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Prospective validation of antibody-based diagnostic procedures for paediatric coeliac disease - the AbCD study

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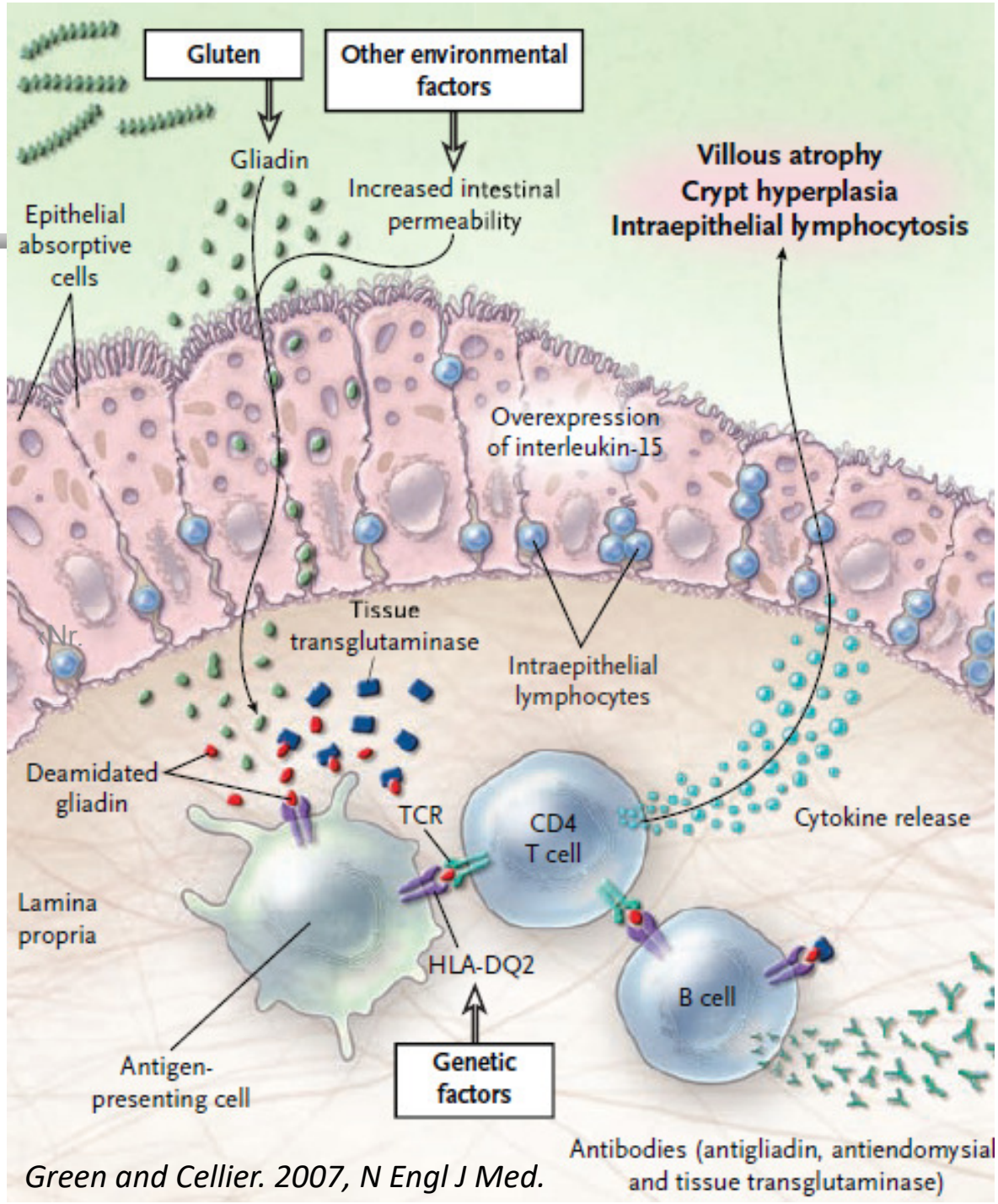


Zöliakie

- Gluten-sensitive Enteropathie
- **Autoimmunerkrankung des Dünndarms**
- Ursache:
  - **Gluten** aus Weizen und verwandte Proteine aus Gerste (Hordein) oder Roggen (Secalin) die mit der Nahrung aufgenommen werden
  - genetische Prädisposition der Betroffenen (HLA-DQ2, DQ8)

    → >98% bei Zöliakie, aber auch 20-40% in Normalbevölkerung

→ Prävalenz Weltweit zwischen 0,1% und 5,5%



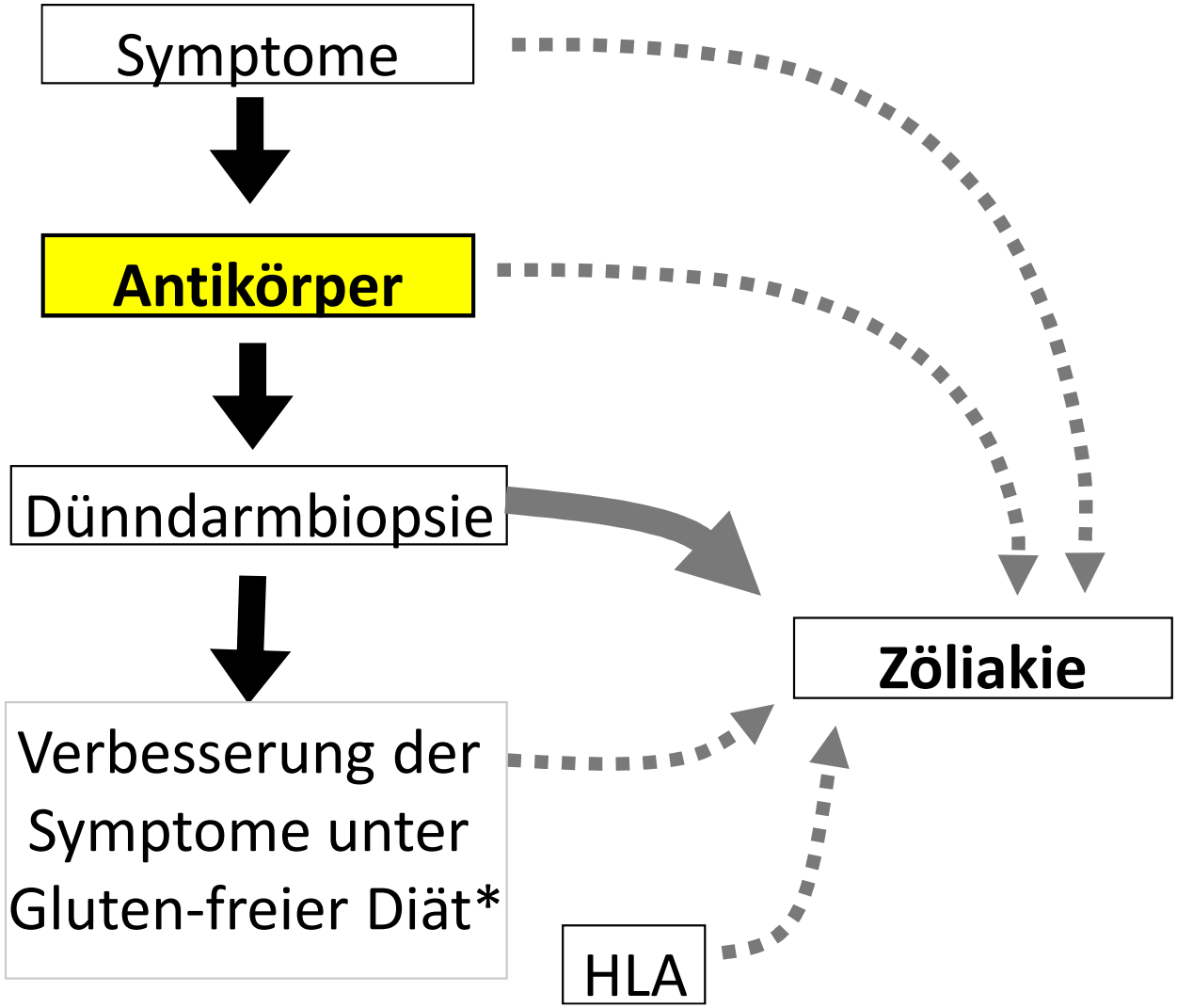
Green and Cellier. 2007, N Engl J Med.

Klinik und „Diagnose“

- Klassisch/Typisch:
  - Chronische Diarrhoe
  - Chronische Bauchschmerzen
  - Gewichtsverlust
  - Mangelerscheinungen
- Atypisch:
  - Müdigkeit
  - Anämie
  - Arthritis
  - Osteoporose
  - Leberdefekt
  - Unfruchtbarkeit

Zimmer, Monatsschr Kinderheilk. 1999





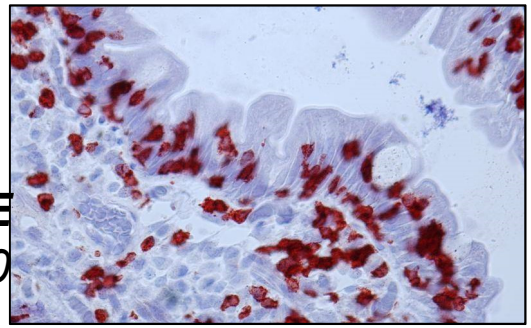
Histologie: Marsh-Oberhofer Klassifikation

Normal
(HE)



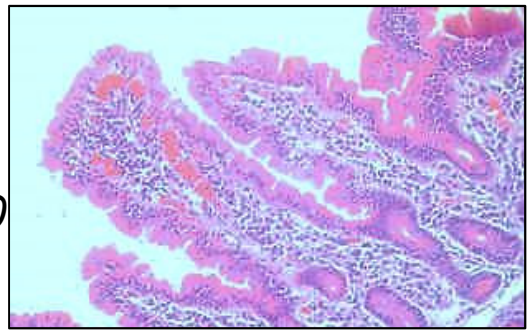
Marsh 1
(CD3+)

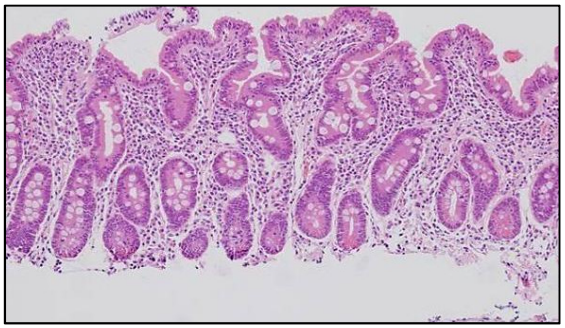
IEL/E
≥ 25/100



Marsh 2
(HE)

≥ 25/100





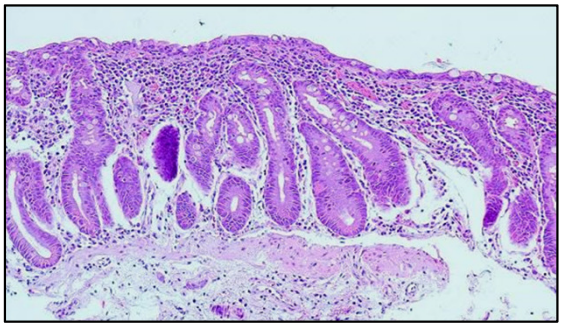
Marsh 3A
(HE)

≥ 25/100



Marsh 3B
(HE)

≥25/100



Marsh 3C
(HE)

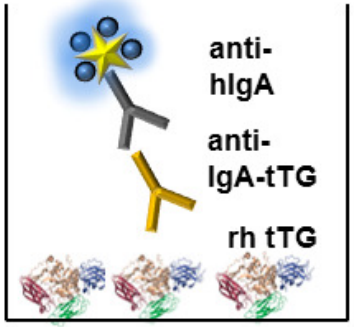
≥25/100

E = Enterozyten
IEL =Intraepitheale Lymphozyten
HE = Hämatoxylin-Eosin-Färbung

A. Werner, Thesis. Univ. Leipzig.2012

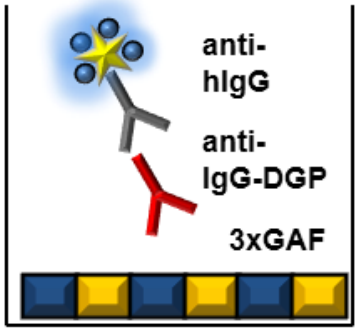
Serum-Antikörperdiagnostik

IgA-/ IgG-anti-tTG



Indirekter ELISA zur Detektion von Antikörper gegen tTG

IgA-/ IgG-anti-DGP

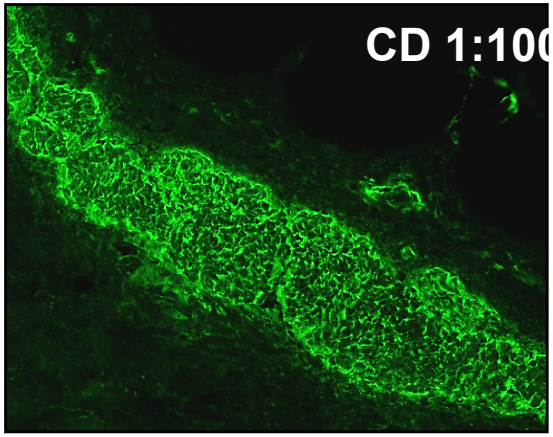


Indirekter ELISA zur Detektion von Antikörper gegen DGP

Gesamt IgA

Antikörper

IgA-/ IgG-EmA



IFT,Affenoesophagus, IgA anti-EmA

IgA-/ IgG-anti-nGlia

CD = Zöliakie
tTG = Gewebstransglutaminase
DGP = deamidierte Gliadinpetide
nGlia = natives Gliadin
EmA = Endomysium
IFT = indirekte Immunfluoreszenz

Antibodies in the Diagnosis of Coeliac Disease: A Biopsy-Controlled, International, Multicentre Study of 376 Children with Coeliac Disease and 695 Controls

Johannes Wolf[1☯], Dirk Hasenclever[2☯], David Petroff[3], Thomas Richter[4], Holm H. Uhlig[5], Martin W. Laaβ[6], Almuthe Hauer[7], Martin Stern[8], Xavier Bossuyt[9], Jan de Laffolie[10], Gunter Flemming[11], Danilo Villalta[12], Wolfgang Schlumberger[13], Thomas Mothes[1*]

Plos One 2014

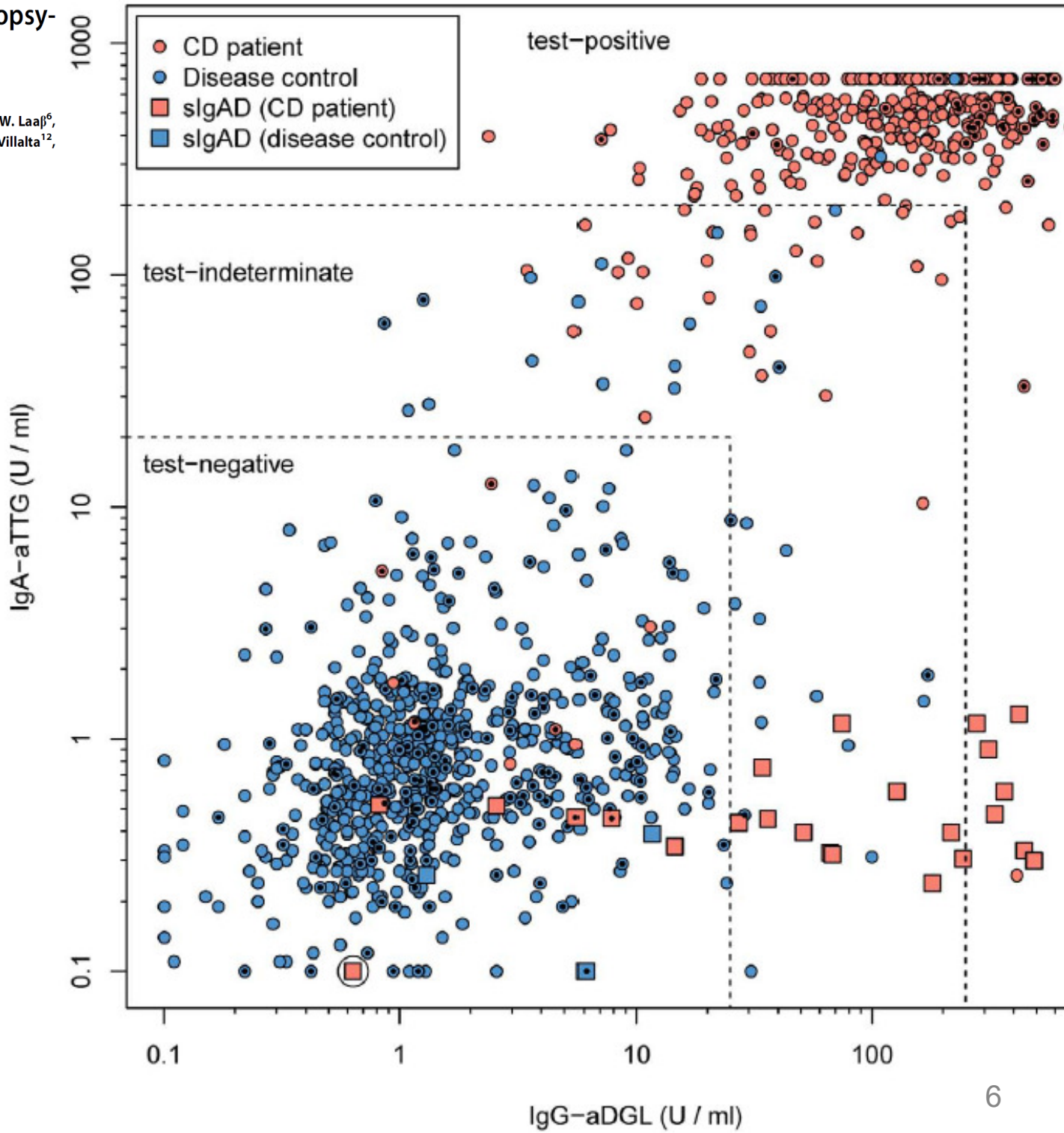
Sensitivität: 0,887

Spezifität: 0,953

PPW: **0,997** (*OC = 0,983*)

NPW: **0,986** (*OC = 0,977*)

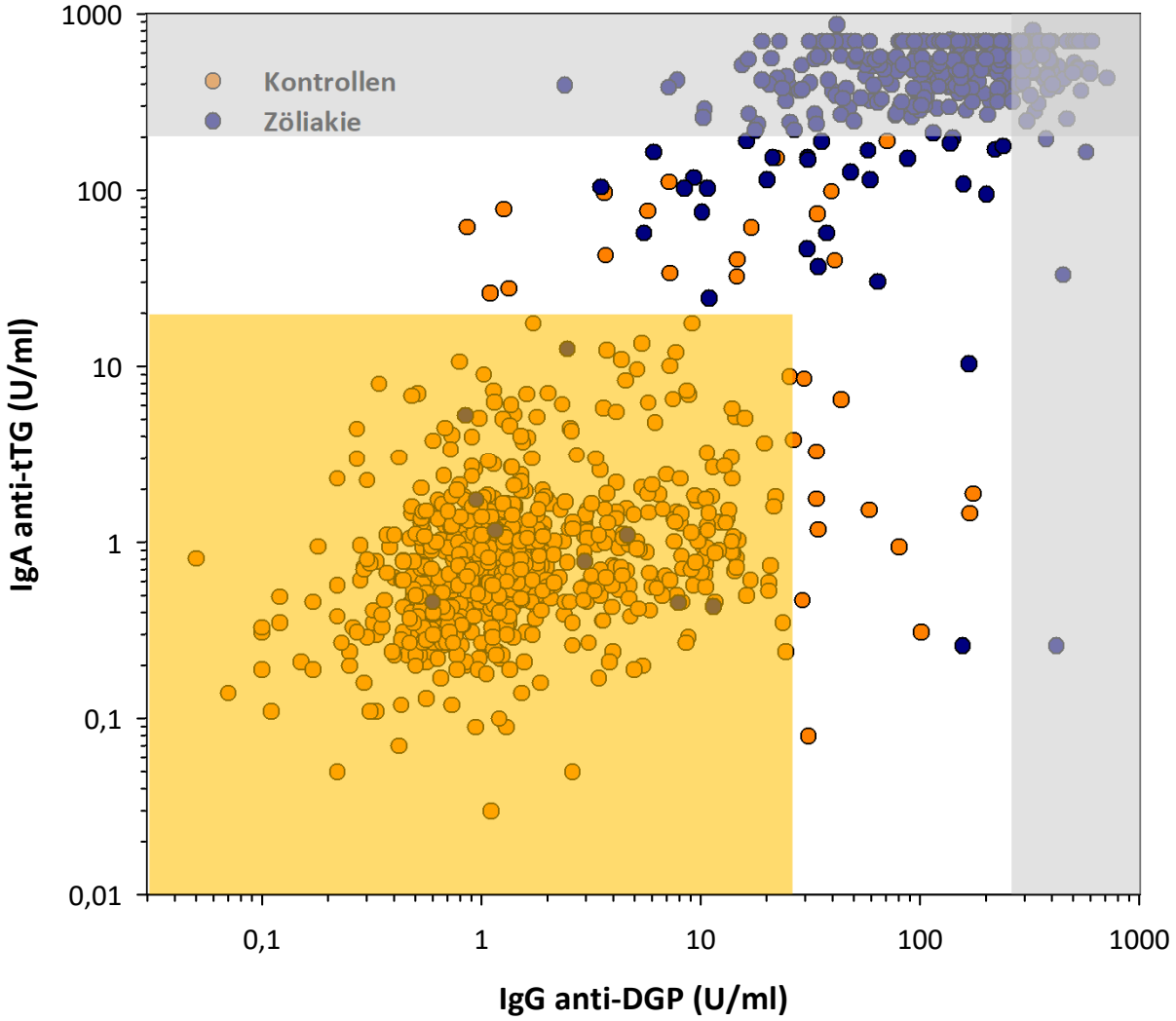
Prävalenz: 35.5%



Three valued logic for test results

Introduce a grey-zone=Test unclear=No decision

- Disease cases and controls are nicely separated by antibody titters
- There are
  - clearly negative cases,
  - clearly positive cases,
  - sporadic cases in between in which additional information is required for diagnosis.
- PPV high in clear cases



Characteristics of diagnostic tests
With grey-zone – three valued logic (T+, T-, T?)

	T-	T+	T?		
D-	p00	p01	p02	P0.	**1-Präv**
D+	p10	p11	p12	P1.	**Präv**
	p.0	p.1	p.2	1	

Sensitivity = **pr (T+ | D+) =** **p11 / p1.**
Anti-Sensitivity = **pr (T- | D+) =** **p10 / p1.**

Specifity = **pr (T- | D-) =** **p00 / p0.**
Anti-Specifity = **pr (T+ | D-) =** **p01 / p0.**

Prevalence= **pr (D+) =** **p1.**

Grey-zone= **pr (T?)=** **p.2 =** **pr (D+) * (1-Sens - aSens) + pr (D-)* (1-Spec-aSpec)**

PPV= **pr (D+|T+)=** **p11/p.1 =** **pr (D+) * Sens / (pr (D+) * Sens + pr(D-)*aSpec)**
NPV= **pr (D-|T-)=** **p00/p.0 =** **pr (D-) * Spec / (pr (D-) * Spec + pr(D+)*aSens)**

Given 5 characteristics
all others can be calculated
using the Bayes formula

Table 2. Comparison of the diagnostic procedures.

	Subjects without known sIgAD (352 CD and 692 control patients)		All subjects (376 CD and 695 control patients)	
	One-test-Procedure	Two-test-procedure	One-test-Procedure	Two-test-procedure
True positives	310	314	310	321
True negatives	673	659	676	662
False positives	2	2	2	2
False negatives	10	8	4	14
Number in grey zone	49	61	49	72
Sensitivity	0.881	0.892	0.824	0.854
Specificity	0.973	0.952	0.973	0.953
Anti-sensitivity	0.028	0.023	0.090	0.037
Anti-specificity	0.003	0.003	0.003	0.003
Prevalence range for reliable test	0.086–0.643	0.085–0.688	0.091–0.361	0.088–0.574
Proportion in grey zone	0.030–0.067	0.048–0.073	0.030–0.046	0.050–0.082

The prevalence range provides the interval for which the test procedure meets the reliability requirements of as defined in the statistics section. The proportion of children in the grey zone was calculated for the endpoints of the prevalence interval from the row above it. CD, coeliac disease; sIgAD, selective IgA deficiency.
doi:10.1371/journal.pone.0097853.t002

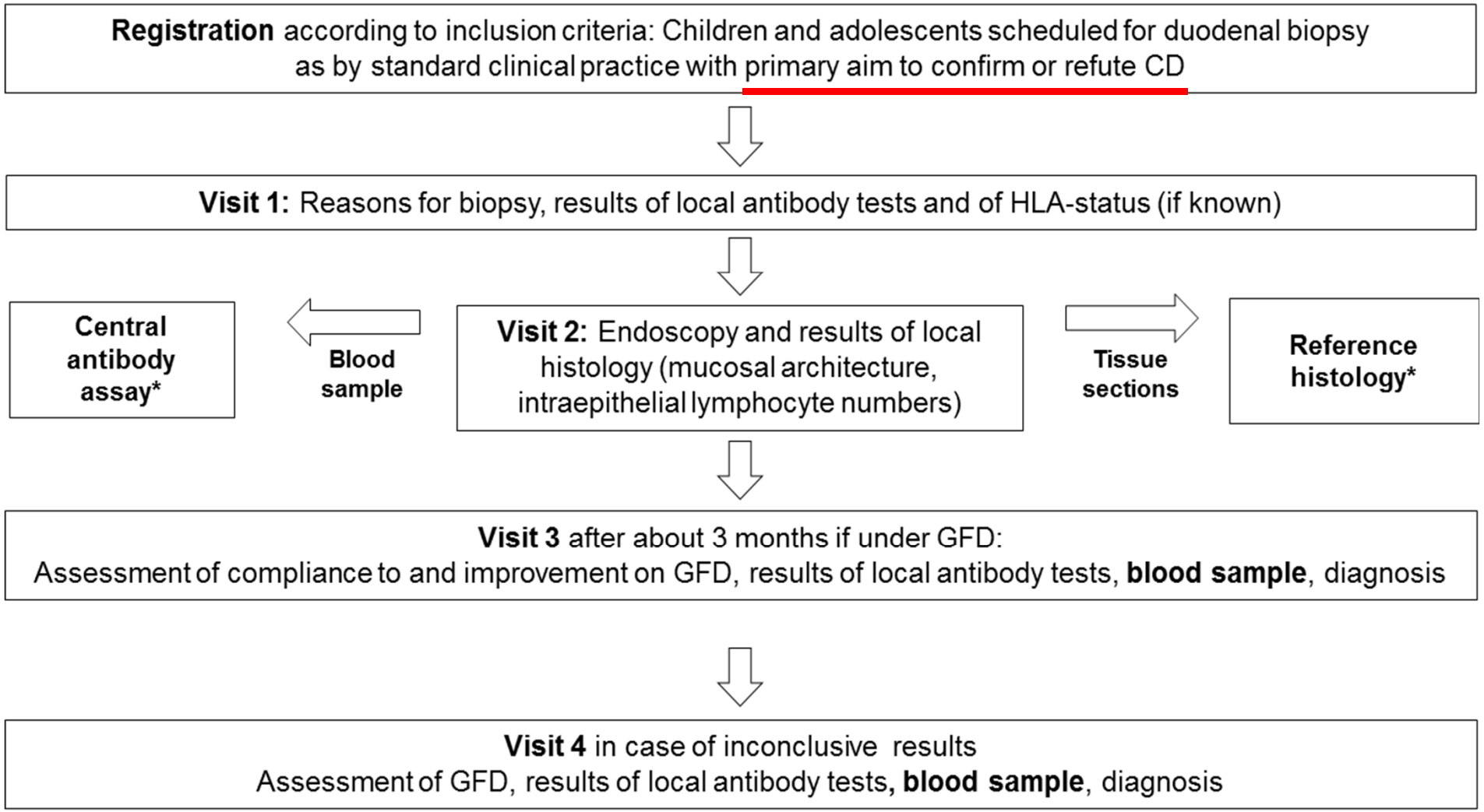
Advantages of three valued test Logic

- Diagnostic decision **only if sufficient information**.
- No decision in unclear cases.

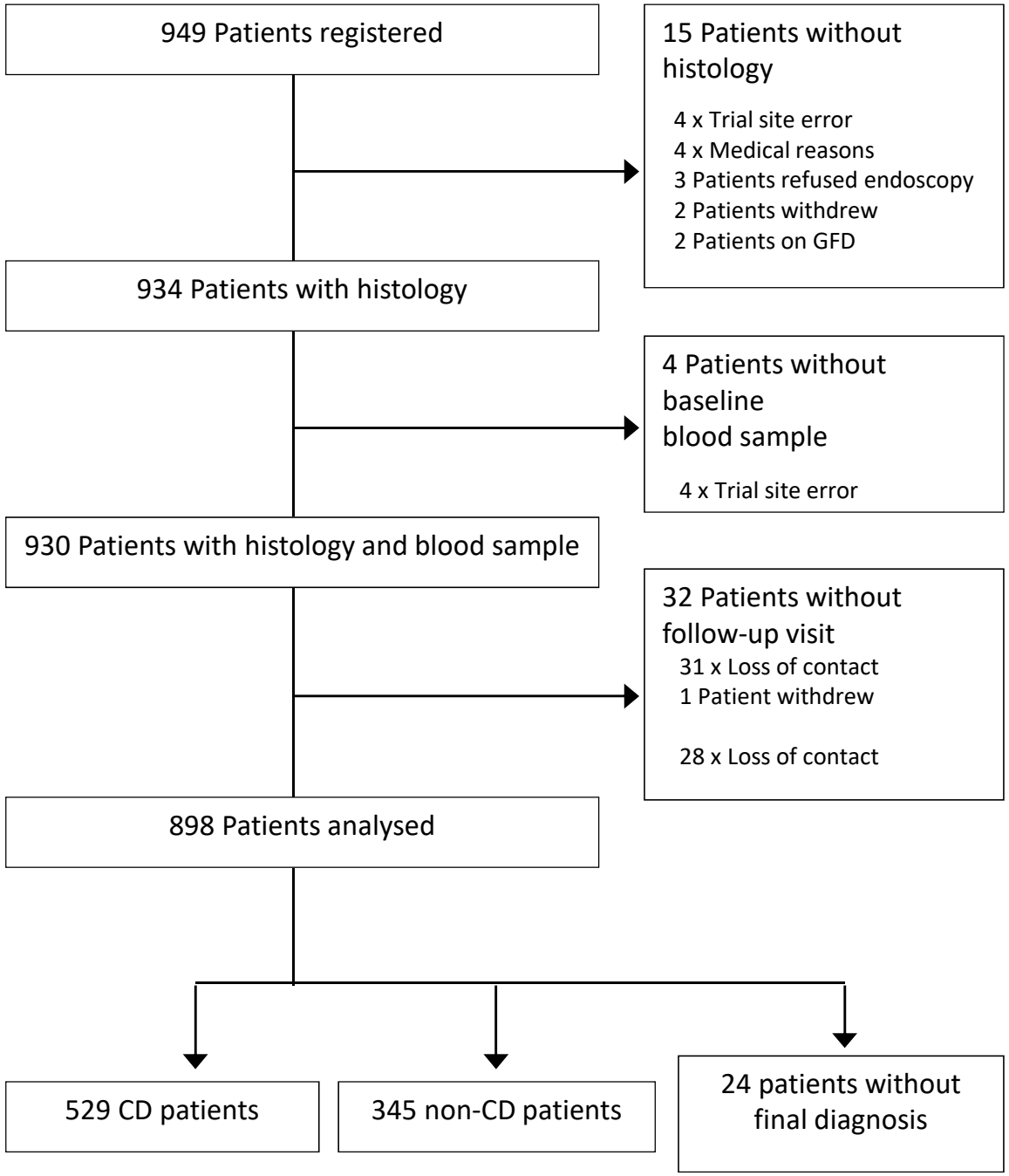
- Reduced sensitivity and (possibly) specificity
- But: **improved PPV** and (possibly) NPV through restriction to cases with clear test result.

- Makes test characteristics **more robust against admixture of difficult cases**.

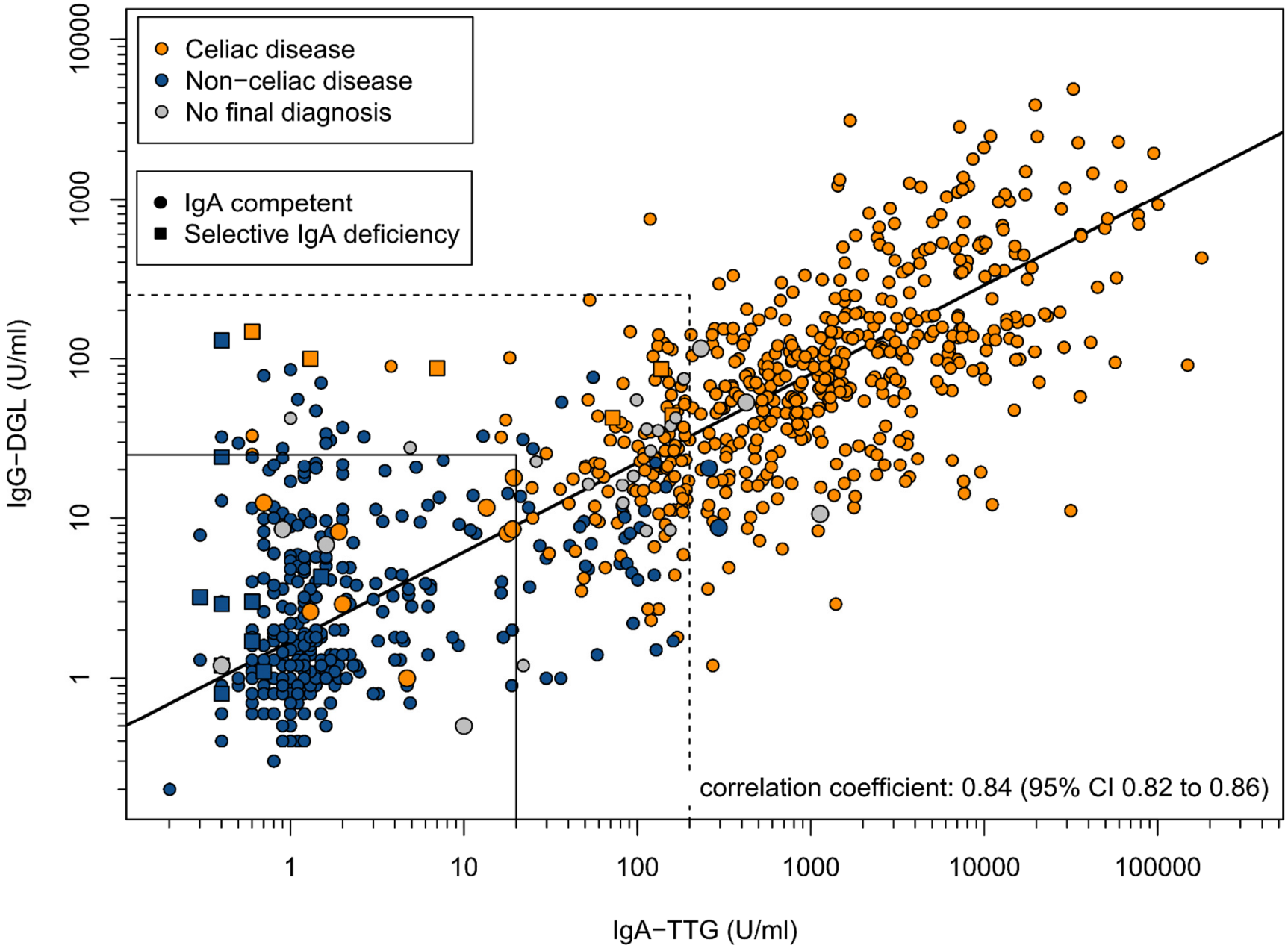
Study Design AbCD phase III



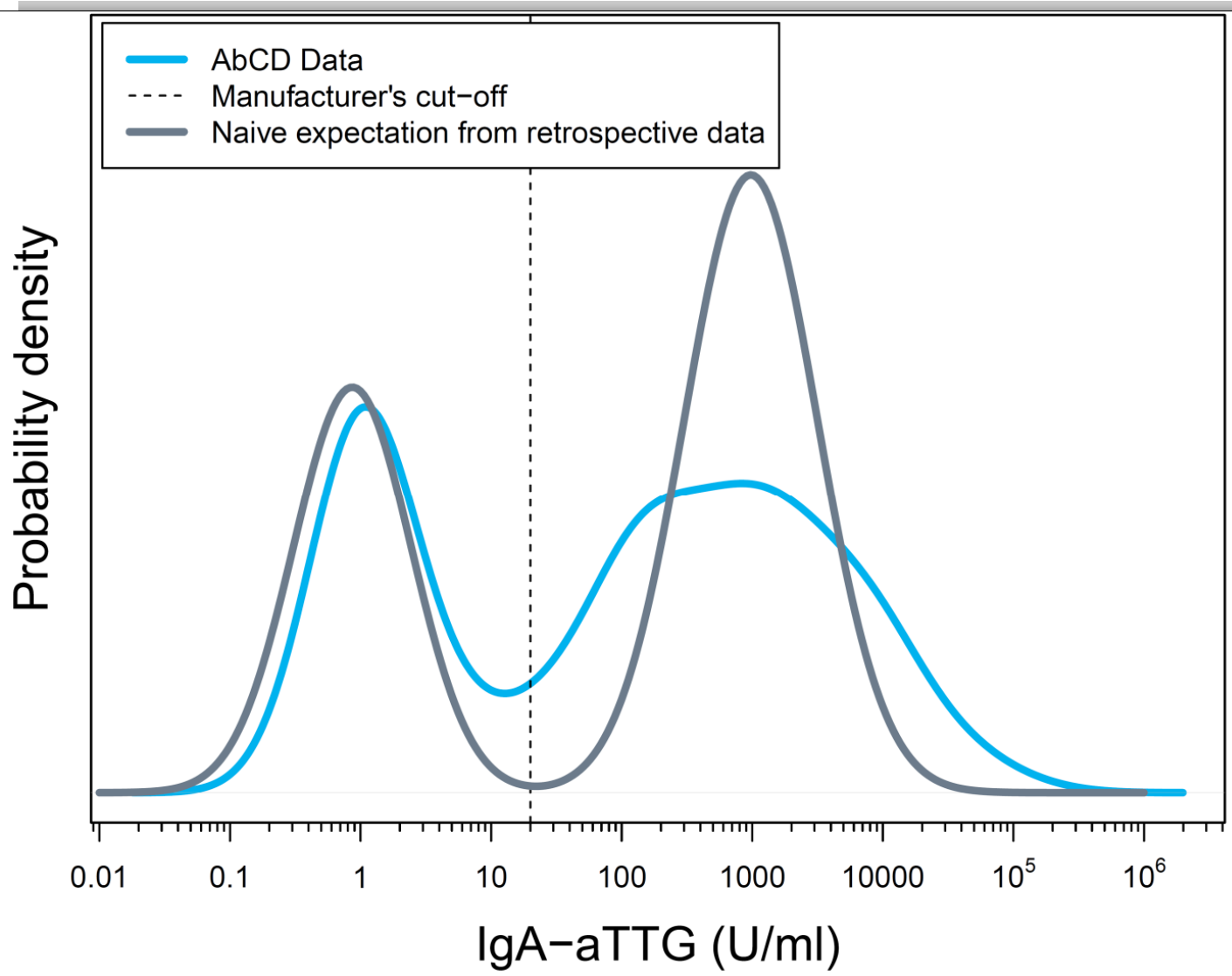
Flow-Chart



Not excluded


Celiac disease
Non−celiac disease
No final diagnosis
IgA competent
Selective IgA deficiency
IgG−DGL (U/ml)
IgA−TTG (U/ml)
correlation coefficient: 0.84 (95% CI 0.82 to 0.86)
1
10
100
1000
10000
100000

Retro- versus prospective



Retro: Registry of cases with diagnosis

Prospective: Admixture of unclear cases

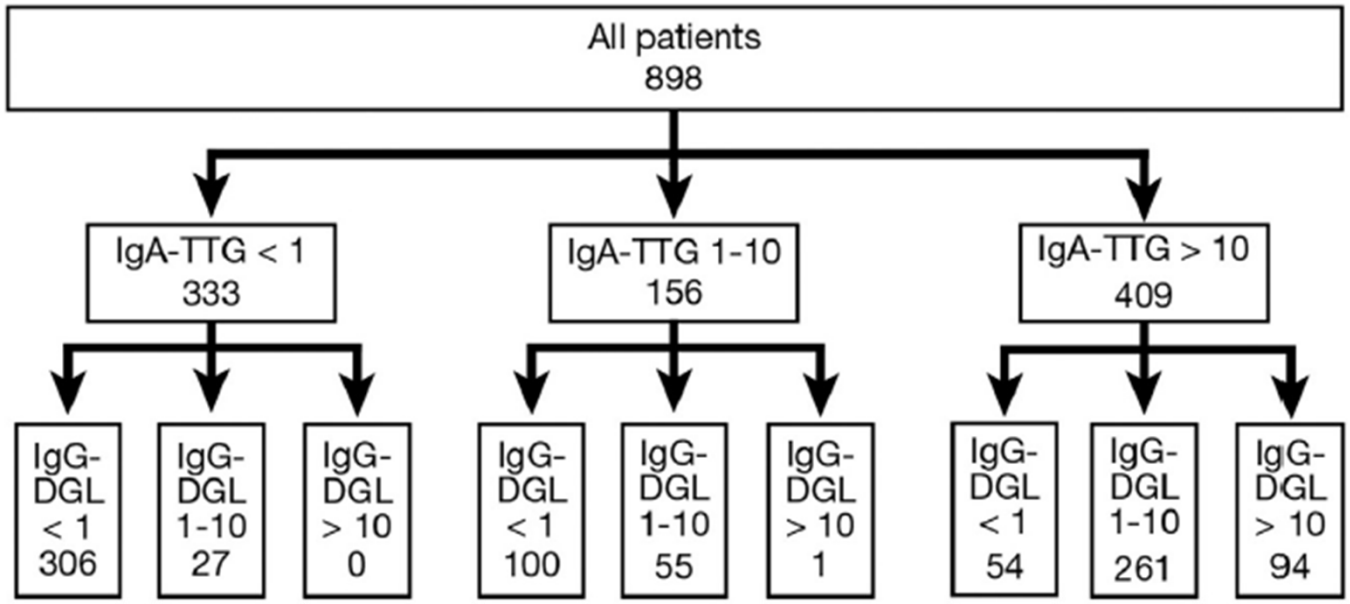
Performance at manufacturer's cut-off

	IgA-aTTG*	IgG-aDGL**	IgA-EMA*
Sensitivity	0.971 (0.953 to 0.983)	0.769 (0.732 to 0.803)	0.958 (0.937 to 0.972)
Specificity	0.893 (0.855 to 0.921)	0.942 (0.912 to 0.962)	0.940 (0.910 to 0.961)
PPV	0.904 [0.881]	0.929 [0.906]	0.931 [0.911]
NPV	0.934 [0.908]	0.707 [0.670]	0.916 [0.888]
PPV§	0.934 [0.914]	0.953 [0.933]	0.962 [0.945]
NPV§	0.952 [0.928]	0.727 [0.691]	0.935 [0.909]

Predictive values of the procedures to be validated

	TTG/IgA-procedure*	TTG/DGL-procedure**
PPV	0.988 [0.975]	0.988 [0.975]
NPV	0.934 [0.908]	0.958 [0.934]
PPV[§]	0.995 [0.984]	0.995 [0.984]
NPV[§]	0.952 [0.929]	0.970 [0.950]
Proportion of patients to be biopsied[#]	0.173 (0.150 to 0.200)	0.203 (0.178 to 0.230)

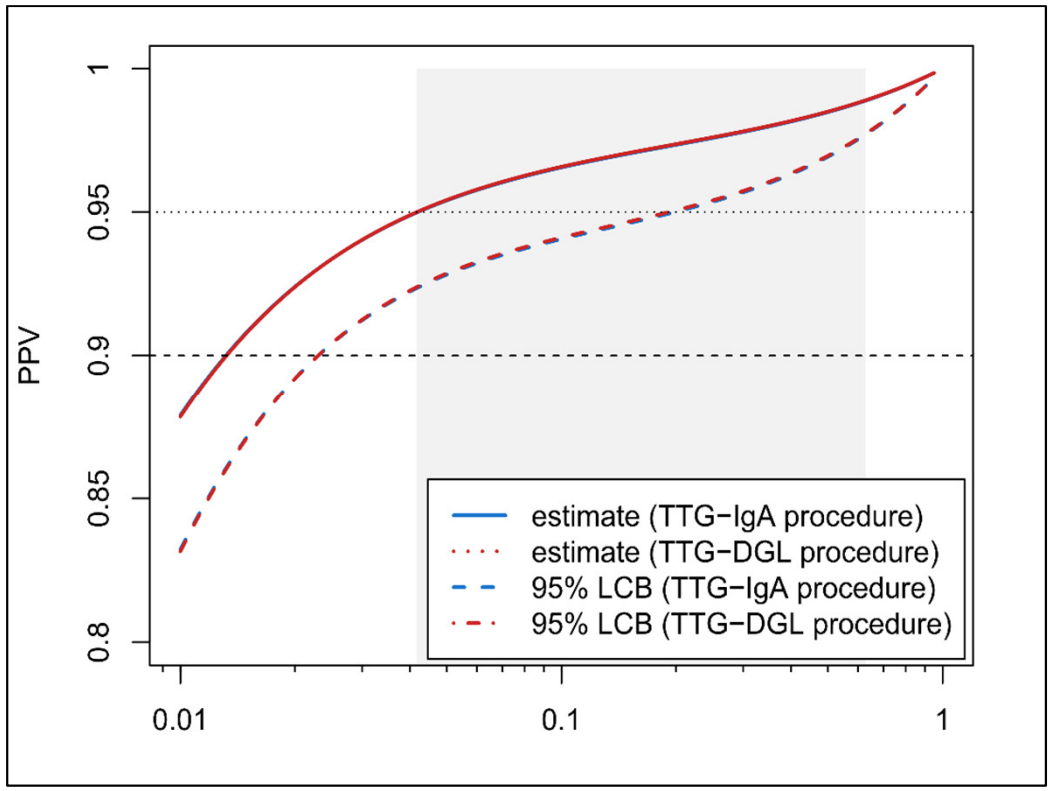
BUT: Prevalence 59%

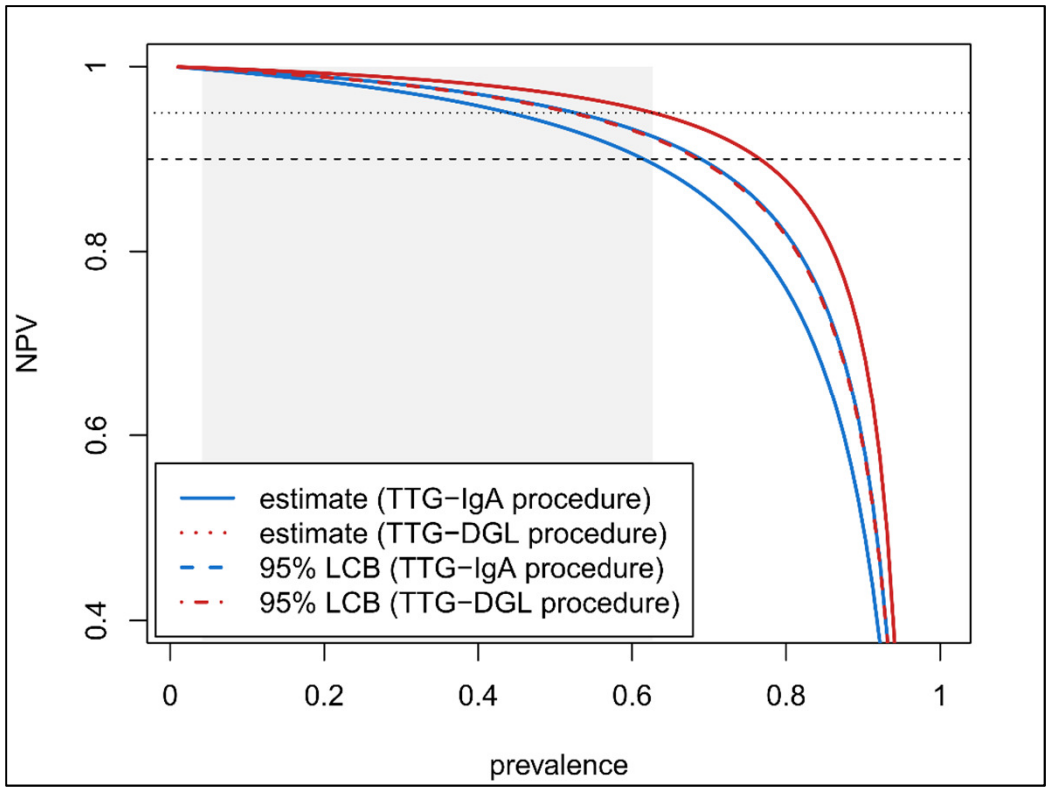


No celiac disease	293	16	0	30	4	0	2	0	0
+IgA-EMA†	1	0	0	17	0	0	2	0	0
sIgAD	9	1	0	0	0	0	0	0	0
Celiac disease	9	9	0	62	44	1	51	259	94
+IgA-EMA†	0	1	0	55	43	1	51	258§	94
sIgAD	0	3	0	0	3	0	0	0	0
No final diagnosis	4	2	0	8	7	0	1	2	0
+IgA-EMA†	0	0	0	7	7	0	1	2	0
sIgAD	0	0	0	0	0	0	0	0	0

Figure 2. Results of antibody assays. Antibody concentration range as multiples of upper limit of normal with number of patients indicated in *boxes*; IgA-EMA+, number of IgA-EMA positive patients. § One IgA-EMA test was unavailable NFD, no final diagnosis possible; sIgAD, number of selective IgA-deficient patients.

Model based extrapolation





PPV and NPV as a function of prevalence
taking selection into the account

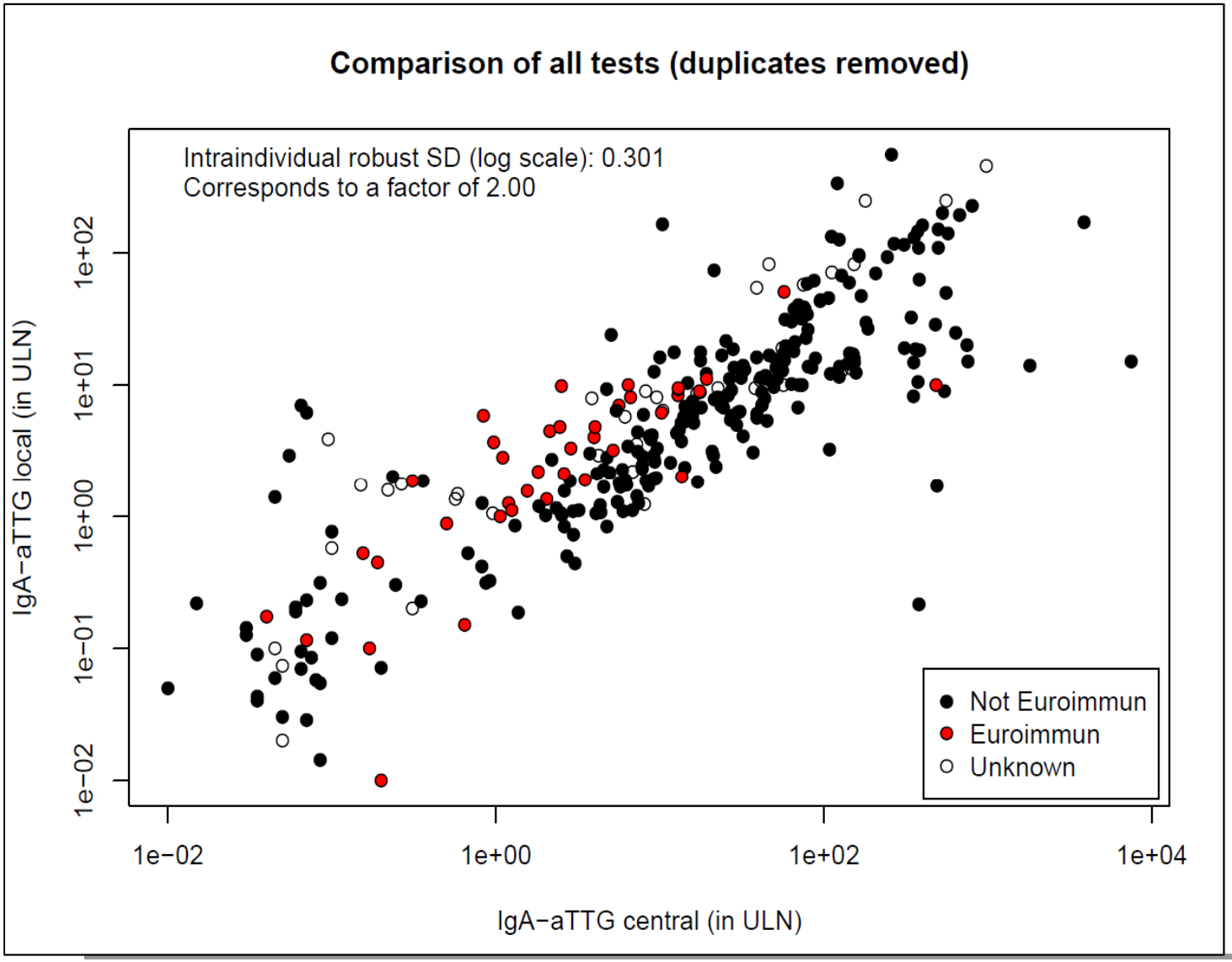
SENS and SPEC are not independent of prevalence in case of selection

- Standard dogma: SENS and SPEC are independent of prevalence
  - TRUE if random sampling from controls and disease
- BUT:
  - Screening Population (~1% CD)
  - Clinical Population (~10% CD)
  - Study population (~70% CD)

enriches for difficult cases.

- Many study patients had prior positive antibody test!
- -> SPEC is underestimated in study population
  - SPEC=89% in study, but 97% in blood donors

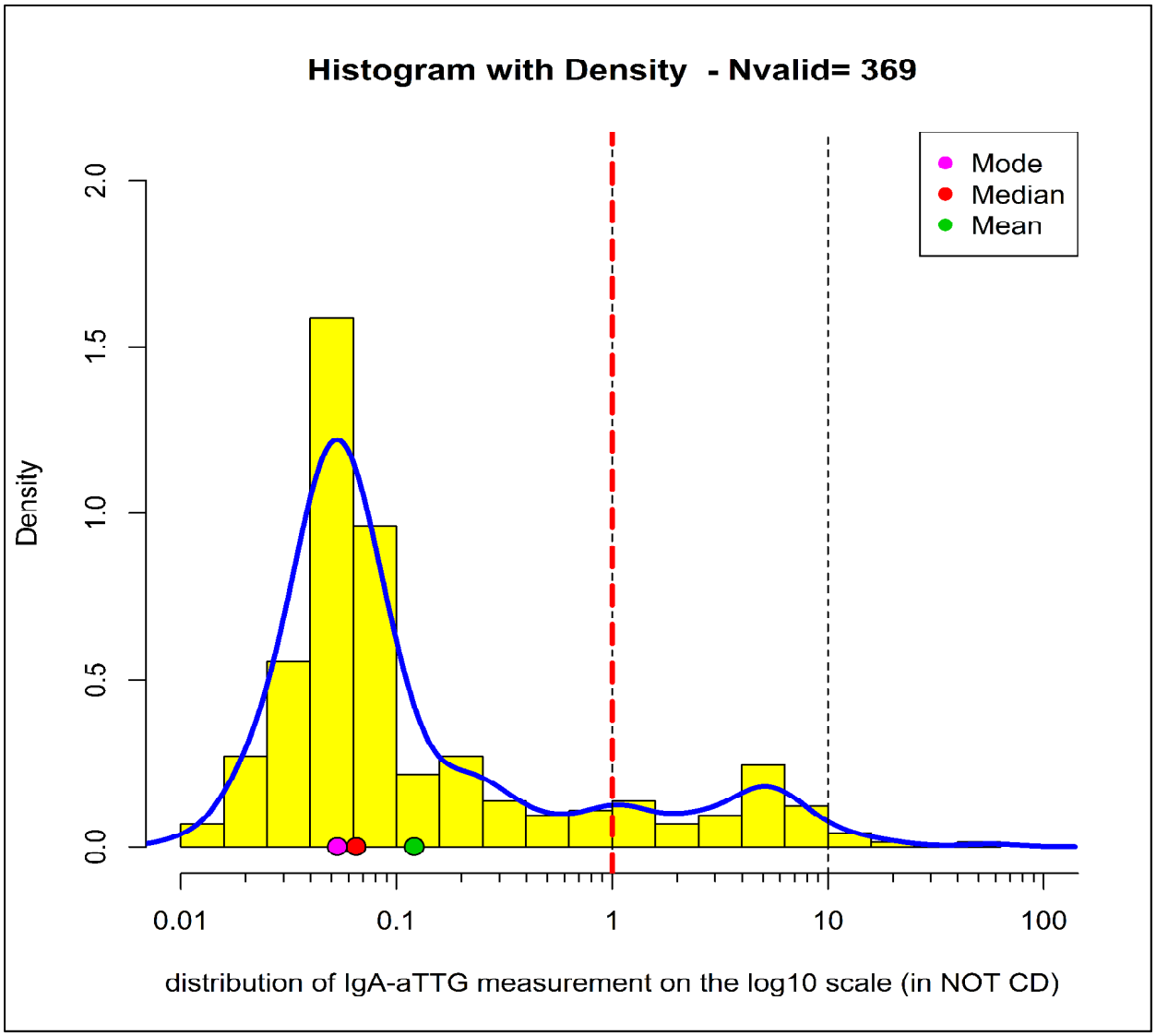
Intra-individual variance



Repeat measurement within a week:

Variation by about 2x

Antibody distribution for non-CD patients



Assume
distribution (of true levels)
in low prevalence
non CD cases
corresponds to peak
on the left.

Discard admixture.

Combined model:
SPEC (> 10UN) = 0.9987

Confirmed in Literature

Evaluation of the ESPGHAN Celiac Guidelines in a North American Pediatric Population

Dominica Gidrewicz, MD, MSc[1], Kathryn Potter, MD, PhD[1], Cynthia L. Trevenen, MD[2], Martha Lyon, MSc, PhD[3] and J. Decker Butzner, MD[1]

OBJECTIVES: We retrospectively examined the performance of the tissue transglutaminase (TTG), endomysial antibody (EMA) tests, and the ESPGHAN (European Society of Paediatric Gastroenterology, Hepatology and Nutrition) nonbiopsy criteria in a pediatric population.

METHODS: Consecutive celiac serologies and corresponding intestinal biopsy results were obtained on children <18 years old over 3.5 years. Patients were classified into three categories: positive TTG, negative TTG, and IgA deficiency.

RESULTS: Of the 17,505 patients with celiac serology performed, 775 had a positive TTG, 574 with a negative TTG were biopsied, and 25 were IgA deficient. Of the patients with a TTG ≥10× upper limit of normal (ULN), positive EMA, and symptoms, 98.2% had biopsies consistent with celiac disease (CD). Four human leukocyte antigen (HLA) DQ2/DQ8-positive patients who met the ESPGHAN nonbiopsy criteria did not have CD. In the group with a TTG 3–10× ULN, 75.7% EMA-positive patients and only 40% EMA-negative patients had CD ($P<0.001$). Of those with a TTG 1–3× ULN, 52.2% EMA-positive patients vs. only 13.3% EMA-negative patients had CD ($P<0.01$). Of the patients with bulbar and duodenal biopsies, 9.8% had CD confined only in the bulb, especially those with a low titer TTG ($P<0.01$). CD prevalence in our cohort was 34.6%. Sensitivity, specificity, and positive predictive value of the TTG were 98.7%, 86.4%, and 79.4%, respectively.

CONCLUSIONS: The TTG is a very sensitive screen for CD, but positive predictive value improves with a positive EMA titer. To apply the new ESPGHAN guidelines, clinicians must understand the performance of their celiac serology tests.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

Am J Gastroenterol advance online publication, 31 March 2015; doi:10.1038/ajg.2015.87

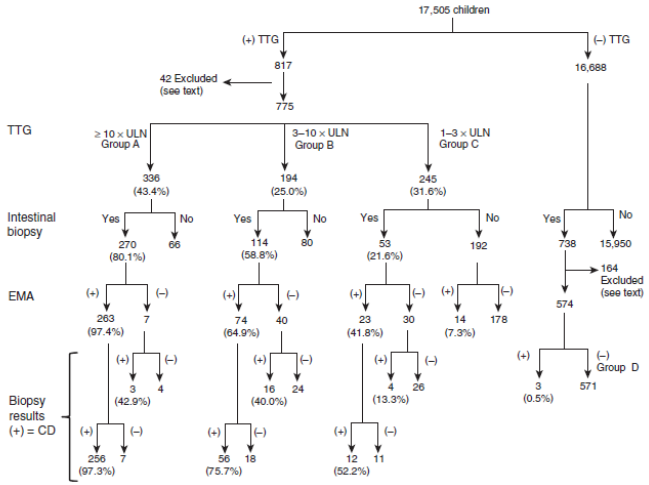


Figure 1. All patients with tissue transglutaminase (TTG) done during the study period. CD, celiac disease; EMA, endomysial antibody; ULN, upper limit of normal.

- Retrospective study, Gidrewicz et al. present IgA-aTTG data from 17,505 consecutive patients.
- Prevalence of CD : about 6%.
- 336 patients with antibody concentrations above 10 ULN,
  - 270 of which were followed up with biopsies.
  - 11 of these were diagnosed as not having CD.
- SPEC (for 10 ULN) is about 1 − 14/16390 = **0.9991**
- Good agreement with model based guesstimate.

Conclusion

- The AbCD-trial demonstrates that coeliac disease
  can be diagnosed or excluded reliably
  with a particular combination of antibody assays

thus **avoiding duodenal biopsy in a substantial proportion of children**.

Antibody Concentrations Decrease 14-Fold in Children With Celiac Disease on a Gluten-Free Diet but Remain High at 3 Months

David Petroff,[*,a] Johannes Wolf,[‡,a] Thomas Richter,[§] Marcus K. H. Auth,[‖] Holm H. Uhlig,[¶,#] Martin W. Laass,[**] Peter Lauenstein,[‡‡] Andreas Krahl,[§§] Norman Händel,[‖‖] Jan de Laffolie,[¶¶] Almuthe C. Hauer,[##] Matthias Heiduk,[***] Gunter Flemming,[‡‡‡] Antje Schmidt,[§§§] Dirk Hasenclever,[‖‖‖,b] and Thomas Mothes[‡,b]

BACKGROUND & AIMS: Celiac disease can be identified by a serologic test for IgA against tissue transglutaminase (IgA-TTG) in a large proportion of children. However, the increased concentrations of antibody rarely normalize within the months after children are placed on a gluten-free diet (GFD). Early serologic predictors of sufficient adherence to GFD are required for optimal treatment.

METHODS: In a prospective study, we observed the response to a GFD in 345 pediatric patients (67% girls; mean age, 8.4 y) who underwent duodenal biopsy to confirm or refute celiac disease from October 2012 through December 2015. Baseline serum samples were tested centrally for IgA-TTG and IgG against deamidated gliadin. Follow-up serologic analyses of children on a GFD were performed about 3 months later.

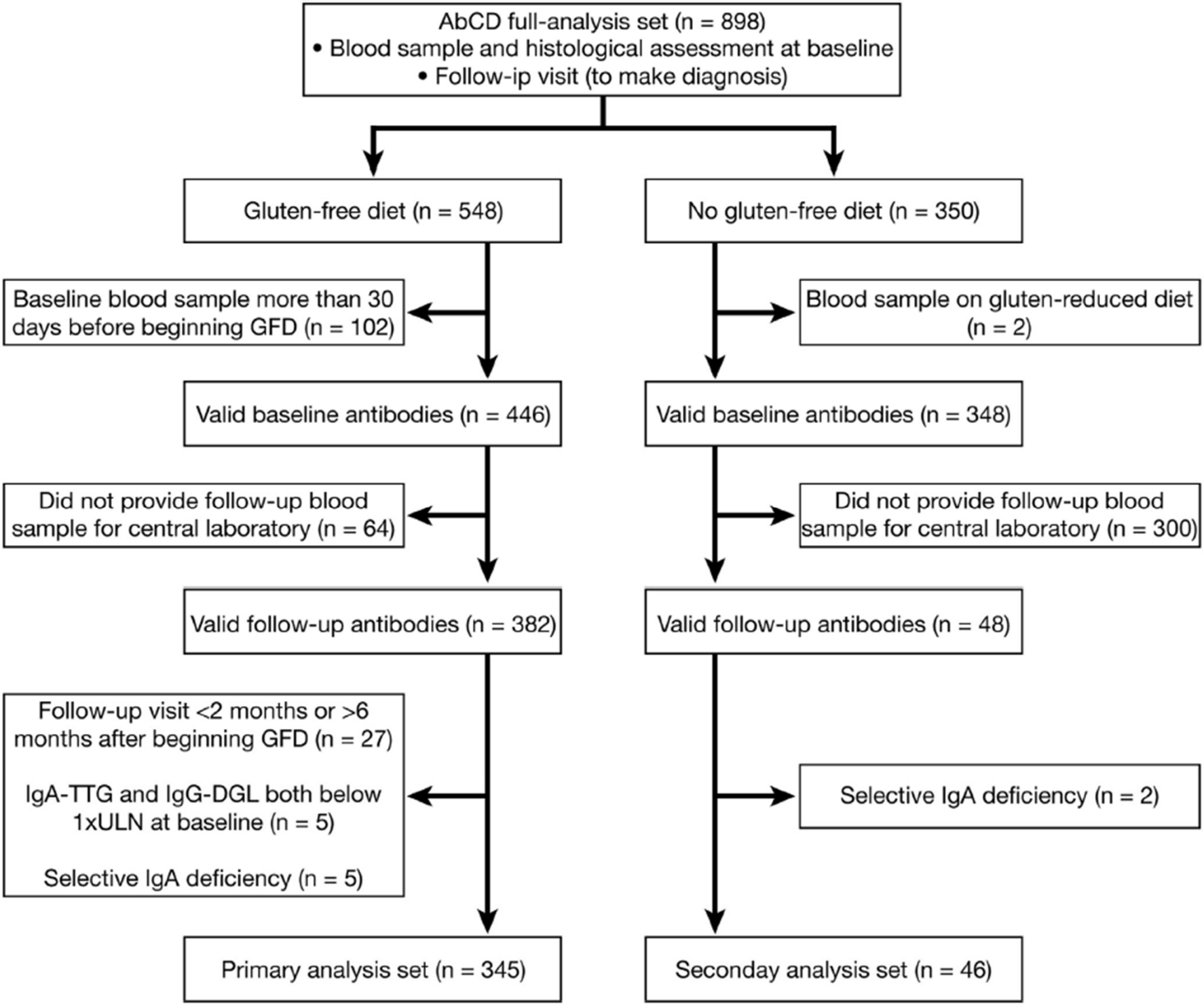
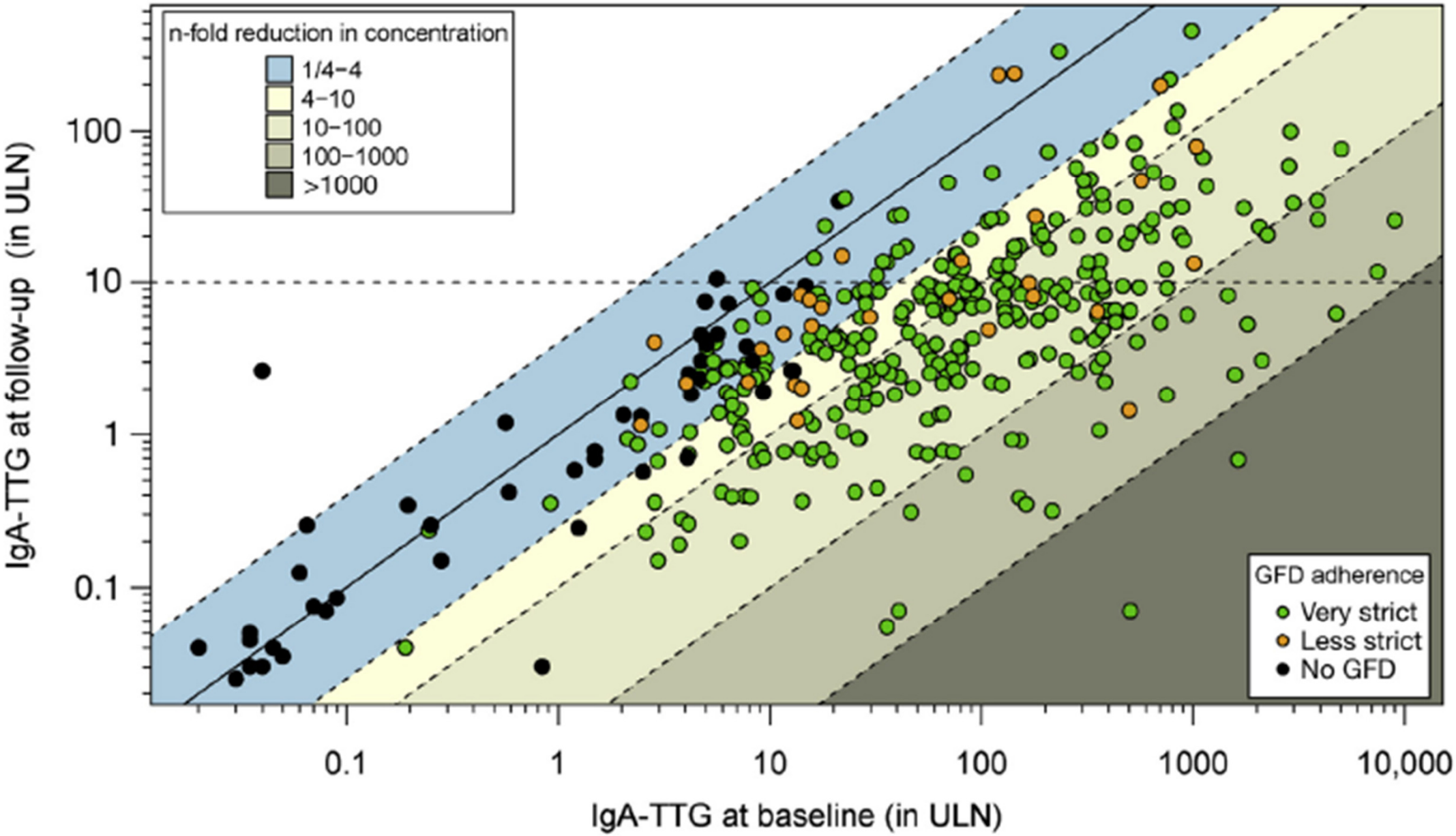


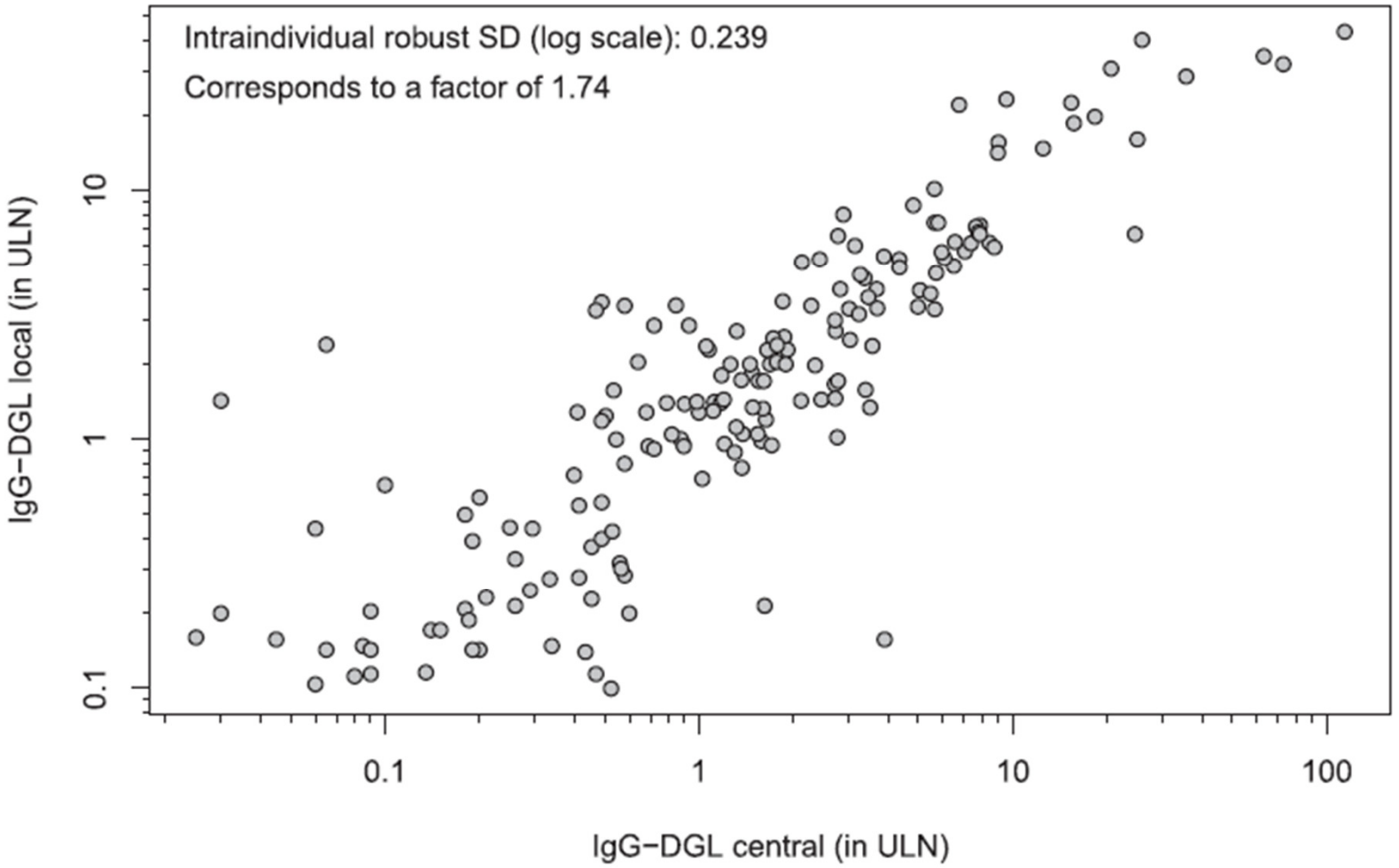
Figure 1. Patient flowchart.


n-fold reduction in concentration
1/4–4
4–10
10–100
100–1000
>1000
GFD adherence
Very strict
Less strict
No GFD
IgA-TTG at follow-up (in ULN)
IgA-TTG at baseline (in ULN)
100
10
1
0.1
0.1
1
10
100
1000
10,000

Definition substantial response

When can a decrease in IgA-TTG concentration be interpreted as a response to a GFD? To distinguish a response from chance fluctuations, we require that the observed decrease after beginning GFD be clearly larger than differences observed when antibodies were measured twice within a short period of time. Such double measurements were analyzed in the Supplementary material to the main AbCD publication.[3] The SD of local and central measurements of IgA-TTG in the same patients before GFD was approximately a factor of 2. The corresponding estimate for IgG-DGL (Supplementary Figure 1) was 1.74. For parsimony, we used the factor 2 for the SD of both IgA-TTG and IgG-DGL. We define a change of 2 SDs (ie, a factor of 4), to be a substantial response.

Intraindividual fluctuations



Supplementary Figure 1. Intraindividual variance on a gluten-containing diet. The last available local antibody concentration is plotted against the measured value in the central laboratory in units of the ULN. The robust estimate for SD is based on mean absolute deviations. A slightly different version of the upper panel was presented by Wolf et al as Supplementary Figure 4.[3]

RESULTS: The geometric mean concentration of IgA-TTG decreased from 72.4-fold to 5.2-fold the upper limit of normal (ULN), or by a factor of 14.0 (95% CI, 12.0–16.4). A substantial response (defined as a larger change than the typical variation in patients not on a GFD) was observed in 80.6% of the children. Only 28.1% of patients had a substantial response in the concentration of IgG against deamidated gliadin. Concentration of IgA-TTG remained above 1-fold the ULN in 83.8% of patients, and above 10-fold the ULN in 26.6% of patients with a substantial response.

CONCLUSIONS: Serum concentration of IgA-TTG decreases substantially in most children with celiac disease within 3 months after they are placed on a GFD, but does not normalize in most. This information on changes in antibody concentrations can be used to assess patient response to the diet at short-term follow-up evaluations. Patients with a substantial response to a GFD often still have high antibody levels after 3 months. German Clinical Trials Registry no. DRKS00003854.

Table 3. Results of a Linear Model for Covariates Affecting IgA-TTG Response to GFD

	Estimate[a]	95% CI	*P* value
Baseline concentration,[b] per factor 10 higher	2.76	2.34–3.25	<.001
Age, per year younger	1.068[c]	1.038–1.098	<.001
GFD adherence, very strict vs less strict	2.15	1.39–3.33	<.001
Sex, male vs female	1.32	1.02–1.71	.036
Type I diabetes, present vs absent	1.57	0.99–2.51	.058