Recent progress in Coeliac Disease

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- Diagnosis
- Prevention
- Potential coeliac disease

Diagnosis

Diagnosis of coeliac disease
ESPGHAN guidelines 2012

Major advances

- New definition
- Histology not gold standard
- Possibility to skip biopsy based on the evidence of high quality serology

Critical issues

- Two algorhytms
- Ambiguous definition of symptomatic
- Accompanying evidence report only on serology, mainly retrospective studies
- No enough evidence for the need of symptoms, EMA and HLA positivity to skip biopsy

Recommendation ↑

Symptoms, signs and conditions requiring testing for CD in children and adolescents

Symptoms and signs suggesting coeliac disease	(*) Common symptoms
Gastrointestinal	chronic or intermittent diarrhea* chronic constipation not responding to usual treatment, abdominal pain distended abdomen* nausea, vomiting
Extraintestinal symptoms	weight loss, failure-to-thrive*, stunted growth/ short stature* delayed puberty, amenorrhea irritability, chronic fatigue neuropathy arthritis/arthralgia chronic iron-deficiency anaemia decreased bone mineralization (osteopenia/osteoporosis), repetitive fractures recurrent aphthous stomatitis, dermatitis herpetiformis–type rash dental enamel defects abnormal liver biochemistry
Specific conditions	first-degree relatives with CD autoimmune conditions: T1DM, thyroid disease, liver disease Down syndrome, Turner syndrome William's syndrome IgA deficiency

What will HLA-DQ2 and DQ8 determination add to the diagnostic certainty of CD-diagnosis?

HLA-DQ2 and/or DQ8 typing does not add to the certainty of the diagnosis if the other criteria for CD diagnosis are fulfilled

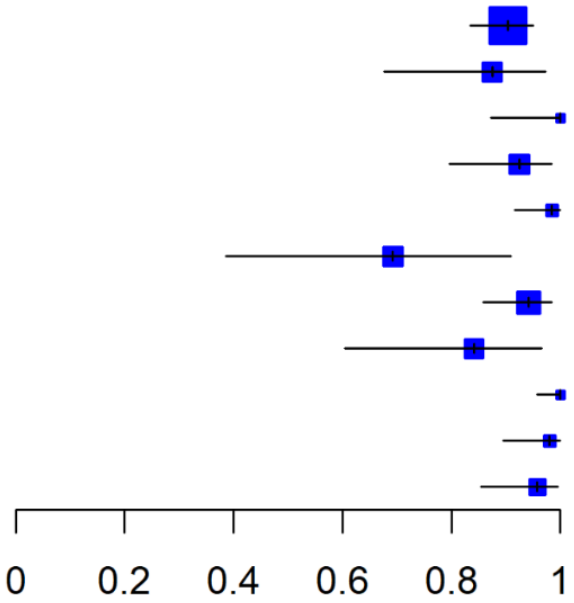
Recommendation ↑↑

HLA DQ2 and DQ8 typing is not required in patients with positive TGA-IgA, if

- **they qualify for CD diagnosis with biopsies or**
- **have high serum TGA-IgA (≥10xULN) and EMA positivity**

Does the algorithm proposed to avoid biopsies in symptomatic patients work in asymptomatic subjects?

Study	TP	TP + FP	PPV (95%CIs)
Nevoral 2013	103	114	0.90 [0.83; 0.95]
Lionetti 2014	21	24	0.88 [0.68; 0.97]
Vriezinga 2014	27	27	1.00 [0.87; 1.00]
Trovato 2015	37	40	0.92 [0.80; 0.98]
Webb 2015	63	64	0.98 [0.92; 1.00]
Cilleruelo 2016	9	13	0.69 [0.39; 0.91]
Donat 2016	65	69	0.94 [0.86; 0.98]
Jansen 2017	16	19	0.84 [0.60; 0.97]
Paul 2017	84	84	1.00 [0.96; 1.00]
Werkstetter 2017	50	51	0.98 [0.90; 1.00]
Wolf 2017	45	47	0.96 [0.85; 0.99]



However, in asymptomatic children the positive predictive value (PPV) of high TGA≥10xULN may be lower than in symptomatic children

Recommendation ↑

A conditional recommendation can be given to diagnose CD without duodenal biopsies in asymptomatic children, using the same criteria as in patients with symptoms.

The decision whether or not to perform diagnostic small bowel biopsies should be made during a **shared decision making process** together with the parents and, if appropriate, with the children.

Which serological test is the most appropriate to diagnose CD?

	Sensitivity	Specificity	Youden's J statistic
TG2	0.936 (0.904 0.958)	0.957 (0.912 0.979)	0.893
DGP	0.907 (0.802 0.959)	0.929 (0.708 0.986)	0.836
EMA	0.983 (0.959 0.993)	0.827 (0.681 0.915)	0.810

Recommendation ↑↑

In subjects with normal serum IgA values for age TGA-IgA should be used as initial serological test **regardless of age**

Should more than one serological test be used and, if so, what should be the sequence of testing?

Current evidence indicates that that adding DGP-IgG, DGP-IgA or AGA-IgA testing to TGA-IgA testing seldom improves sensitivity after excluding patients with low total IgA concentrations

Specificity markedly decreases, especially in children below 4 years of age, in which isolated DGP or AGA positivity is a common transient phenomenon

Recommendation ↑↑

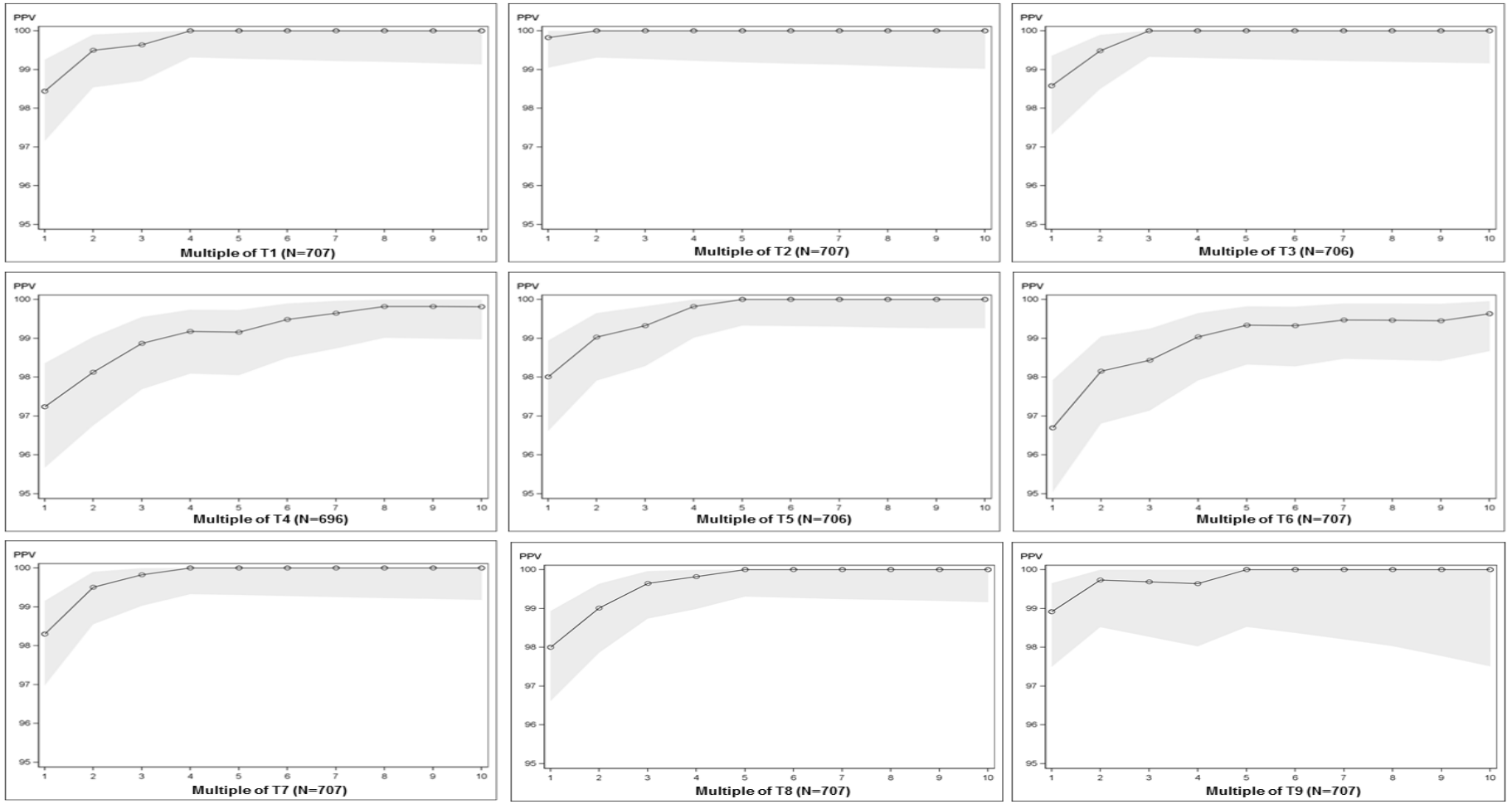
We recommend testing for total IgA and TGA-IgA as initial screening in children with suspected CD

We recommend against testing for EMA, DGG or AGA antibodies (IgG and IgA) as initial screening in clinical practice

In patients with low total IgA concentrations an IgG-based test (DGP, EMA or TGA) should be performed as a second step

At which cut-off for TGA-IgA may a diagnosis of CD safely be done with omission of biopsy?

Inter-test variability makes positive TGA IgA levels < 10xULN not sufficient for the no-biopsy approach



Werkstetter et al, *Gastroenterology* 2017;153:924-935

Recommendation ↑↑

We recommend that for CD diagnosis without biopsies TGA- IgA serum concentration of at least 10xULN should be obligatory

Only antibody tests with proper calibrator curve-based calculation and having the 10xULN value within their measurement range should be used

We recommend against omitting biopsies in IgA deficient cases with positive IgG based serological tests

Is endomysial antibody (EMA) testing necessary in every case to diagnose CD without biopsy?

Although high TGA-IgA (>10xULN) results are rare in children with normal histopathology a positive EMA result will further decrease the rate of false positive results

Recommendation ↑↑

In children with TGA >10X ULN, in whom parents/patient agree to the no-biopsy approach, the CD diagnosis should be confirmed by a positive EMA test in a second blood sample

What is the inter- and intra-observer variability of histopathology results of duodenal and bulb biopsies? Do duodenal bulb biopsies increase the detection rate of CD? Is a reference pathologist needed in clinical practice?

The inter-observer variability of the grading of small-bowel histopathology lesions is high

A higher detection rate for CD may be achieved with more duodenal samples including at least one from the bulb

Histopathology reading can be improved by validated standard operating procedures (SOPs)

Recommendation ↑↑

At least four biopsies from the duodenum (distal to the papilla of Vater) and at least one from the duodenal bulb should be taken for histology assessment

Reading of biopsies should be performed on optimally orientated biopsies. A villous to crypt ratio of <2 indicates mucosal lesions

In cases of discordant results between TGA results and histopathology, re-cutting of biopsies and/or second opinion of an experienced pathologist should be requested

Does Marsh 1 compared to Marsh 0 have a different long-term outcome regarding diagnosis of CD in children with coeliac autoimmunity (positive TGA or EMA) ?

The term potential CD identifies subjects with positive TGA and EMA and no or minor histological changes, such as Marsh 1.

Marsh 1 is not considered sufficient to diagnose CD, but some observations suggest that Marsh 1 small bowel lesions have a higher chance to evolve to villous atrophy in comparison to Marsh 0.

Recommendation ↑

Before diagnosing potential CD it is recommended to check the gluten content of the diet and the correct orientation of biopsies

Potential CD requires clinical and laboratory surveillance to monitor possible evolution to villous atrophy. For follow-up it is important to refer the patient to tertiary care centers with expertise in CD

How often are other clinically relevant diagnoses missed if upper (oesophageal-gastro-duodenal) endoscopy is not performed in patients diagnosed by the non-biopsy approach?

There is no evidence to support that relevant diagnoses (such as HP, EE) are missed if upper endoscopy with biopsies are omitted to diagnose CD

Recommendation ↑↑

The decision to omit upper endoscopy with biopsies can be taken without the consideration of missing other pathologies or diagnoses

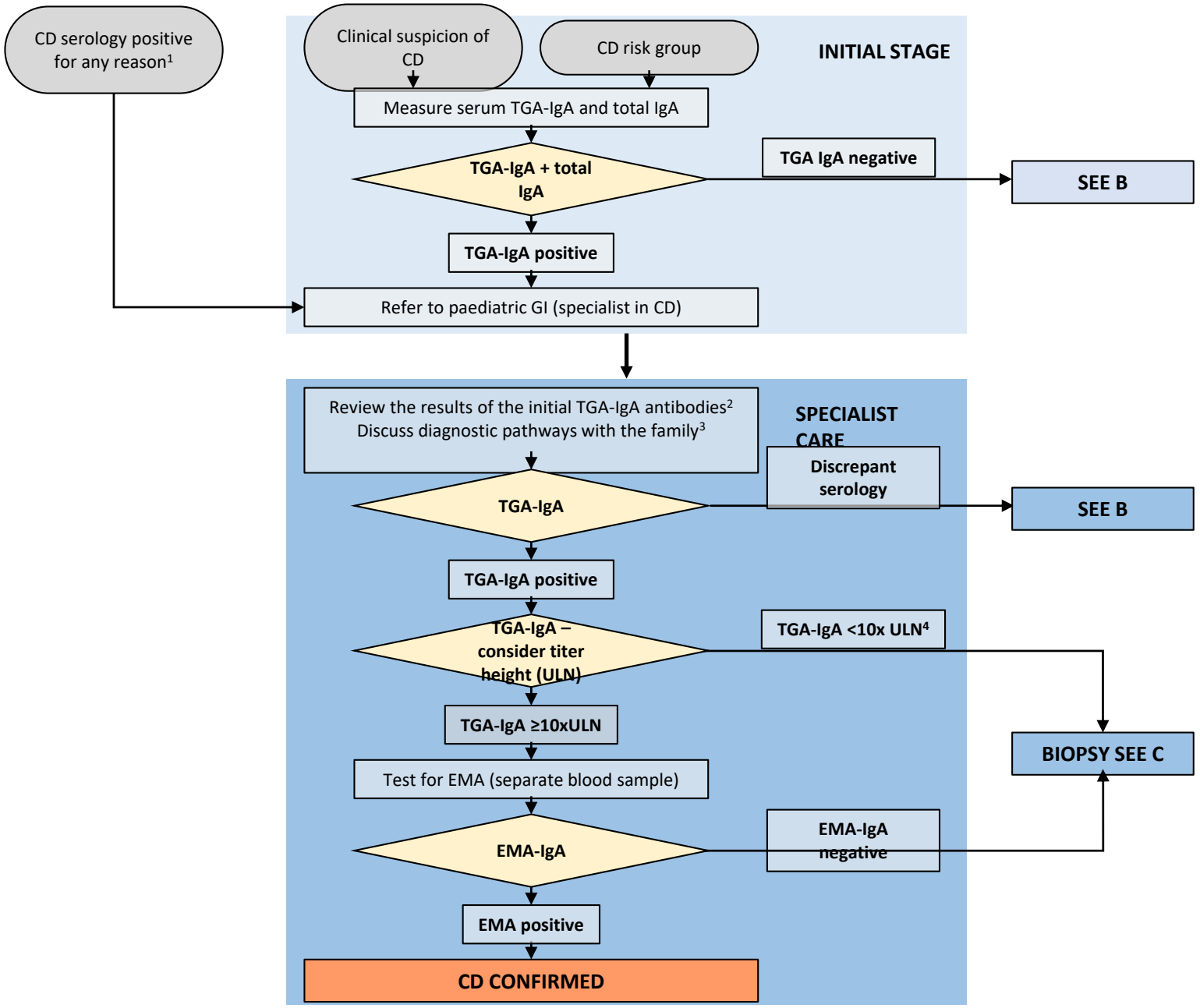
Major changes in comparison to 2012

TGA-IgA is the primary test, irrespective of age.
No DGP IgG/IgA for initial testing

Titres TGA >10xUNL for the no biopsy approach.
No need HLA needed, but EMA in a second serum sample

One algorythm for symptomatic and non-symptomatic patients

A


CD serology positive for any reason[1]
Clinical suspicion of CD
CD risk group
INITIAL STAGE
Measure serum TGA-IgA and total IgA
TGA-IgA + total IgA
TGA IgA negative
SEE B
TGA-IgA positive
Refer to paediatric GI (specialist in CD)
Review the results of the initial TGA-IgA antibodies[2]
Discuss diagnostic pathways with the family[3]
SPECIALIST CARE
TGA-IgA
Discrepant serology
SEE B
TGA-IgA positive
TGA-IgA – consider titer height (ULN)
TGA-IgA <10x ULN[4]
TGA-IgA ≥10xULN
BIOPSY SEE C
Test for EMA (separate blood sample)
EMA-IgA
EMA-IgA negative
EMA positive
CD CONFIRMED

Prevention

Prevention: Who is the target? Which intervention?

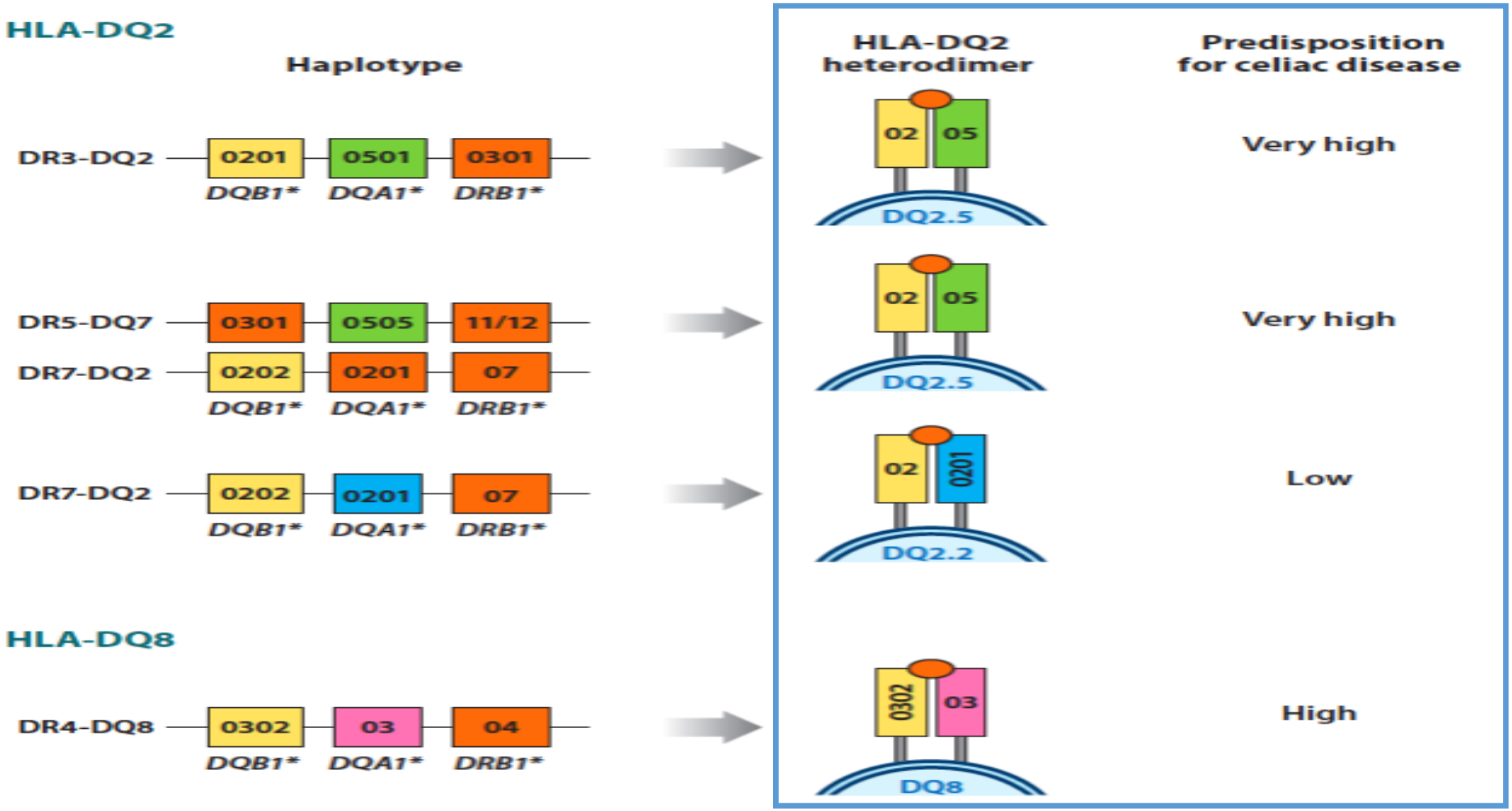
- Genetic factors
- Environmental factors

Natural history: Through which steps the disease progresses?

- Predictive biomarkers

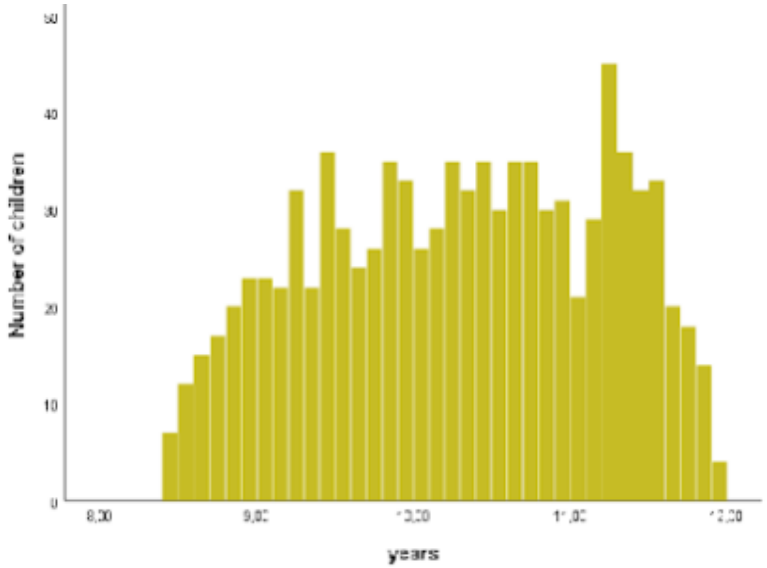
Which strategies for prevention?

HLA-DQ2 is the strongest genetic risk factor for CD



Abadie et al. Annu Rev Immunol 2011

PreventCD cohort at December 2018



Distribution of age n=944

Mean: 10,3 yrs

Range: 8,4-12,0 yrs

Boys: 525

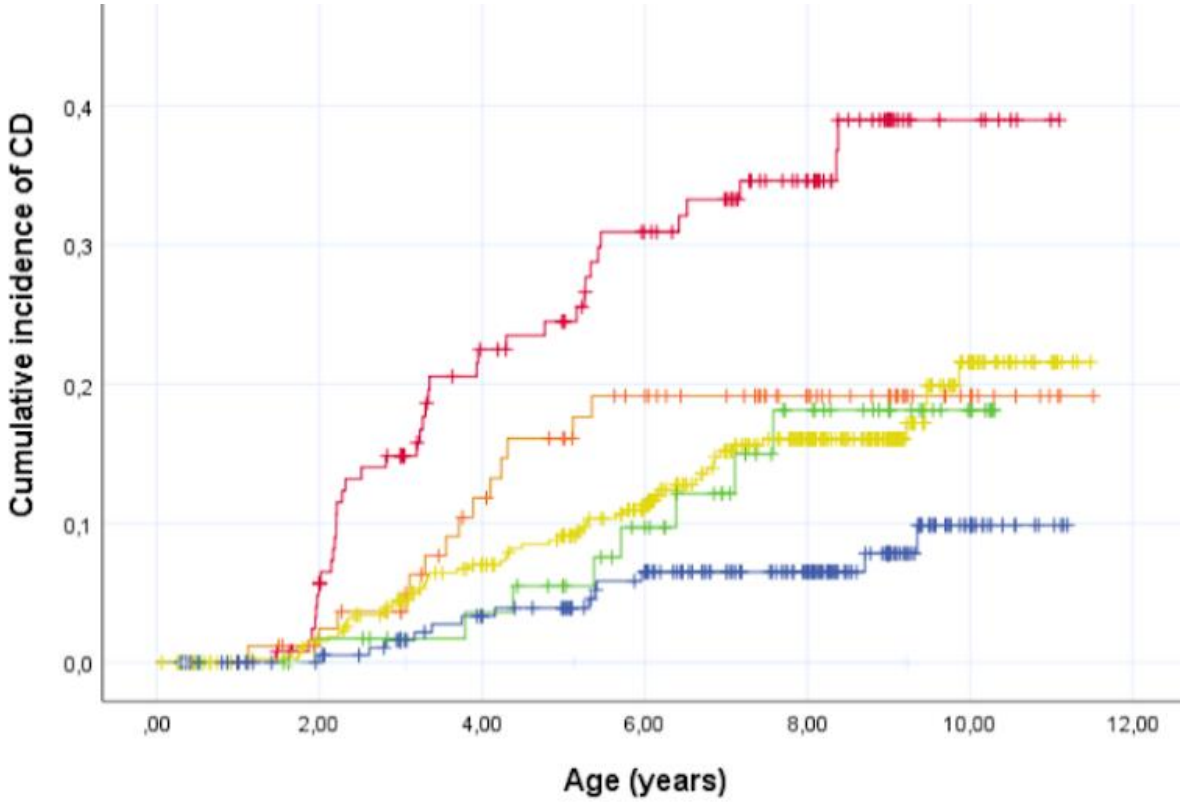
Diagnostic workup for Coeliac Disease	Number of children
	December 2018
Biopsied children	149
CD	133 (5 no biopsy)
No-CD	13
Potential CD	3
Unclear	3

Unpublished data

Cumulative incidence of CD according to HLA-haplotype

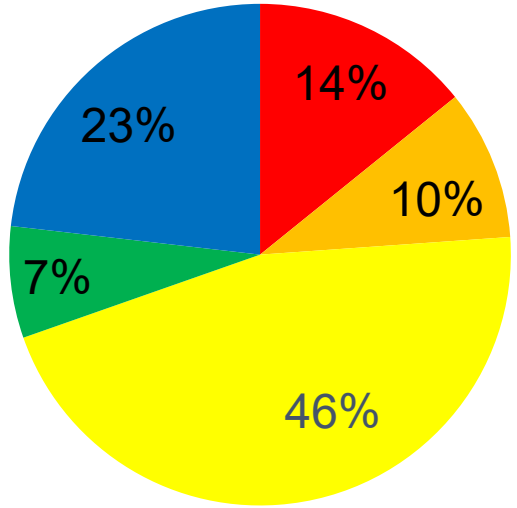
N=911

P<0.001



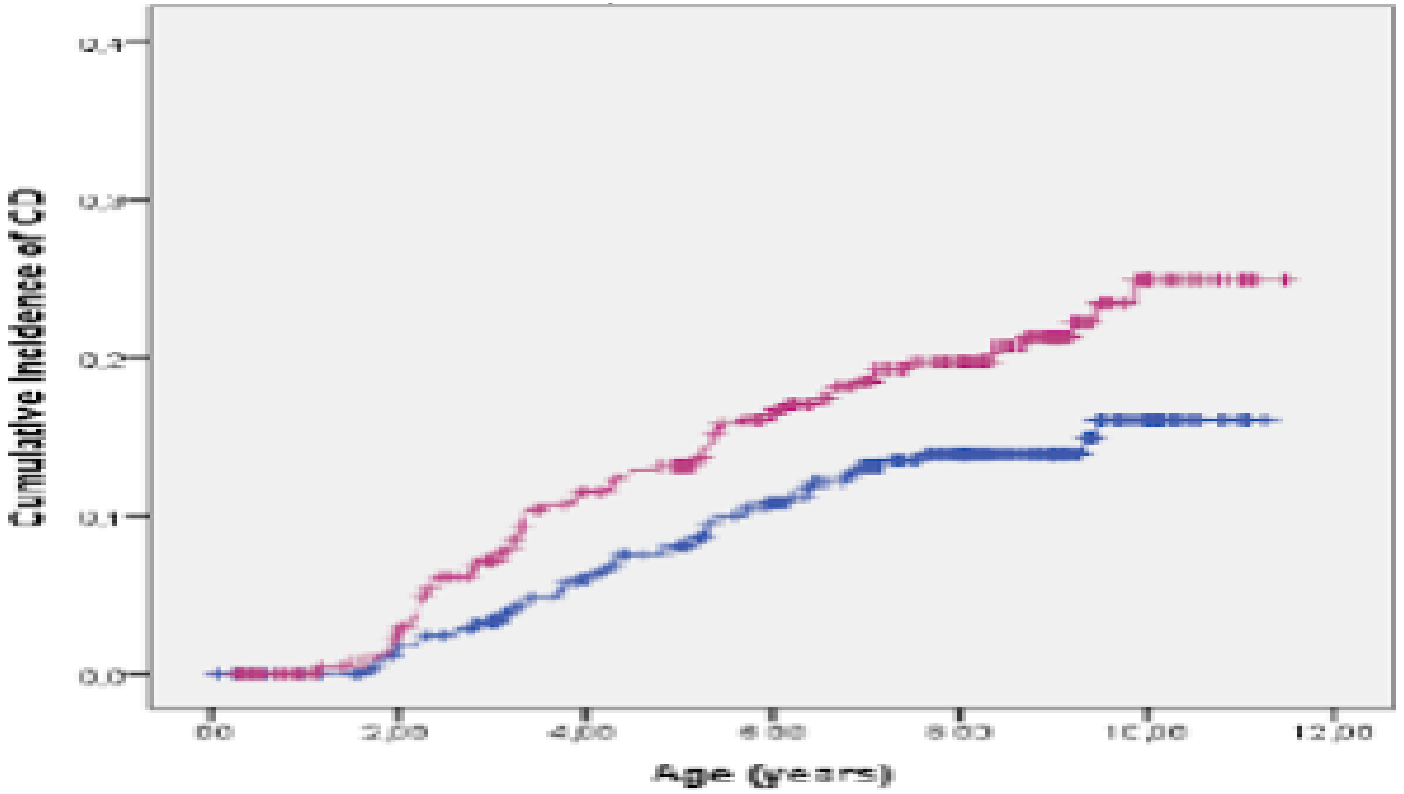
1 = DR3-DQ2/DR3-DQ2, DR3-DQ2/DR7-DQ2
2 = DR7-DQ2/DR5-DQ7
3 = DR3-DQ2/DR5-DQ7, DR3-DQ2/DR4-DQ8, DR3-DQ2/other
4 = DR7-DQ2/DR7-DQ2, DR7-DQ2/DR4-DQ8, DR4-DQ8/DR4-DQ8
5 = DR7-DQ2/other, DR4-DQ8/DR5-DQ7, DR4-DQ8/other

HLA distribution in cohort:



Cumulative incidence of CD according to gender

p=0,016



Cumulative Incidence (%)			
Age Years	**Boys**	**Girls**	Δ gender (%)
3	3,3	6,7	3,4
5	7,9	12,7	4,8
7	12,9	18,1	5,2
8	**13,6**	**19,3**	**5,7**

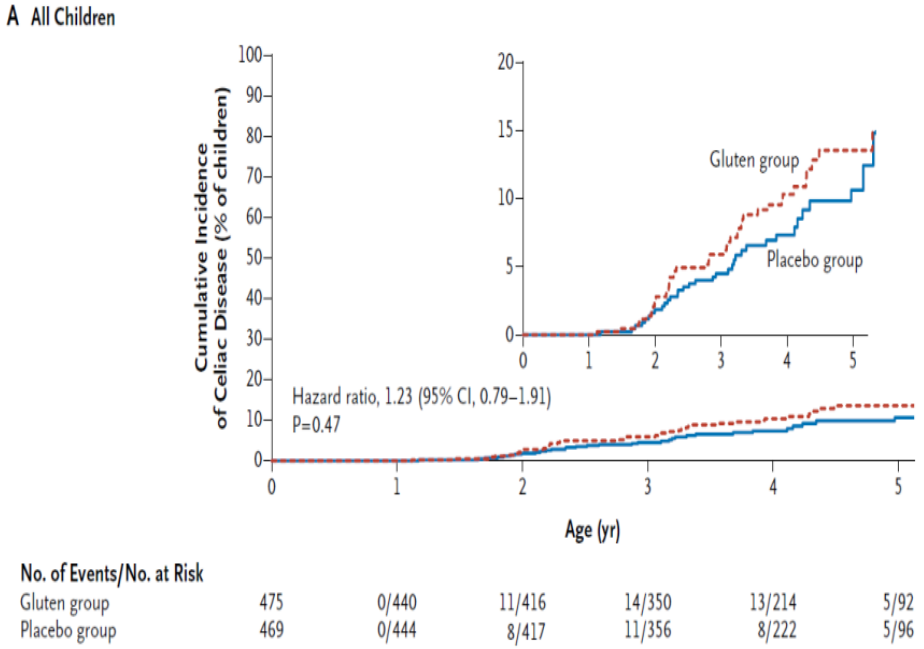
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Randomized Feeding Intervention in Infants at High Risk for Celiac Disease

S.L. Vriezinga, R. Auricchio, E. Bravi, G. Castillejo, A. Chmielewska, P. Crespo Escobar, S. Kolaček, S. Koletzko, I.R. Korponay-Szabo, E. Mummert, I. Polanco, H. Putter, C. Ribes-Koninckx, R. Shamir, H. Szajewska, K. Werkstetter, L. Greco, J. Gyimesi, C. Hartman, C. Hogen Esch, E. Hopman, A. Ivarsson, T. Koltai, F. Koning, E. Martinez-Ojinaga, C. te Marvelde, A. Mocic Pavic, J. Romanos, E. Stoopman, V. Villanacci, C. Wijmenga, R. Troncone, and M.L. Mearin

80 children developed CD/ Gluten vs. placebo: no significant difference



Development of CD **NOT** Related to

Breast Feeding

Daily gluten intake

Country of origin

Family characteristics

Rotavirus vaccination

ORIGINAL ARTICLE

Introduction of Gluten, HLA Status, and the Risk of Celiac Disease in Children

Elena Lionetti, M.D., Stefania Castellaneta, M.D., Ruggiero Francavilla, M.D., Ph.D., Alfredo Pulvirenti, Ph.D., Elio Tonutti, M.D., Sergio Amarri, M.D., Maria Barbato, M.D., Cristiana Barbera, M.D., Graziano Barera, M.D., Antonella Bellantoni, M.D., Emanuela Castellano, M.D., Graziella Guariso, M.D., Maria Giovanna Limongelli, M.D., Salvatore Pellegrino, M.D., Carlo Polloni, M.D., Claudio Ughi, M.D., Giovanna Zuin, M.D., Alessio Fasano, M.D., Ph.D., and Carlo Catassi, M.D., Ph.D., for the SIGENP (Italian Society of Pediatric Gastroenterology, Hepatology, and Nutrition) Working Group on Weaning and CD Risk

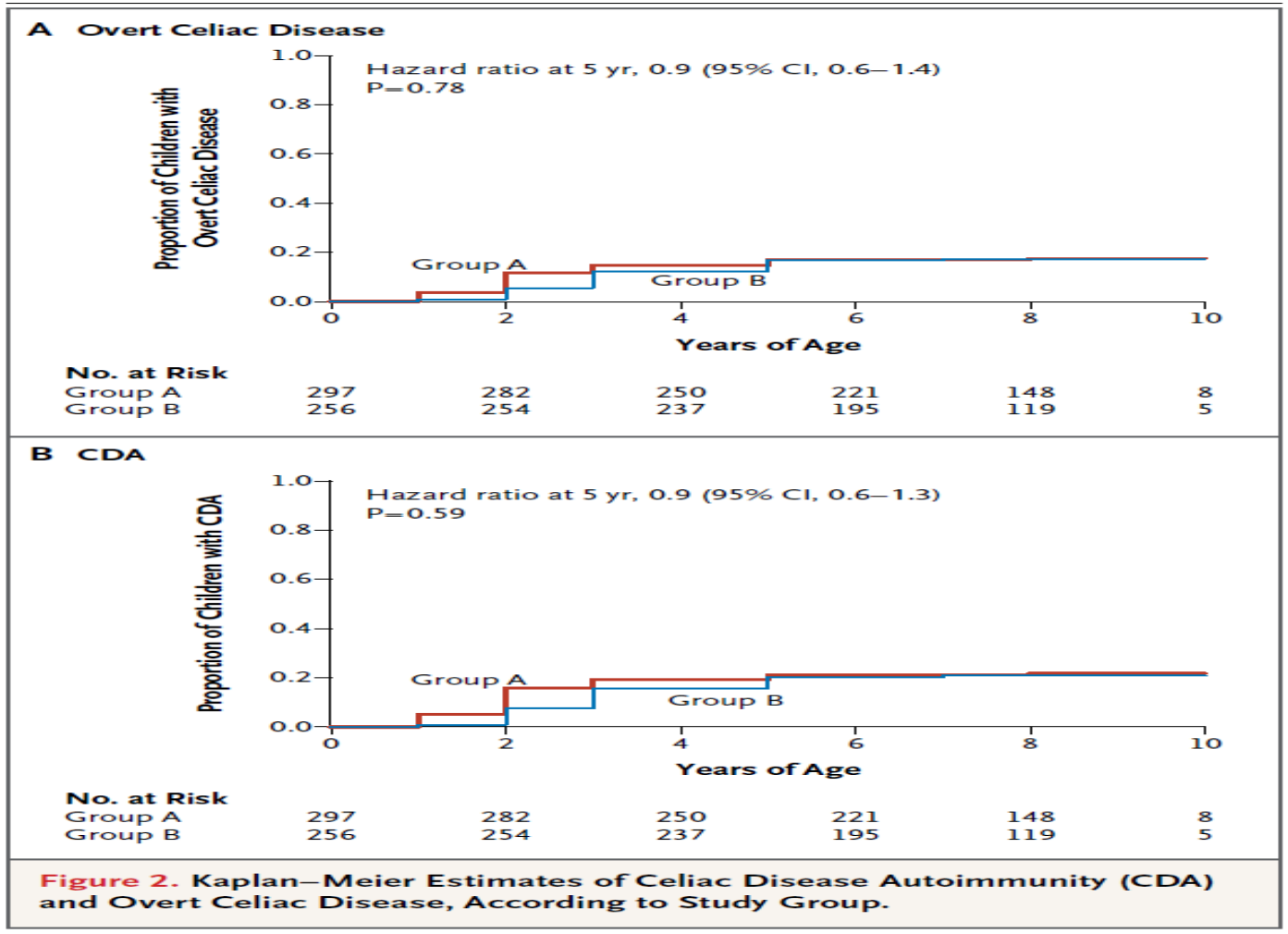
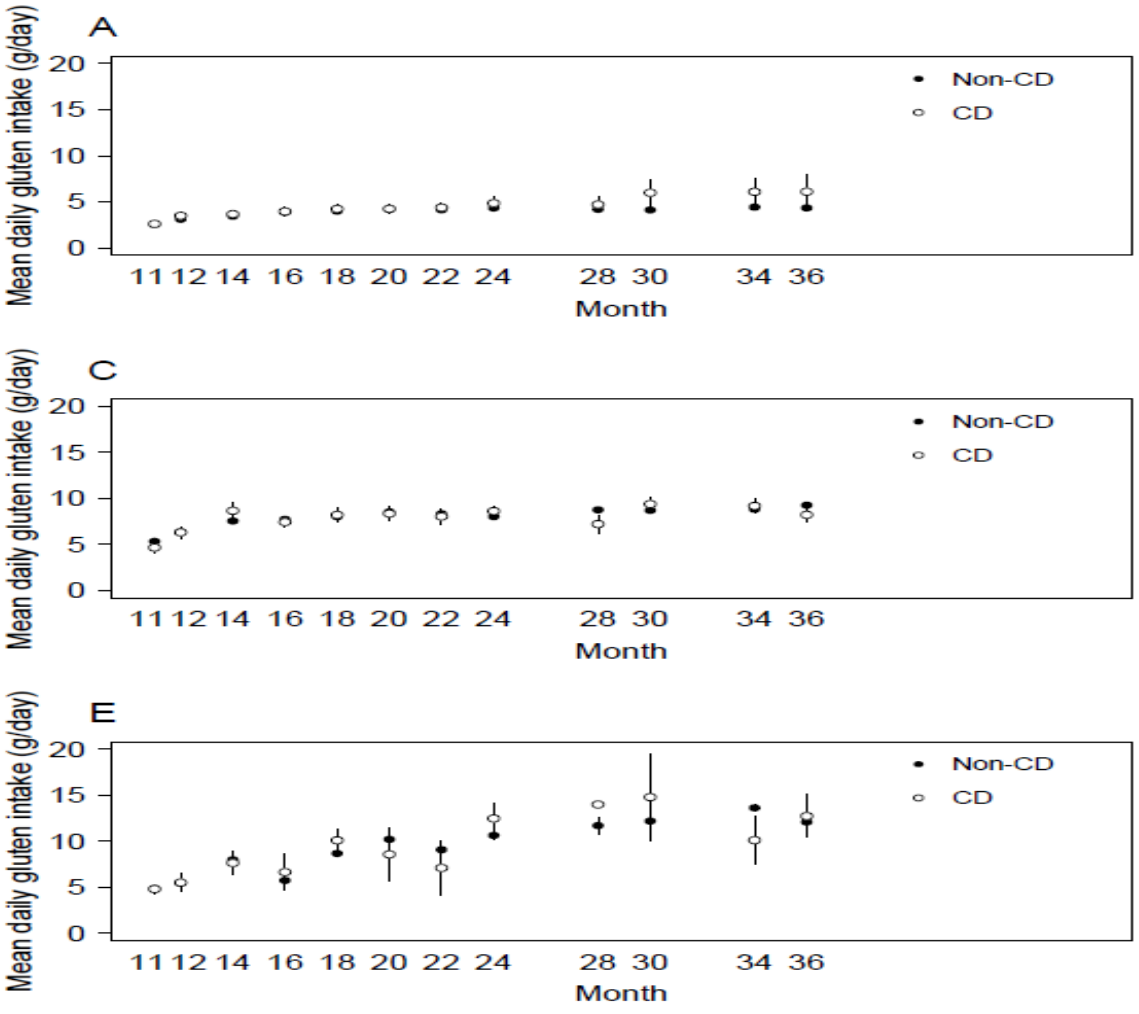


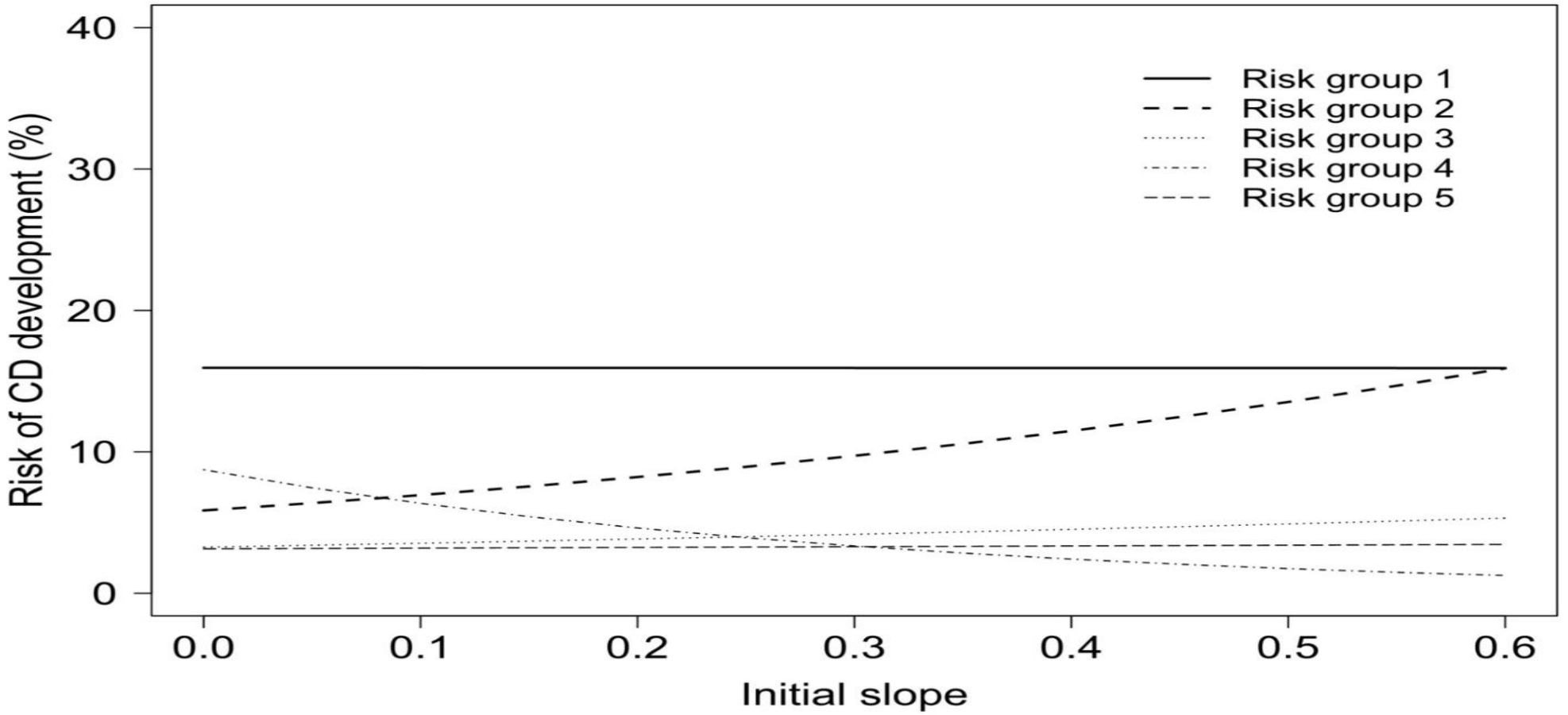
Figure 2. Kaplan–Meier Estimates of Celiac Disease Autoimmunity (CDA) and Overt Celiac Disease, According to Study Group.

Amount of gluten in the first years of life and risk to develop CD



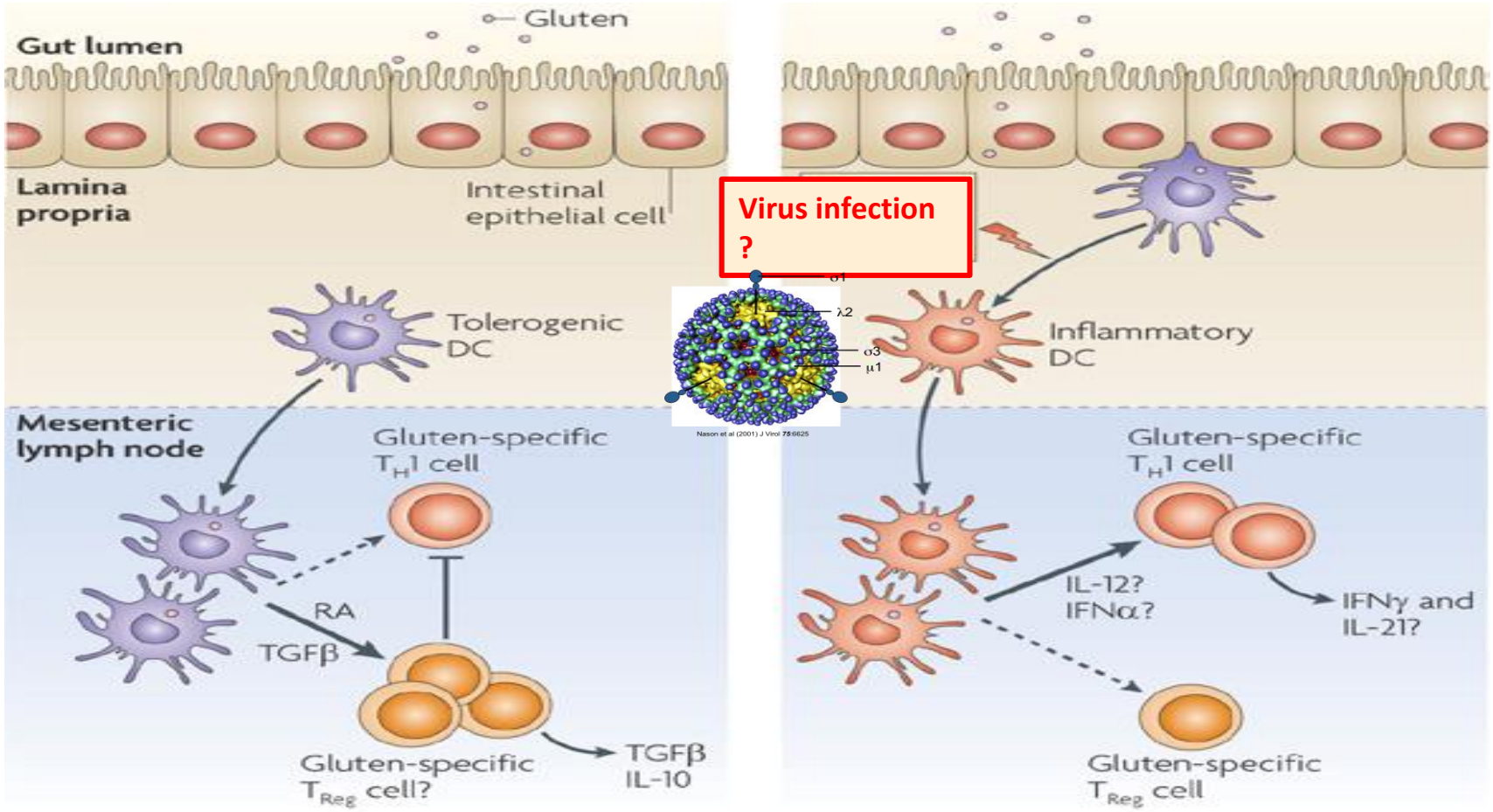
Crespo Escobar P et al, Am J Clin Nutr 2017; 105:890-6

Amount of gluten in the first years of life and risk to develop CD



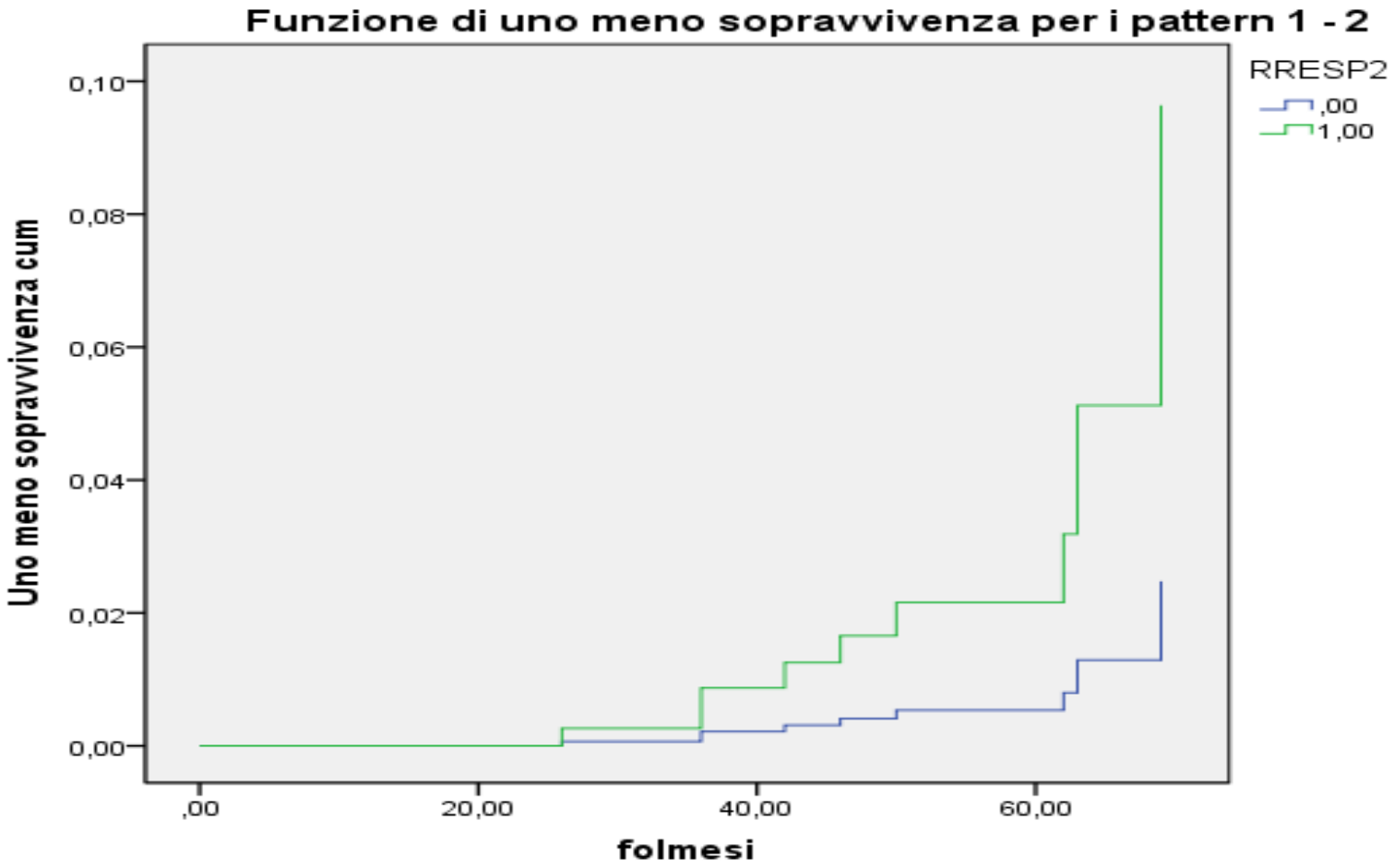
Crespo Escobar P et al, Am J Clin Nutr 2017; 105:890-6

Virus may contribute to break oral tolerance in CD



Fom Jabri & Sollid, Nature Review Immunology 2009

Cumulative incidence of CD in relation to the number of respiratory infections in the first two years of life



Auricchio R et al , Pediatrics 2017: 140(4):e20164102

Other environmental factors candidate to increase risk for CD (exposome)

- Birth delivery mode
- Antibiotics (pregnancy, first 6 months of life)
- Other medications (PPI, maternal iron supplementation)
- Altered microbioma

Outline

Prevention: Who is the target? Which intervention?

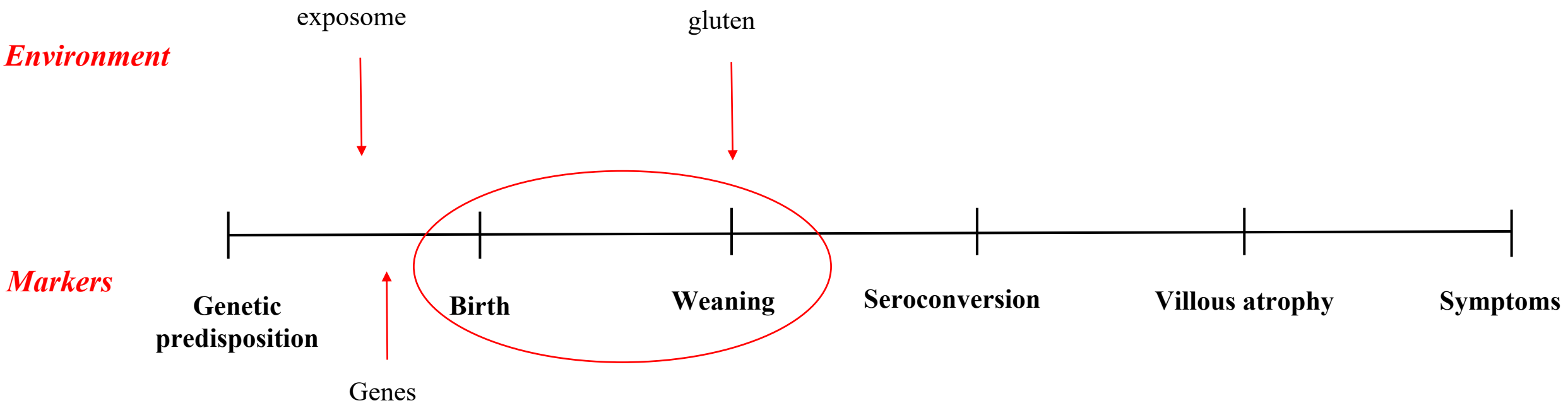
- Genetic factors
- Environmental factors

Natural history: Through which steps the disease progresses?

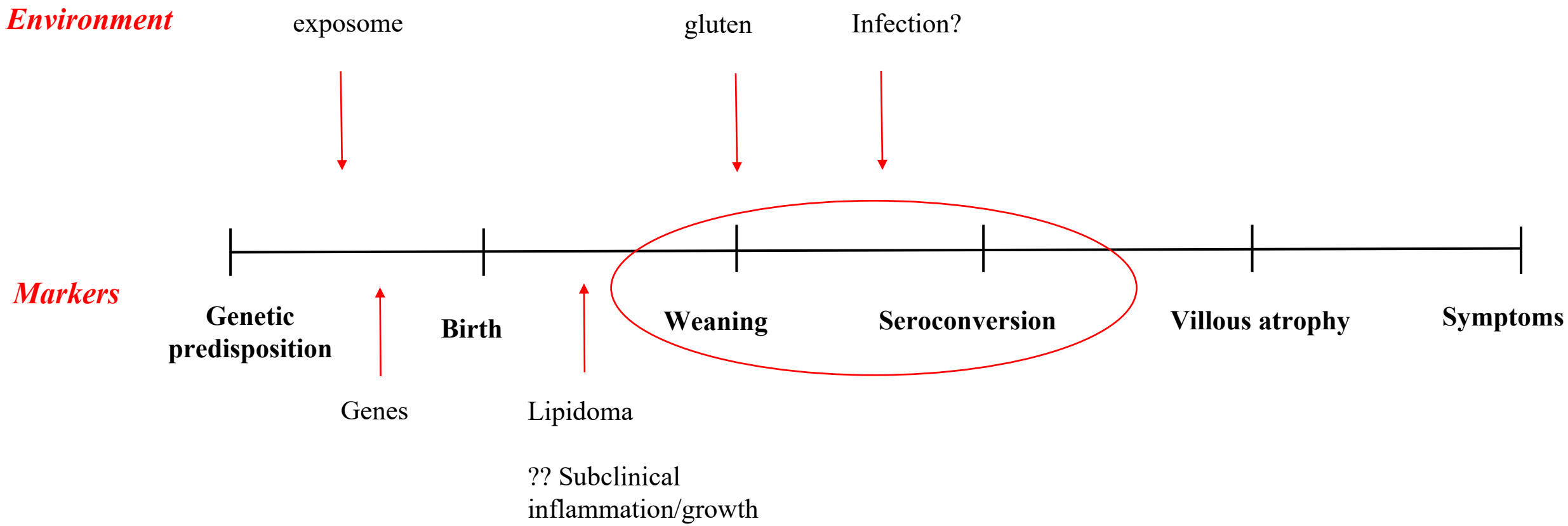
- Predictive biomarkers

Which strategies for prevention?

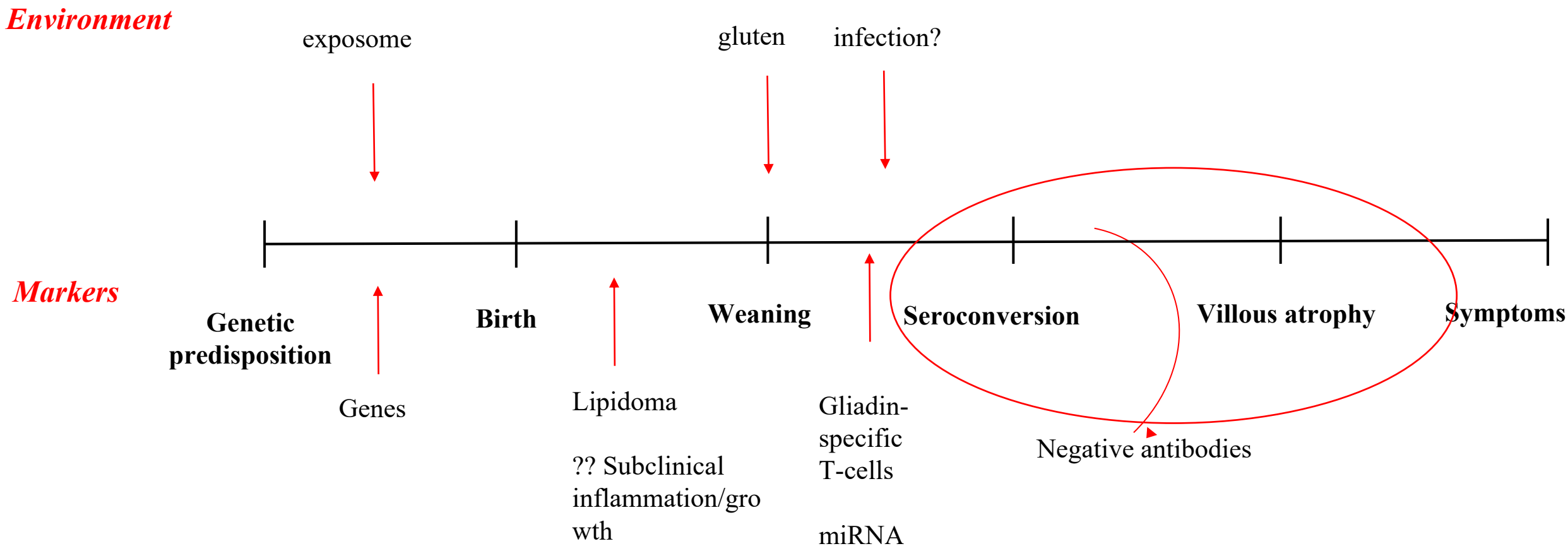
Natural history of coeliac disease



Natural history of coeliac disease



Natural history of coeliac disease



After seroconversion
Is it possible to reverse it?

- High rate of spontaneous normalization of coeliac serology in type 1 diabetes *(Castellaneta et al, Diabetes Care 2015)*

- Anti-TG2 positivity lost in 49% of children carrying genetic risk prospectically followed from birth *(Simell et al, Am J GE 2007)*

- In CELIPREV 19/23 potential CD subjects at 10 years from biopsy were serologically negative *(Lionetti et al, J Clin Med 2019)*

- Coeliac disease: a multifactorial disease
- Progression through different stages
- Biomarkers mark the different stages helping to predict those at risk of progression and amenable to intervention
- Possible strategies for prevention

Which strategies for prevention?

- Intervention on “external exposome”
- Intervention on “internal exposome” (microbioma)
- Protection from infections/delay in gluten introduction
- Lower gluten load in early life: low-gluten grains? enzymes?

Potential Coeliac Disease

Potential coeliac disease (PCD)

Normal small intestinal mucosa, at increased risk of developing CD, as indicated by positive CD serology

(Ludvigsson et al, Gut 2012)

Presence of CD-specific antibodies and compatible HLA, but without histological abnormalities in duodenal biopsies. May or may not have symptoms and signs. May not develop a gluten-dependent enteropathy later.

(Husby et al, JPGN 2012)

Questions

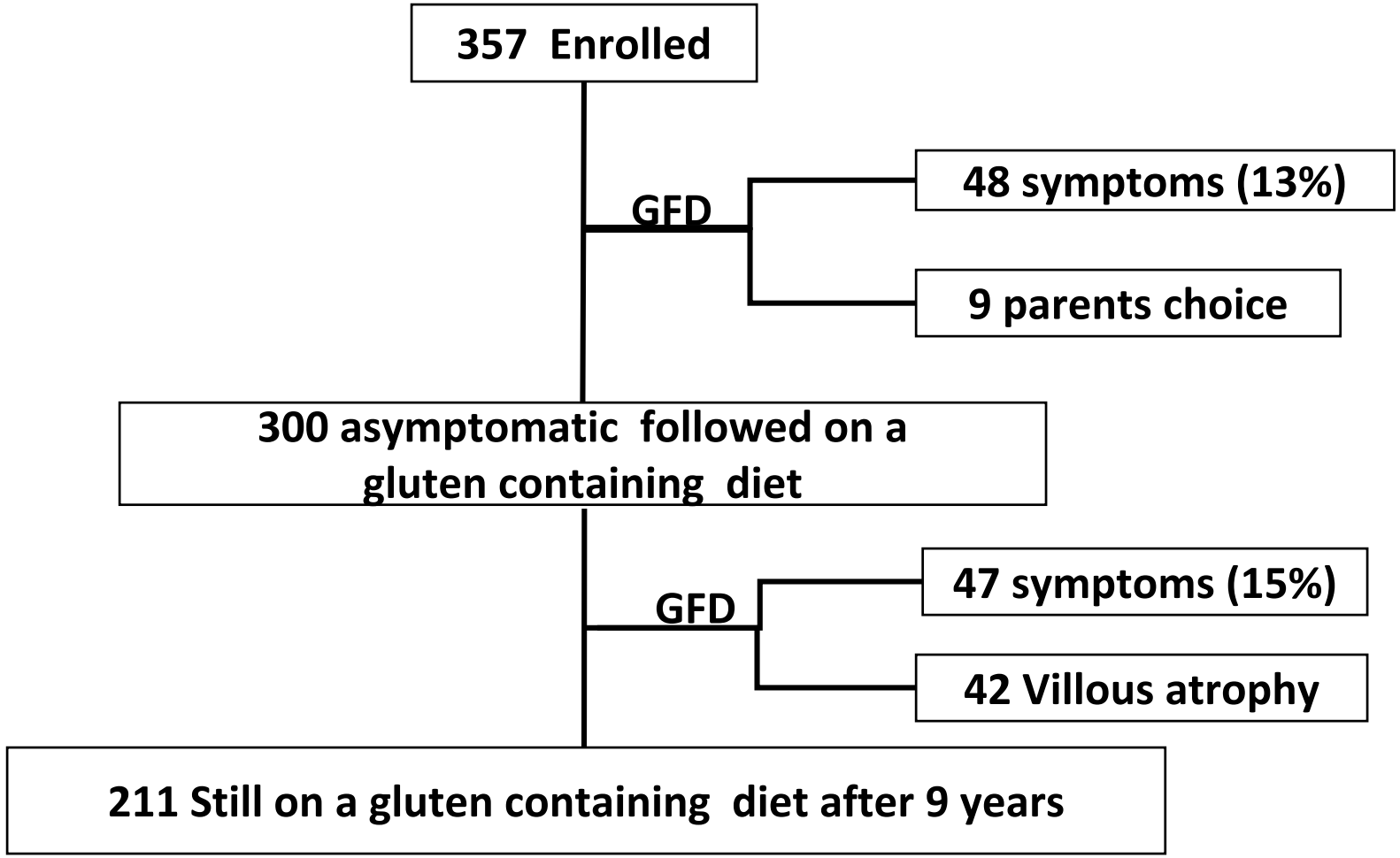
1. Which are the clinical and laboratory features of potential CD?

1. Which is the natural history of this condition?

1. Which biomarkers are predictive of the evolution?

1. To treat or not to treat with a GFD?
    - What is disease: autoantibodies, enteropathy, clinical symptoms?
    - Outcome in those treated

Progression of the study PCD cohort



Clinical, histological and serological features (357 PCD patients)

- 14,6% of patients with CD diagnosis 2001-2016
- Age: 6,4 years (range: 1.1-17,8)
- 239 girls (67 %)
- Clinical: 48 symptomatic (13%),
- 178 (50%) at-risk groups (82 autoimmune, 96 first degree relatives)
- Histology: 41% Marsh 0, 59% Marsh 1
- Anti-TG2 median titer: 1,5-2 x ULN
- Diet time 0: normal daily gluten intake

Laboratory features in Potential Coeliac Disease

- Genetics
- Autoantibodies
- Histology

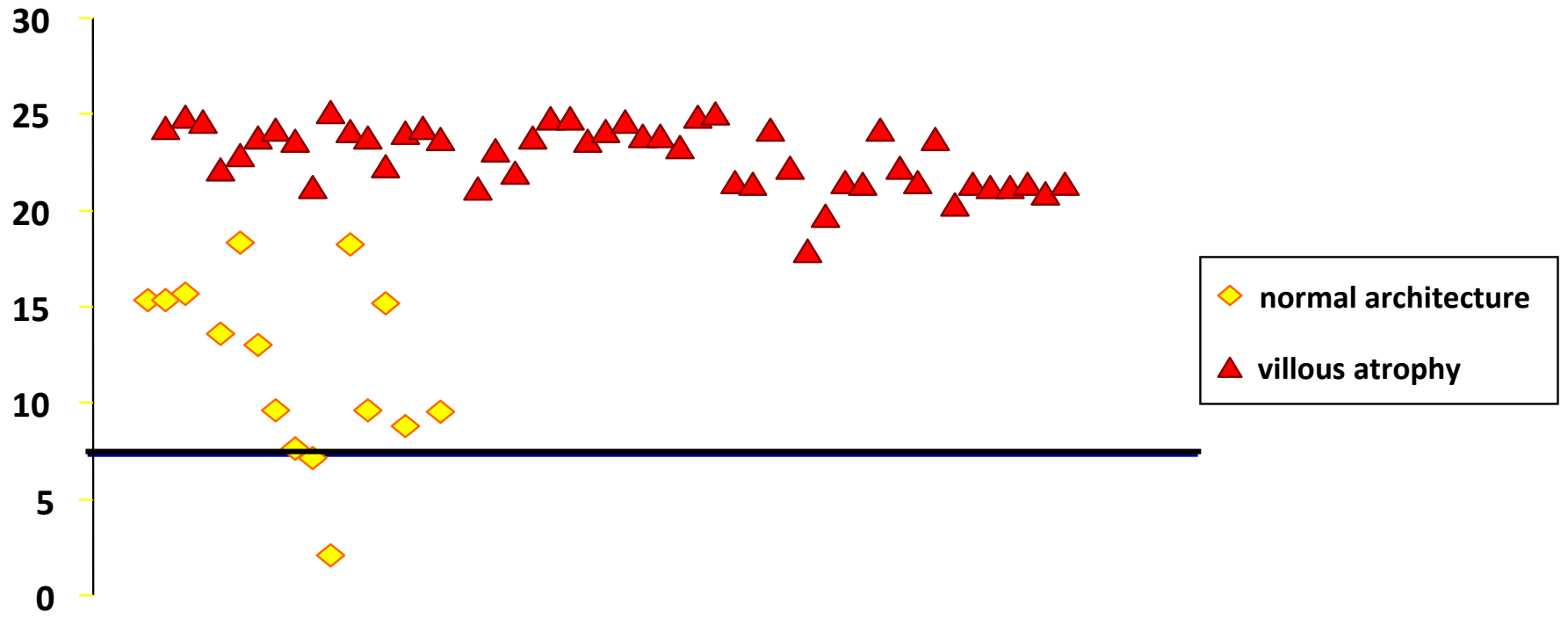
HLA risk classes distribution

HLA HAPLOTYPE RISK CLASS	311 OVERT CELIAC CASES	105 POTENTIAL CELIACS
Double DQ2	74 (24%)	12 (11,5%)
DQ2 in trans	117 (38%)	22 (21%)
DQ2 single	79 (25%)	39 (37,1%)
DQ8 or DQB1*02 (DQA1*05 negative)	13 (4%)	18 (17,1%)
No DQ2 / no DQ8	28 (9%)	14 (13,3%)
Chi Square = 35,31 p= 0.0000004		

Association results for 11 non-HLA celiac risk variants

SNPs	GENE		Potential vs. atrophic			
			Potential	Atrophic	χ^2	*P*
rs2816316	RGS1	AA	123 (74.1%)	12 (60%)	2.817	0.024
		AC	41 (24.7%)	7 (35%)		
		CC	2 (1.2%)	1 (5%)		
rs6441961	CCR	AA	29 (17.5%)	3 (15%)	1.033	0.597
		AG	80 (48.2%)	12 (60%)		
		GG	57 (34.3%)	5 (25%)		
rs17810546	IL12A/SCHIP	AA	135 (81.3%)	18 (90%)	1.002	0.606
		AG	29 (17.5%)	2 (10%)		
		GG	2 (1.2%)	—		
rs9811792	IL12A	AA	50 (30.1%)	12 (60%)	7.773	0.021
		AG	83 (50%)	7 (35%)		
		GG	33 (19.9%)	1 (5%)		
rs1464510	LPP	AA	34 (20.5%)	5 (25%)	0.87	0.275
		AC	92 (55.4%)	10 (50%)		
		CC	40 (24.1%)	5 (25%)		
rs6822844	IL2/IL21	AA	5 (3.0%)	0	1.552	0.460
		AC	30 (18.%)	2 (10%)		
		CC	131 (78.9%)	18 (90%)		
rs2327832	OLIG3-TNFAIP3	AA	108 (65.1%)	10 (50%)	1.747	0.417
		AG	52 (31.3%)	9 (45%)		
		GG	6 (3.6%)	1 (5%)		
rs1738074	TAGAP	AA	34 (20.5%)	3 (15%)	0.337	0.845
		AG	78 (47.0%)	10 (50%)		
		GG	54 (32.5%)	7 (35%)		
rs3184504	SH2B3	AA	50 (30.1%)	8 (40%)	1.154	0.561
		AG	78 (47.0%)	7 (35%)		
		GG	38 (22.9%)	5 (25%)		
rs842647	REL	AA	94 (56.6%)	8 (40%)	2.134	0.344
		AG	57 (34.3%)	10 (50%)		
		GG	15 (9.0%)	2 (10%)		
rs917997	IL18RAP	AA	12 (7.2%)	—	1.547	0.461
		AG	61 (36.7%)	8 (40%)		
		GG	93 (56.0%)	12 (60%)		

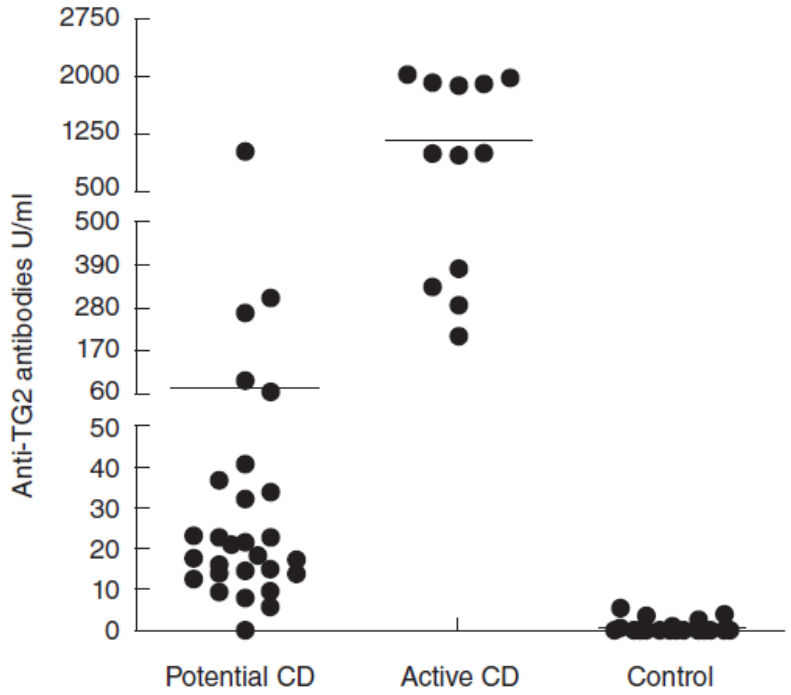
Anti-TG2 serum levels in EMA+ subjects with normal jejunal architecture

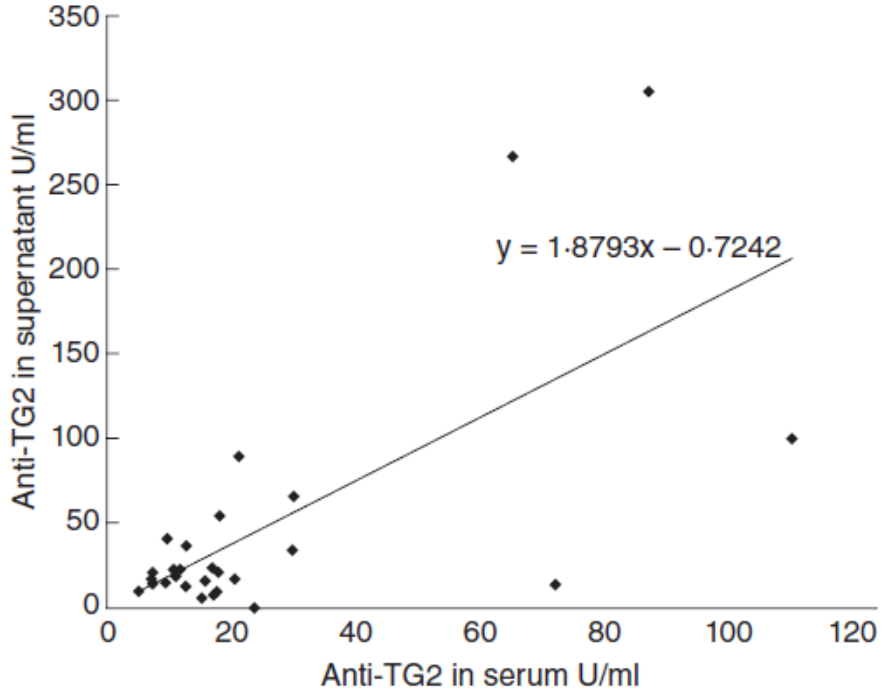


IgA anti-TG2 intestinal deposits

Patients	Positive IgA anti-TG2 deposits	Positive serum Anti-TG2
Active CD >2 years age	35/35 (100%)	35/35 (100%)
Active CD <2 years age	38/52 (73%)	35/38 (92%)
Potential CD	43/59 (73%)	59/59 (100%)
In remission CD	2/12 (17%)	0/0 (0%)
Controls	10/68 (15%)	0/0 (0%)

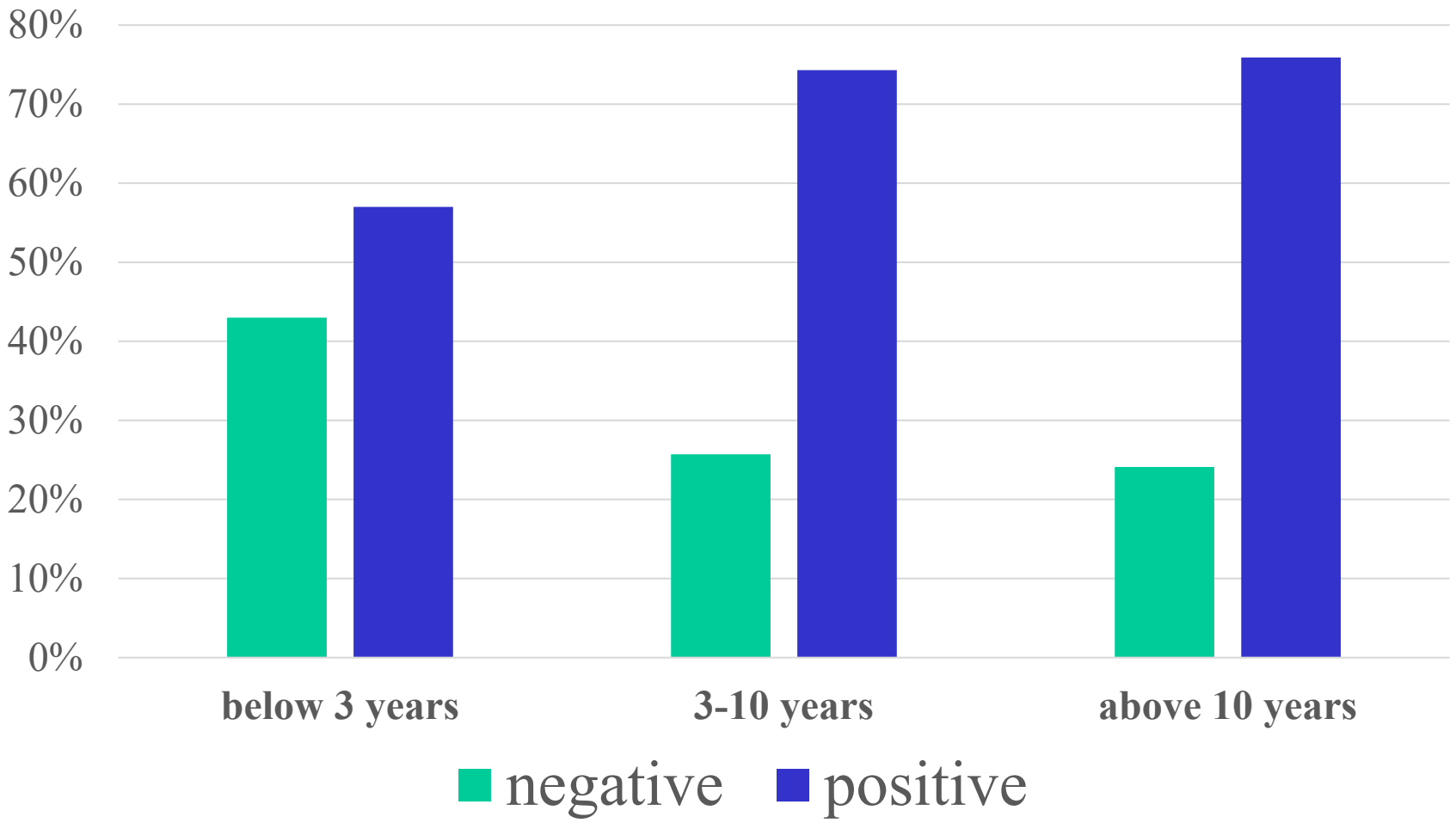
Intestinal auto-antibodies in potential CD





Tosco A et al Clin Exp Immunol 2013

Serology trend in relation to age at diagnosis



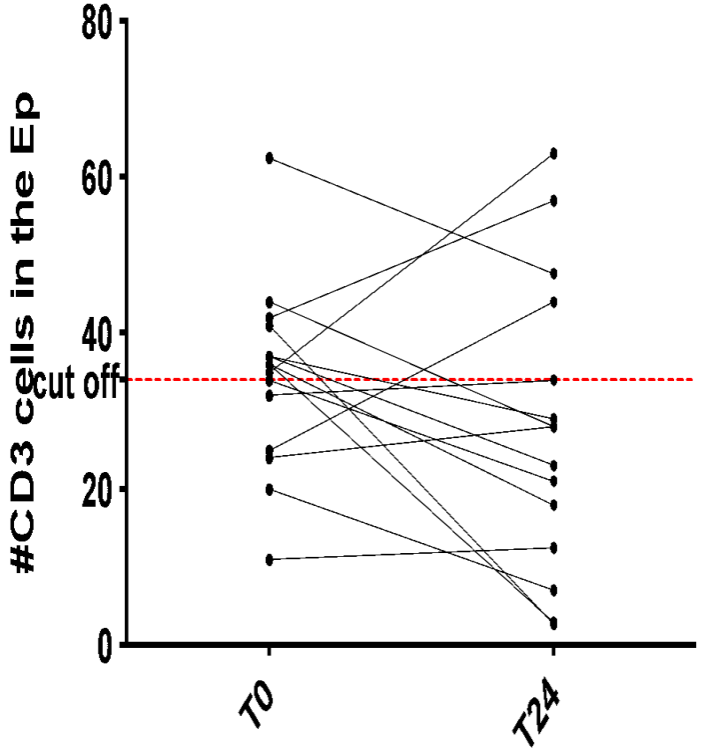
Younger children at diagnosis showed a trend of serology towards negativization more than in other age group

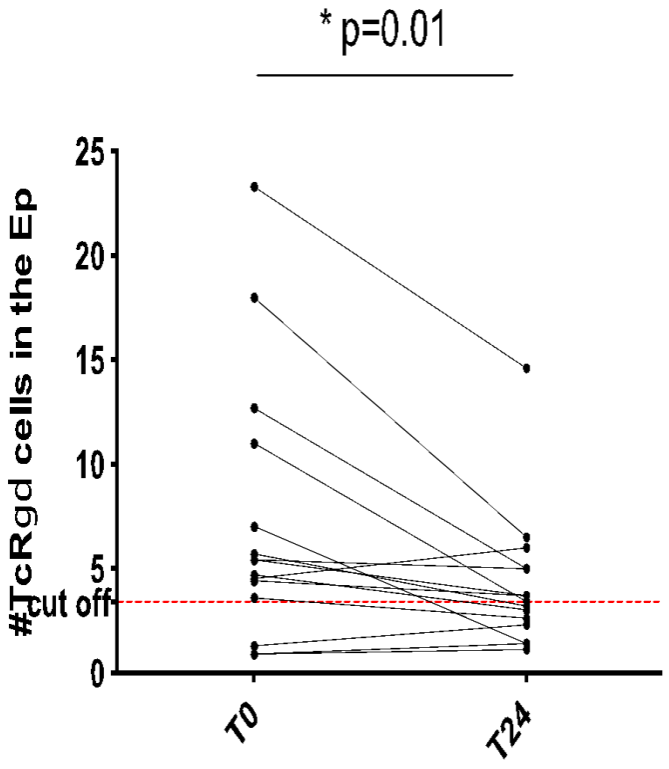
Features predicting disappearance of anti-TG2 antibodies from serum in potential CD

- anti-TG2 titer at T0 (p=0.04)
- HLA class, lower risk (p=0.001)
- γδ infiltration at T0 (p=0.05)

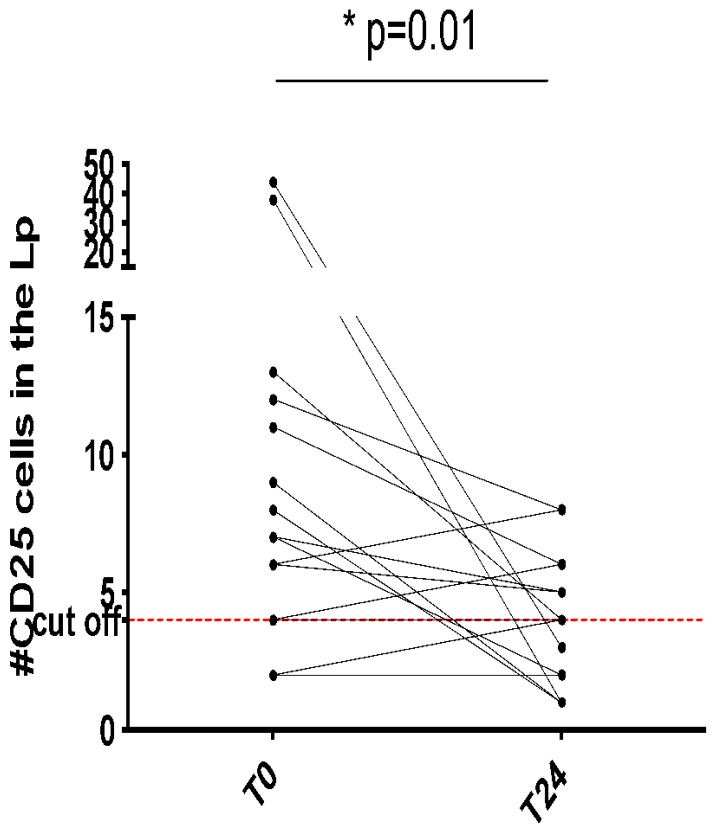
51/86 (59%) became negative in the first 2 years of follow-up

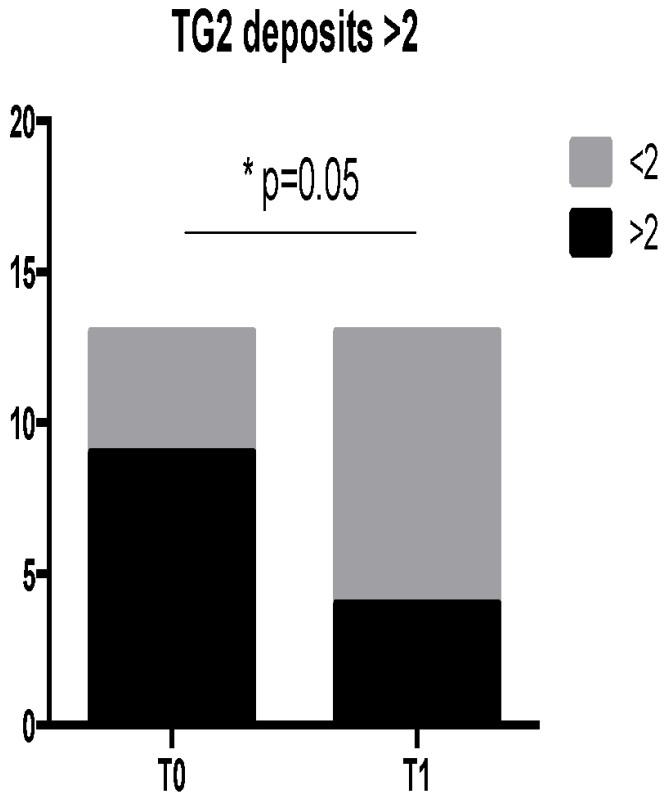
Epithelial infiltration in potential CD patients who have become seronegative





Signs of lamina propria inflammation and intestinal deposits of antiTG2 antibodies in potential CD patients who have become seronegative

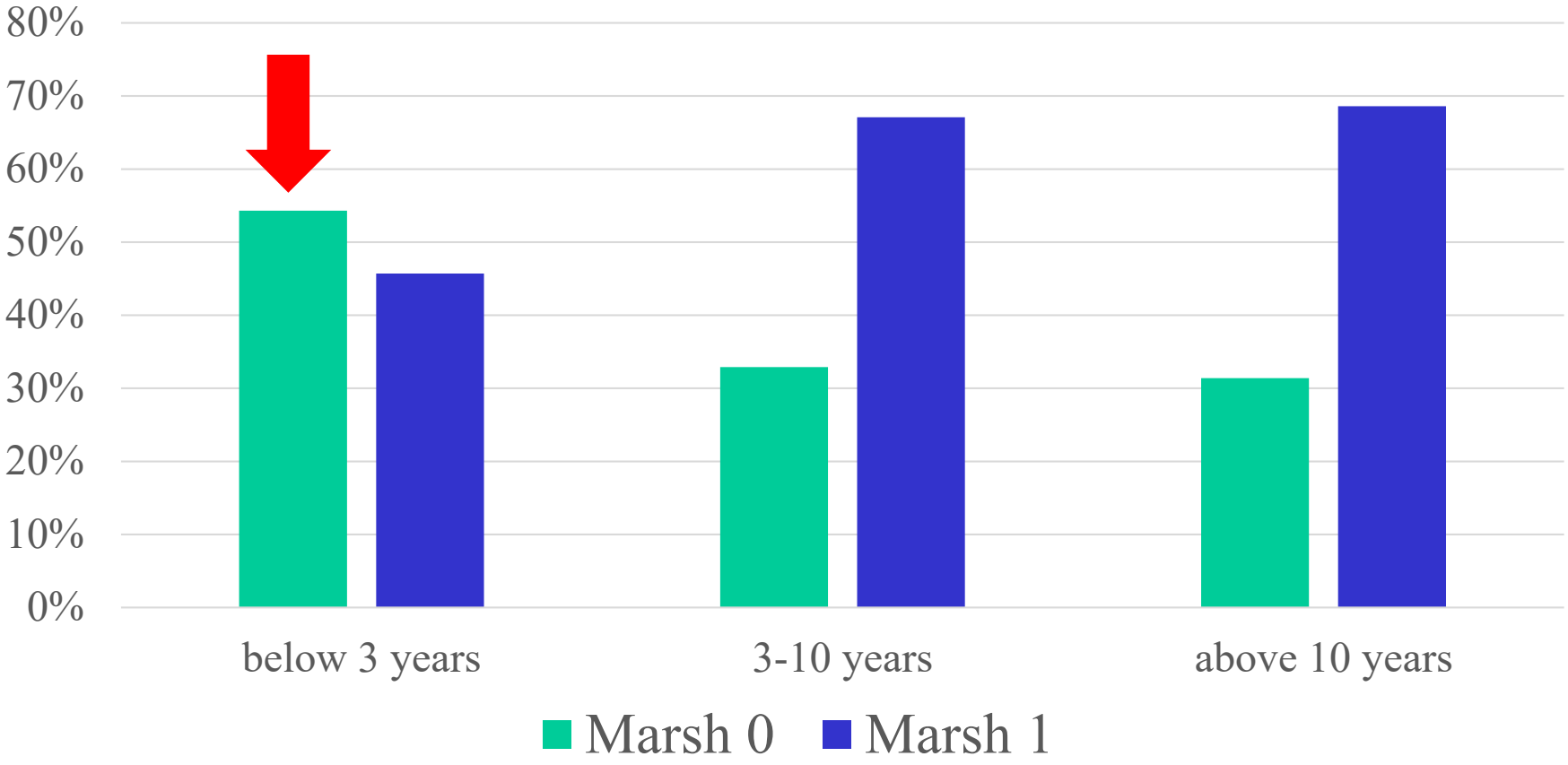




Heterogeneity among potential CD patients

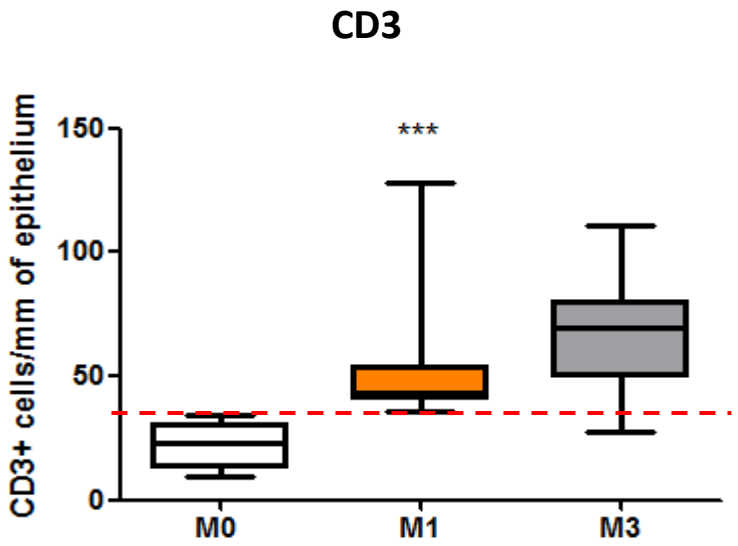
Marsh 0 vs Marsh 1

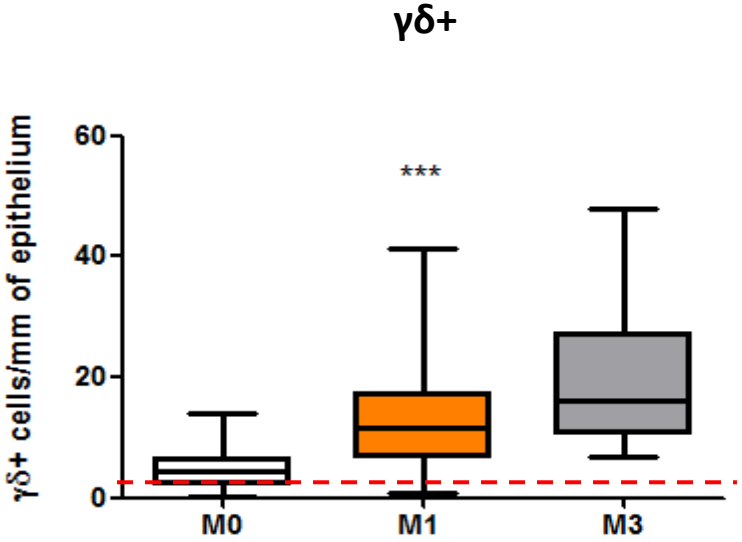
Duodenal mucosa infiltration in different age group at diagnosis

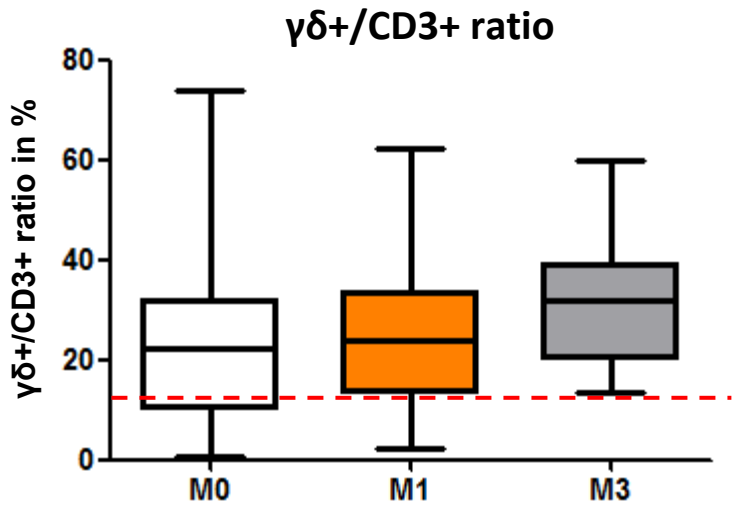


Younger children at diagnosis showed less duodenal mucosal infiltration than other age group.

Potential CD biopsies: Marsh 0 vs Marsh 1
γδ IELs

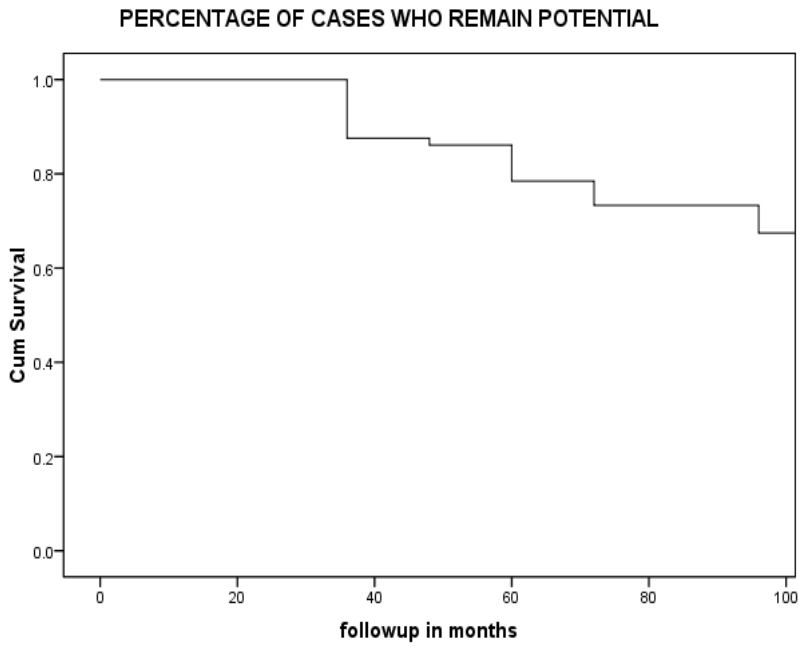


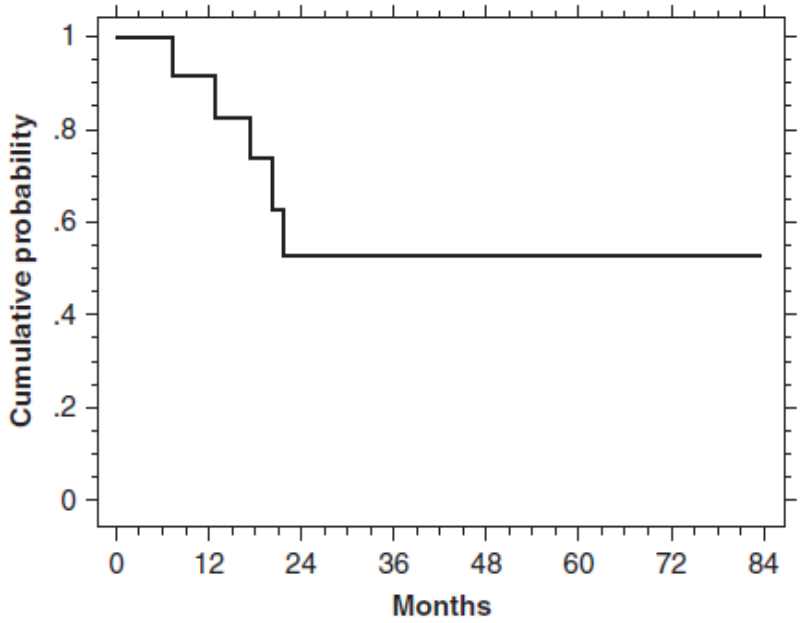




Will all become coeliac?

Survival curves

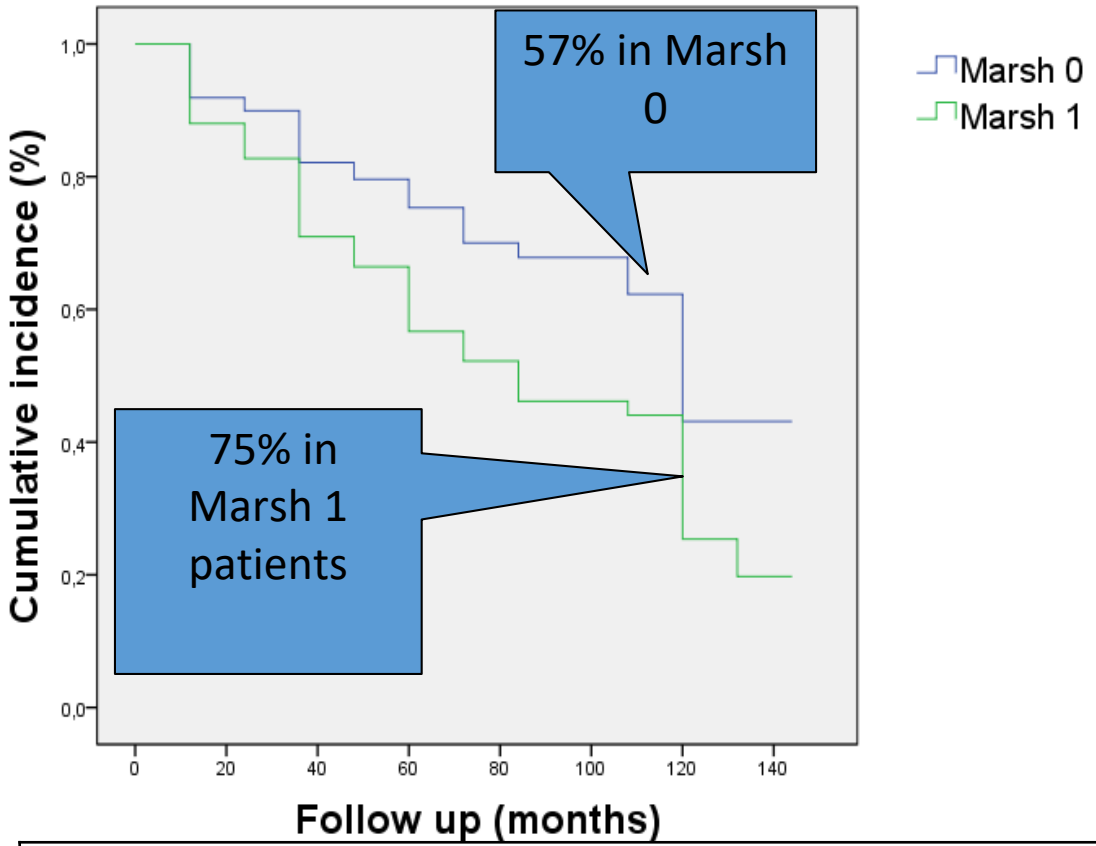




Auricchio R et al. Am J Gastroenterol 2014

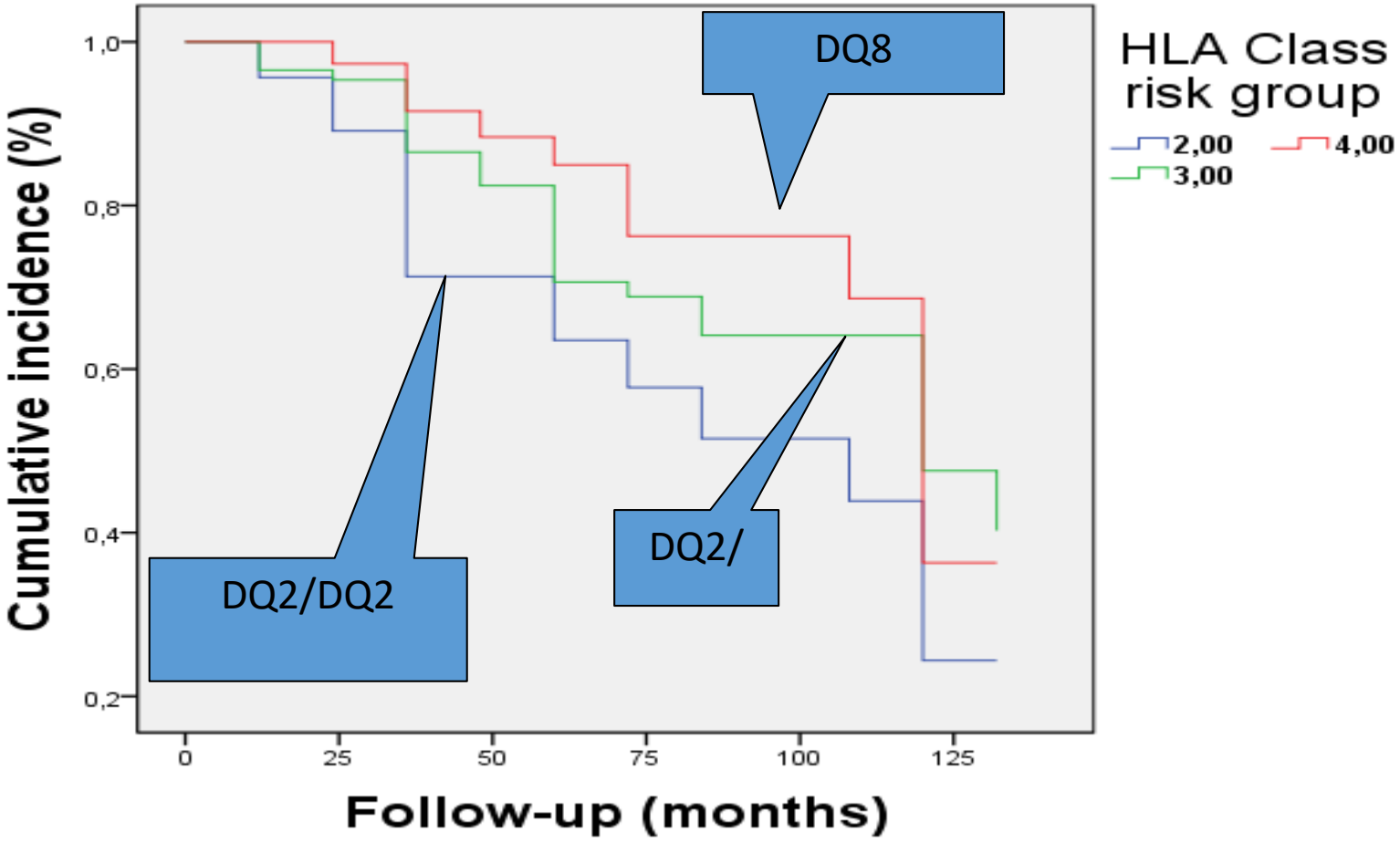
Biagi F et al. Scand J Gastroenterol 2013

γδ infiltration is the best predictor of evolution to villous atrophy



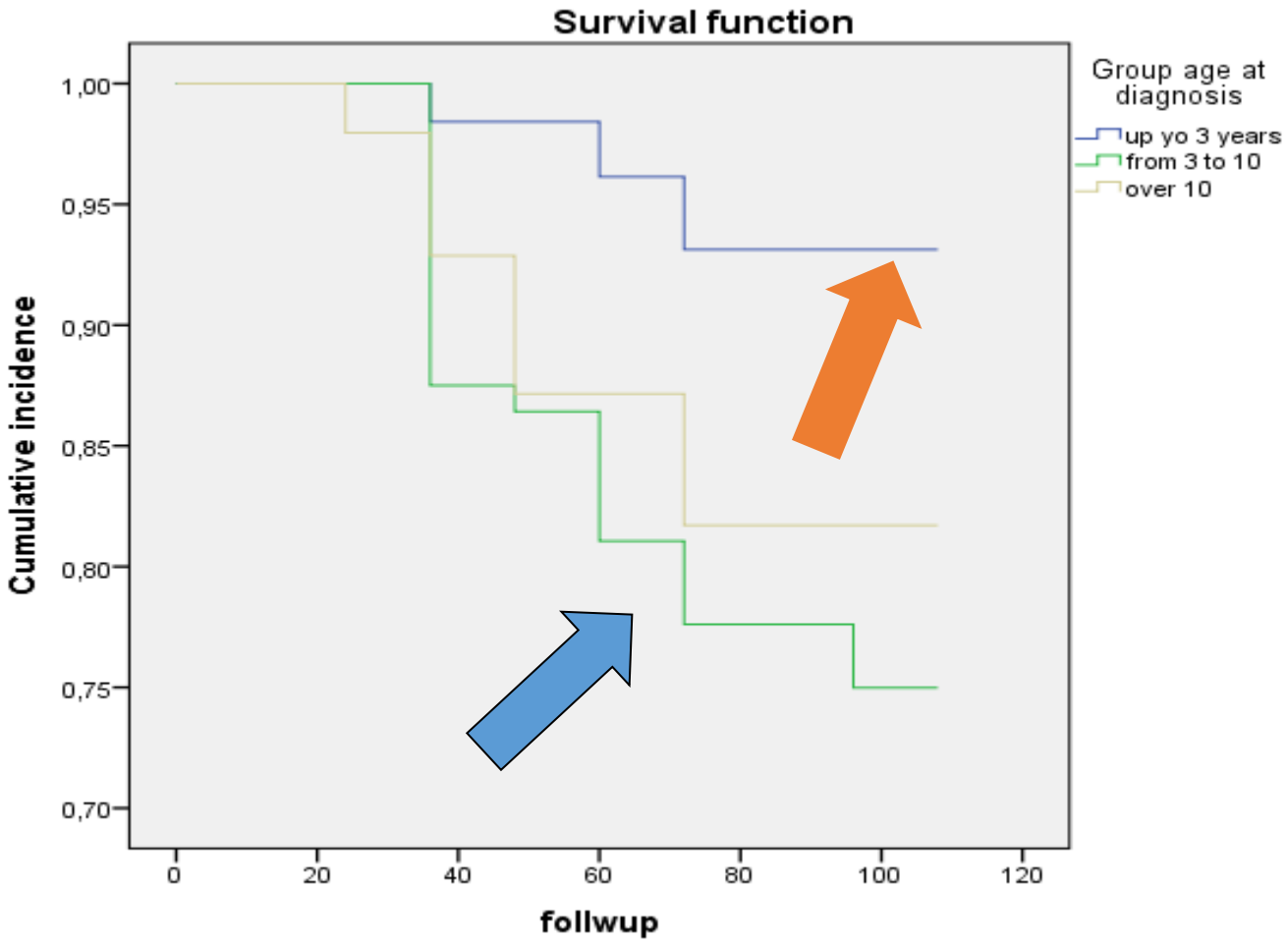
γδ IEL biopsy at diagnosis		
	Cases	**Potential**
	11,9	**6,44**
CI	**8,3-15,5**	**5,5-7,3**
p	**0.05**	

Risk factors: HLA doses



All have at risk HLA, but still **there is a dose-effect (p = 0,04)**

Risk factors: age at diagnosis



Children recruited at older ages (above 10 years old) have an increased risk to become celiac, compared to children enrolled younger (< 3 years)

This effect is not related to the length of follow up

Discriminant analysis at time of diagnosis

Variable at enrollement	Wilks Lambda	Variance Ratio F	p
γδ Lympho	,916	11,693	,001
Age at diagnosis	,882	8,465	,000
IL12a	,854	7,193	,000
SH2B3	,815	7,089	,000
RGS1	,792	6,532	,000
CCR	,770	6,120	,000
IL2_IL21	,757	5,601	,000
HLA Haplotype	,739	5,349	,000

By this model the outcome of about 80% of cases might be predicted at time of enrollment.

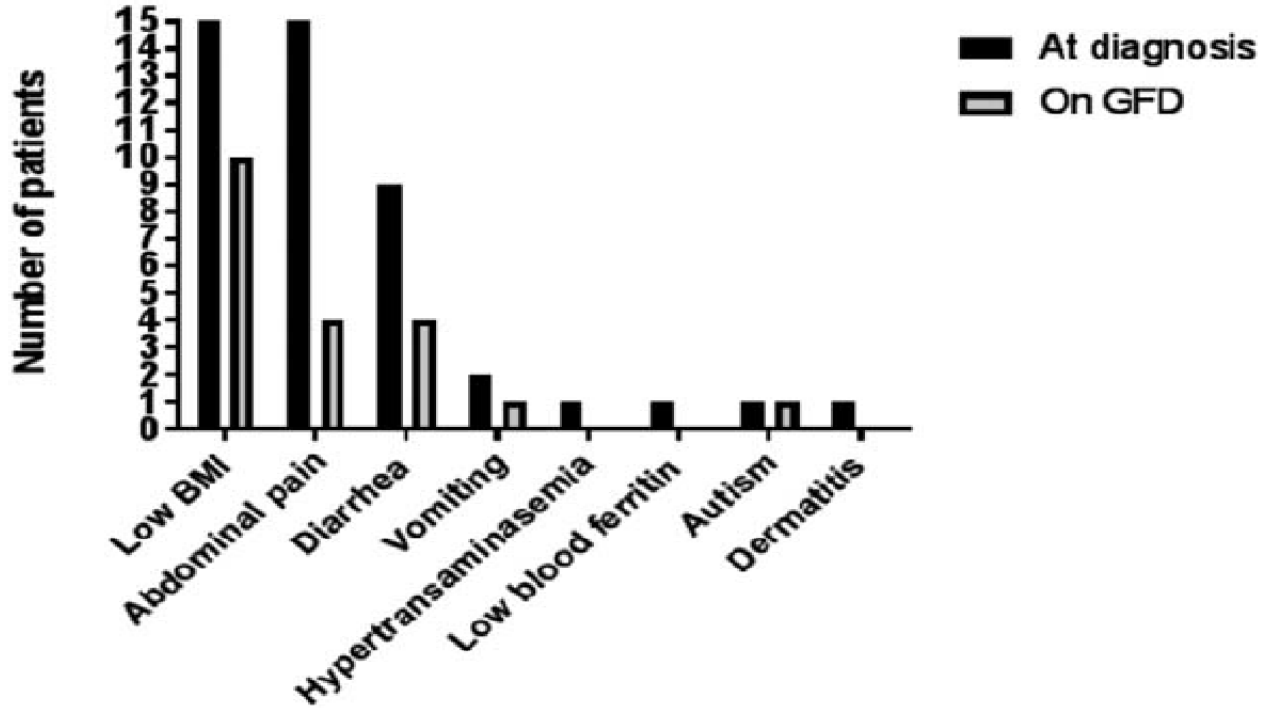
Add serology at 24 and 36 months of follow-up, we can improve prediction of developing villous atrophy to 86,8%!!!

Management

To treat or not to treat?

The Effect of Gluten-free Diet on Clinical Symptoms and the Intestinal Mucosa of Patients With Potential Celiac Disease

Roberta Mandile, Valentina Discepolo, Serena Scapaticci, Maria Rosaria Del Vecchio, Maria Antonia Maglio, Luigi Greco, Riccardo Troncone, and Renata Auricchio



Most frequent symptoms are low BMI(36%), abdominal pain (34%) and diarrea (19%)

Symptoms improve in only half of cases (19/35) in 12 months of GFD (GI>no GI symptoms)

(JPGN 2018)

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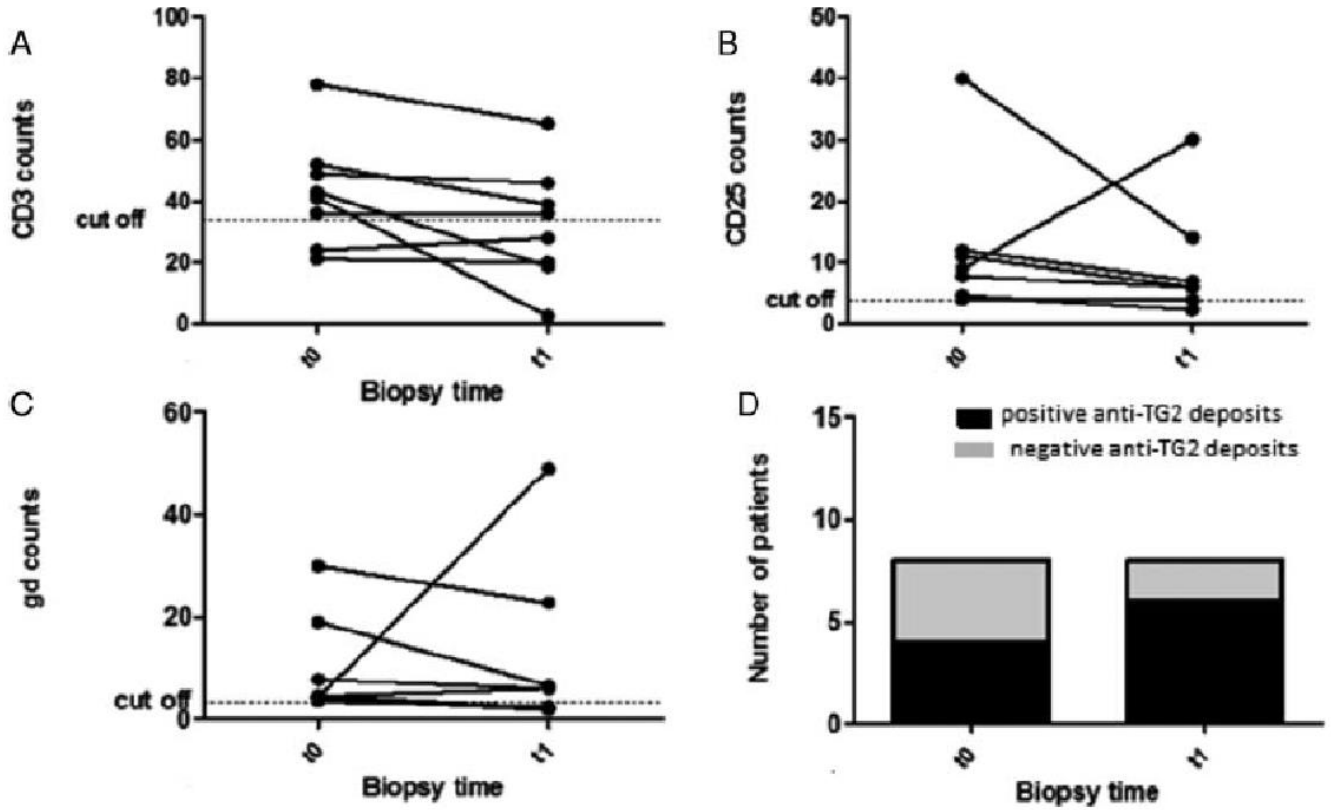

The intestinal inflammation did not always improve after 1 years of GFD

(JPGN 2018)

Conclusions

“Potential” Coeliac Disease is increasingly diagnosed

Most are (apparently) asymptomatic. Only half of symptomatic patients improve on a GFD

With a follow-up up to 10 years, 42 children progress to villous atrophy; most in the first 2 years of follow up

Risk factors for progression are:

- Age at enrollment
- Genetic profile (HLA and no HLA)
- Infiltrative lesion (Marsh 1) at diagnosis
- Persistence of increased levels of anti-tTG2 (T24!!!)

TCR-γδ+ IEL density best predictor of evolution to villous atrophy