwide. HLA analysis is not required for accurate diagnosis. Clinical Trial Registration no: DRKS00003555

KEY WORDS: ESPGHAN; non-biopsy approach; autoimmunity; ProCeDE study

Introduction:

Celiac disease (CD) is an autoimmune disorder triggered by gluten and related prolamines in genetically susceptible individuals carrying the HLA-DQ2 and/or -DQ8 alleles[1]. CD is characterized by enteropathy and presence of CD-specific autoantibodies against tissue-transglutaminase (transglutaminase type 2, TGA) and endomysium (EMA). The prevalence of CD in Europe and North-America is about 1-2%[2], with higher rates in first-degree relatives of CD patients and individuals with associated disorders such as type 1 diabetes mellitus or trisomy 21[3].

Until 2012 the histological proof of villous atrophy on small bowel biopsies was obligatory for the diagnosis of CD. During the last decade unambiguousness of histopathology was questioned[4-6], while a strong correlation between TGA titer levels and severity of mucosal lesions was recognized[7].

In 2012 the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) published new diagnostic criteria for CD[1].

These criteria gave pediatric gastroenterologists the option to diagnose CD without biopsies in children with symptoms indicative for CD, serum TGA-IgA titers above 10 times upper limit of normal (≥10xULN) in a calibration-curve-based test, positive EMA-IgA in a second blood sample and positive HLA-risk alleles. The evidence for this approach was mostly based on retrospective data or small single-center studies.

Our Prospective Celiac Disease Diagnostic Evaluation study (ProCeDE) aimed to evaluate in a multi-center setting whether this non-biopsy approach allows a correct diagnosis in clinical practice with a positive predictive value above 99% when all required conditions are fulfilled.

Secondary aims included determining the accuracies of various TGA-tests and their reliability to predict CD if levels are ≥10xULN as well as the impact of HLA-typing, EMA-IgA, and type of symptoms on CD diagnosis without biopsies.

Methods:

Study design and participants:

From November 2011 to May 2014, 33 pediatric gastroenterology units from 21 countries (Europe, Middle East) recruited consecutive patients <19 years on a gluten-containing diet, with positive TGA results analyzed in their own or external laboratories. Exclusion criteria comprised refusal to duodenal biopsies, primary or secondary immunodeficiency, malignancy or previous diagnosis of CD. Recruited patients were excluded from the analysis if local and central HLA-results were unavailable, serum or histology slides were not provided for central assessment, biopsies were unreadable due to poor quality, total IgA was low, inclusion criteria were violated or consent was withdrawn.

Local work up

Obligatory diagnostic work-up at the local site included serology (total IgA, TGA, EMA) and histopathology from duodenal biopsies. Collected data comprised family, medical and dietary history, symptoms, physical examination, basic laboratory parameters, most recent local TGA- and EMA-IgA results including date of measurement and name of test-kit/manufacturer with respective upper limit of normal (ULN) (***supplementary tables S1, S2***), local HLA-typing for DQ2/DQ8 if performed, endoscopy findings, histopathology including Marsh-Oberhuber staging[8,9] and local diagnosis (CD, no CD, unclear). Data entry was completed into study database before central analysis started. Local serology should have been done maximum two weeks prior to or at biopsy. Serum for central laboratory, DNA, and histology slides were collected at time of biopsy.

A child was considered to have low/deficient total IgA if serum concentration was <0.25 g/l, negative TGA-IgA but positive IgG-based antibodies ***(see supplement 1.8).***

According to clinical presentation patients were stratified in three groups: malabsorption symptoms, other clinical symptoms and no symptoms.

Malabsorption was considered with at least one of the following symptoms: chronic diarrhea, weight loss or insufficient gain, growth failure and anemia (hemoglobin below reference value for age and sex).

Central analyses

All investigators performing central analyses were blinded towards available local and central results. Overall 16 antibody tests (eight TGA-IgA, one TGA-IgG, 6 DGP-IgG, and one EMA) from five different manufacturers were analyzed head-to-head (***supplementary 1.5.2, tables S3, S4)***. Details and results on DGP-IgG tests are shown in the supplementary tables only.

Immunofluorescent analysis of EMA-IgA was performed by one experienced technician (G.H.) with serum dilutions of 1:5, 1:10, 1:100, 1:1000, and 1:2.5 if 1:5 dilution was negative. A signal in 1:2.5 dilution or higher was considered positive **(*supplementary 1.5.1*)**.

All tests were performed according to manufacturers' instructions in a single run either on automated, calibrated ELISA systems (EUROIMMUN Analyzer I) or on the respective automatized systems (Phadia250, Thermo Fisher; QuantaFlash, INOVA). Standard curves were available for all tests. Two different HLA-DQ2/DQ8-typing approaches were applied (***supplementary 1.6***) and results stratified in five HLA risk groups[10,11]. Negative HLA-status was defined if none of the CD related risk alleles or only alleles encoding the α-subunit (without the corresponding β-subunit) of DQ2 and/or DQ8 were present[12]. In patients with negative HLA status but positive central serology and histopathology, a 3rd HLA-typing for rare risk alleles was performed from a new blood sample. If central HLA-typing was not possible for ethical or technical reasons, local results were used.

The reference pathologist reported histology on provided slides (hematoxylin-eosin and CD3+ immunostaining) including Marsh-Oberhuber-staging[8,9]. Unclear cases were blindly reviewed by a 2nd reference pathologist. If specimens were non-evaluable the paraffin embedded biopsy blocks were requested for reoriented cuttings and blindly evaluated including morphometry.

Central diagnosis

The final central diagnosis for each patient was 1) proven CD or 2) no CD or 3) inconclusive case. CD was proven if HLA-DQ2/DQ8, local TGA-IgA, local and/or central EMA-IgA were all positive, and both, local and reference pathologists reported at least Marsh 2 staging.

CD was excluded if HLA-DQ2/DQ8 was negative, local TGA-IgA below 3xULN, local and central EMA-IgA were negative and local and central pathologists reported Marsh 0 or 1.

Patients not meeting these criteria were initially considered as unclear and histopathology was revised as described above. The diagnostic committee reviewed each unclear case and voted in a Delphi process (***supplementary methods 1.9, figure S2****.).* If this did not allow a clear diagnosis, cases were finally regarded as inconclusive.

Criteria for CD diagnosis without biopsies

For local and central TGA levels the multiple of the respective upper limit of normal (ULN) was calculated and stratified into high positive (≥10xULN) or low to moderate positive (>1 to <10xULN). For tests with a given grey zone the lower bound was used as ULN. To evaluate whether the non-biopsy approach would contradict the final central diagnosis, we considered the combination of high local TGA, positive local EMA-IgA, positive central HLA-status, and symptoms. Furthermore, the diagnostic accuracies of high central TGA (≥10xULN) for each included commercial kit alone and in combinations with HLA-status, EMA results and symptoms were calculated against the central diagnosis as reference.

Study oversight

The study was approved by the ethics committees of each participating center. Written informed consent was obtained by legal guardians and patients as appropriate for age. The study was co-funded by industry (EUROIMMUN Medizinische Labordiagnostika AG, Eurospital, INOVA Diagnostics, R-Biopharm, Phadia/Thermo-Fisher, Dr. Schär GmbH) and non-profit organizations (ESPGHAN, AOK Bayern health insurance, Celiac Disease patient organizations from Denmark, Finland, Germany,

Hungary, Italy, The Netherlands and The United Kingdom). Funding partners were not involved in study design, recruitment, data collection, analysis and interpretation or writing of the manuscript.

All authors had access to the study data and reviewed and approved the final manuscript. ProCeDE is registered at the German Registry for Clinical Trials, Reg-No DRKS00003555.

Statistical analyses

With 701 participants the study had 80% power at 5% significance level to detect a PPV of more than 97% for most test scenarios. Assuming an estimated ratio (PPV) ≥99% and using the exact binomial distribution a sample size of 348 with power of 86.1% was calculated.

When sequential test design was considered (by ADDPlan Software, Cologne, Germany), the needed number increased to 357. The interim analysis with the first 200 patients showed that the proportion of cases potentially qualifying for omitting biopsies with local parameter ranged between 50-65%. Therefore we planned to recruit a minimum of 700 patients.

Mean and standard deviation (SD) or median and range and frequency in % were indicated.

For main analysis of diagnostic accuracies, all inconclusive cases were considered as no CD, or were excluded in a subsample analysis.

Sensitivity, specificity, PPVs and positive likelihood ratios for different scenarios (TGA ≥10xULN alone and in combination with other criteria) were calculated with 95% confidence intervals (CI) using binominal distribution (Copper-Pearson CI). Sensitivity expresses the proportion of patients qualifying for the non-biopsy approach.

All statistical analyses were done by B.F. and K.W. using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

Results:

Of 968 eligible patients 803 (83.0%) were recruited. Ninety-six patients were excluded, thereof 36 due to non-evaluable histology and 17 due to low total IgA (***figure 1, supplementary tables S5, S6, S7***). From one center, all 12 patients had to be excluded due to incomplete sample sets. In the final cohort (N=707), 399 patients (56.4%) qualified for the non-biopsy approach according to ESPGHAN-guidelines. Basic characteristics are shown in ***table 1***.

In 29 patients, local TGA-IgA was negative at time of biopsy but all had positive TGA-IgA before referral (***supplementary table S8***). Local EMA-IgA was available in 681 and central EMA-IgA in 704 patients. Forty-five patients (7.6%) were biopsied with capsule. In those undergoing endoscopy macroscopic findings were reported on a standardized questionnaire in 653 patients. Erosive esophagitis was present in 3.7%, but no case of eosinophilic esophagitis was reported. Gastric erosions were found in 3.2%, duodenal erosions in 6.3% and a duodenal ulcer in 0.3% of the patients. *Helicobacter pylori* status was available in 441 patients, thereof 21 (4.5%) were positive. The local pathologist provided Marsh classification in 676 cases. Compared to the central pathologist there was disagreement regarding the histological judgement of CD (Marsh 2 or 3) and no CD (Marsh 0 or 1) in 48/676 patients (7.1%) (***supplementary table S19, S20***). EurGenRisk-typing for HLA-DQ2/DQ8 was successful in 697 and EuroArray-typing in 696/698 DNA samples. For the other nine patients without central DNA sample, local HLA-typing was available and considered for analysis. In total, 18/707 patients were HLADQ2/DQ8 negative. For 2/18 patients with high suspicion of CD, the 3rd typing with new DNA material was HLAD2/DQ8-positive; the remaining 16 patients had no CD (***supplementary table S9***).

Central diagnosis in the final cohort (N=707) was proven CD in 645 (91.2%), no CD in 46 (6.5%) and inconclusive case in 16 (2.3%) patients (***supplementary table S10)***.

Sixty-four patients had tentatively started a gluten-free diet before the diagnostic work-up of CD, thereof 32 within 12 months prior to biopsy. All of them had a clear diagnosis of CD. None of the inconclusive patients had been on gluten-free diet before.

Diagnostic accuracy in clinical practice

Using the central diagnosis as reference, the diagnostic accuracies of local TGA-IgA ≥10xULN in combination with other criteria (scenarios) are shown in ***table 2***. Considering all 16 inconclusive cases as no CD, high local TGA-IgA as single criterion (scenario 1) revealed four false positive patients (0.56%), thereof 2 had T1DM. If EMA-IgA was included (scenario 4), two false positive patients remained (0.28%). HLA-results did not improve accuracies (scenario 4).

If all ESPGHAN criteria for the non-biopsy approach were fulfilled (***table 2***, scenario 5, 56.4% of the cohort) one patient with unspecific symptoms remained false positive. If only malabsorption symptoms would qualify (39.3% of the patients, scenario 6), the PPV increased to 100%.

In the subsample analysis excluding 16 inconclusive cases, one patient was false positive for TGA ≥10xULN (scenario 7 and 8). If malabsorption and/or EMA-IgA were included in the diagnostic decision no false positives were found (scenario 9-12).

Details on false positive patients are summarized in ***supplementary table S11.***

Diagnostic accuracy of central serology evaluations

PPVs for each central TGA result ≥10xULN (N=696 to 707) ranged between 99.63 [98.67; 99.96] and 100.00 [99.23; 100.00] (***figure 2)***. The prevalence of high TGA results varied between 22.64 [19.46; 26.06] and 83.57 [80.48; 86.34] ***(supplementary table S12)***. Tests T4 and T6 did not reach a PPV of ≥99% for the lower bound of the 95% CI due to respectively one and two additional false positive patients, thereof one child with T1DM. If malabsorption symptoms were considered for the decision, or if inconclusive cases were excluded, no false positive was found.

For the DGP-IgGs ≥10xULN the specificity was high (one false positive) but sensitivity was low, for details see ***supplementary table S13, figure S3***.

Discussion:

The results of our prospective multi-center diagnostic evaluation study ProCeDE show that the ESPGHAN non-biopsy approach allows a correct diagnosis of CD. At least 50% of affected children in clinical practice will benefit from this non-biopsy approach which reduces burden and risks of endoscopy and anesthesia while saving costs for health care systems[13]. This ensuring conclusion was achieved in spite of using local results of a large variety of different TGA and EMA tests, which were performed in many laboratories in very different settings and countries.

Since the publication of the current ESPGHAN-guidelines, several studies investigated if CD can be correctly diagnosed without biopsies, both in children and adults[7,13-29]. The majority were of retrospective nature, done by single centers, applied only one or few TGA tests and used histopathology as only reference standard for diagnosis. These studies had a high risk of selection bias excluding inconclusive cases and not acknowledging the limited inter-pathology agreement[4-6,30]. Our finding with discordance regarding CD diagnosis between local and central pathologists questions histopathology as reference standard in validation studies and supports our approach to build the reference diagnosis on concordant results of different diagnostic tests. There are concerns regarding the concept of using the same threshold (10xULN) of non-standardized tests with recognized inter- and intra-test variability as criterion to omit biopsies for CD diagnosis[31]. As this approach gives quantification of TGA concentrations a large weight, type and quality of serology tests are crucial and calibration curves allowing linear calculation of results are obligatory[1]. In the ProCeDE-study nine different TGA tests were centrally used, seven of them reliably predicted CD with a PPV of 100% with titers ≥10xULN and at even lower levels. This raises the question to further lower the threshold. However, the central lab had one standardized system following all manufacturers' instructions, using the same calibration curves on automatized machines with fixed settings, involving the same lab technicians. In practice, inter-lab variability is high[15,32] which we confirmed when comparing central and local results of the same manufacturer (***supplementary fig S6, table***

S15). In our study 10 different TGA-IgA-tests were used by the local laboratories of the 32 centers, with only four patients with high TGA-IgA levels ≥10xULN being false positive. This strongly supports that the current ESPGHAN criteria are robust in clinical practice. However, accounting for the inter- and intra-lab variabilities and the lack of standardization among TGA-IgA-tests and laboratories[15], we recommend against lowering this threshold and keeping the 10xULN as one criterion for the non-biopsy approach.

Our data revealed that HLA-typing for DQ2/DQ8 does not improve accuracy of CD diagnosis without biopsies and can be omitted for this purpose. All patients with TGA-IgA ≥10xULN and positive EMA carried HLA risk alleles. Only two of 645 CD patients had initially a negative HLA-status, both were later reliably identified as having initially false negative HLA-results. Inter-test agreement was close to perfect between the two HLA tests used (***supplementary S16)***. Negative results for HLA DQ2/DQ8 in patients with TGA or EMA positivity are most likely false negative caused by mixing up blood samples or due to very rare risk allele combinations not recognized by the test systems[1,33,34].

A positive EMA result as obligatory criterion for the non-biopsy approach is still debated. EMA is more specific than TGA and DGP testing[35], but immunofluorescence requires an experienced examiner[36]. As expected, sensitivity (proportion of patients qualifying for the non-biopsy approach) varied between participating centers. In concordance with previous studies[18,19,21,37] inclusion of EMA improved the positive LR and the PPV. Our results support the use of EMA as confirmatory test when CD is diagnosed without biopsies.

The ESPGHAN criteria also request the presence of symptoms for the non-biopsy approach. Symptoms of malabsorption increase the pre-test probability for CD compared to less specific complains such as abdominal pain and thereof the post-test probability of a given serological result. This is indicated by a higher PPV and positive LR as shown in the scenarios 1, 2 and 3 (***table 2***)[16,17,21,23]. Transient TGA-IgA positivity occurs in persons at genetic risk for CD, particularly those with T1DM[38], although TGA-IgA levels are mostly low. False positive moderate or even high titer levels are more

likely when serologic tests with a steeper calibration curve are applied (T4 and T6 in the central lab). A recent population based screening study in Swedish schoolchildren suggested that the non-biopsy approach is also safe to diagnose CD in the absence of symptoms[24]. The number of 80 asymptomatic children in our study, particularly those with T1DM, was too low to draw valid conclusions.

There is some concern that the non-biopsy approach may result in clinically relevant missed co-morbidities such as gastroesophageal reflux disease, eosinophilic esophagitis or *Helicobacter pylori* infection related complications [39]. However, our data suggest that the frequency of pathologic findings unrelated to untreated celiac disease is rare and most likely not higher than in the general population (*supplementary manuscript*).

The main strength of our study is the large prospective cohort recruited in a variety of clinical centers from different countries and settings, which truly reflects clinical practice. Further advantages comprise detailed assessment of medical history and clinical symptoms, the large panel of local and central laboratory tests including central EMA-IgA, two HLA-typing tests, and central reference pathology. In contrast to previous studies we did not rely on local histopathology as "gold standard", we based the diagnosis on concordant diagnostic test results and implemented a careful work-up and review process of initially unclear cases including re-cuttings and a blinded morphometric analysis. Our study showed the complexity and pitfalls occurring in the diagnostic work-up of children with suspected CD. We considered inconclusive cases as a separate group to transparently reflect that a clear diagnosis or exclusion of CD is not always possible.

As a limitation, not all eligible patients were recruited, the majority due to general concerns towards study participation (n=81). Eleven patients with initially positive TGA-IgA in external laboratories were re-tested for TGA-IgA before considering endoscopy and not confirmed to have autoimmunity and therefore not included. In only 22 patients the reason for not being recruited was refusal towards biopsy, which may bear a risk for bias but does overall minimally influence the proportion of children qualifying for the non-biopsy approach. Furthermore, some recruited children were

excluded due to missing samples or data (n=24) or insufficient quality of histology specimen (n=36). Re-evaluation of initially inconclusive cases was only possible when paraffin blocks were available. As the main reasons for non-recruiting or excluding patients seem to be random and independent from our main outcome, we consider a low risk for selection bias within our cohort.

We conclude from our results that the new ESPGHAN diagnostic criteria allowing omission of biopsies enables a correct diagnosis of CD in symptomatic children if TGA-IgA levels exceed 10xULN and positive EMA-IgA confirms celiac disease autoimmunity in a second blood sample. If one of these criteria is not fulfilled, biopsy should be performed to confirm the diagnosis. HLA-typing for DQ2/DQ8 does not contribute to the accuracy of this two-step approach and therefore is not necessary in these children.

Figure 1: *Flow-chart of eligible, recruited and excluded patients and central diagnosis of final cohort (N=707); for the non-biopsy approach local serology results have been considered; in total 96 patients had been excluded, thereof 36 due to non-evaluable histopathology and 60 due to other reasons*

Figure 2: *Positive predictive value (PPV) with 95% confidence interval (grey shaded) for CD diagnosis for each central TGA-serology, including eight TGA-IgA tests (T1 to T8) and one TGA-IgG test (T9), all with calibration curve based result calculations. The x-axis shows the multiple of the respective limit of normal according to the manufacturers' instructions (all truncated at 10xULN), the y-axis shows the PPV. Please see table 3 for the names and manufacturer of each test.*

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Table 1: *General characteristics of the final cohort (N=707), frequencies in %, age [yrs] in median (minimum; maximum); stratified in three groups according to clinical manifestation: patients with malabsorption symptoms[#], other clinical signs and symptoms* or no symptoms;*

	Patients by clinical manifestation			**Total**	
Basic characteristics	**Malabsorption symptom(s)[#] N=384-405[&]**	**Other symptom(s)* N=208-222[&]**	**No symptoms N=76-80[&]**	**N**	
Age [yrs] median (min;max)	5.0 (0.7;18.0)	7.6 (1.1;18.5)	8.4 (2.4;18.6)	**707**	**6.2 (0.7;18.6)**
Female [%]	61.2	72.1	65.0	**707**	**65.1**
Risk factors of CD				**N**	**%**
1st degree relative [%]	13.0	14.5	53.2	**693**	**18.0**
2nd degree relative [%]	7.6	11.1	9.2	**668**	**8.8**
Type 1 Diabetes mellitus [%]	4.7	12.6	22.5	**705**	**9.2**
Autoimmune Thyroiditis [%]	1.3	4.2	2.5	**690**	**2.3**
Down Syndrome [%]	1.5	0.0	2.5	**705**	**1.1**
Turner Syndrome [%]	0.0	0.4	1.3	**707**	**0.3**
Gluten consumption				**N**	**%**
Daily [%]	95.2	92.3	94.9	**677**	**94.2**
≥ 3 to 4 times / week [%]	4.1	6.8	3.8	**677**	**4.9**
1 to 2 times / week [%]	0.8	1.0	1.3	**677**	**0.9**
Basic laboratory parameters				**N**	**%**

Hemoglobin < reference for age [%]	28.6	0.0	0.0	**686**	**16.5**
Albumin < reference for age [%]	10.0	5.8	2.0	**531**	**7.9**
ALT > reference for age [%]	9.8	5.5	7.0	**613**	**8.2**
TPO > reference for age [%]	12.0	13.4	5.6	**160**	**11.9**
HLA-risk group[‡§]				**n**	**%**
1	32.4	23.4	27.5	**205**	**29.0**
2	8.6	10.4	2.5	**60**	**8.5**
3	44.2	45.9	42.5	**315**	**44.5**
4	6.2	4.1	6.3	**39**	**5.5**
5[$]	8.6	16.2	21.2	**88**	**12.5**

[#] *malabsorption symptoms: diarrhea, weight loss or insufficient weight gain, growth failure, iron deficiency anemia*

** other clinical signs and symptoms: abdominal pain, constipation, abdominal distention, flatulence, vomiting, anorexia, fatigue, irritability/moodiness, lack of concentration, and in children >12 yrs: delayed puberty, amenorrhea*

[&] *N of patients for whom data are available vary between the different listed characteristics*

[‡] *HLA risk groups were defined as follows: group 1 is associated with the lowest risk and included DR3–DQ2/DR3–DQ2 (DQ2.5/DQ2.5) and DR3–DQ2/DR7–DQ2 (DQ2.5/DQ2.2); group 2 DR7–DQ2/DR5–DQ7 (DQ2.2/DQ7); group 3 DR3–DQ2/DR5–DQ7 (DQ2.5/DQ7), DR3–DQ2/DR4–DQ8 (DQ2.5/DQ8), and DR3–DQ2/other (DQ2.5/other); group 4 DR7–DQ2/DR7–DQ2 (DQ2.2/DQ2.2), DR7–DQ2/DR4–DQ8 (DQ2.2/DQ8), and DR4–DQ8/DR4–DQ8 (DQ8/DQ8); and group 5 which is associated with a very low or no risk for CD includes DR7–DQ2/other (DQ2.2/other), DR4–DQ8/DR5–DQ7 (DQ8/DQ7),and DR4–DQ8/other (DQ8/other); "other" refers to any HLA-DQ haplotype except DR3–DQ2, DR7–DQ2, DR4–DQ8,or DR5–DQ7. F*

[§] *based on results from Eu-Gen-typing (Eurospital) for 697 patients, on EUROarray (Euroimmun) for one patient and for local HLA typing results for nine patients*

[$] *thereof in 16 patients none of the CD related risk alleles or only alleles encoding the α-subunit (without the corresponding β-subunit) of DQ2 and/or DQ8 were present and were therefore regarded as HLA-DQ2/DQ8 negative*

Table 2: *Diagnostic accuracies with 95% CIs to diagnose CD based on local TGA-IgA tests in combination with other criteria, either considering inconclusive cases as no CD (scenario 1-6, N=707) or excluding inconclusive cases (scenario 7-12, N=691); scenario 5 and 11 correspond to the current ESPGHAN criteria for the non-biopsy approach; TP=true positive, FP=false positive, FN=false negative, TN=true negative, PPV=positive predictive value, LR+=positive likelihood ratio*

Scenario	N	Combination	TP	FP	FN	TN	Sensitivity° [95%CI]	Specificity [95%CI]	PPV [95%CI]	LR+ [95%CI]
1	707	**Local TGA≥10xULN**	458	4	187	58	71.01 [67.34; 74.48]	93.548 [84.30; 98.21]	99.134 [97.80; 99.76]	11.01 [4.26; 28.43]
2	707	+ any symptom(s)	408	3	237	59	63.26 [59.40; 66.99]	95.161 [86.50; 98.99]	99.270 [97.88; 99.85]	13.07 [4.33; 39.49]
3	707	+ malabsorption[#]	286	1	359	61	44.34 [40.46; 48.27]	98.387 [91.34; 99.96]	99.652 [98.07; 99.99]	27.49 [3.93; 192.50]
4	707	**Local TGA≥10xULN + EMA* (+/- HLA[§])**	447	2	198	60	69.30 [65.58; 72.84]	96.774 [88.83; 99.61]	99.555 [98.40; 99.95]	21.48 [5.49; 84.07]
5	707	+ any symptom(s)	398	1	247	61	61.71 [57.83; 65.47]	98.387 [91.34; 99.96]	99.749 [98.61; 99.99]	38.26 [5.47; 267.60]
6	707	+ malabsorption[#]	278	0	367	62	43.10 [39.24; 47.02]	100.0 [94.22; 100.00]	100.00 [98.68; 100.00]	∞
Excluding all inconclusive cases										
7	691	**Local TGA≥10xULN**	458	1	187	45	71.01 [67.34; 74.48]	97.826 [88.47; 99.95]	99.782 [98.79; 99.99]	32.66 [4.70; 227.10]
8	691	+ any symptom(s)	408	1	237	45	63.26 [59.40; 66.99]	97.826 [88.47; 99.95]	99.756 [98.65; 99.99]	29.10 [4.18; 202.40]
9	691	+ malabsorption[#]	286	0	359	46	44.34 [40.46; 48.27]	100.00 [92.29; 100.00]	100.00 [98.72; 100.00]	∞

Table 2: *Diagnostic accuracies with 95% CIs to diagnose CD based on local TGA-IgA tests in combination with other criteria, either considering inconclusive cases as no CD (scenario 1-6, N=707) or excluding inconclusive cases (scenario 7-12, N=691); scenario 5 and 11 correspond to the current ESPGHAN criteria for the non-biopsy approach; TP=true positive, FP=false positive, FN=false negative, TN=true negative, PPV=positive predictive value, LR+=positive likelihood ratio*

Scenario	N	Combination	TP	FP	FN	TN	Sensitivity° [95%CI]	Specificity [95%CI]	PPV [95%CI]	LR+ [95%CI]
10	691	**Local TGA≥10xULN + EMA* (+/- HLA[§])**	447	0	198	46	69.30 [65.58; 72.84]	100.00 [92.29; 100.00]	100.00 [99.18; 100.00]	∞
11	691	+ any symptom(s)	398	0	247	46	61.71 [57.83; 65.47]	100.00 [92.29; 100.00]	100.00 [99.08; 100.00]	∞
12	691	+ malabsorption[#]	278	0	367	46	43.10 [39.24; 47.02]	100.00 [92.29; 100.00]	100.00 [98.68; 100.00]	∞

°Sensitivity: proportion of patients qualifying for the non-biopsy approach

**EMA-IgA: results of local clinical centers were considered, except for 25 patients without local EMA-IgA result for whom the central EMA-IgA was used*

[§]HLA: central HLA-typing results were considered, except for nine patients with local but without central HLA-typing (eight due to ethical reasons, one due to sample contamination), however, including HLA outcomes had no effect on the accuracies

[#] Malabsorption symptoms comprise any of the following: diarrhea, weight loss or insufficient weight gain, growth retardation, iron deficiency anaemia

Table 3: *Specifications of central serology tests, including test number, name and manufacturer, type of analysis and machine, limit of normal and performing laboratory*

Test No.	Trade name	Manufacturer	Type of analysis	Machine	Limit of normal	Limit of normal (upper, if any range)	Performing laboratory
EMA-test							
E1	Anti-Endomysium-IIFT IgA (or IgG) Tissue: monkey esophagus and liver	EUROIMMUN	Immunofluorescence	Fluorescence microscope Zeiss	1:2.5^{+}	1:5	Munich*
TGA-tests							
T1	EliA Celikey IgA	Thermo Fisher	Fluoescence Enzyme Immunoassay	Phadia 250	7 U/ml	10 U/ml	Odense
T2	VarelisA Celikey® tTG-IgA ELISA	Thermo Fisher	ELISA	EUROIMMUN Analyzer I	5 U/ml	8 U/ml	Odense
T3	QUANTA Lite tTG IgA	Inova diagnostics	ELISA	EUROIMMUN Analyzer I	4 U/ml	10 U/ml	Odense
T4	QUANTA Flash tTG IgA	Inova diagnostics	Chemiluminescenc	BioFlash	20 U	30 U	Munich

			e				
T5	Eu-tTG IgA New - *code 9105*	Eurospital	ELISA	EUROIMMUN Analyzer I	9 U/ml	16 U/ml	Odense
T6	Anti-Gewebstransglutaminase-ELISA (IgA)	EUROIMMUN	ELISA	EUROIMMUN Analyzer I	20 RU/ml	---	Odense
T7	Anti-TG2-IgA (open form)	R-Biopharm/Zedira	ELISA	EUROIMMUN Analyzer I	2.6 U/ml	3.5 U/ml	Odense
T8	Anti-TG2-IgA (closed form/standard)	R-Biopharm/Zedira	ELISA	EUROIMMUN Analyzer I	2.6 U/ml	3.5 U/ml	Odense
T9	Anti-TG2-Ig**G** (open form)	R-Biopharm/Zedira	ELISA	EUROIMMUN Analyzer I	2.6 U/ml	3.5 U/ml	Odense
DGP-tests							
D1	EliA GliadinDP IgG	Thermo Fisher	Fluoescence Enzyme Immunoassay	Phadia 250	7 U/ml	10 U/ml	Odense
D2	QUANTA Lite DGP IgG	Inova diagnostics	ELISA	EUROIMMUN	20-30 U	30 U	Odense

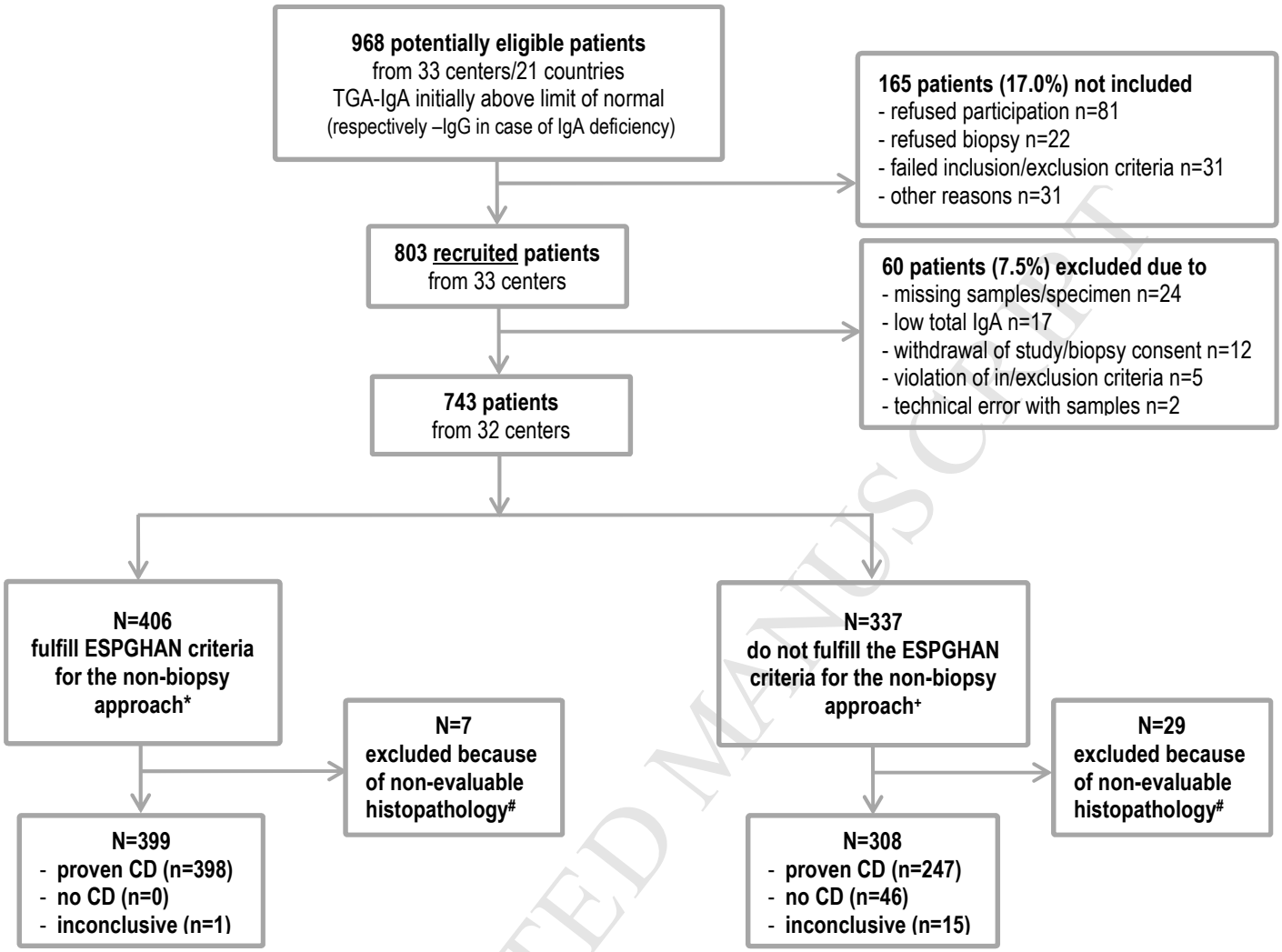
				Analyzer I			
D3	QUANTA Flash DGP IgG	Inova diagnostics	Chemiluminescence	BioFlash	20-30 U	30 U	Munich
D4	a-Gliapep-IgG - *code 9138*	Eurospital	ELISA	EUROIMMUN Analyzer I	10 U/ml	---	Odense
D5	Anti-Gliadin(GAF-3X)-ELISA IgG	EUROIMMUN	ELISA	EUROIMMUN Analyzer I	25 RU/ml	---	Odense
D6	Anti-DGPx1-IgG	R-Biopharm/Zedira	ELISA	EUROIMMUN Analyzer I	5.8 U/ml	8.4 U/ml	Odense

[+] *1:2.5 dilutions done in patients (n=16) with negative central EMA at 1:5 due to with discrepant results or negative HLA*

**immunofluorescence evaluations were exclusively done by one experienced bioanalyst*

 # only done in IgA-deficient cases or if exclusion of IgA deficiency needed to be confirmed

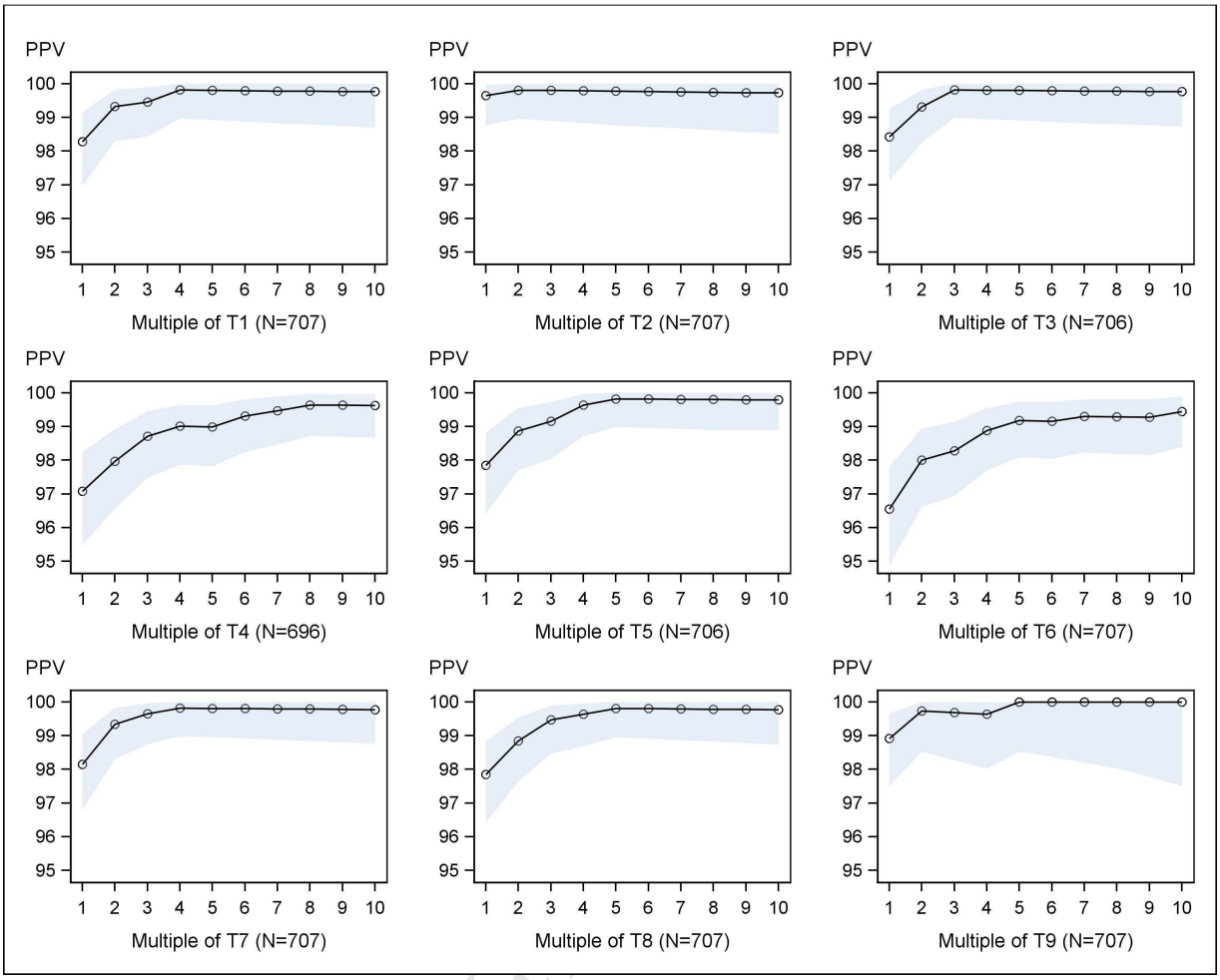
Figure 1



* *high local TGA-IgA ≥10x ULN plus positive local EMA-IgA plus HLA plus any symptom*

\+ *low local TGA-IgA <10xULN and/or negative local EMA-IgA and/or negative HLA and/or no symptoms*

\# *non-evaluable as considered by the reference pathologist*



Supplementary Manuscript

Accuracy in Diagnosis of Celiac Disease without Biopsies in Clinical Practice

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1 Supplementary methods

1.1 Inclusion and exclusion criteria

Children between > 6 months to 18 years were included if they had positive TGA-IgA (any titer above limit of normal) or positive EMA-IgA performed in or outside the study center. In children with low total IgA, increased IgG-antibodies were considered. Patients had to be on gluten containing diet at time of biopsy. CD specific antibodies were measured because they had clinical signs or symptoms indicative for CD or an increased risk for CD[1]. Clinical symptoms indicative for CD comprised chronic or intermittent diarrhoea, failure to thrive, weight loss, growth retardation, delayed puberty, amenorrhoea, iron-deficiency anaemia, recurrent nausea or vomiting, chronic abdominal pain, cramping or distension, chronic constipation, chronic fatigue, recurrent aphthous stomatitis (mouth ulcers), dermatitis herpetiformis-like rash, fracture with inadequate traumas/osteopenia/osteoporosis, abnormal liver biochemistry. Conditions associated with increased risk for CD included type 1 diabetes mellitus, Down syndrome, autoimmune thyroid disease, Turner syndrome, Williams syndrome, selective IgA deficiency, autoimmune liver disease, and having a 1st degree relative with CD.

Exclusion criteria comprise negative TGA and EMA antibodies, contraindications for endoscopy or biopsies or refusal of the parents to perform the biopsies, malignancy, serious chronic infections such as HIV or tuberculosis or congenital immunodeficiency, immune suppressive drugs, language barriers which did not allow to give informed consent or no signed informed consent form.

1.2 Pre-Screening-Log

The clinical center listed all patients that were considered to be eligible for study participation due to positive TGA-IgA (or TGA-/EMA/DGP-IgG in case of low total IgA) on the pre-screening-log. The log-file was submitted to the study management (K.W.) at the end of each month and reviewed for consistency.

The basic information on the pre-screening-log included the date when the patients were considered to be eligible, the TGA-IgA level, and if the in/exclusion were fulfilled and the patient recruited. If not, the reason why the patient was not recruited (e.g. “refused to biopsy”) was briefly indicated. If the patient met all inclusion criteria and was recruited, additional information on month and year of birth and potential date for biopsy were listed and the patient-ID was assigned.

1.3 Exclusion process of recruited patients

Patients were retrospectively excluded if it became evident that in- or exclusion criteria were not fulfilled, if the dataset was incomplete for relevant information from the clinical center (no information on clinical presentation, local serology or local histopathology), if serum for central serology was not available, if neither local nor central HLA-typing was done, if histology specimen were not provided for the reference histology, if the first or second reference pathologist and morphometry analysis revealed that the histology specimen was non-evaluable due to low quality or orientation problem, or if mix-up of serum samples was obvious.

1.4 Local serology

Local serology should have been done maximum two weeks prior to or at time of biopsy. There have been ten different TGA-IgA tests applied in the laboratories of the local clinical centers ***(table S1)***,

however, even if the same test was used the limits of normal (locally applied cut off) varied in between the laboratories.

For EMA, there were 14 different tests ***(table S2)***. In 13/32 laboratories titration to the highest dilution still giving a positive results was performed and the report indicated this dilution. All other laboratories only reported if the sample was negative or positive or gave a grading for positivity (+, ++, +++).

1.5 Central serology

In total, 16 tests from 5 different manufacturers have been included in the panel for central serology analyses.

1.5.1 Central EMA-IgA

Immunofluorescent analysis of EMA-IgA (EMA-IgG if low total IgA) was performed by one experienced bioanalyst (G.H., Dr. von Hauner Children's Hospital, Munich, Germany). Serum was diluted 1:5, 1:10, 1:100, 1:1000 in phosphate-buffered saline and 1:2.5 if the 1:5 dilution gave a negative result. The diluted sera were incubated for 30 minutes on EUROIMMUN IIF mosaic slides combining BIOChips of monkey esophagus and liver tissue (FA 1911-3 A/G, EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) according to manufacturer's instructions. After washing with PBS-Tween the BIOCHIPs were incubated for another 30 minutes with FITC-labelled anti-human-IgA or IgG (goat) conjugate and washed again with PBS-Tween. The BIOCHIPs were examined on a fluorescence-microscope (Standard 16, serial number 1047060, Zeiss, Jena, Germany). A signal in 1:2.5 dilution or higher was considered positive.

1.5.2 Central TGA-IgA and DGP-IgG

All ELISA-based tests were run in single determinations by the same bioanalysts in the central laboratory (Dept. of Clinical Immunology, Odense University Hospital, Denmark) on one automatized ELISA platform (EUROIMMUN Analyzer I, EUROIMMUN Medizinische Labordiagnostika AG, Luebeck, Germany). Please find the details on the analysis and calibration for all ELISA tests in ***table S3***.

There were four non-ELISA-based tests which need to run on specialized automatized systems, thereof two tests from Thermo Fisher/Phadia which were run on the Phadia250 machine (Fluoescence Enzyme Immunoassay; Dept. of Clinical Immunology, Odense University Hospital, Denmark) and two tests from INOVA diagnostics which were run on the BIO-FLASH machine (chemiluminescence technique; done by G.H. at Werfen GmbH, Kirchheim, Germany). Please find the details on these tests in ***table S4.***

1.6 Central HLA-typing

An EDTA-full blood sample was taken at the clinical center around the time of biopsy and DNA was either directly isolated by the lab of the clinical center and frozen at -20°C or the full blood was stored at -20°C until shipment to the central laboratory for DNA isolation in Munich, Dr. von Hauner Children's hospital, with the Qiagen Minikit on the QiaCube (Qiagen GmbH, Hilden, Germany). In Munich, all DNA samples were aliquoted and patient IDs were re-coded to guarantee data security before sending the samples to Eurospital SpA (Trieste, Italy) and Euroimmun GmbH (Lübeck, Germany) for HLA typing.

The Eu-Gen-risk test (Eurospital SpA) is based on two series of eight PCR tubes where primers for amplification of individual DQA1, DQB1 and DR alleles are present. Each tube provides with a single results with the only exceptions of tubes #7 (B1*03:01 and B1*03:03) and #8 (B1*03:01 and B1*03:04) where two alleles are present. Doing so it is always known whether B1*03:01 or B1*03:03 or B1* 03:04

alleles are present. If there is a positive result is in both tubes, this means that the B1*03:01 allele is present. If the positive result is only found in one position (e.g. tube #7) the positivity will refer to the B1*03:03 allele. Vice versa if the positivity is only in tube #8, then it refers to B1*03:04. In total, the following 16 alleles were typed: DQA1*01, DQA1*02:01, DQA1*03, DQA1*05, DQA1*06, DQB1*02, DQB1*03:01 and DQB1*03:03, DQB1*03:01 and DQB1*03:04, DQB1*03:02, DQB1*03:05, DQB1*04, DRB1*03, DRB1*04, DRB1*07, DRB1*11; results were expressed in terms of: DQ and DR genotypes, complete Haplotypes and Homo- or Heterozygosis status.

HLA-DQ2.2, -DQ2.5 and -DQ8 determination by EUROArray HLA-DQ2/DQ8 was performed according to manufacturer's instructions (EUROIMMUN Medizinische Labordiagostika AG, Luebeck, Germany) for HLA-typing. Briefly, sequence specific amplification of all relevant HLA-DQ alleles was achieved by multiplex PCR with simultaneous fluorescence labelling of the reaction products. PCR products were resolved by hybridization to specific probes on a EUROArray slide using TITERPLANE incubation technique. Spot intensities were analyzed by the EUROIMMUN Microarray Scanner. Finally, celiac disease associated HLA-DQ alleles and genotypes were automatically deduced by the EUROArrayScan software.

In patients with negative HLA-risk alleles but with positive central serology and reference histopathology, a 3rd HLA-typing with DNA material from a new blood sample to exclude sample confusion was performed by SNP tests[2], the Olerup low resolution DQ typing kit, and the B*03 subtyping kits to identify rare risk alleles.

1.7 Endoscopy findings

The clinical centers reported on a standardized questionnaire all macroscopic findings if upper endoscopy was performed, including erosive esophagitis indicating reflux disease , findings of eosinophilic esophagitis (furrors, rings, narrowing), gastric and duodenal nodularity, erosions and ulcer and if available also the *Helicobacter pylori* status based on histology, rapid urea test and/or culture.

1.8 Central reference pathology and small-intestinal biopsy morphometry analysis

The clinical centers were asked to take at least five forceps biopsies during upper endoscopy, at least four from the pars descendens duodeni and at least one from the duodenal bulb (Husby). One center still performed capsule biopsies resulting in only one or two tissue samples per case. All local pathologists gave written agreement before inclusion of the center to fill a structured histology report including Marsh staging. They were asked to provide four hematoxylin and eosin (H&E) stained slides (including at least one biopsy from the duodenal bulb if available), and one or two unstained slides which to the referral center for immunohistochemical evaluation of the number of intraepithelial T lymphocytes using anti-CD3 monoclonal antibodies. The central histopathology report was based on the Marsh-Oberhuber Classification [3,4] and included information on number of IEL (< or ≥25 IEL /100 enterocytes) and orientation of the biopsies. If there were more than 1 biopsy fragment (usually 5-6) and with different degrees of villous atrophy, the highest grade was attributed to the case. To fulfill the criterion of CD enteropathy Marsh 2 or higher was required.

In patients with inconsistent histopathology outcomes or with biopsy specimen that were non-evaluable by standard histopathological techniques, the original biopsies (paraffin blocks) were requested if possible. Morphology of these biopsies was then again evaluated using validated quantitative morphometry according to standard operating procedures (SOP)[5,6]. The assessment was performed on hematoxylin-eosin stained 2µm sections of paraffin embedded mucosal samples. The investigator (A.P.,

Bucharest and Tampere) identified correctly oriented sections where reliable morphometric measurements could be obtained, i.e. perpendicular sectioning as to the mucosal surface so that crypts were cut longitudinally and not in cross sections. At least three readable villus-crypt units were demanded for the evaluations according to the SOP. The villus heights (VH, µm) and corresponding crypt depths (CrD, µm) were measured and results were given as the mean ratio of the measurements (VH:CrD). A VH:CrD equal or more than 2 was considered normal. For the samples with poor orientation additional cuttings were requested and reassessment was done. If no units had been measured even after re-cuttings, the sample was classified as not measurable. If one or two units only were measured, the VH:CrD was given but the evaluator stated this was not according to the SOP.

1.9 Definition of patients with low total IgA

Since patients with deficient or low total IgA may not produce sufficient TGA-IgA, we tested patients with total IgA below 0.25 g/l and negative TGA-IgA for IgG-based antibodies (both in local and central lab). If local total IgA was not available but TGA-IgA was >3x ULN, the patient was considered to have normal total IgA. If local total IgA was not available but TGA-IgA was ≤3x ULN, then the total IgA was repeated in central lab (Odense or Munich).
All patients with low total IgA were excluded from the main data analysis but separately analyzed as a subgroup.

1.10 Central diagnosis

The process how initially unclear patients were finally classified into the categories “proven CD”, “no CD” and “inconclusive” is described in the following. Please also see figure S2 which provides an overview on this process.

1.10.1 Initial approach to classify the patients

Proven CD was considered if the local TGA-IgA was above the respective limit of normal, the local and the central EMA-IgA were positive, the HLA-genotype was associated with CD risk and if both the local and the reference pathologist gave at least ≥ Marsh 2.
Celiac disease was excluded if none of the above criteria were fulfilled. All other patients were initially regarded as unclear.
Patients with low total IgA (see 1.8) were analyzed as a separate group.

1.10.2 Second approach to classify the patients

The diagnostic committee decided to also classify patients as proven CD patients if 1) only the local EMA-IgA was negative but all other criteria were positive or 2) if the local pathologist did not report any Marsh staging but stated that the histology findings were indicative for CD and this was in line with the reference pathologist.
CD was also excluded if only the local TGA-IgA was positive but below the 3x ULN and if all other outcomes including the central serology were negative.

1.10.3 Review of unclear cases and 1st and 2nd voting by the diagnostic committee

All remaining unclear cases were reviewed by the diagnostic committee (reviewers S.K., I.K., C.R., L.M, R.T, S.H., A.T.).
The Center-Patient IDs (identification numbers) were re-coded to avoid that the reviewers might be biased by the origin of the patient or the responsible clinical center.

For each patient a summary were available for the reviewers, including symptoms, risk factors and relevant findings in physical examination, local and central serology and histopathology results. However, for the central serology (TGA and DGP) not the raw numbers (titer levels) were shown but a summary of how many TGA-IgA or DGP-IgG tests were positive, meaning above limit of normal according to the manufacturer's instructions (e.g. 4/8 TGA-IgA tests positive).

The voting was done anonymously and the reviewers chose one of the following options: proven CD, no CD, and unclear case. If the majority (at least four of seven reviewers) agreed on the diagnosis of CD or the exclusion of CD, the unclear case was solved. If there was no agreement, the patient was still regarded as unclear.

If the committee considered that any relevant information was missing or that there was doubt about the current results, the clinical center or the central laboratories were approached and the new information was included in the summary and a second review process was done.

In the 2nd review process, unclear cases were also distinguished in potential CD (seropositivity but normal histology) or unclear cases due to inconsistent clinical picture due to either inconsistency between serology and histology or between the local and the reference pathologist.

1.10.4 Second reference pathology and 3rd voting of the diagnostic committee

For all potential and unclear cases, the centrally available histology sections were examined by a second reference pathologist (D.A., Dresden) who was blinded to any clinical information on the patients. The new pathology report was included in the 3rd voting of the diagnostic committee.

1.10.5 Morphometry and 4th and final voting of the diagnostic committee

For the remaining cases, the original biopsies (paraffin blocks) were collected from the pathology department of the clinical centers and morphometry analysis including measuring villous-crypt-ratio was done as described above by a pediatric gastroenterologist (A.P. Bucharest and Tampere) who is specialised in CD morphometry (***see 1.7***). Again, the investigator was blinded for all clinical data of the patients.

Afterwards, the 4th and final voting was done by six of initially seven members of the committee (S.K., I.K., C.R., L.M, R.T, S.H.). If histology specimen were finally considered as non-evaluable, the patients were excluded for this reason.

If the final classification into "proven" or "no CD" was still not possible after the 4th review process, the remaining unclear patients were then considered to be inconclusive.

1.10.6 Diagnosis of patients with low total IgA

The diagnostic approach of patients with low total IgA was similar to the process described above for patients with sufficient total IgA titers, however, TGA-IgG, DGP-IgG and EMA-IgG antibodies were considered. Morphometry analysis was not done in these patients but was also not necessary.

1.11 Statistics

Mean±SD and/or median and minimum to maximum were indicated for continuous variables. Proportions were given in percent. Differences between the excluded patients and the final cohort were tested with the Wilcoxon sum rank test.

For each central TGA-IgA and DGP-IgG tests the optimal ULN to diagnose CD was determined if the lower bound of the PPV's 95% CI reached ≥ 99%.

The probability for positive disease was estimated by logistic regression and the predicted line with 95% confidence limits and the observations were displayed.

Inter-observer agreements between local and central outcomes for histopathology and EMA and for the two HLA-typing tests were given as simple kappa coefficient with 95% CI. A kappa coefficient of 0 to 0.20 was considered as slight, 0.21 to 0.40 as fair, between 0.41 to 0.60 as moderate, 0.61 to 0.80 as substantial, and 0.81 to 1.00 as (almost) perfect agreement. Level of significance was α=0.05 for all tests. The manuscript was written in accordance with the STARD-statement[7].

2 Supplementary results

2.1 Exclusion of patients and final cohort

All excluded patients and the reasons for exclusion are summarized in ***table S5***.

Compared to the patients included in the final cohort, the excluded patients (except patients with total IgA deficiency) tended to be younger with a median age of 4.3 versus 6.2 years (p=0.09, Wilcoxon sum rank test) and the median for the multiple of the ULN for the local TGA-IgA was significantly lower with a 5.3 versus 11.8 (p<0.001). For details see ***table S6***.
The distribution of all patients, stratified in patients in the final cohort and excluded patients, is shown for all centers in ***figure S1***.

2.2 Symptoms and stool behaviour

Parents and patients were asked to report symptoms and stool pattern, considering the period four weeks prior to study visit. Abdominal pain was the most frequent gastrointestinal (50.6%) and weight loss respectively insufficient weight gain the most commonly reported extraintestinal symptom (30%). The frequency of all reported symptoms is summarized in ***table S7***.

According to the Bristol stools scale (BSS), 13.0% reported hard stools (BSS type 1-2), 68.6% normal consistency (BSS type 3-5) and 18.4% mushy to liquid stool (BSS type 6-7). Stool frequency was ≤ 2 stools per week in 8.6%, 3 to 4 stools per week in 15.8%, 1-2 stools per day in 61.4% and ≥3 stools per day in 14.2% of patients.

2.3 Final central diagnosis

After the initial approach to classify the patients into proven and no CD, 189 patients remained unclear, thereof 4 with negative HLA. As a result of the diagnostic review process, 16 patients were finally classified as finally inconclusive and 29 had to be excluded from analysis mostly due to non-evaluable histology. For details see ***figure S2***.

2.4 Patients with low total IgA

In total, 17 patients fulfilled the criteria for low total IgA, thereof one patient had a total IgA of 0.23g/l, negative TGA-IgA but positive DGP-IgG, positive central EMA-IgG (1:1000) and positive local and central histopathology. Eleven patients were diagnosed as proven CD, in five CD was excluded and one patient was considered to have potential CD and remained inconclusive. The summary of all patients with low total IgA is shown in ***table S8***.

2.5 Patients with initially positive TGA-IgA but negative autoantibodies at time of biopsy

Twenty-nine patients had negative TGA-IgA at time of biopsy; thereof 10 had also negative local EMA-IgA. All of them had a previously positive TGA-IgA titer either done outside or in the clinical center. The characteristics of these patients are summarized in ***table S9***.

2.6 HLA-negative patients

In total, 18/707 patients were initially HLA-negative (***table S10***). For 2/18 patients with high suspicion of CD, the 2nd typing from newly drawn blood samples were HLA-positive in both. One of these two patients was positive for DQ2 in the second sample and the initial negative sample was DNA from the father, in whom blood was initially drawn the same day and was mixed up with the son's blood. In the second child, the typing was positive in SNP and DQB1*03 subtyping analysis for one copy of the CD-related DQ9 allele (DQB1*0303)[8] and a rare haplotype with an unusual hybrid allele DR7-DQA1*0201-B1*0301/0303 and thus a HLA status resembling to DQ2.2/DQ7 (DQ2 in trans) was found. None of the remaining 16 patients with negative HLA or DQA1*05 was diagnosed with CD. Details on all HLA-negative patients are summarized in ***table S10***. All of them had local TGA-IgA below 3xULN, four patients had at least one positive central TGA-IgA results and five at least one positive DGP-IgG titer. Of note, central and local EMA-IgA were negative in all 16 patients, indicating a higher specificity of EMA compared to TGA and DGP antibodies.

2.7 Endoscopy and macroscopic findings

Information on macroscopic findings at endoscopy was reported in 653 who underwent endoscopy, 54 (7.6%) were biopsied with capsule. Information on *H. pylori* status from gastric biopsies was available in 442.

Esophageal findings

Erosive esophagitis indicating gastroesophageal reflux disease (GERD) was identified in 24/653 (3.7%) patients. No case of eosinophilic esophagitis was reported.

Gastric findings

H. pylori infection was found in 20 of 442 (4.5%) patients with known *H. pylori* status. Erosions were found in 21/653 (3.2%) patients, 2/21were *H. pylori* infected, 16 were not infected and in the remaining three the *H. pylori* status was unknown. No gastric ulcer was reported in the 653 patients.

Duodenal findings

Erosions were found in 41/653 (6.3%) patients, with only one of them being infected with *H. pylori*, 33 were not infected and in the remaining the infectious status was unknown. Two patients had a duodenal ulcer (0.3%), one was not infected with *H. pylori*, and in the second *H. pylori* status was not assessed.

2.8 Inconclusive cases

Finally 16 patients remained inconclusive and had no clear diagnosis, thereof nine were classified as potential CD with positive serology but negative histology and seven patients were considered as inconclusive due to contradictory results. Details on all inconclusive cases are summarized in ***table S11***.

2.9 Time between local TGA-IgA and biopsy

In 563 patients, the time gap between local TGA-IgA and biopsy was <14 days. The median time between local serology and biopsy was 0 days (minimum 0 days, maximum 304 days). Of 144 patients with time gap >13 days, 135 patients had proven CD, 7 patients were finally considered as no CD and two patients were inconclusive, thereof one with a time gap of 16 days and one with 54 days (see supplementary ***table S12***.

2.10 False positive patients

Details on the eight patients with high TGA-IgA (>10xULN) either at the local or at least one central test but with either no CD (N=2) or inconclusive diagnosis (n=6) are summarized in ***table S12.*** In six patients, there was no villous atrophy seen by neither the local nor the reference pathologists. For one patient, villous atrophy was found in the bulb only in morphometry analysis and for two other patients the biopsy specimen and therefore the morphometry analysis were not available. For another patient, both the local and the reference pathologist graded Marsh 1, the second reference pathologist gave Marsh 3 while morphometry analysis showed normal crypt-height ratio. One patient had clear villous atrophy (Marsh 3) in all histology examinations but all central TGA-IgA and EMA-IgA were negative with normal total IgA and 4/6 positive DGP-IgG results. The high discrepancy between local TGA level (20xULN) and none of eight central TGA-IgA tests giving a level above cut off, is not to explain. A wrong labelling of local or central serum samples or biopsies cannot be excluded but in contrast to other cases, there was no prove for sample mix-up and therefore the diagnostic committee decided to not exclude this patient.

2.11 Accuracies and predicted probabilities of central TGA-IgA/-IgG and DGP-IgG tests

The diagnostic accuracies including sensitivity, specificity, positive predictive value (PPV) and positive likelihood ratio (LR+) for the central TGA results are summarized in ***table S13***. For the central DGP-tests the accuracies are summarized in ***table S14*** and the PPV with 95% CI band in ***figure S3***.

The predicted probabilities for the multiples of each central TGA and DGP test are shown in ***figure S4 and S5***.

2.12 Agreement between local and central EMA-IgA

Comparing the local and the central EMA-IgA results (categorized in “positive” and “negative” according to the respective test instructions), there was an agreement in 93.75% of the patients (N=704) and the kappa coefficient was 0.65 [0.56;0.75]. For details, see ***table S15.***

2.13 Agreement between local and central TGA-IgA

In 334 patients the local TGA-IgA was analyzed with a test that was also included in the TGA-IgA test panel of the central serology, and the sera that were used for local and central serology were taken on the same day. In ***figure S6*** the correlation between the local and the corresponding central TGA-IgA titer are shown.

Comparing the ≥10 ULN for the local TGA-IgA and the corresponding TGA-IgA result of the central lab (***table S16***), the agreement was 86.5% and the kappa coefficient was kappa -0.597 [-0.6966; -0.4978]. If local laboratories have applied different levels of normal than those suggested by the manufacturer and applied for central test, the 10x ULN was calculated with the level of normal that was indicated by the local laboratory, as the real situation should be given.

2.14 Agreement between Eu-Gen and EUROArray HLA-typing outcomes

In 695 patients, there were valid typing results available both for EU-Gen-risk and EUROArray HLA-DQ2/DQ8. The agreement was 97.6% with a high kappa coefficient of 0.93 [0.90;0.96]. Particularly depending on two different variants for the definition of HLA-DQ8 positivity of the EUROArray, the agreement may vary. For details see ***table S17***.

2.15 Agreement between local and central HLA-typing outcomes

In 151 patients both local and central HLA-typing was available. For the comparison between the local typing and the EU-Gen-risk (Eurospital S.p.A), the agreement was 92.1 % and the kappa coefficient 0.74 [0.61;0.88], for details please see ***table S18.*** For the comparison between the local typing and the EUROArray HLA-DQ2/DQ8 (EUROIMMUN Medizinische Labordiagnostika AG, Luebeck, Germany), the agreement was 92.1 % and the kappa coefficient 0.74 [0.60;0.88], for details please see ***table S19.***

2.16 Agreement between local and central histopathology

The agreement in the Marsh-Oberhuber (0 to 3C) staging between local histopathology versus reference histopathology is 41.7 % with a kappa coefficient of 0.20 [0.15; 0.25]. However, in 31 patients no local Marsh staging was available as the local pathologist considered it not feasible. For details please see ***table S20***.

When summarizing the Marsh-Oberhuber stagings in the two main outcomes indicative for CD (Marsh 2 or 3A-C) or not (Marsh 0 and 1), the kappa coefficient for the agreement between local and central histopathology was 92.9% with a kappa coefficient of 0.66 [0.57;0.75]. For details please see ***table S21***.

2.17 Agreement between local histopathology and final diagnosis

Table S22 shows the comparison between the summarized Marsh-Oberhuber staging in the two main outcomes indicative for Celiac Disease (Marsh 2 or 3A-C) or not (Marsh 0 and 1), we found 3 patients in whom the local pathologist considered CD but the final diagnosis was “no CD”. Vice versa, there were 37 patients in whom the local pathologist evaluation resulted in Marsh 0 or Marsh 1 but the final central diagnosis was “proven CD”. Overall, there were 40 cases in whom the local histopathology gave the wrong statement when considering the central diagnosis as reference.

Table S23 also includes the patients for whom the local pathologist did not indicate a Marsh staging but gave a general statement if the specimen is normal or pathologic but not indicative with CD or is compatible with CD.

2.18 Agreement between local and central diagnosis

When comparing local and final central diagnosis, there was one patient false positive for proven CD in the local clinical center and “proven CD” in the central diagnosis. Furthermore, eight patients were classified false negative as “no CD” by the clinical center but were considered to be “proven CD” in the final central diagnosis. For more details, see ***Table S24.*** As the local clinical centers had less information on the patient than the central diagnostic committee, no kappa coefficient was calculated.

3 Supplementary discussion

Our prospective multicentre study with extensive local and central diagnostic work up demonstrates that in the real life situation the diagnosis of CD may be complex with a high percentage of cases showing discordant results for TGA-IgA (negative vs positive, any positivity vs high positive), EMA (positive vs negative) and histology (indicative for CD vs no CD). The concordance regarding EMA results

was surprisingly high in spite of the low experience with EMA measurement in some centers, which only started EMA testing for the purpose of the ProCeDE study. In contrast, the inter-observer agreement between the local and the central reference pathologist regarding the diagnosis of CD "Yes" or "No" was not satisfactory. In 48 of 676 cases in which the local pathologist provided Marsh staging, there was disagreement regarding the final diagnosis of CD between the pathologists (Marsh 2 or 3 vs Marsh 0 or 1 (***table S21***). Our results confirm that there is not a single diagnostic test with a sufficiently high sensitivity and specificity which could serve as reference standard. Therefore, we had planned from the very beginning of the study that the final diagnoses "CD" or "no CD" had to be based on a combination of test results. Each of the different diagnostic tests had to be tested for its accuracy against this final diagnosis. Our results clearly demonstrate that previous studies cannot be considered as reliable in which laboratory tests (HLA-typing, TGA, EMA or DGP serology) were validated against histology as only reference [9-18].

The ESPGHAN-guidelines do not consider DGPs for the criteria of the non-biopsy approach[1]. Some publications suggest combining TGA and DGP tests [19,20], while others concluded that adding DGP does not improve accuracy [21,22]. The 10xULN of the DGP tests analyzed in our central lab showed PPVs of 100% for almost all six tests. However, the proportion of CD cases having DGP-IgG titers >10xULN was much lower (range 2.48 - 48.9%) compared to TGA-IgA (57.8 - 84.7%). This study was not designed to evaluate the sensitivity or specificity of DGP-IgG or TGA tests for case findings. Our results are biased towards TGA testing since a positive TGA results was one of the inclusion criteria. Therefore, we may have missed some CD cases with negative TGA-IgA results but positive DGP results. This is certainly true for IgA deficient patients, and may rarely occur in others with low TGA titers. Further, this study did not evaluate occurrence of DGP positivity in other conditions or healthy people. However, we are not aware of any published case of an IgA competent patient with negative TGA-IgA results but very high (>10xULN) DGP results and biopsy proven CD. Future studies testing patients with suspected CD with the combination of TGA-IgA and DGP-IgG instead of total IgA including a cost calculation considering is necessary to decide on the recommendation for initial testing.

Potential risk of missing other endoscopy findings

Patients with untreated celiac disease commonly have esophageal dysmotility and delayed gastric emptying which may increase their risk for GERD. These motility alterations resolve on a gluten free diet [23]. The prevalence of *H. pylori* infection in our cohort was surprisingly low considering that we recruited in countries with high prevalence of *H. pylori* infection in children such as Israel, Iran, Russia and some Eastern and Southern European countries. *H. pylori* infection is mostly acquired within the first 5 years of life and persists without therapy. Epidemiological studies showed an inverse relationship between *H. pylori* infection and immune mediated disorders including inflammatory bowel disease [24], childhood asthma [25], and celiac disease [26] suggesting a protective role of *H. pylori* infection. Macroscopic duodenal erosions and shallow ulcerations are well known findings in celiac enteropathy. They resolve with a gluten free diet. In summary, endoscopic pathological findings were unfrequently reported in this cohort. Most of the pathologies were likely to be related to untreated celiac disease and expected to resolve on a gluten free diet. The *H. pylori* infection rate was low. Erosions were not more frequently observed in infected compared to non-infected children. No peptic ulcer disease was found in *H. pylori* positive children. *H. pylori* gastritis in the absence of ulcer disease in children is no indication for therapy [27]. Our findings indicate that the non-biopsy approach is not resulting in disadvantages for these children due to missed co-morbidities.

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5 Supplementary figures and tables

Figure S1: *number of patients (n) from final cohort (N=707) and all recruited but excluded patients (N=96) per clinical study center*

Figure S1: *Number of patients (n) from final cohort (N=707) per clinical study center plus all recruited but excluded patients due to non-evaluable histopathology, low total IgA or other reasons (N=96)*

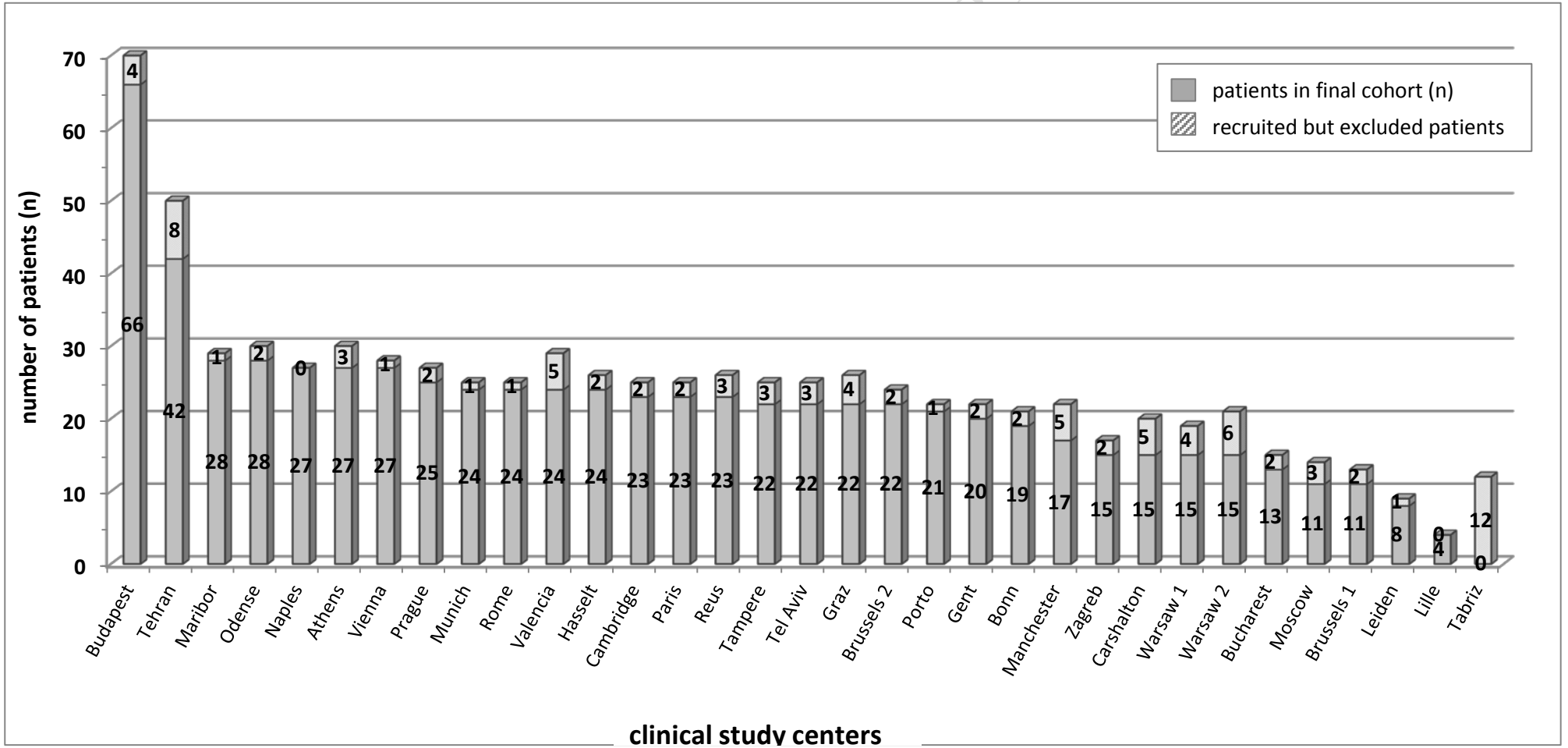

Figure S2: *Flow chart on the review process of the unclear patients*

Figure S2: *Flow-chart of the diagnostic review process of unclear cases from the first approach until the final decision after the 4th review; when this review process was completed, patients without definitive final central diagnosis were then named inconclusive.*

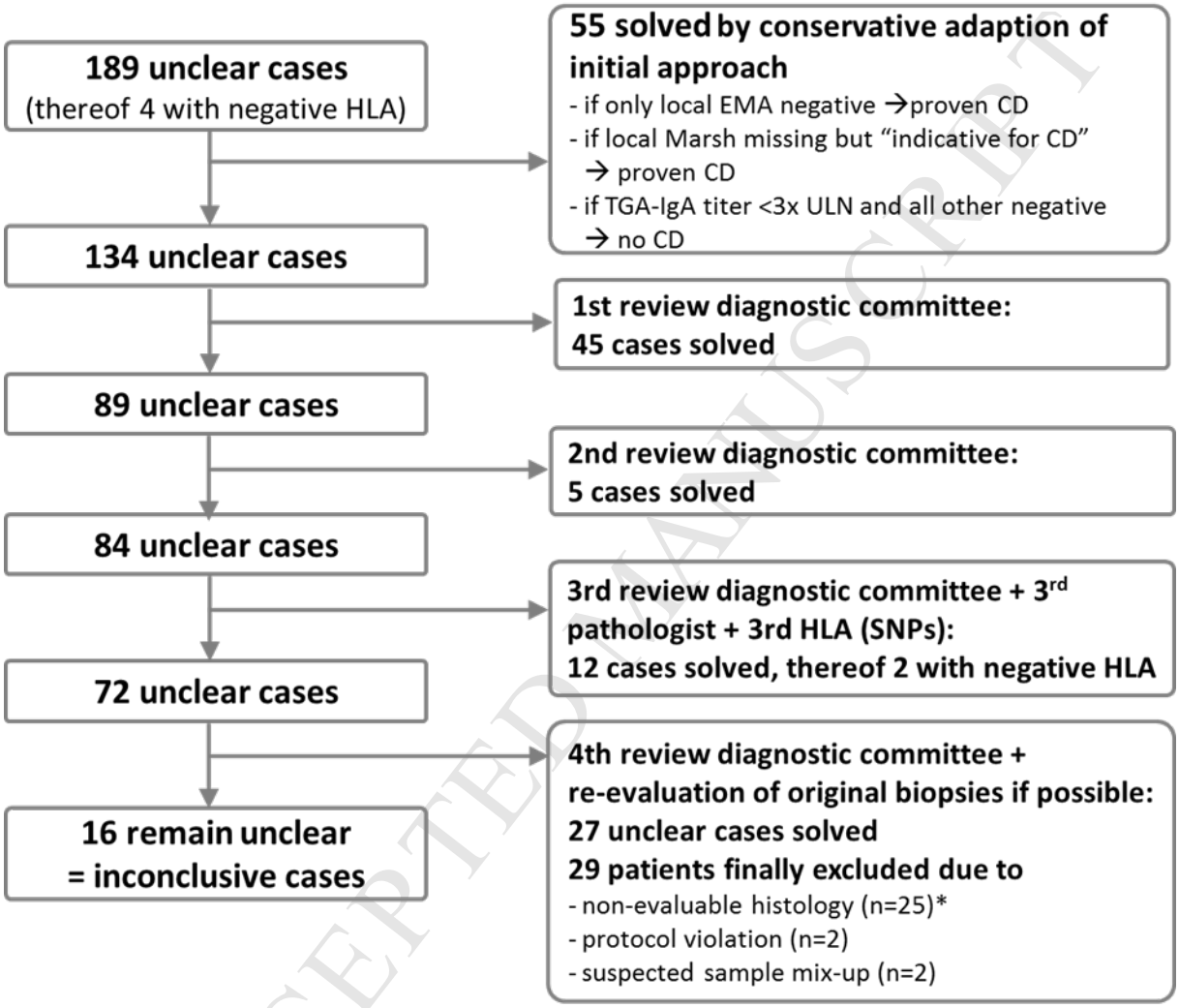

Figure S3: *Positive predictive value (PPV) for each central DGP test*

Figure S3: *Positive predictive value (PPV) with 95% CI band (grey shaded) for CD diagnosis for each of the six central DGP-IgG tests (D1 to D6); the x-axis shows the multiple of the respective limit of normal according to the manufacturers' instructions (all truncated at 10xULN except test D5 which is truncated at 8xULN due to limited maximum measuring range of the antibodies in the central laboratory). The y-axis shows the PPV. Please see table 3 for the names and manufacturer of each test.*

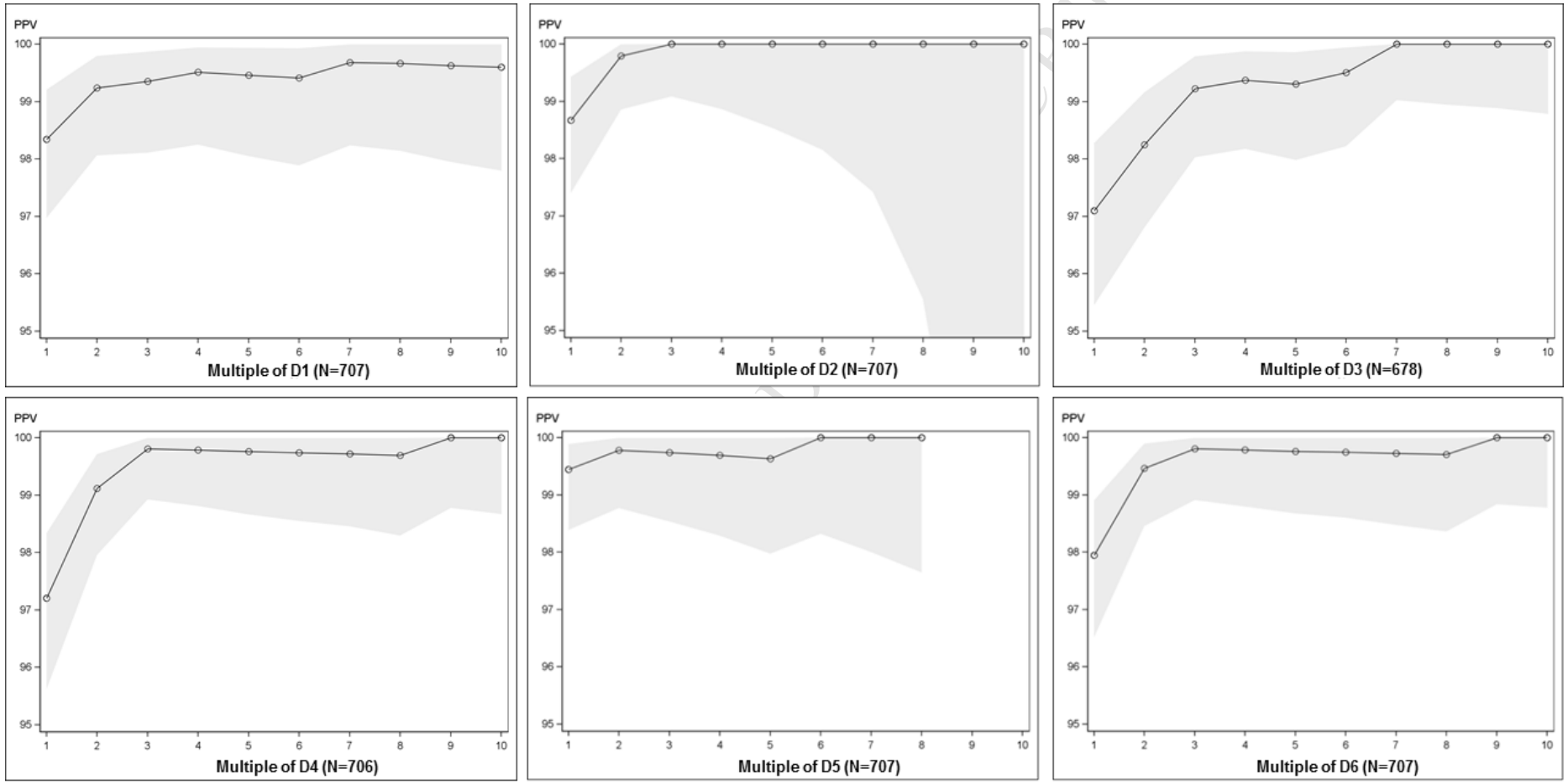

Figure S4: *Predicted probabilities for each central TGA test*

Figure S4: *Predicted probabilities with 95% CI band (grey shaded) of the CD diagnosis (yes=1, no=0) for central TGA-tests, including eight TGA-IgA tests (T1 to T8) and one TGA-IgG test (T9); the x-axis shows the multiple of the respective limit of normal according to the manufacturers' instructions (all truncated at 11xULN). The y-axis shows the probabilities for CD diagnosis. Please see table 3 for the names and manufacturer of each test.*

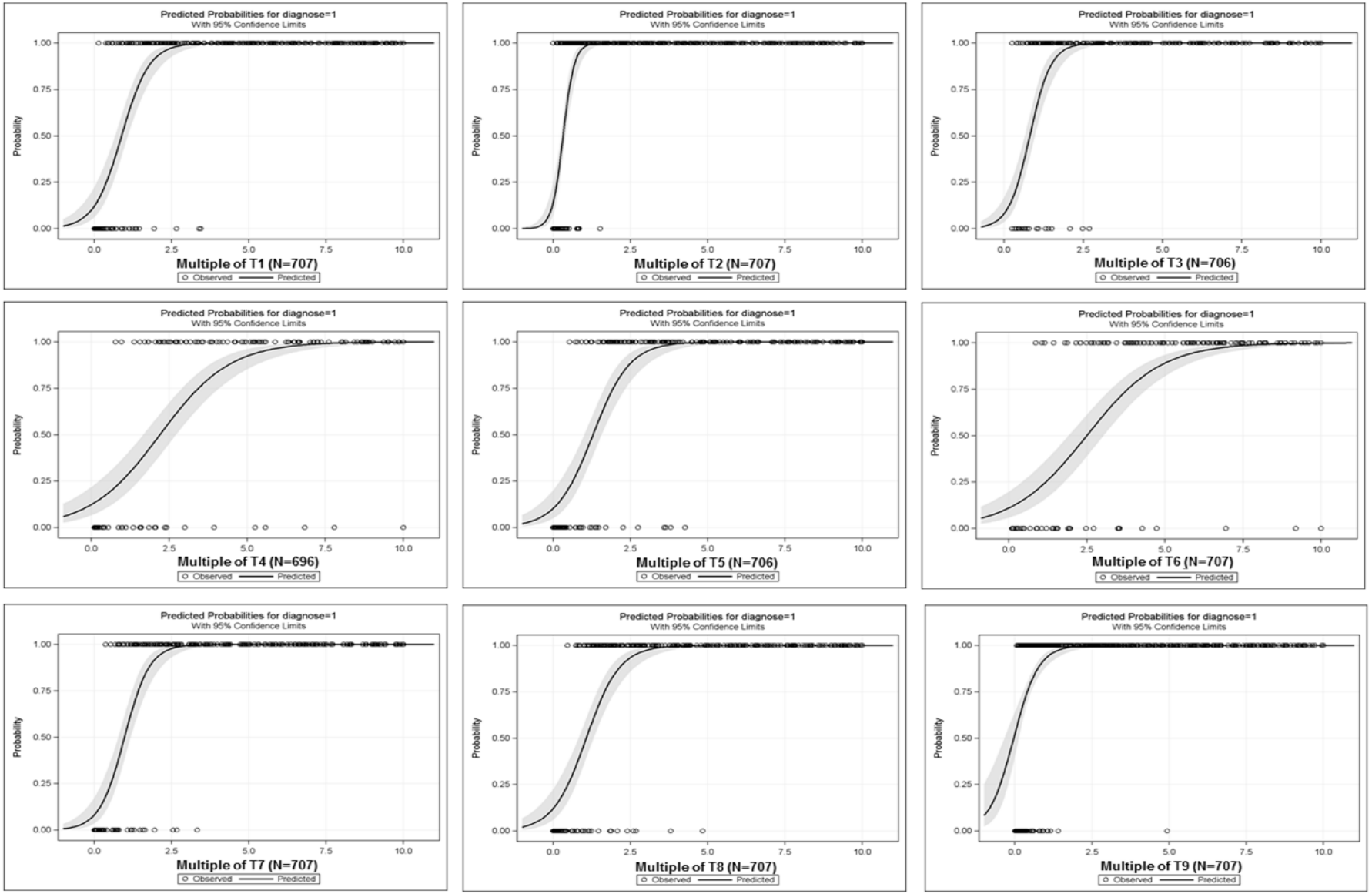

Figure S5: *Predicted probabilities for each central DGP tests*

Figure S5: *Predicted probabilities with 95% CI band of the CD diagnosis (yes=1, no=0) for central DGP-IgG tests (D1 to D6); the x-axis shows the multiple of the respective limit of normal according to the manufacturers' instructions (all truncated at 11xULN). The y-axis shows the probabilities for CD diagnosis. Please see table 3 for the names and manufacturer of each test.*

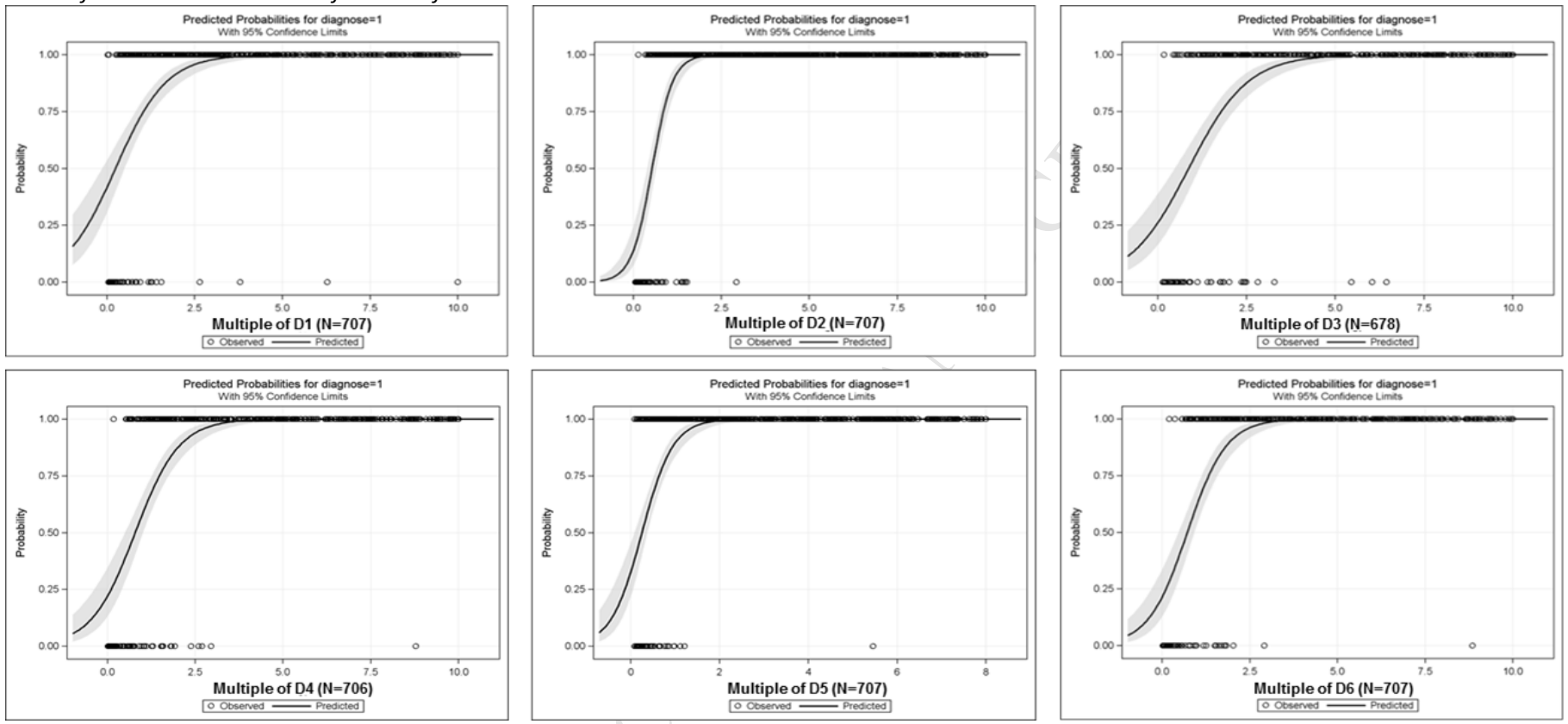

Figure S6: *Comparison of central TGA-IgA and corresponding local TGA-IgA*

Figure S6: *Correlation between TGA-IgA-titers of the local laboratory and the titer of the corresponding TGA-IgA test in the central laboratory panel if included, and if both local and central test were analyzed from serum taken at the same day (N=334); as the local laboratories usually only measured up to the maximum measuring range for a certain test (100 U/ml, 128 U/ml or 200 U/ml), the central results were truncated to the same maximum titer level.*

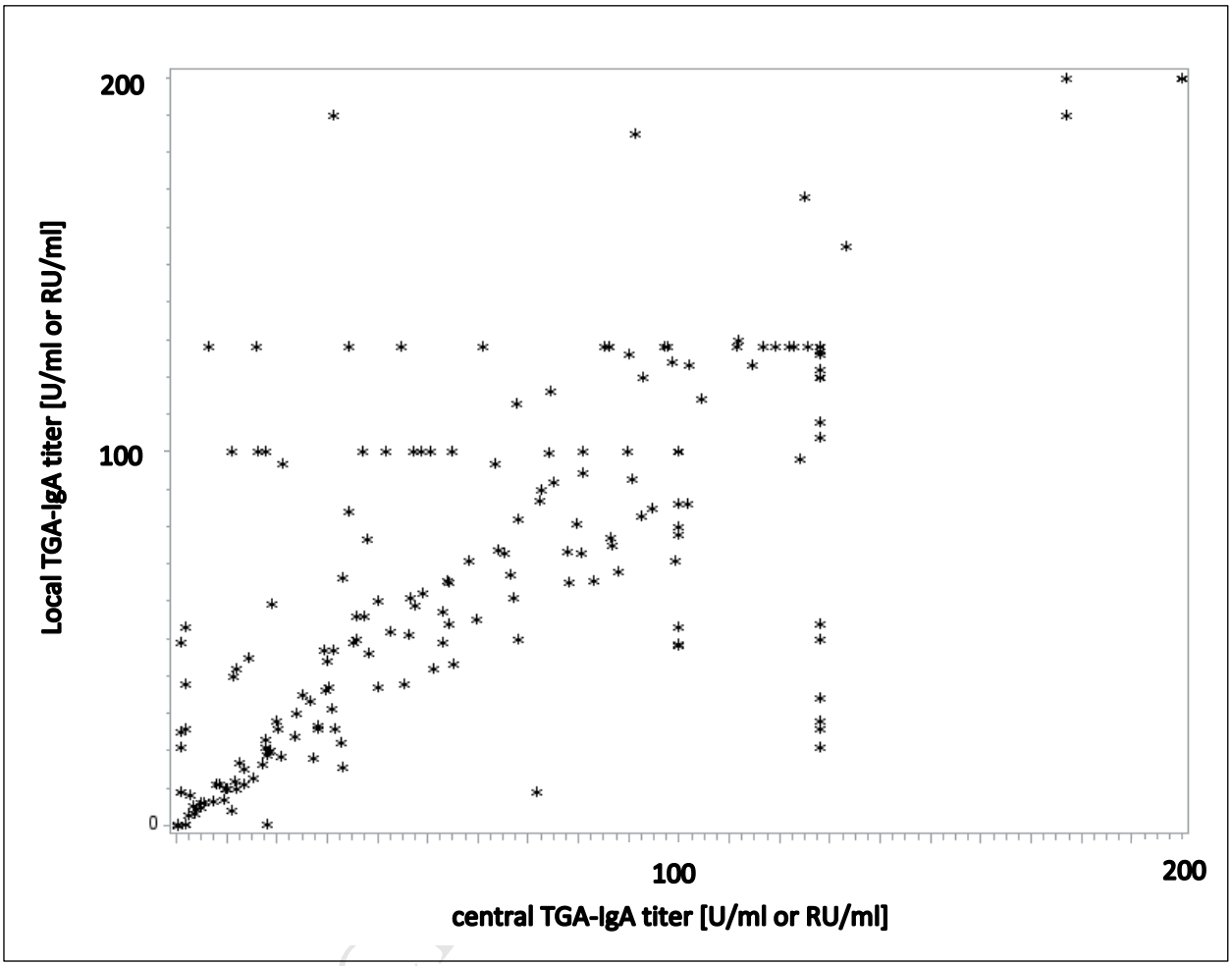

Table S1: Specifications of Local TGA-IgA and DGP-IgG

Table S1: *Specifications of local TGA-IgA and DGP-IgG tests (as applied in and reported by the laboratories of the clinical centers), including manufacturer and test-kit, applied limits of normal, maximum measuring range (if any) and information if calibration curves were available or not*

manufacturer	name of test-kit	settings			values assigned to samples above the maximum of measuring range (if any)	Calibration curve (yes/no)	number of centers using the same test and settings
		unit	limit of normal	limit of normal (upper, if any range)			
TGA-IgA tests							
Biosystems, S.A.	Anti-Tranglutaminase IgA	U/ml	<10	>14	>100	unknown	1
Delta BiologicalsDIA Medix	Anti-Tissue Transglutaminase IgA	U/ml	<1	>7	>200	yes	1
EUROIMMUN Polska	recombinant, expression with baculovirus vector in insect cells	U/ml	<20	≥20	>200	unknown	1
EUROIMMUN AG	Anti-Tissue Transglutaminase ELISA (IgA)	RU/ml	<20	≥20	>200	yes	1
	Anti-Gewebs-Transglutaminase ELISA (IgA)	U/ml	<8	>=12	none	yes	1
Eurospital	Eu-tTG	U/ml	<7	>16	none	yes	1
		U/ml	<9	>=16	>100	yes	5
in house	ultra-sensitive liquid-phase capture RBC ELISA	U/ml	<3	>=5	>100	yes	1
in house	Radioligand assay	cpm	<150 cpm	>=150	4000	no	1
Inova Diagnostics	Quanta Lite h-tTGIgA	U	<20	>=20	N/A	no	3
Phadia-Thermofisher	Varelisa-Celikey	U/ml	<5	>8	>100	yes	1
	EliA Celikey IgA ImmunoCAP	U/mL	<4	>10	>100	yes	1
		U/ml	<6	>6	>128	yes	1
		U/ml	<7	>=10	>128	yes	13
DGP-IgG tests							
EUROIMMUN AG	Anti-Gliadin-(GAF3X) ELISA (IgG)	RU/ml	<25	---	none	yes	2
Inova Diagnostics	QUANTA LiteTM Gliadin IgG II	U/ml	20	30	none	no	4
Phadia	EliA Giadin DP IgG ImmunoCAP	U/ml	7	10	>100 / none	yes	5
Orgentec	Anti-DGP IgG	U/ml	8	12	none	unknown	1

Table S2: *Specifications of Local EMA-IgA*

Table S2: *Specifications of local EMA tests as applied in and indicated by the laboratories of the clinical centers, including name of manufactuerer and test kit, type of result (titration to lowest dilution or ordinal scale) and lowest dilution regarded positive if titration to lowest dilution was done*

manufacturer	Name of test-kit	Type of result (titration to highest dilution, e.g. 1:10, OR ordinal scale, e.g. "negative", "mild pos.", "pos." OR "+" "++" "+++")	lowest dilution regarded as positive	number of centers using the same test and settings
Alphadia	Immunofluorescence	ordinal scale	na	3
Bio-Diagnostics	NOVA Lite Monkey Oesophagus IFA KIT EMA-IgA and IgG	ordinal scale	na	1
BioSystems	Monkey Oesophagus EMA-IgA and -IgG	titration	≥1:5	1
Delta BiologicalsDIA Medix	Primate Distal Esophagus Kit EMA-IgA and -IgG	ordinal scale	na	1
EUROIMMUN AG	Monkey Oesophagus EMA-IgA and -IgG	titration	≥ 1:10	5
Eurospital	Antiendomysium IgA and IgG Monkey Oesophagus	ordinal scale	na	4
	NOVA Lite Monkey Oesophagus IFA KIT EMA-IgA and IgG	titration	≥ 1:5	1
Inova Diagnostics		titration	≥ 1:10	4
		ordinal scale	na	5
IMMCO Diagnostics	EMA IgA and IgG	titration	na	1
In house	EMA IgA and IgG	titration	1:2.5	1
In house	EMA IgA and IgG	ordinal scale	na	1
In-house	EMA IgA and IgG	ordinal scale	na	1
Scimedx	EMA-IgA	ordinal scale	na	2
Orgentec	Monkey Oesophagus EMA-IgA and -IgG	titration	≥ 1:5	1

Table S3: *Specifications of central ELISA based tests (TGA, DGP)*

Table S3: *Specifications of centrally analysed TGA-IgA/IgG and DGP-IgG ELISA tests; all these tests were run on the same automatized platform (Euroimmun Analyzer I) and processed by the same bioanalysts.*

Test	Thermofisher/ Phadia Varelisa Celikey IgA	Inova R h-tTG IgA	Inova DGP IgG	Eurospital tTG IgA	Eurospital Glia Pep IgG	Euroimmun tTG IgG	Euroimmun GAF-3X	Zedira tTG IgA closed	Zedira DGP IgG	Zedira tTG IgA open	Zedira tTG IgG open
REF no.	181 96	704605	704520	9105	9138	EA 1910-9601 A	EV 3011-9601 G	E001	E020	E006	E007
Instrument	Euroimmun Analyzer I										
Standards	6 standards in duplicates	5 standards in duplicates	1 standard in duplicates	5 standards in duplicates	5 standards in duplicates	3 standards in single determ.	3 standards in single determ.	6 standards in duplicates	6 standards in duplicates	6 standards in duplicates	6 standards in duplicates
Measuring range	0 - 100 U/ml	0 - 100 U/ml	0 - >150 U	0 - 100 AU/ml	0 - 100 AU/ml	2 - 200 RU/ml	2 - 200 RU/ml	0 - 100 U/ml	0 - 100 U/ml	0 - 100 U/ml	0 - 100 U/ml
Curve drawing / calculation	4 parameter lin-log	cubic spline lin-log	ratio	Linear regression	Linear regression	point-to-point, linear	point-to-point, linear	4 parameter lin-log	4 parameter lin-log	4 parameter lin-log	4 parameter lin-log
Kit controls	1 pos + 1 neg in duplicates	1 pos + 1 neg in duplicates	1 pos + 1 neg in duplicates	1 pos + 1 neg in duplicates	1 pos + 1 neg in duplicates	1 pos + 1 neg in single determ.	1 pos + 1 neg in single determ.	1 pos + 1 neg in duplicates	1 pos + 1 neg in duplicates	1 pos + 1 neg in duplicates	1 pos + 1 neg in duplicates
In house control	1 in duplicates	1 in duplicates	1 in duplicates	1 in duplicates	1 in duplicates	1 in single determ.	1 in single determ.	1 in duplicates	1 in duplicates	1 in duplicates	1 in duplicates
Inter-assay-variation, validation data	N=9, Mean = 10, CV% = 14	N=6, Mean = 17, CV% = 5	N=7, Mean = 11, CV% = 6	N=5, Mean = 25, CV% = 9	N=5, Mean = 20, CV% = 6	N=5, Mean = 25, CV% = 19	N=5, Mean = 23, CV% = 7	N=6, Mean = 5, CV% = 10	N=6, Mean = 2, CV% = 22	N=6, Mean = 5, CV% = 10	N=6, Mean = 16, CV% = 8
	N=6, Mean = 55, CV% = 12	N=6, Mean = 79, CV% = 9	N=6, Mean = 64, CV% = 6	N=5, Mean = 85, CV% = 5	N=5, Mean = 80, CV% = 8	N=5, Mean = 115, CV% = 4	N=5, Mean = 101, CV% = 4	N=5, Mean = 64, CV% = 8	N=6, Mean = 48, CV% = 4	N=5, Mean = 51, CV% = 3	N=5, Mean = 50, CV% = 4
Intra-assay-variation, validation data	N=4, Mean = 10, CV% = 13	N=4, Mean = 15, CV% = 9	N=6, Mean = 11, CV% = 5	N=5, Mean = 20, CV% = 6	N=5, Mean = 29, CV% = 4	N=5, Mean = 29, CV% = 5	N=5, Mean = 20, CV% = 5	N=4, Mean = 5, CV% = 11	N=4, Mean = 3, CV% = 18	N=4, Mean = 6, CV% = 10	N=4, Mean = 15, CV% = 5
	N=4, Mean = 58, CV% = 17	N=4, Mean = 67, CV% = 16	N=4, Mean = 68, CV% = 6	N=5, Mean = 86, CV% = 3	N=5, Mean = 87, CV% = 3	N=5, Mean = 105, CV% = 3	N=5, Mean = 79, CV% = 6	N=4, Mean = 55, CV% = 5	N=4, Mean = 54, CV% = 7	N=4, Mean = 49, CV% = 8	N=4, Mean = 45, CV% = 5

Table S4: *Specifications of central non-ELISA tests (TGA, DGP)*

Table S4: *Specification of the four centrally analysed non-ELISA-based TGA-IgA and DGP-IgG tests from Thermofisher/Phadia and Inova Diagnostics; the tests were run on the specific instruments*

Test	Thermofisher/Phadia EliA Celikey DGP IgG	Thermofisher/Phadia EliA Celikey tTG IgA	QUANTA Flash t-TG IgA	QUANTA Flash DGP IgG
REF no.	na	na	701103	701173
Instrument	Phadia250	Phadia250	BIO-FLASH	BIO-FLASH
Standards	6 standards in duplicates	6 standards in duplicates	*lot-specific calibration	*lot-specific calibration
Measuring range	0 - 600 µg/l	0 - 80 µg/l	1,9 CU - 4965,5 CU	2,8 CU - 1936,7 CU
Curve drawing / calculation	Rodbard 4-parameter	Rodbard 4-parameter	lot specific working calibration curve	lot specific working calibration curve
Kit controls	1 pos + 1 neg in single determ. + 1 curve control	1 pos + 1 neg in single determ. + 1 curve control	1 pos + 1 neg	1 pos + 1 neg
In house control	1 in single determ.	1 in single determ.	-	-
Inter-assay-variation, validation data	N=5, Mean = 19, CV% = 6	N=5, Mean = 13, CV% = 4	N=6, Mean = 10, CV% = 6	N=6, Mean = 11, CV% = 7
	N=5, Mean = 190, CV% = 4	N=5, Mean = 123, CV% = 6	N=6, Mean = 67, CV% = 5	N=6, Mean = 56, CV% = 2
Intra-assay-variation, validation data	N=4, Mean = 20, CV% = 0	N=4, Mean = 13, CV% = 5	N=3, Mean = 10, CV% = 4	N=3, Mean = 11, CV% = 5
	N=4, Mean = 199, CV% = 6	N=4, Mean = 127, CV% = 6	N=3, Mean = 67, CV% = 4	N=3, Mean = 56, CV% = 1

Table S5: *Summary of recruited but excluded patients including reasons for exclusion*

Table S5: *Summary of recruited but excluded patients including reasons for exclusion and sorted by time of exclusion (n=79); patients which have been excluded due to low total IgA are listed separately in table S8 (n=17), in total 96 recruited patients had to be excluded. Some patients listed below have not been entered into the database and therefore have no ID (n=21;*

ID	Time of exclusion	Reason for exclusion
*not in database**	after recruitment	withdrew consent for study
*not in database**	after recruitment	withdrew consent for study
*not in database**	after recruitment	withdrew consent for study
*not in database**	after recruitment	withdrew consent for study
*not in database**	after recruitment	withdrew consent for biopsy
*not in database**	after recruitment	withdrew consent for biopsy
*not in database**	after recruitment	withdrew consent for biopsy
*not in database**	after recruitment	withdrew consent for biopsy
*not in database**	after recruitment	withdrew consent for biopsy
*not in database**	after recruitment	withdrew consent for biopsy
*not in database**	after recruitment	withdrew consent for biopsy
*not in database**	after recruitment	withdrew consent for biopsy
*not in database**	after recruitment	violation of exclusion criteria "immunodeficiency"
*not in database**	after recruitment	violation of exclusion criteria "previous CD diagnosis"
*not in database**	after recruitment	violation of exclusion criteria "previous CD diagnosis"
1017	after recruitment	incomplete - no histology slides available
151	after recruitment	incomplete - no HLA-typing (neither central nor local)
182	after recruitment	incomplete - no HLA-typing (neither central nor local)
203	after recruitment	incomplete - no HLA-typing (neither central nor local)
205	after recruitment	incomplete - no HLA-typing (neither central nor local)
241	after recruitment	incomplete - no HLA-typing (neither central nor local)
273	after recruitment	incomplete - no HLA-typing (neither central nor local)
326	after recruitment	incomplete - no HLA-typing (neither central nor local)
1005	after recruitment	incomplete - no HLA-typing (neither central nor local)
832	after recruitment	incomplete - no serum sample
973	after recruitment	incomplete - no serum sample
45	after recruitment	incomplete - several samples/data missing
623	after recruitment	incomplete - several samples/data missing
954	after recruitment	incomplete - several samples/data missing
955	after recruitment	incomplete - several samples/data missing
956	after recruitment	incomplete - several samples/data missing
957	after recruitment	incomplete - several samples/data missing
958	after recruitment	incomplete - several samples/data missing
*not in database**	after recruitment	incomplete - several samples/data missing
*not in database**	after recruitment	incomplete - several samples/data missing
*not in database**	after recruitment	incomplete - several samples/data missing
*not in database**	after recruitment	incomplete - several samples/data missing

*not in database**	after recruitment	incomplete - several samples/data missing
*not in database**	after recruitment	incomplete - several samples/data missing
162	after reference pathology	non evaluable histology
209	after reference pathology	non evaluable histology
398	after reference pathology	non evaluable histology
414	after reference pathology	non evaluable histology
465	after reference pathology	non evaluable histology
478	after reference pathology	non evaluable histology
571	After reference pathology	non evaluable histology
679	after reference pathology	non evaluable histology
817	after reference pathology	non evaluable histology
885	after reference pathology	non evaluable histology
1021	after reference pathology	non evaluable histology
66	after 4th review	non evaluable histology
88	after 4th review	non evaluable histology
90	after 4th review	non evaluable histology
116	after 4th review	non evaluable histology
181	after 4th review	non evaluable histology
192	after 4th review	non evaluable histology
221	after 4th review	non evaluable histology
224	after 4th review	non evaluable histology
267	after 4th review	non evaluable histology
384	after 4th review	non evaluable histology
437	after 4th review	non evaluable histology
489	after 4th review	non evaluable histology
516	after 4th review	non evaluable histology
598	after 4th review	non evaluable histology
628	after 4th review	non evaluable histology
638	after 4th review	non evaluable histology
648	after 4th review	non evaluable histology
655	after 4th review	non evaluable histology
698	after 4th review	non evaluable histology
703	after 4th review	non evaluable histology
740	after 4th review	non evaluable histology
755	after 4th review	non evaluable histology
844	after 4th review	non evaluable histology
875	after 4th review	non evaluable histology
944	after 4th review	non evaluable histology
729	4th review	protocol violation – gluten-free diet at time of biopsy is confirmed
541	4th review	protocol violation – negative TGA- and EMA-IgA, inclusion was based on positive TGA-IgG only and no total IgA had been done in the local center, but in the central lab IgA deficiency was excluded
733	4th review	technical error - sample mix-up is very likely
840	4th review	technical error - sample mix-up (confirmed for DNA, likely for serum)

* *for patients who were only submitted via the pre-screening log but no entered into the database, only basic information on age, gender TGA-IgA is available*

Table S6: *Excluded patients compared to the patients in the final cohort*

Table S6: *Excluded patients (except patients in low total IgA group) compared to patients in the final cohort for gender, age and the x-fold ULN of TGA-IgA*

Basic characteristics	Excluded patients (except low total IgA) (N=79)	Patients included in final cohort (N=707)
Female [%]	59.7%	65.1%
Age [yrs] mean ± SD (median; min-max)	6.3±4.4 (4.3; 1.1-17.9)	7.1±4.2 (6.2;0.7-18.6)
TGA-IgA x-fold ULN mean ± SD (median; min-max)*	23.9±120.4 (5.3;0.0-1018.3)	25.8±61.8 (11.8; 0.0-985.8)

**please note: some local laboratories only measured up to a certain maximum measuring range*

Table S7: *Gastrointestinal and extraintestinal symptoms*

Table S7: Gastrointestinal and extraintenstinal symptoms associated with celiac disease which were present within 4 weeks prior to study visit, listed according to frequency in descending order.

Symptom	Number of patients (%)	Total number of patients (N)
Gastrointestinal symptoms		
Abdominal pain*	355 (50.6)	701
- mild	161 (22.9)	
- moderate	132 (18.8)	
- severe	34 (4.9)	
- child cannot describe	28 (4.0)	
Diarrhea[#]	190 (27.1)	702
Abdominal distention	179 (25.3)	707
Flatulence	127 (18.2)	698
Constipation[§]	116 (16.5)	703
Recurrent vomiting	61 (8.7)	704
Extraintestinal symptoms		
Weight loss or insufficient gain[+]	213 (30.7)	693
Growth retardation[+]	113 (16.5)	684
Anemia	117 (16.6)	686
Anorexia	127 (18.19)	698
Fatigue / lack of energy	159 (22.8)	696
Irritability / moodiness	168 (24.2)	694
Lack of concentration (in children ≥6yrs of age)	49 (10.2)	481
Delayed puberty (in adolescents ≥12yrs of age)	7 (2.0)	343
Amenorrhea (in girls post-menarche)	5 (1.8)	277

*severity of abdominal pain was assessed as follows: mild = no influence on daily activity; moderate = some influence on daily activity, e.g. child interrupts play or homework; severe = major influence on daily activity

#chronic or intermittent

[§]only one stool every 3 days / less than 3 stools per week

[+] decrease of at least 2 percentiles

Table S8: *Characteristics of patients with low total IgA*

Table S8: *Characteristics of patients with low total IgA, both from local and from central laboratories and pathologists, including basic characteristics, local and central serology outcomes and histopathology (Marsh staging) as well as symptoms and final central diagnosis*

ID	Gender	Age [yrs]	HLA risk group	Total IgA [g/l]	Local TGA-IgA [x-fold above ULN]	Local TGA-IgG [x-fold above ULN]	No. of positive TGA-IgA in central lab	Local DGP-IgG [x-fold above ULN]	No. of positive DGP-IgG in central lab	Local EMA-IgA	Local EMA-IgG	Central EMA-IgG	Local histology Marsh staging	Reference histology Marsh staging	Symptoms	Final central diagnosis
5	male	17.1	1	0.06	0.22	4.1	1/8	-	6/6	negative	1:20	1:1000	M3	M3	none	proven CD
187	male	3.9	5	0.02	0.00	-	0/8	-	0/6	negative	-	negative	M0	M1	diarrhea	no CD
429	male	4.2	1	0.08	0.22	-	0/8	13.3	6/6	-	-	1:1000	M3	M3	diarrhea, flatulence, fatigue	proven CD
451	female	12.5	3	0.08	0.22	-	0/8	6.0	6/6	-	-	1:1000	M3	M3	severe abd. pain, fatigue, amenhorrea	proven CD
592	female	13.5	1	0.06	0.00	4.8	0/8	4.3	6/6	negative	positive	1:1000	M3	M3	constipation, weight loss, growth failure	proven CD
621	female	3.2	2	0.06	0.00	5.0	0/8	-	6/6	negative	1:1280	1:1000	M3	M3	diarrhea, growth failure, weight loss	proven CD
649	female	6.4	5	0.00	0.00	2.3	0/8	-	0/6	-	negative	negative	M0	M1	abd. pain, diarrhea	no CD
727	female	6.0	3	0.06	-	75.4	0/8	-	6/6	-	positive	1:1000	M3	M3	diarrhea, fatigue	proven CD
816	male	2.7	5	0.09	-	2.9	0/8	-	5/6	-	-	negative	M0	M0	abd. pain, diarrhea, anorexia, weight loss	no CD
912	male	5.2	4	0.06	0.57	8.1	1/8	-	5/6	negative	weakly positive	negative	M3	M3	abd. pain, diarrhea	proven CD
918	male	2.6	3	0.06	0.00	5.6	0/8	-	0/6	negative	-	negative	M0	M3	abd. distention	no CD
924	male	1.0	5	0.12	0.14	1.3	0/8	-	5/6	negative	-	negative	M0	M0	diarrhea, anorexia	no CD
941	male	7.0	1	0.6	-	65.6	0/8	0.2	2/6	-	1:640	1:1000	M2	M3	diarrhea	proven CD
945	male	6.2	3	0.6	-	27.1	0/8	-	6/6	-	1:320	1:1000	M2	M3	irritability	proven CD
992	female	14.6	3	0.7	0.01	-	0/8	-	4/6	positive	-	1:1000	M0	M0	abd. pain, diarrhea, constipation, vomiting	Inconclusive /potential
994	female	13.2	1	0.7	-	18.29	0/8	-	6/6	negative	-	1:1000	M3	M3	abd. pain, diarrhea, constipation	proven CD
1023	female	3.3	1	0.23	0.01	10.0	0/8	-	6/6	negative	positive	1:1000	M3	M3	fatigue	proven CD

Table S9: *Characteristics of patients with negative TGA-IgA at time of biopsy*

Table S9: *Characteristics of patients with negative TGA-IgA at time of biopsy but previously positive TGA-IgA in external laboratory which lead to referral to clinical center and biopsy*

ID	Gender	Age	Gluten-free diet prior to biopsy?	Gluten intake	HLA risk group	Total IgA [g/l]	Initial TGA-IgA#	Cut-off initial TGA-IgA testkit	Local TGA-IgA [x-fold above ULN]	No. of positive TGA-IgA in central lab	No. of positive DGP-IgG in central lab	Local EMA-IgA	Central EMA-Iga	Local pathology	Reference pathology	Risk for CD and/or symptoms	Final central diagnosis
55	female	12.5	no	daily	1	1.05	14 U/l	<7 U/ml	0.4	1/8	5/6	negative	1:5	M0	M0	type 1 diabetes, 1st degree relative	no CD
124	female	5.6	yes*	daily	3	0.53	31	<9 U/ml	0.77	5/8	4/6	1:10	1:5	M3 only in bulb	M0	abd. pain, diarrhea, vomiting	proven CD
129	female	6.1	no	daily	5	1.10	28.7	-	0.2	0/8	1/6	-	negative	M0	M0	growth failure, abd. distention	no CD
236	male	9.3	no	daily	5	0.81	"positive"	-	0.9	8/8	5/6	positive	1:100	M3	M3	severe abd. pain	proven CD
237	male	5.3	no	daily	5	1.06	"positive"	-	0.9	3/8	0/6	negative	negative	M0	M0	none	no CD
239	female	3.2	no	daily	4	0.65	"positive"	-	0.2	0/8	0/6	negative	negative	M0	M0	abd. distention	no CD
277	female	6.0	no	3-4x/week	3	1.45	"positive"	-	0.7	6/8	1/6	1:5	1:10	M0	M2	1st degree relative; abd. pain	Inconclusive
286	female	4.5	no	daily	4	0.96	"positive"	-	0.5	6/8	4/6	mild pos	1:10	M3 only in bulb	M0	abd. pain, diarrhea, weight loss	proven CD
288	male	6.7	yes*	daily	5	0.92	"positive"	-	0.1	0/8	0/6	negative	negative	M0	M0	abd. pain, diarrhea	no CD
298	female	4.7	no	daily	3	0.54	29 U/ml	<20 U/ml	0.4	0/8	0/6	negative	negative	M0	M0	abd . pain	no CD
301	male	5.3	no	daily	5	1.68	49 U/ml	<20 U/ml	0.4	0/8	2/6	negative	negative	M0	M0	abd. pain	no CD
364	female	10.8	no	daily	1	1.48	5.3 U/ml	<5 U/ml	0.8	6/8	6/6	++	1:100	M3	M3	none	proven CD
394	female	9.3	no	daily	5	1.36	34.9 U/ml	<18 U/ml	0.0	0/8	0/6	negative	negative	M0	M0	autoimmune thyroid disease; abd. pain, weight loss, growth failure	no CD
408	female	6.8	no	daily	3	1.84	24 U/ml	<20 U/ml	0.0	0/8	0/6	negative	negative	M0	M0	severe abd. pain	no CD
409	female	10.5	no	daily	5	1.03	24 U/ml	<20 U/ml	0.0	0/8	0/6	negative	negative	M0	M0	severe abd. pain, abd. distention	no CD
428	female	1.06	no	daily	5	0.27	26 U/ml	<18 U/ml	0.0	0/8	5/6	negative	negative	M3	M0	weight loss, growth failure	no CD
433	female	7.1	no	daily	5	1.73	23.2 U/ml	<18 U/ml	0.0	0/8	0/6	negative	negative	M1	M1	none	no CD
459	female	2.2	no	daily	3	0.48	35 U/ml	<20 U/ml	0.0	0/8	1/6	negative	negative	M0	M0	abd. pain, diarrhea, vomiting	no CD
493	female	7.2	no	daily	5	0.55	33.8 U/ml	<7 U/ml	0.0	0/8	0/6	-	negative	M0	M3	abd. pain, diarrhea, fatigue	no CD
524	male	14.9	no	daily	5	4.04	23 U/ml	20 U/ml	0.0	0/8	0/6	negative	negative	M0	M1	abd. pain	no CD
642	female	3.3	no	3-4x/week	3	0.33	7 U/l	<7 U/ml	0.6	1/8	0/6	negative	negative	M0	M1	abd. pain, diarrhea, constipation	no CD
651	female	13.3	no	3-4x/week	3	1.21	8.9 U/ml	<7 U/ml	0.9	8/8	6/6	1:200	1:100	M3	M2	abd. pain, weight loss, fatigue	proven CD
659	female	4.2	no	daily	3	0.57	"positive"	-	0.8	3/8	2/6	negative	1:10	M0	M2	abd. pain	proven CD
667	male	10.2	no	daily	3	0.72	"positive"	-	0.4	1/8	2/6	1:5	1:10	M0	M1	abd. pain	proven CD
732	female	2.7	no	daily	3	0.97	17 U/ml	<7 U/ml	0.1	8/8	6/6	1:2560	1:10	M0	M3	diarrhea	proven CD
747	female	5.5	no	daily	3	0.40	9.1 U/ml	<9 U/ml	0.4	4/8	6/6	++	1:10	M2	M1	none	proven CD
757	female	14.3	no	daily	5	1.07	60 U/ml	<20 U/ml	0.1	1/8	0/6	negative	negative	M0	M1	none	no CD
1022	female	12.9	no	daily	3	1.29	"positive"	-	0.4	2/8	1/6		negative	M0	M0	type 1 diabetes	no CD
1025	male	3.7	no	daily	1	0.23	"positive"	-	0.0	0/8	1/6		negative	M0	M0	growth failure, 1st degree relative	no CD

** patient 124 was on tentative gluten-free diet for 2 weeks, diet was stopped 11 months prior to biopsy; patient 288 was on gluten-free diet for 6 months, diet was stopped 7 months prior to biopsy;*
 # initial TGA-IgA was initially done in the clinical center or elsewhere (pediatric practice or another hospital) and the patient was forwarded to the clinical center due to the positive TGA-IgA result

Table S10: *Characteristics of patients with initially negative HLA risk alleles*

Table S10: *Characteristics of 18 patients with initially negative HLA risk alleles, thereof two with 3rd typing due to clinical suspicion of CD, and 16 patients in which CD was clearly excluded (N=707); basic characteristics, details on HLA-typing, local and central serology and histology (Marsh staging), symptoms and final diagnosis*

ID	Age [yrs]	Gender	HLA-DQ genotype Eurospital Positive alleles	HLA-DQ genotype EUROIMMUN Positive alleles	3rd typing (SNPs)	Total IgA [g/l]	Local TGA-IgA [x-fold above ULN]	No. of positive TGA-IgA in central lab	No. of positive DGP-IgG in central lab	Local EMA-IgA	Central EMA-IgA	Marsh staging of pathologists		Symptoms	Final central diagnosis
												Local	Reference		
507	1.8	male	DQA1*05	α-subunit DQ2.5	DQ2.2/DQ7[$]	1.06	33.3	8/8	6/6	1:320	1:1000	M3	M3	diarrhea, weight loss, anorexia	proven CD
761	1.5	female	rare allele combination[#]	α-subunit DQ2.2	DQ9/variant DQ2.2#	1.55	1.3	8/8	6/6	1:1280	1:000	M3	M3	diarrhea, weight loss, vomiting, anorexia	proven CD
237	5.3	male	DQA1*05	α-subunit DQ2.5	-	1.06	0.85	3/8	0/6	negative	negative	M0	M0	none	no CD
211	1.5	male	all negative	all negative	-	0.53	1.05	0/8	0/6	negative	negative	M0	M0	diarrhea, growth retardation	no CD
829	4.6	female	DQA1*05	α-subunit DQ2.5	-	0.43	1.30	0/8	2/6	negative	negative	M0	M0	abd. pain, weight loss, growth failure	no CD
301	5.3	male	DQA1*05	α-subunit DQ2.5	-	1.68	0.44	0/8	2/6	negative	negative	M0	M0	abd. pain	no CD
412	15.7	female	DQA1*05	α-subunit DQ2.5	-	1.84	1.50	0/8	0/6	negative	negative	M0	M0	abd. pain	no CD
839	1.3	male	DQA1*05	α-subunit DQ2.5	-	0.25	2.65	0/8	0/6	negative	negative	M0	M2	Flatulence, anorexia	no CD
757	14.4	female	all negative	all negative	-	1.07	0.04	1/8	0/6	negative	negative	M0	M1	abd. pain	no CD
493	7.1	female	all negative	all negative	-	0.55	0.01	0/8	0/6	negative	negative	M0	M3A	abd. pain, diarrhea,	no CD
213	5.7	male	all negative	α-subunit DQ8 A1*03:01	-	1.06	1.24	1/8	0/6	negative	negative	M3	M0	recurrent arthritis	no CD
355	8.0	female	DQA1*05	α-subunit DQ2.5	-	1.11	2.45	0/8	1/6	negative	negative	M1	M0	abd. distention	no CD
766	14.9	male	all negative	α-subunit DQ8 A1*03:02/03	-	1.64	2.95	0/8	1/6	negative	negative	M1	M0	abd. pain, growth retardation,	no CD
394	9.3	female	all negative	all negative	-	1.36	0.03	0/8	0/6	negative	negative	M0	M0	abd. pain, weight loss, constipation, growth failure	no CD
838	3.1	female	DQA1*05	α-subunit DQ2.5	-	0.51	1.90	0/8	0/6	negative	negative	M0	M1	anorexia, weight loss, growth failure	no CD
128	11.4	female	DQA1*05	α-subunit DQ2.5	-	2.43	1.65	3/8	1/6	negative	negative	M1	M0	constipation, growth failure, irritability	no CD
288	6.7	male	DQA1*05	α-subunit DQ2.5	-	0.92	0.13	0/8	0/6	negative	negative	M0	M0	abd. pain, abd. distention, diarrhea	no CD
130	1.1	male	all negative	all negative	-	0.37	1.64	0/8	0/6	negative	negative	M3	M0	constipation, irritability	no CD

[$] *mix-up of initial sample with sample of the child's father was proven; 3rd typing was done with new sample and the result was confirmed by re-typing with EU-Gen-risk and EUROarray*

*# Eurospital found DRB1*07 DRB1*04, DQA1*0201 DQA1*03, DQB1*0303/01, SNP typing and DQB1*03 subtyping in University of Debrecen showed DQ9 (DQA1*03-DQB1*0303) and a rare allele DR7-DQA1*0201-B1*0303/01*

Table S11: *Characteristics of inconclusive patients*

Table S11: *Characteristics of the 16 patients that were finally considered to be inconclusive (N=707), including final voting outcome of the diagnostic committee*

ID	Gender	Age	Gluten-free diet prior to biopsy?	Gluten intake	HLA risk group	Total IgA [g/l]	Local TGA-IgA [x-fold above ULN]	No. of positive TGA-IgA in central lab	No. of positive DGP-IgG in central lab	Local EMA-IgA	Central EMA-IgA	Marsh staging of pathologist			Morphometry analysis: Villous-crypt-ratio	Symptoms or at risk for CD	Final voting of the diagnostic committee (6 voters)	Reason why the case remained inconclusive
												Local	Reference					
													1	2				
22	male	2.6	no	daily	3	0.32	1.5	8/8	6/6	positive	positive	M0	M0	M0	2.08 but only 2 units measurable	fatigue, irritability	potential (6)	no villous atrophy
115	female	7.0	no	daily	3	1.94	10.0	1/8	0/6	1:10	negative	M0	M0	-	1.25; villous atrophy in bulb	T1DM; abd. pain, constipation	(5) potential (1)	inconsistent serology & histology
190	male	9.8	no	daily	3	0.98	4.7	4/8	2/6	mild positive	negative	M1	M1	M2	1:4; patchy	1st degree relative	unclear (3) proven (3)	inconsistent serology & histology
201	male	15.6	no	daily	1	1.79	5.1	7/8	6/6	mild positive	positive	M0	M1	M1	2.9; normal	none	potential (6)	o villous atrophy
240	female	1.1	no	daily	3	1.16*	20.0	0/8	4/6	negative	negative	M3	M3	M3	0.3; clear atrophy	diarrhea, weight loss, rowth retardation,	unclear (3) proven CD (3)	negative EMA and central serology
255	male	3.7	no	daily	1	0.42	1.5	2/8	4/6	negative	negative	M0	M0	M0	Not measurable	abdominal pain, vomiting	potential (5) exclude (1)	Inconsistent serology & histology
268	female	11.7	no	daily	3	2.11	2.9	7/8	1/6	negative	negative	pathologic but no Celiac	M0	-	2.3, but only 1 unit measurable	T1DM, autoim. thyroid disease; abd. pain	potential (6)	positive TGA but negative EMA, no villous atrophy
277	female	6.0	no	3-4x/week	3	1.45	0.7	6/8	1/6	1:5	positive	M0	M2	M0	2.19; normal	1st degree relative,; abd. pain	potential (5) proven CD (1)	inconsistent serology & histology
370	male	3.9	no	daily	3	0.27	1.9	0/8	0/6	+	negative	M3	M3	M3	0.53; villous atrophy	abd. pain, anorexia, weight loss	unclear (4) proven (2)	negative central serology
498	female	4.6	no*	daily	2	1.03	5.2	7/8	5/6	1:10	positive	M3	M0	M2	2.2; normal	growth retardation	potential (6)	inconsistent histology
518	female	8.3	no	daily	5	1.05	12.3	4/8	3/6	1:2.5	negative	M0	M0	-	2.9; normal	none	potential (6)	inconsistent serology & no villous atrophy
520	male	2.7	no	daily	3	0.70	5.5	7/8	3/6	negative	positive	M0	M1	M1	2.2 but only 2 units measurable	weight loss	potential (6)	no villous atrophy
644	male	5.1	no	daily	3	0.62	3.4	7/8	4/6	+	positive	M1	M1	M3	2.5, normal on measurable sites	abdominal pain	potential (3) proven CD (3)	inconsistent histology
777	female	5.2	no	daily	3	1.87	3.0	6/8	0/6	negative	negative	M0	M0	-	1.78 signs of crypt hyperplasia	type 1 diabetes; abd. pain, anorexia, irritability	potential (5) proven CD (1)	no villous atrophy
813	female	3.2	no	3-4x/week	2	0.55	2.2	7/8	4/6	1:2.5	positive	M1	M1	-	2.3; normal	none	potential (6)	no villous atrophy
831	female	6.0	no	daily	3	1.12	2.7	5/8	2/6	negative	positive	M0	M3	M3	Not measurable	abd. pain, anorexia, weight loss	proven CD (3) unclear (2) potential (1)	inconsistent serology & histology

**suspicion of wrong total IgA value (low total low total IgA as explanation) not confirmed by the center clinical center; no serum was left after central serology was done, and no follow-up available.*

Table S12: *Characteristics of false positive patients*

Table S12: *Characteristics of false positive patients, either for local TGA-IgA ≥10fold ULN or for central TGA-IgA / DGP-IgG ≥10fold ULN;*

ID	gender	age	HLA risk group	Total IgA [g/l]	Local TGA-IgA [x-fold above ULN]	No. of positive TGA-IgA in central lab	No. of positive DGP-IgG in central lab	Local EMA-IgA	Central EMA-IgA	Marsh staging of pathologist			Morphometry analysis: Villous-crypt-ratio	Risk factors or symptoms indicative for CD	Final diagnosis	Name of false positive local TGA-IgA or central TGA/DGP test ≥10xULN
										Local	Reference 1	Reference 2				
115	female	7.0	3	1.94	10.0	1/8	0/6	1:10	negative	M0	M0	-	1.25; villous atrophy in bulb	type 1 diabetes; moderate abdominal pain, constipation	Inconclusive case	**local TGA-IgA:** Euroimmun Polska (recombinant expression with baculovirus vector in insect cells), limit of normal <20 U/ml
240*	female	1.1	3	1.16#	20.0	0/8	4/6	negative	negative	M3	M3	M3	0.3; clear atrophy	diarrhea, weight loss, growth failure, abdominal distention	Inconclusive case	**local TGA-IgA:** done in external lab, test unknown, limit of normal <15 U/ml
518	female	8.3	1	1.05	12.3	4/8	3/6	1:2.5	negative	M0	M0	-	2.9, normal	none	Inconclusive case	**local TGA-IgA:** home-made RBC ELISA, limit of normal < 3 U/ml
796	male	6.9	3	1.15	10.3	2/8	0/6	negative	negative	M0	M0	-	-	type 1 diabetes; abdominal distention	no CD	**local TGA-IgA:** In-house radioligand assay, limit of normal <150cpm
22	male	2.7	3	0.32	1.5	8/8	6/6	positive	1:10	M0	M0	M0	2.08, but only 2 units measurable	fatigue, irritability	Inconclusive case	**central test T4:** Quanta Flash TG2-IgA
268	female	11.7	3	2.11	2.9	7/8	1/6	negative	1:10	M0	M0	-	2.3; but only 1 unit measurable	type 1 diabetes, autoimmune thyroid disease; mild abdominal pain, flatulence	Inconclusive case	**central test T6:** Euroimmun Anti-TG2-IgA
644*	female	5.1	3	0.62	3.4	7/8	4/6	positive	1:10	M1	M1	M3	2.5, normal on measurable sites	mild abdominal pain	Inconclusive case	**central test T6:** Euroimmun Anti-TG2-IgA
1022	female	12.9	3	1.29	0.4	2/8	1/6	*not available*	negative	M0	M0	-	-	type 1 diabetes	no CD	**central test D1:** Thermofisher EliA Gliadin DGP IgG

**time gap between local TGA-IgA and biopsy was >14 days: for patient with ID 240, the gap was 16 days, for patient with ID 644 the gap was 54 days.*

#suspicion of wrong total IgA value (low total low total IgA as explanation) not confirmed by the center clinical center; no serum was left after central serology was done, and no follow-up available.

Table S13: *Diagnostic accuracies measures for central TGA tests*

Table S13: *Diagnostic accuracies measures and 95% CI for central TGA tests T1 toT9 ≥10xULN ± combinations with EMA and/or symptoms to diagnose CD without biopsy , either considering inconclusive cases as no CD (Method M1, N=696-707) or excluding inconclusive cases (M2, N=680-691);*

Test	M	N	Combination	TP	FP	FN	TN	False %	Sensitivity[$] % [95%CI]			Specificity % [95%CI]			PPV % [95%CI]			LR+ [95%CI]		
T1	M1	707	**TGA-IgA ≥10xULN**	424	0	221	62	31.26	65.74	61.931	69.40	100.00	94.22	100.00	100.00	96.84	100.00	∞	∞	∞
T1	M1	707	+ any symptoms	382	0	263	62	37.20	59.23	55.32	63.05	100.00	94.22	100.00	100.00	99.12	100.00	∞	∞	∞
T1	M1	707	+ malabsorption	261	0	384	62	54.31	40.47	36.65	44.37	100.00	94.22	100.00	100.00	99.02	100.00	∞	∞	∞
T1	M1	707	TGA-IgA + EMA-IgA	415	0	230	62	32.53	64.34	60.51	68.04	100.00	94.22	100.00	100.00	96.84	100.00	∞	∞	∞
T1	M1	707	+ any symptoms	374	0	271	62	38.33	57.98	54.07	61.83	100.00	94.22	100.00	100.00	99.12	100.00	∞	∞	∞
T1	M1	707	+ malabsorption	255	0	390	62	55.16	39.54	35.74	43.43	100.00	94.22	100.00	100.00	99.02	100.00	∞	∞	∞
T1	M2	691	**TGA-IgA ≥10xULN**	424	0	221	46	31.98	65.74	61.93	69.40	100.00	92.29	100.00	100.00	96.84	100.00	∞	∞	∞
T1	M2	691	+ any symptoms	382	0	263	46	38.06	59.23	55.32	63.05	100.00	92.29	100.00	100.00	99.12	100.00	∞	∞	∞
T1	M2	691	+ malabsorption	261	0	384	46	55.57	40.47	36.65	44.37	100.00	92.29	100.00	100.00	99.02	100.00	∞	∞	∞
T1	M2	691	TGA-IgA + EMA-IgA	415	0	230	46	33.29	64.34	60.51	68.04	100.00	92.29	100.00	100.00	96.84	100.00	∞	∞	∞
T1	M2	691	+ any symptoms	374	0	271	46	39.22	57.98	54.07	61.83	100.00	92.29	100.00	100.00	99.12	100.00	∞	∞	∞
T1	M2	691	+ malabsorption	255	0	390	46	56.44	39.54	35.74	43.43	100.00	92.29	100.00	100.00	99.02	100.00	∞	∞	∞
T2	M1	707	**TGA-IgA ≥10xULN**	373	0	272	62	38.47	57.83	53.91	61.68	100.00	94.22	100.00	100.00	99.02	100.00	∞	∞	∞
T2	M1	707	+ any symptoms	340	0	305	62	43.14	52.71	48.78	56.62	100.00	94.22	100.00	100.00	98.92	100.00	∞	∞	∞
T2	M1	707	+ malabsorption	245	0	400	62	56.58	37.98	34.22	41.86	100.00	94.22	100.00	100.00	98.51	100.00	∞	∞	∞
T2	M1	707	TGA-IgA + EMA-IgA	366	0	279	62	39.46	56.74	52.82	60.61	100.00	94.22	100.00	100.00	99.00	100.00	∞	∞	∞
T2	M1	707	+ any symptoms	334	0	311	62	43.99	51.78	47.85	55.70	100.00	94.22	100.00	100.00	98.90	100.00	∞	∞	∞
T2	M1	707	**TGA-IgA ≥10xULN**	373	0	272	62	38.47	57.83	53.91	61.68	100.00	94.22	100.00	100.00	99.02	100.00	∞	∞	∞
T2	M2	691	**TGA-IgA ≥10xULN**	373	0	272	46	39.36	57.83	53.91	61.68	100.00	92.29	100.00	100.00	99.02	100.00	∞	∞	∞
T2	M2	691	+ any symptoms	340	0	305	46	44.14	52.71	48.78	56.62	100.00	92.29	100.00	100.00	98.92	100.00	∞	∞	∞
T2	M2	691	+ malabsorption	245	0	400	46	57.89	37.98	34.22	41.86	100.00	92.29	100.00	100.00	98.51	100.00	∞	∞	∞
T2	M2	691	TGA-IgA + EMA-IgA	366	0	279	46	40.38	56.74	52.82	60.61	100.00	92.29	100.00	100.00	99.00	100.00	∞	∞	∞
T2	M2	691	+ any symptoms	334	0	311	46	45.01	51.78	47.85	55.70	100.00	92.29	100.00	100.00	98.90	100.00	∞	∞	∞
T2	M2	691	+ malabsorption	240	0	405	46	58.61	37.21	33.47	41.07	100.00	92.29	100.00	100.00	98.48	100.00	∞	∞	∞
T3	M1	706	**TGA-IgA ≥10xULN**	436	0	208	62	29.46	67.70	63.94	71.30	100.00	94.22	100.00	100.00	99.16	100.00	∞	∞	∞
T3	M1	706	+ any symptoms	393	0	251	62	35.55	61.03	57.14	64.81	100.00	94.22	100.00	100.00	99.07	100.00	∞	∞	∞
T3	M1	706	+ malabsorption	273	0	371	62	52.55	42.39	38.54	46.31	100.00	94.22	100.00	100.00	98.66	100.00	∞	∞	∞
T3	M1	706	TGA-IgA + EMA-IgA	428	0	216	62	30.59	66.46	62.67	70.10	100.00	94.22	100.00	100.00	99.14	100.00	∞	∞	∞
T3	M1	706	+ any symptoms	386	0	258	62	36.54	59.94	56.04	63.75	100.00	94.22	100.00	100.00	99.05	100.00	∞	∞	∞
T3	M1	706	+ malabsorption	267	0	377	62	53.40	41.46	37.62	45.37	100.00	94.22	100.00	100.00	98.63	100.00	∞	∞	∞
T3	M2	690	**TGA-IgA ≥10xULN**	436	0	208	46	30.14	67.70	63.94	71.30	100.00	92.29	100.00	100.00	99.16	100.00	∞	∞	∞
T3	M2	690	+ any symptoms	393	0	251	46	36.38	61.03	57.14	64.81	100.00	92.29	100.00	100.00	99.07	100.00	∞	∞	∞
T3	M2	690	+ malabsorption	273	0	371	46	53.77	42.39	38.54	46.31	100.00	92.29	100.00	100.00	98.66	100.00	∞	∞	∞
T3	M2	690	TGA-IgA + EMA-IgA	428	0	216	46	31.30	66.46	62.67	70.10	100.00	92.29	100.00	100.00	99.14	100.00	∞	∞	∞
T3	M2	690	+ any symptoms	386	0	258	46	37.39	59.94	56.04	63.75	100.00	92.29	100.00	100.00	99.05	100.00	∞	∞	∞
T3	M2	690	+ malabsorption	267	0	377	46	54.64	41.46	37.62	45.37	100.00	92.29	100.00	100.00	98.63	100.00	∞	∞	∞

Table S13: *Diagnostic accuracies measures and 95% CI for central TGA tests T1 toT9 ≥10xULN ± combinations with EMA and/or symptoms to diagnose CD without biopsy , either considering inconclusive cases as no CD (Method M1, N=696-707) or excluding inconclusive cases (M2, N=680-691);*

Test	M	N	Combination	TP	FP	FN	TN	False %	Sensitivity[§] % [95%CI]			Specificity % [95%CI]			PPV % [95%CI]			LR+ [95%CI]		
T4	M1	696	**TGA-IgA ≥10xULN**	539	1	97	59	14.08	84.75	81.72	87.46	98.333	91.06	99.96	99.82	98.97	100.00	50.85	7.28	355.20
T4	M1	696	+ any symptoms	479	1	157	59	22.70	75.31	71.77	78.62	98.333	91.06	99.96	99.79	98.85	100.00	45.19	6.47	315.70
T4	M1	696	+ malabsorption	325	0	311	60	44.68	51.10	47.14	55.05	100.00	94.04	100.00	100.00	98.87	100.00	∞	∞	∞
T4	M1	696	TGA-IgA + EMA-IgA	524	1	112	59	16.24	82.39	79.20	85.27	98.333	91.06	99.96	99.81	98.94	100.00	49.43	7.08	345.30
T4	M1	696	+ any symptoms	466	1	170	59	24.57	73.27	69.65	76.67	98.333	91.06	99.96	99.79	98.81	100.00	43.96	6.29	307.20
T4	M1	696	+ malabsorption	315	0	321	60	46.12	49.53	45.57	53.49	100.00	94.04	100.00	100.00	98.84	100.00	∞	∞	∞
T4	M2	680	**TGA-IgA ≥10xULN**	539	0	97	44	14.26	84.75	81.72	87.46	100.00	91.96	100.00	100.00	99.32	100.00	∞	∞	∞
T4	M2	680	+ any symptoms	479	0	157	44	23.09	75.31	71.77	78.62	100.00	91.96	100.00	100.00	99.23	100.00	∞	∞	∞
T4	M2	680	+ malabsorption	325	0	311	44	45.74	51.10	47.14	55.05	100.00	91.96	100.00	100.00	98.87	100.00	∞	∞	∞
T4	M2	680	TGA-IgA + EMA-IgA	524	0	112	44	16.47	82.39	79.20	85.27	100.00	91.96	100.00	100.00	99.30	100.00	∞	∞	∞
T4	M2	680	+ any symptoms	466	0	170	44	25.00	73.27	69.65	76.67	100.00	91.96	100.00	100.00	99.21	100.00	∞	∞	∞
T4	M2	680	+ malabsorption	315	0	321	44	47.21	49.53	45.57	53.49	100.00	91.96	100.00	100.00	98.84	100.00	∞	∞	∞
T5	M1	706	**TGA-IgA ≥10xULN**	478	0	166	62	23.51	74.22	70.66	77.56	100.00	94.22	100.00	100.00	99.23	100.00	∞	∞	∞
T5	M1	706	+ any symptoms	431	0	213	62	30.17	66.93	63.14	70.55	100.00	94.22	100.00	100.00	99.15	100.00	∞	∞	∞
T5	M1	706	+ malabsorption	299	0	345	62	48.87	46.43	42.52	50.37	100.00	94.22	100.00	100.00	98.77	100.00	∞	∞	∞
T5	M1	706	TGA-IgA + EMA-IgA	467	0	177	62	25.07	72.52	68.89	75.93	100.00	94.22	100.00	100.00	99.21	100.00	∞	∞	∞
T5	M1	706	+ any symptoms	422	0	222	62	31.44	65.53	61.72	69.20	100.00	94.22	100.00	100.00	99.13	100.00	∞	∞	∞
T5	M1	706	+ malabsorption	291	0	353	62	50.00	45.19	41.30	49.12	100.00	94.22	100.00	100.00	98.74	100.00	∞	∞	∞
T5	M2	690	**TGA-IgA ≥10xULN**	478	0	166	46	24.06	74.22	70.66	77.56	100.00	92.29	100.00	100.00	99.23	100.00	∞	∞	∞
T5	M2	690	+ any symptoms	431	0	213	46	30.87	66.93	63.14	70.55	100.00	92.29	100.00	100.00	99.15	100.00	∞	∞	∞
T5	M2	690	+ malabsorption	299	0	345	46	50.00	46.43	42.52	50.37	100.00	92.29	100.00	100.00	98.77	100.00	∞	∞	∞
T5	M2	690	TGA-IgA + EMA-IgA	467	0	177	46	25.65	72.52	68.89	75.93	100.00	92.29	100.00	100.00	99.21	100.00	∞	∞	∞
T5	M2	690	+ any symptoms	422	0	222	46	32.17	65.53	61.720	69.20	100.00	92.29	100.00	100.00	99.13	100.00	∞	∞	∞
T5	M2	690	+ malabsorption	291	0	353	46	51.16	45.19	41.30	49.12	100.00	92.29	100.00	100.00	98.74	100.00	∞	∞	∞
T6	M1	707	**TGA-IgA ≥10xULN**	539	2	106	60	15.28	83.57	80.48	86.34	96.77	88.83	99.61	99.63	98.67	99.96	25.91	6.62	101.30
T6	M1	707	+ any symptoms	478	2	167	60	23.90	74.11	70.55	77.45	96.77	88.83	99.61	99.58	98.50	99.95	22.97	5.87	89.88
T6	M1	707	+ malabsorption	324	0	321	62	45.40	50.23	46.30	54.16	100.00	94.22	100.00	100.00	98.87	100.00	∞	∞	∞
T6	M1	707	TGA-IgA + EMA-IgA	523	1	122	61	17.40	81.09	77.85	84.04	98.39	91.34	99.96	99.81	98.94	100.00	50.27	7.19	351.40
T6	M1	707	+ any symptoms	464	1	181	61	25.74	71.94	68.30	75.38	98.39	91.34	99.96	99.79	98.81	100.00	44.60	6.38	311.80
T6	M1	707	+ malabsorption	313	0	332	62	46.96	48.53	44.61	52.46	100.00	94.22	100.00	100.00	98.83	100.00	∞	∞	∞
T6	M2	691	**TGA-IgA ≥10xULN**	539	0	106	46	15.34	83.57	80.48	86.34	100.00	92.29	100.00	100.00	99.32	100.00	∞	∞	∞
T6	M2	691	+ any symptoms	478	0	167	46	24.17	74.11	70.55	77.45	100.00	92.29	100.00	100.00	99.23	100.00	∞	∞	∞
T6	M2	691	+ malabsorption	324	0	321	46	46.45	50.23	46.30	54.16	100.00	92.29	100.00	100.00	98.87	100.00	∞	∞	∞
T6	M2	691	TGA-IgA + EMA-IgA	523	0	122	46	17.66	81.09	77.85	84.04	100.00	92.29	100.00	100.00	99.30	100.00	∞	∞	∞
T6	M2	691	+ any symptoms	464	0	181	46	26.19	71.94	68.30	75.38	100.00	92.29	100.00	100.00	99.21	100.00	∞	∞	∞
T6	M2	691	+ malabsorption	313	0	332	46	48.05	48.53	44.61	52.46	100.00	92.29	100.00	100.00	98.83	100.00	∞	∞	∞

[§] *Sensitivity: proportion of patients qualifying for non-biopsy approach;*

**TP=true positive, FP=false positive, FN=false negative, TN=true negative, PPV=positive predictive value, LR+=positive likelihood ratio*

Table S13: *Diagnostic accuracies measures and 95% CI for central TGA tests T1 toT9 ≥10xULN ± combinations with EMA and/or symptoms to diagnose CD without biopsy , either considering inconclusive cases as no CD (Method M1, N=696-707) or excluding inconclusive cases (M2, N=680-691);*

Test	M	N	Combination	TP	FP	FN	TN	False %	Sensitivity[§] % [95%CI]			Specificity % [95%CI]			PPV % [95%CI]			LR+ [95%CI]		
T7	M1	707	**TGA-IgA ≥10xULN**	446	0	199	62	28.15	69.15	65.42	72.70	100.00	94.22	100.00	100.00	99.18	100.00	∞	∞	∞
T7	M1	707	+ any symptoms	401	0	244	62	34.51	62.17	58.30	65.93	100.00	94.22	100.00	100.00	99.08	100.00	∞	∞	∞
T7	M1	707	+ malabsorption	279	0	366	62	51.77	43.26	39.39	47.18	100.00	94.22	100.00	100.00	98.69	100.00	∞	∞	∞
T7	M1	707	TGA-IgA + EMA-IgA	437	0	208	62	29.42	67.75	63.99	71.35	100.00	94.22	100.00	100.00	99.16	100.00	∞	∞	∞
T7	M1	707	+ any symptoms	393	0	252	62	35.64	60.93	57.04	64.72	100.00	94.22	100.00	100.00	99.07	100.00	∞	∞	∞
T7	M1	707	+ malabsorption	272	0	373	62	52.76	42.17	38.33	46.09	100.00	94.22	100.00	100.00	98.65	100.00	∞	∞	∞
T7	M2	691	**TGA-IgA ≥10xULN**	446	0	199	46	28.80	69.15	65.42	72.70	100.00	92.29	100.00	100.00	99.18	100.00	∞	∞	∞
T7	M2	691	+ any symptoms	401	0	244	46	35.31	62.17	58.30	65.93	100.00	92.29	100.00	100.00	99.08	100.00	∞	∞	∞
T7	M2	691	+ malabsorption	279	0	366	46	52.97	43.26	39.39	47.18	100.00	92.29	100.00	100.00	98.69	100.00	∞	∞	∞
T7	M2	691	TGA-IgA + EMA-IgA	437	0	208	46	30.10	67.75	63.99	71.35	100.00	92.29	100.00	100.00	99.16	100.00	∞	∞	∞
T7	M2	691	+ any symptoms	393	0	252	46	36.47	60.93	57.04	64.72	100.00	92.29	100.00	100.00	99.07	100.00	∞	∞	∞
T7	M2	691	+ malabsorption	272	0	373	46	53.98	42.17	38.33	46.09	100.00	92.29	100.00	100.00	98.65	100.00	∞	∞	∞
T8	M1	707	**TGA-IgA ≥10xULN**	435	0	210	62	29.70	67.44	63.67	71.05	100.00	94.22	100.00	100.00	99.16	100.00	∞	∞	∞
T8	M1	707	+ any symptoms	391	0	254	62	35.93	60.62	56.73	64.41	100.00	94.22	100.00	100.00	99.06	100.00	∞	∞	∞
T8	M1	707	+ malabsorption	268	0	377	62	53.32	41.55	37.72	45.46	100.00	94.22	100.00	100.00	98.63	100.00	∞	∞	∞
T8	M1	707	TGA-IgA + EMA-IgA	428	0	217	62	30.69	66.36	62.56	70.00	100.00	94.22	100.00	100.00	99.14	100.00	∞	∞	∞
T8	M1	707	+ any symptoms	384	0	261	62	36.92	59.54	55.63	63.35	100.00	94.22	100.00	100.00	99.04	100.00	∞	∞	∞
T8	M1	707	+ malabsorption	262	0	383	62	54.17	40.62	36.80	44.52	100.00	94.22	100.00	100.00	98.60	100.00	∞	∞	∞
T8	M2	691	**TGA-IgA ≥10xULN**	435	0	210	46	30.39	67.44	63.67	71.05	100.00	92.29	100.00	100.00	99.16	100.00	∞	∞	∞
T8	M2	691	+ any symptoms	391	0	254	46	36.76	60.62	56.73	64.41	100.00	92.29	100.00	100.00	99.06	100.00	∞	∞	∞
T8	M2	691	+ malabsorption	268	0	377	46	54.56	41.55	37.72	45.46	100.00	92.29	100.00	100.00	98.63	100.00	∞	∞	∞
T8	M2	691	TGA-IgA + EMA-IgA	428	0	217	46	31.40	66.36	62.56	70.00	100.00	92.29	100.00	100.00	99.14	100.00	∞	∞	∞
T8	M2	691	+ any symptoms	384	0	261	46	37.77	59.54	55.63	63.35	100.00	92.29	100.00	100.00	99.04	100.00	∞	∞	∞
T8	M2	691	+ malabsorption	262	0	383	46	55.43	40.62	36.80	44.52	100.00	92.29	100.00	100.00	98.60	100.00	∞	∞	∞
T9	M1	707	**TGA-IgA ≥10xULN**	146	0	499	62	70.58	22.64	19.46	26.06	100.00	94.22	100.00	100.00	97.51	100.00	∞	∞	∞
T9	M1	707	+ any symptoms	135	0	510	62	72.14	20.93	17.85	24.28	100.00	94.22	100.00	100.00	97.30	100.00	∞	∞	∞
T9	M1	707	+ malabsorption	99	0	546	62	77.23	15.35	12.65	18.37	100.00	94.22	100.00	100.00	96.34	100.00	∞	∞	∞
T9	M1	707	TGA-IgA + EMA-IgA	143	0	502	62	71.00	22.17	19.02	25.58	100.00	94.22	100.00	100.00	97.45	100.00	∞	∞	∞
T9	M1	707	+ any symptoms	132	0	513	62	72.56	20.47	17.42	23.79	100.00	94.22	100.00	100.00	97.24	100.00	∞	∞	∞
T9	M1	707	+ malabsorption	96	0	549	62	77.65	14.88	12.23	17.87	100.00	94.22	100.00	100.00	96.23	100.00	∞	∞	∞
T9	M2	691	**TGA-IgA ≥10xULN**	146	0	499	46	72.21	22.64	19.46	26.06	100.00	92.29	100.00	100.00	97.51	100.00	∞	∞	∞
T9	M2	691	+ any symptoms	135	0	510	46	73.81	20.93	17.85	24.28	100.00	92.29	100.00	100.00	97.30	100.00	∞	∞	∞
T9	M2	691	+ malabsorption	99	0	546	46	79.02	15.35	12.65	18.37	100.00	92.29	100.00	100.00	96.34	100.00	∞	∞	∞
T9	M2	691	TGA-IgA + EMA-IgA	143	0	502	46	72.65	22.17	19.02	25.58	100.00	92.29	100.00	100.00	97.45	100.00	∞	∞	∞
T9	M2	691	+ any symptoms	132	0	513	46	74.24	20.47	17.42	23.79	100.00	92.29	100.00	100.00	97.24	100.00	∞	∞	∞
T9	M2	691	+ malabsorption	96	0	549	46	79.45	14.88	12.23	17.87	100.00	92.29	100.00	100.00	96.23	100.00	∞	∞	∞

[§] *Sensitivity: proportion of patients qualifying for non-biopsy approach;*

**TP=true positive, FP=false positive, FN=false negative, TN=true negative, PPV=positive predictive value, LR+=positive likelihood ratio*

Table S14: *Diagnostic accuracies measures for central DGP tests*

Table S14: *Diagnostic accuracies measures and 95% CI for central DGP tests D1 toT6 ≥10xULN ± combinations with EMA and/or symptoms to diagnose CD without biopsy , either considering inconclusive cases as no CD (Method M1, N=678-707) or excluding inconclusive cases (M2, N=678-691);*

Test	M	N	Combination	TP	FP	FN	TN	False %	Sensitivity[§] % [95%CI]			Specificity % [95%CI]			PPV % [95%CI]			LR+ [95%CI]		
D1	M1	707	**DGP-IgG ≥10xULN**	249	1	396	61	56.15	38.61	34.83	42.49	98.39	91.34	99.96	99.60	97.79	99.99	23.93	3.42	167.60
D1	M1	707	+ any symptoms	235	0	410	62	57.99	36.43	32.71	40.28	100.00	94.22	100.00	100.00	98.44	100.00	∞	∞	∞
D1	M1	707	+ malabsorption	180	0	465	62	65.77	27.91	24.48	31.54	100.00	94.22	100.00	100.00	97.97	100.00	∞	∞	∞
D1	M1	707	DGP-IgG + EMA-IgA	245	0	400	62	56.58	37.98	34.22	41.86	100.00	94.22	100.00	100.00	98.51	100.00	∞	∞	∞
D1	M1	707	+ any symptoms	231	0	414	62	58.56	35.81	32.11	39.65	100.00	94.22	100.00	100.00	98.42	100.00	∞	∞	∞
D1	M1	707	+ malabsorption	176	0	469	62	66.34	27.29	23.88	30.90	100.00	94.22	100.00	100.00	97.93	100.00	∞	∞	∞
D1	M2	691	**DGP-IgG ≥10xULN**	249	1	396	45	57.45	38.61	34.83	42.49	97.83	88.47	99.95	99.60	97.79	99.99	17.76	2.55	123.70
D1	M2	691	+ any symptoms	235	0	410	46	59.33	36.43	32.71	40.28	100.00	92.29	100.00	100.00	98.44	100.00	∞	∞	∞
D1	M2	691	+ malabsorption	180	0	465	46	67.29	27.91	24.48	31.54	100.00	92.29	100.00	100.00	97.97	100.00	∞	∞	∞
D1	M2	691	DGP-IgG + EMA-IgA	245	0	400	46	57.89	37.98	34.22	41.86	100.00	92.29	100.00	100.00	98.51	100.00	∞	∞	∞
D1	M2	691	+ any symptoms	231	0	414	46	59.91	35.81	32.11	39.65	100.00	92.29	100.00	100.00	98.42	100.00	∞	∞	∞
D1	M2	691	+ malabsorption	176	0	469	46	67.87	27.29	23.88	30.90	100.00	92.29	100.00	100.00	97.93	100.00	∞	∞	∞
D2	M1	707	**DGP-IgG ≥10xULN**	16	0	629	62	88.97	2.48	1.42	4.00	100.00	94.22	100.00	100.00	79.41	100.00	∞	∞	∞
D2	M1	707	+ any symptoms	16	0	629	62	88.97	2.48	1.42	4.00	100.00	94.22	100.00	100.00	79.41	100.00	∞	∞	∞
D2	M1	707	+ malabsorption	16	0	629	62	88.97	2.48	1.42	4.00	100.00	94.22	100.00	100.00	79.41	100.00	∞	∞	∞
D2	M1	707	DGP-IgG + EMA-IgA	16	0	629	62	88.97	2.48	1.42	4.00	100.00	94.22	100.00	100.00	79.41	100.00	∞	∞	∞
D2	M1	707	+ any symptoms	16	0	629	62	88.97	2.48	1.42	4.00	100.00	94.22	100.00	100.00	79.41	100.00	∞	∞	∞
D2	M1	707	+ malabsorption	16	0	629	62	88.97	2.48	1.42	4.00	100.00	94.22	100.00	100.00	79.41	100.00	∞	∞	∞
D2	M2	691	**DGP-IgG ≥10xULN**	16	0	629	46	91.03	2.48	1.42	4.00	100.00	92.29	100.00	100.00	79.41	100.00	∞	∞	∞
D2	M2	691	+ any symptoms	16	0	629	46	91.03	2.48	1.42	4.00	100.00	92.29	100.00	100.00	79.41	100.00	∞	∞	∞
D2	M2	691	+ malabsorption	16	0	629	46	91.03	2.48	1.42	4.00	100.00	92.29	100.00	100.00	79.41	100.00	∞	∞	∞
D2	M2	691	DGP-IgG + EMA-IgA	16	0	629	46	91.03	2.48	1.42	4.00	100.00	92.29	100.00	100.00	79.41	100.00	∞	∞	∞
D2	M2	691	+ any symptoms	16	0	629	46	91.03	2.48	1.42	4.00	100.00	92.29	100.00	100.00	79.41	100.00	∞	∞	∞
D2	M2	691	+ malabsorption	16	0	629	46	91.03	2.48	1.42	4.00	100.00	92.29	100.00	100.00	79.41	100.00	∞	∞	∞
D3	M1	678	**DGP-IgG ≥10xULN**	302	0	316	60	46.61	48.87	44.86	52.89	100.00	94.04	100.00	100.00	98.79	100.00	∞	∞	∞
D3	M1	678	+ any symptoms	279	0	339	60	50.00	45.15	41.17	49.17	100.00	94.04	100.00	100.00	98.69	100.00	∞	∞	∞
D3	M1	678	+ malabsorption	209	0	409	60	60.32	33.82	30.09	37.70	100.00	94.04	100.00	100.00	98.25	100.00	∞	∞	∞
D3	M1	678	DGP-IgG + EMA-IgA	297	0	321	60	47.35	48.06	44.06	52.08	100.00	94.04	100.00	100.00	98.77	100.00	∞	∞	∞
D3	M1	678	+ any symptoms	274	0	344	60	50.74	44.34	40.37	48.35	100.00	94.04	100.00	100.00	98.66	100.00	∞	∞	∞
D3	M1	678	+ malabsorption	204	0	414	60	61.06	33.01	29.31	36.87	100.00	94.04	100.00	100.00	98.21	100.00	∞	∞	∞
D3	M2	662	**DGP-IgG ≥10xULN**	302	0	316	44	47.73	48.87	44.86	52.89	100.00	91.96	100.00	100.00	98.79	100.00	∞	∞	∞
D3	M2	662	+ any symptoms	279	0	339	44	51.21	45.15	41.17	49.17	100.00	91.96	100.00	100.00	98.69	100.00	∞	∞	∞
D3	M2	662	+ malabsorption	209	0	409	44	61.78	33.82	30.09	37.70	100.00	91.96	100.00	100.00	98.25	100.00	∞	∞	∞
D3	M2	662	DGP-IgG + EMA-IgA	297	0	321	44	48.49	48.06	44.06	52.08	100.00	91.96	100.00	100.00	98.77	100.00	∞	∞	∞
D3	M2	662	+ any symptoms	274	0	344	44	51.96	44.34	40.37	48.35	100.00	91.96	100.00	100.00	98.66	100.00	∞	∞	∞
D3	M2	662	+ malabsorption	204	0	414	44	62.54	33.01	29.31	36.87	100.00	91.96	100.00	100.00	98.21	100.00	∞	∞	∞

Table S14: *Diagnostic accuracies measures and 95% CI for central DGP tests D1 toT6 ≥10xULN ± combinations with EMA and/or symptoms to diagnose CD without biopsy , either considering inconclusive cases as no CD (Method M1, N=678-707) or excluding inconclusive cases (M2, N=678-691);*

Test	M	N	Combination	TP	FP	FN	TN	False %	Sensitivity[§] % [95%CI]			Specificity % [95%CI]			PPV % [95%CI]			LR+ [95%CI]		
D4	M1	706	**DGP-IgG ≥10ULN***	275	0	369	62	52.27	42.70	38.85	46.64	100.00	94.22	100.00	100.00	98.67	100.00	∞	∞	∞
D4	M1	706	+ any symptoms	256	0	388	62	54.96	39.75	35.95	43.65	100.00	94.22	100.00	100.00	98.57	100.00	∞	∞	∞
D4	M1	706	+ malabsorption	193	0	451	62	63.88	29.97	26.45	33.67	100.00	94.22	100.00	100.00	98.11	100.00	∞	∞	∞
D4	M1	706	DGP-IgG + EMA-IgA	270	0	374	62	52.97	41.93	38.08	45.84	100.00	94.22	100.00	100.00	98.64	100.00	∞	∞	∞
D4	M1	706	+ any symptoms	252	0	392	62	55.52	39.13	35.34	43.02	100.00	94.22	100.00	100.00	98.55	100.00	∞	∞	∞
D4	M1	706	+ malabsorption	189	0	455	62	64.45	29.35	25.86	33.03	100.00	94.22	100.00	100.00	98.07	100.00	∞	∞	∞
D4	M2	690	**DGP-IgG ≥10xULN***	275	0	369	46	53.48	42.70	38.85	46.63	100.00	92.29	100.00	100.00	98.67	100.00	∞	∞	∞
D4	M2	690	+ any symptoms	256	0	388	46	56.23	39.75	35.95	43.65	100.00	92.29	100.00	100.00	98.57	100.00	∞	∞	∞
D4	M2	690	+ malabsorption	193	0	451	46	65.36	29.97	26.45	33.67	100.00	92.29	100.00	100.00	98.11	100.00	∞	∞	∞
D4	M2	690	DGP-IgG + EMA-IgA	270	0	374	46	54.20	41.93	38.08	45.84	100.00	92.29	100.00	100.00	98.64	100.00	∞	∞	∞
D4	M2	690	+ any symptoms	252	0	392	46	56.81	39.13	35.34	43.02	100.00	92.29	100.00	100.00	98.55	100.00	∞	∞	∞
D4	M2	690	+ malabsorption	189	0	455	46	65.94	29.35	25.86	33.03	100.00	92.29	100.00	100.00	98.07	100.00	∞	∞	∞
D5	M1	707	**DGP-IgG ≥8ULN**[#]	152	0	493	62	69.73	23.57	20.34	27.04	100.00	94.22	100.00	100.00	97.60	100.00	∞	∞	∞
D5	M1	707	+ any symptoms	122	0	523	62	73.97	18.92	15.96	22.15	100.00	94.22	100.00	100.00	97.02	100.00	∞	∞	∞
D5	M1	707	+ malabsorption	153	0	492	62	69.59	23.72	20.49	27.20	100.00	94.22	100.00	100.00	97.62	100.00	∞	∞	∞
D5	M1	707	DGP-IgG + EMA-IgA	153	0	492	62	69.59	23.72	20.49	27.20	100.00	94.22	100.00	100.00	97.62	100.00	∞	∞	∞
D5	M1	707	+ any symptoms	150	0	495	62	70.01	23.26	20.05	26.71	100.00	94.22	100.00	100.00	97.57	100.00	∞	∞	∞
D5	M1	707	+ malabsorption	120	0	525	62	74.26	18.61	15.67	21.83	100.00	94.22	100.00	100.00	96.97	100.00	∞	∞	∞
D5	M2	691	**DGP-IgG ≥8xULN**[#]	155	0	490	46	70.91	24.03	20.78	27.52	100.00	92.29	100.00	100.00	97.65	100.00	∞	∞	∞
D5	M2	691	+ any symptoms	152	0	493	46	71.35	23.57	20.34	27.04	100.00	92.29	100.00	100.00	97.60	100.00	∞	∞	∞
D5	M2	691	+ malabsorption	122	0	523	46	75.69	18.92	15.96	22.15	100.00	92.29	100.00	100.00	97.02	100.00	∞	∞	∞
D5	M2	691	DGP-IgG + EMA-IgA	153	0	492	46	71.20	23.72	20.49	27.20	100.00	92.29	100.00	100.00	97.62	100.00	∞	∞	∞
D5	M2	691	+ any symptoms	150	0	495	46	71.64	23.26	20.05	26.71	100.00	92.29	100.00	100.00	97.57	100.00	∞	∞	∞
D5	M2	691	+ malabsorption	120	0	525	46	75.98	18.61	15.67	21.83	100.00	92.29	100.00	100.00	96.97	100.00	∞	∞	∞
D6	M1	707	**DGP-IgG ≥10xULN**	300	0	345	62	48.80	46.51	42.61	50.45	100.00	94.22	100.00	100.00	98.78	100.00	∞	∞	∞
D6	M1	707	+ any symptoms	278	0	367	62	51.91	43.10	39.24	47.02	100.00	94.22	100.00	100.00	98.68	100.00	∞	∞	∞
D6	M1	707	+ malabsorption	208	0	437	62	61.81	32.25	28.65	36.01	100.00	94.22	100.00	100.00	98.24	100.00	∞	∞	∞
D6	M1	707	DGP-IgG + EMA-IgA	293	0	352	62	49.79	45.43	41.54	49.36	100.00	94.22	100.00	100.00	98.75	100.00	∞	∞	∞
D6	M1	707	+ any symptoms	272	0	373	62	52.76	42.17	38.33	46.09	100.00	94.22	100.00	100.00	98.65	100.00	∞	∞	∞
D6	M1	707	+ malabsorption	202	0	443	62	62.66	31.32	27.75	35.05	100.00	94.22	100.00	100.00	98.19	100.00	∞	∞	∞
D6	M2	691	**DGP-IgG ≥10xULN**	300	0	345	46	69.31	24.03	20.78	27.52	100.00	94.22	100.00	100.00	97.65	100.00	∞	∞	∞
D6	M2	691	+ any symptoms	278	0	367	46	53.11	43.10	39.24	47.02	100.00	92.29	100.00	100.00	98.68	100.00	∞	∞	∞
D6	M2	691	+ malabsorption	208	0	437	46	63.24	32.25	28.65	36.01	100.00	92.29	100.00	100.00	98.24	100.00	∞	∞	∞
D6	M2	691	DGP-IgG + EMA-IgA	293	0	352	46	50.94	45.43	41.54	49.36	100.00	92.29	100.00	100.00	98.75	100.00	∞	∞	∞
D6	M2	691	+ any symptoms	272	0	373	46	53.98	42.17	38.33	46.09	100.00	92.29	100.00	100.00	98.65	100.00	∞	∞	∞
D6	M2	691	+ malabsorption	202	0	443	46	64.11	31.32	27.75	35.05	100.00	92.29	100.00	100.00	98.19	100.00	∞	∞	∞

[§] *Sensitivity: proportion of patients qualifying for non-biopsy approach; *TP=true positive, FP=false positive, FN=false negative, TN=true negative, PPV=positive predictive value, LR+=positive likelihood ratio*

[#] for DGP-IgG test D5, the 10x ULN is 250 U/ml but the maximum measuring range in the central laboratory was 200 U/ml, therefore the 8x ULN was applied

Table S15: *Agreement between local and central EMA-IgA*

Table S15: *Agreement in EMA-IgA outcome categorized in negative and positive between the results by the laboratory of the clinical study centers and the results from the central study laboratory*

Local EMA-IgA	Central EMA-IgA		
	Negative	positive	Total
Negative	46	37	83
Positive	6	589	595
Total	52	626	678*

**in 26 patients, local EMA-IgA and in 3 patients central EMA-IgA was not available*

Table S16: *Agreement between local and central TGA-IgA*

Table S16: *Agreement in ≥10xULN TGA-IgA between the local and the corresponding central TGA-IgA of the central lab if available and if both serum samples for local and central analyses were taken on the same day (N=334). As the real-life situation should be shown, it was not taken into account if the level of normal in the local laboratory differed from the manufacturer's instructions and therefore from the level of normal in the central lab.*

Local TGA-IgA ≥10ULN	Corresponding central TGA-IgA ≥10ULN		
	No	Yes	Total
No	95	18	113
Yes	25	196	221
Total	120	214	334

Table S17: *Agreement between Eu-Gen-risk and EUROArray HLA-typing*

Table S17: *Agreement between Eu-Gen risk and EUROArray HLA-typing*

Eur-Gen-Risk	EUROArray HLA-DQ2/DQ8				
	Negative	**Positive DQ2**	**Positive DQ8**	**Positive DQ2/DQ8**	**Total**
Negative	**17**	0	0	0	17
Positive DQ2	1**	**543 (534)***	1	3 (12)*	548
Positive DQ8	0	0	**49**	0	49
Positive DQ2/DQ8	0	12 (0)*	0	**69 (81)***	81
Total	18	555	50	72	**695**

*()*if definition of HLA-DQ8 positivity of EUROArray includes not only DQB1*03:02 and DQA1*03:01 but also DQB1*03:02 and DQA1*03:02/03 allele as "possible DQ8": the 12 discrepant cases disappear and are HLA-DQ2/DQ8 both in EUROArray and in Eu-Gen-Risk; however then 9 additional cases were positive for HLA-DQ2/DQ8 in EUROArray while Eu-Gen-Risk showed DQ2 only.*

*** Both tests detected alleles encoding the ß-subunit of DQ2 but not the corresponding α-subunit - interpreted as DQ2 positive with Eu-Gen-Risk and as DQ2 negative with EUROArray*

Table S18: *Agreement between local HLA-typing and EurGen-risk*

Table S18: *Agreement between local HLA-typing and Eu-Gen-risk*

Eu-Gen-Risk	Local HLA typing				
	Negative	**Positive DQ2**	**Positive DQ8**	**Positive DQ2/DQ8**	**Total**
Negative	**1**	0	0	0	1
Positive DQ2	0	**119**	2	4	127
Positive DQ8	0	0	**8**	0	8
Positive DQ2/DQ8	0	4	2	**11**	15
Total	1	123	12	15	**151**

Table S19: *Agreement between local HLA-typing and EUROArray*

Table S19: *Agreement between local HLA-typing and EUROArray*

EUROArray HLA-DQ2/DQ8	Local HLA typing				
	Negative	**Positive DQ2**	**Positive DQ8**	**Positive DQ2/DQ8**	**Total**
Negative	**1**	0	0	0	1
Positive DQ2	0	**120**	2	5	127
Positive DQ8	0	0	**8**	0	8
Positive DQ2/DQ8	0	3	2	**10**	15
Total	1	123	12	15	**151**

*()*if definition of HLA-DQ8 positivity of EUROArray includes not only DQB1*03:02 and DQA1*03:01 but also DQB1*03:02 and DQA1*03:02/03 allele as "possible DQ8": the 12 discrepant cases disappear and are HLA-DQ2/DQ8 both in EUROArray and in Eu-Gen-Risk; however then 9 additional cases were positive for HLA-DQ2/DQ8 in EUROArray while Eu-Gen-Risk showed DQ2 only.*

*** Both tests detected alleles encoding the ß-subunit of DQ2 but not the corresponding α-subunit - interpreted as DQ2 positive with Eu-Gen-Risk and as DQ2 negative with EUROArray*

Table S20: *Agreement between local and central Marsh-Oberhuber staging*

Table S20: *Agreement between local and central Marsh-Oberhuber staging*

Local histopathology Marsh-Oberhuber	Reference histopathology Marsh-oberhuber staging						
	0	**1**	**2**	**3A**	**3B**	**3C**	**Total**
0	**25**	15	5	7	0	1	53
1	6	**9**	4	5	2	2	28
2	0	3	**1**	7	5	2	18
3A	5	3	5	**37**	22	25	97
3B	0	6	4	65	**61**	80	216
3C	3	2	4	25	81	**149**	264
Total	39	38	23	146	171	259	**676***

* *in 31 patient the local pathologist did not indicate Marsh staging*

Table S21: *Agreement between local and central histology summary*

Table S21: *Comparison between local and reference histopathology summarized into indicative for CD (Marsh 2 to 3A-C) or not (Marsh 0 and 1)*

Local histopathology indicative for CD?	Reference histopathology indicative for CD?		
	No	**Yes**	**Total**
No	**55**	26	81
Yes	22	**573**	595
Total	77	599	**676***

* *in 31 patients the local pathologist did not indicate Marsh staging*

Table S22: *Agreement between local histology summary and the final central diagnosis*

Table S22: *comparison between local histopathology summarized into indicative for CD (Marsh 2 to 3A-C) or not (Marsh 0 and 1) and the final central diagnosis*

Local histopathology indicative for CD?	Final central diagnosis			
	No CD	Inconclusive	Proven CD	Total
No	32	12	37	81
Yes	3	3	589	595
Total	35	15	626	676

** in 31 patients the local pathologist did not indicate Marsh staging*

Table S23: *Agreement between local histology statement and the final central diagnosis*

Table S23: *Comparison between local histopathology statement (biopsy specimen is considered to show normal villi or pathologic signs but not indicative for CD or signs of CD with villous atrophy) and the final central diagnosis; in contrast to tables S21 and S22, this table includes also patients for whom the local pathologist did not give any Marsh staging but only the overall summary.*

Local histopathology statement	Final central diagnosis			
	No CD	Inconclusive	Proven CD	Total
Normal	32	9	20	61
Pathologic but no CD	11	3	18	32
Compatible with active CD	3	4	607	614
Total	46	16	645	707

Table S24: *Agreement between local and central diagnosis*

Table S24: *Comparison between the local diagnosis by the clinical center as reported in the database (no CD, unclear, proven CD) and the final central diagnosis (no CD, inconclusive case or proven CD)*

Local diagnosis	Final central diagnosis			
	No CD	Inconclusive	Proven CD	Total
No CD	31	3	8	42
Unclear / Inconclusive	14	10	37	61
Proven CD	1	3	600	604
Total	46	16	645	707

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