Accuracy in Diagnosis of Celiac Disease Without Biopsies in Clinical Practice

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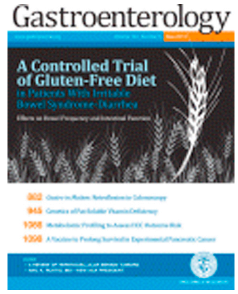

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Accuracy of Tests for Antibodies Against Tissue-transglutaminase in Diagnosis of Celiac Disease, Without Biopsy

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. Symptoms, measurements of total IgA, TGA, and EMA, and histopathology findings from duodenal biopsies (reference standard) were recorded. Children were considered to have malabsorption if they had chronic diarrhea, weight loss (or insufficient gain), growth failure, or anemia. We directly compared 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 analysis (reference). 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 without other features of celiac disease, were classified no celiac disease. Central histo-morphometry analyses were performed on all other biopsies and carefully reviewed in a blinded manner. Inconclusive cases were regarded as not having celiac disease. 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, positive results from the EMA tests of 2 blood samples, and the presence of 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; population; food allergy

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.*

References:

1. Husby S, Koletzko S, Korponay-Szabo IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease. J Pediatr Gastroenterol Nutr 2012;54:136-60.

2. Rewers M. Epidemiology of celiac disease: What are the prevalence, incidence, and progression of celiac disease? Gastroenterology 2005;128:S47-S51.

3. Salardi S, Volta U, Zucchini S, et al. Prevalence of Celiac Disease in Children With Type 1 Diabetes Mellitus Increased in the Mid-1990s: An 18-year Longitudinal Study Based on Anti-endomysial Antibodies. Journal of Pediatric Gastroenterology and Nutrition 2008;46:612-4.

4. Arguelles-Grande C, Tennyson CA, Lewis SK, Green PHR, Bhagat G. Variability in small bowel histopathology reporting between different pathology practice settings: impact on the diagnosis of coeliac disease. J Clin Pathol 2012;65:242-7.

5. Mubarak A, Nikkels P, Houwen R, Ten Kate F. Reproducibility of the histological diagnosis of celiac disease. Scandinavian Journal of Gastroenterology 2011;46:1065-73.

6. Picarelli A, Borghini R, Donato G, et al. Weaknesses of histological analysis in celiac disease diagnosis: new possible scenarios. Scandinavian Journal of Gastroenterology 2014;49:1318-24.

7. Alessio MG, Tonutti E, Brusca I, et al. Correlation Between IgA Tissue Transglutaminase Antibody Ratio and Histological Finding in Celiac Disease. J Pediatr Gastroenterol Nutr 2012;55:44-9.

8. Marsh M. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity Gastroenterology 1992;102:330-54.

9. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol 1999;11:1185-94.

10. Bourgey M, Calcagno G, Tinto N, et al. HLA related genetic risk for coeliac disease. Gut 2007;56:1054-9.

11. Margaritte-Jeannin P, Babron MC, Bourgey M, et al. HLA-DQ relative risks for coeliac disease in European populations: a study of the European Genetics Cluster on Coeliac Disease. Tissue Antigens 2004;63:562-7.

12. Korponay-Szabo IR, Troncone R, Discepolo V. Adaptive diagnosis of coeliac disease. Best Practice & Research Clinical Gastroenterology 2015;29:381-98.

13. Tortora R, Imperatore N, Capone P, et al. The presence of anti-endomysial antibodies and the level of anti-tissue transglutaminases can be used to diagnose adult coeliac disease without duodenal biopsy. Alimentary Pharmacology & Therapeutics 2014;40:1223-9.

14. Barker CC, Mitton C, Jevon G, Mock T. Can Tissue Transglutaminase Antibody Titers Replace Small-Bowel Biopsy to Diagnose Celiac Disease in Select Pediatric Populations? Pediatrics 2005;115:1341-6.

15. Beltran L, Koenig M, Egner W, et al. High-titre circulating tissue transglutaminase-2 antibodies predict small bowel villous atrophy, but decision cut-off limits must be locally validated. Clinical & Experimental Immunology 2014;176:190-8.

16. Di Tola M, Marino M, Goetze S, et al. Identification of a serum transglutaminase threshold value for the noninvasive diagnosis of symptomatic adult celiac disease patients: a retrospective study. Journal of Gastroenterology 2016:1-9.

17. Donat E, Ramos JM, Sánchez-Valverde F, et al. ESPGHAN 2012 Guidelines for Coeliac Disease Diagnosis: Validation Through a Retrospective Spanish Multicentric Study. Journal of Pediatric Gastroenterology and Nutrition 2016;62:284-91.

18. Gidrewicz D, Potter K, Trevenen CL, Lyon M, Butzner JD. Evaluation of the ESPGHAN Celiac Guidelines in a North American Pediatric Population. Am J Gastroenterol 2015;110:760-7.

19. Klapp G, Masip E, Bolonio M, et al. Celiac Disease: The New Proposed ESPGHAN Diagnostic Criteria Do Work Well in a Selected Population. Journal of Pediatric Gastroenterology and Nutrition 2013;56:251-6.

20. Mubarak A, Wolters VM, Gerritsen SA, Gmelig-Meyling FH, Ten Kate FJ, Houwen RH. A Biopsy Is Not Always Necessary to Diagnose Celiac Disease. J Pediatr Gastroenterol Nutr 2011;52:554-7.

21. Nevoral J, Kotalova R, Hradsky O, et al. Symptom positivity is essential for omitting biopsy in children with suspected celiac disease according to the new ESPGHAN guidelines. European Journal of Pediatrics 2014;173:497-502.

22. Trovato CM, Montuori M, Anania C, et al. Are ESPGHAN Biopsy-Sparing Guidelines for Celiac Disease also Suitable for Asymptomatic Patients. Am J Gastroenterol 2015;110:1485-9.

23. Vermeersch P, Geboes K, Mariën G, Hoffman I, Hiele M, Bossuyt X. Defining Thresholds of Antibody Levels Improves Diagnosis of Celiac Disease. Clinical Gastroenterology and Hepatology 2013;11:398-403.

24. Webb C, Norström F, Myléus A, et al. Celiac Disease Can Be Predicted by High Levels of Anti-Tissue Transglutaminase Antibodies in Population-Based Screening. Journal of Pediatric Gastroenterology and Nutrition 2015;60:787-91.

25. Wolf J, Hasenclever D, Petroff D, et al. Antibodies in the Diagnosis of Coeliac Disease: A Biopsy-Controlled, International, Multicentre Study of 376 Children with Coeliac Disease and 695 Controls. PLoS One 2014;9:e97853.

26. Kurppa K, Salminiemi J, Ukkola A, et al. Utility of the New ESPGHAN Criteria for the Diagnosis of Celiac Disease in At-risk Groups. Journal of Pediatric Gastroenterology and Nutrition 2012;54:387-91.

27. Elitsur Y, Sigman T, Watkins R, et al. Tissue Transglutaminase Levels Are Not Sufficient to Diagnose Celiac Disease in North American Practices Without Intestinal Biopsies. Digestive Diseases and Sciences 2017;62:175-9.

28. Smarrazzo A, Misak Z, Costa S, et al. Diagnosis of celiac disease and applicability of ESPGHAN guidelines in Mediterranean countries: a real life prospective study. BMC Gastroenterology 2017;17:17.

29. Fernández-Bañares F, Alsina M, Modolell I, et al. Are positive serum-IgA-tissue-transglutaminase antibodies enough to diagnose coeliac disease without a small bowel biopsy? Post-test probability of coeliac disease. Journal of Crohn's and Colitis 2012;6:861-6.

30. Webb C, Halvarsson B, Norström F, et al. Accuracy in Celiac Disease Diagnostics by Controlling the Small-bowel Biopsy Process. J Pediatr Gastroenterol Nutr 2011;52:549-53.

31. Egner W, Shrimpton A, Sargur R, Patel D, Swallow K. ESPGHAN Guidance on Coeliac Disease 2012: Multiples of the upper limit of normal for decision making do not Harmonise Assay performance across centres. Journal of Pediatric Gastroenterology & Nutrition.

32. Li M, Yu L, Tiberti C, et al. A report on the International Transglutaminase Autoantibody Workshop for Celiac Disease. Am J Gastroenterol 2009;104:154-63.

33.Björck S, Lynch K, Brundin C, Agardh D. Repeated Screening Can Be Restricted to At-Genetic-Risk Birth Cohorts. Journal of Pediatric Gastroenterology and Nutrition 2016;62:271-5.

34.Bodd M, Tollefsen S, Bergseng E, Lundin KEA, Sollid LM. Evidence that HLA-DQ9 confers risk to celiac disease by presence of DQ9-restricted gluten-specific T cells. Human Immunology 2012;73:376-81.

35.Giersiepen K, Lelgemann M, Stuhldreher N, et al. Accuracy of Diagnostic Antibody Tests for Coeliac Disease in Children: Summary of an Evidence Report. J Pediatr Gastroenterol Nutr 2012;54:229-41.

36.Lewis NR, Scott BB. Systematic review: the use of serology to exclude or diagnose coeliac disease (a comparison of the endomysial and tissue transglutaminase antibody tests). Alimentary Pharmacology & Therapeutics 2006;24:47-54.

37.Swallow K, Wild G, Sargur R, et al. Quality not quantity for transglutaminase antibody 2: the performance of an endomysial and tissue transglutaminase test in screening coeliac disease remains stable over time. Clinical and Experimental Immunology 2013;171:100-6.

38.Simell; S, Hoppu; S, Hekkala; A, et al. Fate of Five Celiac Disease-Associated Antibodies During Normal Diet in Genetically At-Risk Children Observed from Birth in a Natural History Study. Am J Gastroenterol 2007;102:2016-35.

39.Guandalini S, Newland C. Can We Really Skip the Biopsy in Diagnosing Symptomatic Children With Celiac Disease. Journal of Pediatric Gastroenterology and Nutrition 2013;57:e24.

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;*

Basic characteristics	Patients by clinical manifestation			Total	
	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

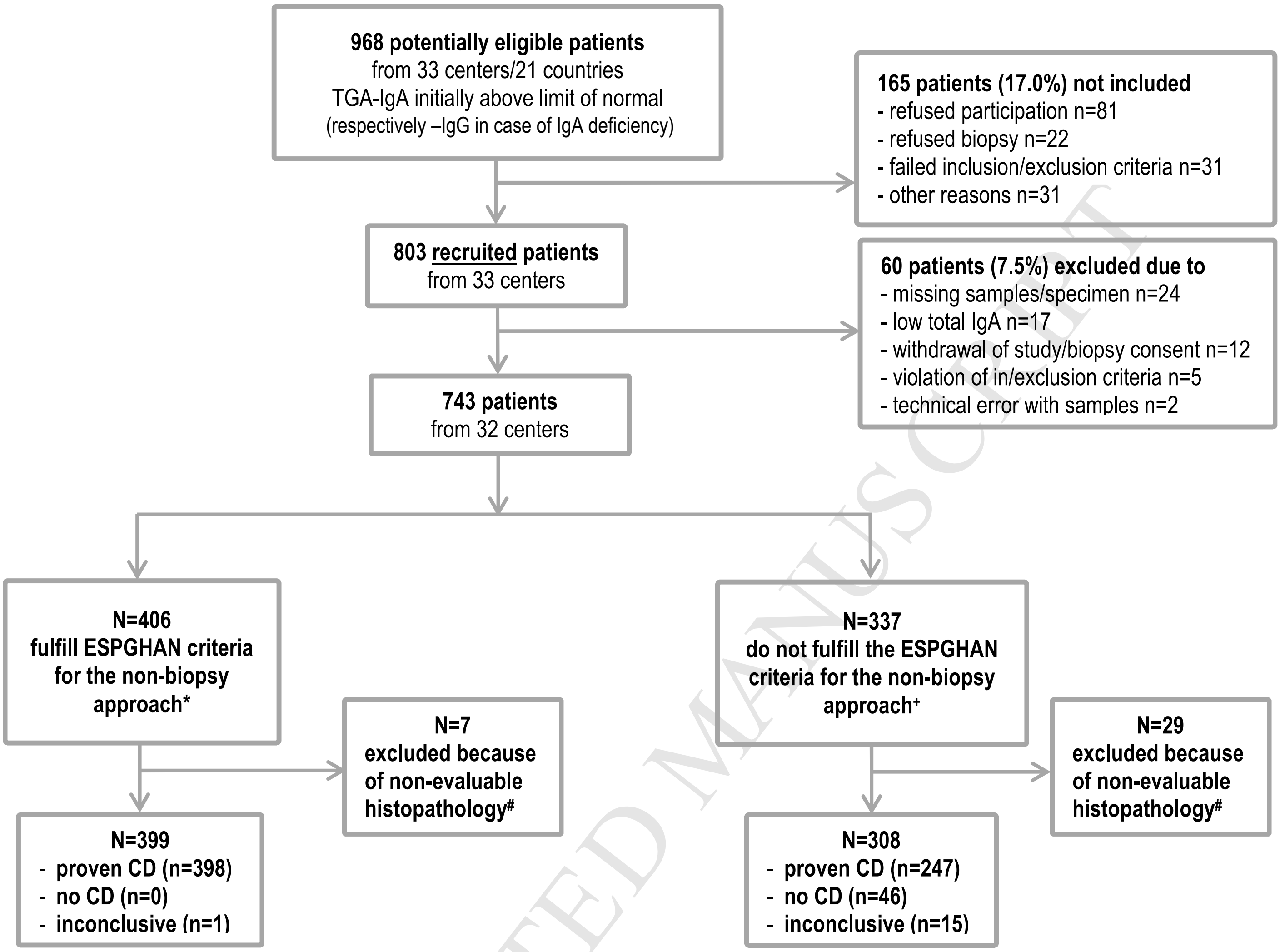
				Analyzer I			
D3	QUANTA Flash DGP IgG	Inova diagnostics	Chemiluminescence	BioFlash	20-30 U	30 U	Munich
D4	a-Gliapep-IgG - *code 9138*	Eurospital	ELISA	EUROIMMUN Analyzer I	10 U/ml	---	Odense
D5	Anti-Gliadin(GAF-3X)-ELISA IgG	EUROIMMUN	ELISA	EUROIMMUN Analyzer I	25 RU/ml	---	Odense
D6	Anti-DGPx1-IgG	R-Biopharm/Zedira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

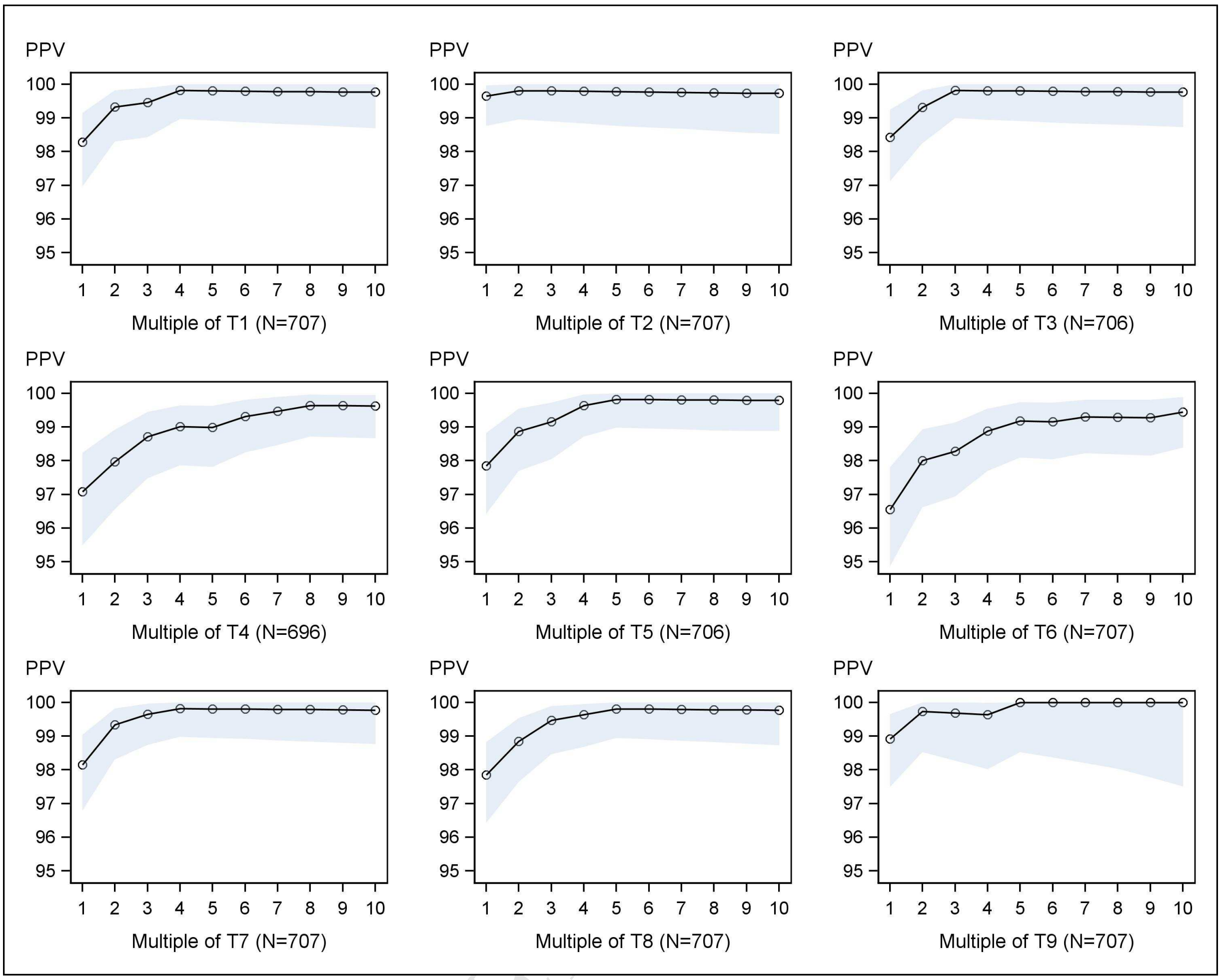
Figure 1



[*] *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*



PPV
100
99
98
97
96
95
1 2 3 4 5 6 7 8 9 10
Multiple of T1 (N=707)
Multiple of T2 (N=707)
Multiple of T3 (N=706)
Multiple of T4 (N=696)
Multiple of T5 (N=706)
Multiple of T6 (N=707)
Multiple of T7 (N=707)
Multiple of T8 (N=707)
Multiple of T9 (N=707)