Enhanced monitoring and screening in pediatric coeliac disease

Wessels, M.M.S.

CHAPTER 6

Raising the cut-off level of anti-tissue transglutaminase antibodies to detect coeliac disease reduces the number of small bowel biopsies in children with type 1 diabetes — a retrospective study

SUBMITTED

Wessels MMS*

Velthuis A*

Lochem van E

Duijndam E

Hoorweg-Nijman E

Kruijff de I

Wolters VM

Berghout E

Meijer J

Boksma JA

Mul D

van Alfen-van der Velden JA

Mearin ML

Setten van P

* Contributed equally

Abstract

OBJECTIVE Our aim was to study the optimal cut-off value for the anti-tissue transglutaminase type 2 IgA antibody in serum (TG2A) to select for diagnostic small bowel biopsies for coeliac disease (CD) in children with type 1 diabetes mellitus (T1DM). In particular, we endeavour an increase in specificity and positive predictive value and more importantly a decrease in normal histology, without losing too much sensitivity.

PATIENTS AND METHODS Children with T1DM who had both elevated TG2A titers during regular screening and duodenal biopsies during the course of their diabetes were included. Anti-endomysial antibodies (EMA) if present were recorded. The optimal TG2A cut-off value was determined using receiver operating characteristics (ROC) curve analysis; and compared with the cut-off value used in the ESPGHAN guidelines in terms of sensitivity, specificity, positive and negative predictive value. TG2A titers were expressed as the ratio between the value obtained and the upper limit of normal (ULN).

RESULTS A total of 63 children were included. The optimal cut-off value for performing a biopsy proved 11xULN. Raising the cut-off value from 3xULN to 11xULN changed the sensitivity from 96% to 87%, increased the specificity from 36% to 73%, the positive predictive value from 88% to 94% and the negative predictive value from 67% to 53%. The percentage of normal histology was reduced from 12% to 6%.

CONCLUSION Our data indicate that increasing the TG2A cut-off value for performing duodenal biopsies in children with T1DM and suspected CD leads to a substantial reduction of unnecessary biopsies. We advocate to adapt the ESPGHAN 2012 guidelines for this group of children, including monitoring patients with TG2A levels below 11xULN over time.

Introduction

Children with type 1 diabetes mellitus (T1DM) are at risk of developing coeliac disease (CD). Both conditions are autoimmune diseases showing strong linkage to the human leukocyte antigen (HLA) system[1]. The prevalence of CD among patients with T1DM is estimated between 3 to 10%[2]. Most children with both T1DM and CD are asymptomatic or present with non-specific symptoms[3]. Duodenal biopsies is the gold standard for diagnosis of CD in children with T1DM[3]. CD is not only believed to cause diminished diabetes control in children with T1DM, but may also result in complications, including decreased bone density and gastrointestinal malignancies[1]. CD is treated with a gluten-free diet (GFD)[4]. Therefore, children with diabetes are regularly screened for CD; at diagnosis of T1DM and subsequently every 1-2 years thereafter[5]. Anti-tissue transglutaminase type 2 IgA antibodies (TG2A) are commonly used for screening and have a sensitivity and specificity above 90%[6]. Despite this high accuracy, the interpretation of the TG2A titers in children with T1DM has proven difficult. Significant quantitative differences exist among different TG2A assays[7], elevated TG2A titers often show spontaneous normalization in children with T1DM[8] and people at genetic risk for CD (like children with T1DM) have more often false-positive TG2A results[9]. In 2012, the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) introduced new guidelines for diagnosing CD[10], including an algorithm for asymptomatic children belonging to a high-risk group, like children with T1DM. In this algorithm, the cut-off value of serum TG2A titers for performing biopsies in these children is 3x upper limit of normal (ULN). This study was prompted by our observation that biopsies performed in children with T1DM and TG2A titers >3xULN are often not consistent with CD. We hypothesize that the cut-off value of 3xULN was chosen too low. The aim of our study was to investigate the optimal cut-off value for the TG2A titers in order to overcome negative biopsies in children with T1DM without losing too much sensitivity.

Patients and methods

Study design and settings

This is a retrospective observational study covering the time period 2002-2015. Data were collected both at University Hospitals and middle to large secondary care clinics: Leiden University Medical Center (LUMC) Leiden, University Medical Center Groningen (UMCG), Rijnstate Hospital Arnhem, Haga Hospital The Hague, Children's Diabetes Centre Nijmegen (CDCN), Maas Hospital Pantein Beugen, Spaarne Hospital Hoofddorp, St. Antonius Hospital Nieuwegein, Zuwe Hofpoort Woerden, MC Zuiderzee Lelystad, Isala Hospital Zwolle, Deventer Hospital and University Medical Center Utrecht (UMCU). Due to the retrospective nature of this study, informed consent was not required. The pro-

cedures followed were in accordance with the ethical standards of the Medical Research Involving Human Subjects Act and the principles of the declaration of Helsinki (59th General assembly, Seoul, October 2008) of the World Medical Association. Formal approval from the local feasibility committee of Rijnstate Hospital Arnhem was obtained.

Study group

The study population consisted of all consecutive children and adolescents (<19 years of age) with T1DM, who underwent esophagogastroduodenoscopy with duodenal biopsies because of elevated TG2A titers>3xULN[10]. Screening with CD specific serology in these children was done every 1-2 years after diagnosis of T1DM according to the ISPAD international guideline for the management of pediatric T1DM, regardless of symptoms[5]. Exclusion criteria were: IgA deficiency, a GFD at the time of duodenal biopsies, CD diagnosed before T1DM and an interval >180 days between measurement of the TG2A titer and duodenal biopsies.

Data collection

Data was retrieved from either patient charts or electronic data systems and entered into standard forms using Research Manager version 5.2.0.5 (Cloud9 Health Solutions,the Netherlands). Clinical, anthropometric and laboratory data were collected. This included several baseline characteristics such as age at first duodenal biopsies, gender, other autoimmune disease(s), family history of CD and other autoimmune diseases and GFD adherence. Presence of symptoms suggestive for CD[10] was also registered: chronic or intermittent diarrhea[11], failure to thrive, weight loss, delayed puberty, amenorrhoea, iron-deficiency anaemia, chronic abdominal pain, chronic constipation, chronic fatigue, dermatitis herpetiformis-like rash and spontaneous fracture/osteopenia.

Serology

Since 13 different hospitals participated in this study, TG2A titers were assessed while using different types of assays, different arbitrary units and different cut-off values. In order to compare the TG2A titers, results were expressed as the ratio between the value obtained and the upper limit of normal. This ratio was rounded to whole numbers. When a TG2A level was written in the file as >50, it was regarded as 50 and >128 was regarded as 128, etcetera. Since some patients were analyzed twice (accompanied by a second biopsy), without starting a GFD in between, the total number of measurements exceeds the total number of patients included in our study. TG2A IgA ELISA's used were obtained from 6 different manufacturers: Aesku (Wendelsheim, Germany), n=4; Euroimmun (Lübeck, Germany), n=2; Inova (San Diego, California), n=4; Orgentec (Mainz, Germany), n=3; Phadia (Freiburg, Germany) n=50; Sanquin (Amsterdam, The Netherlands), n=2. Anti-endomysial antibodies (EMA) as determined by indirect immunofluorescence were recorded in all patients.

Duodenal biopsy

All children included in this study underwent esophagogastroduodenoscopy in order to obtain duodenal biopsies, 4 from duodenum and 1-2 from bulb. Histological findings were revised and classified according to the Marsh criteria[12] by a single pathologist, specialized in Marsh typing. This pathologist was blinded for previous interpretations and clinical and laboratory findings. Definite CD was confirmed by Marsh 2 or 3 histology in combination with the already known positive coeliac serology. CD autoimmunity without histology alterations in the small bowel biopsies was considered potential CD[10].

Statistical analysis

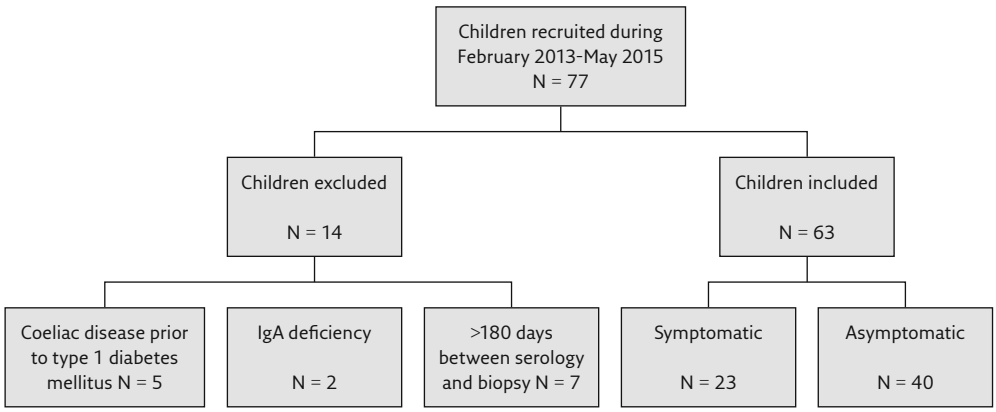
Continuous data are presented as mean ± standard deviation (SD). Differences in means between groups were tested using the Students' T-test. Categorical data are presented as frequencies and percentages. Differences in percentages between groups were tested using the Pearson chi-square test or Fisher's Exact test. Receiver Operating Characteristic (ROC) analyses were performed to determine the optimal TG2A cut-off value. Sensitivity, specificity, positive and negative predictive values were calculated. Sensitivity and specificity were tested by the McNemar test. Statistical analysis was performed using IBM SPSS Statistics (version 18.0). A P-value less than 0.05 was considered to indicate statistical significance.

Results

Study design

In our study, 77 children were eligible. A total of 63 children fulfilled our inclusion criteria. 14 children were excluded (**Figure 1**). The median interval between TG2A measure-

Figure 1 *Flowchart of participants (children with type 1 diabetes mellitus).*



ment and duodenal biopsies was 72 days (range 0-178). The total number of analysed TG2A titers was 65, since 2 patients underwent a second paired antibody determination as well as second biopsies.

Baseline characteristics

The baseline characteristics of all patients are presented in **Table 1**. In our study population, a preference for females was noticed (62%). Symptoms suggestive for CD were present in 37% of the patients. When comparing asymptomatic with symptomatic children, no statistically significant differences were observed in baseline characteristics, neither in associated autoimmune disease, family history of associated autoimmune disease, family history of CD nor in Marsh histology. Three children suffered from autoimmune hypothyroidism. The prevalence of CD in the total group is 83%, with a higher prevalence in the asymptomatic patients when compared to the symptomatic children, 88% and 74% respectively (p>0.05).

Table 1 *The baseline characteristics and differences between the asymptomatic and symptomatic children with type 1 diabetes mellitus and elevated tissue transglutaminase antibodies.*

	Study population n=63	**Asymptomatic children n=40**	**Symptomatic children n=23**	**P-value**
Mean age in years (at time of 1st biopsy)	9.7 (SD 4.7)	10.5 (SD 4.2)	8.2 (SD 5.1)	0.06
Female n(%)	39 (62)	27 (68)	12 (52)	0.23
Other autoimmune disease n(%)	3 (5)	3 (8)	0 (0)	0.31
Family history of other autoimmune disease n(%)	21 (33)	13 (33)	8 (35)	0.85
Family history of coeliac disease n(%)	4 (6)	2 (5)	2 (9)	0.62
CD (Marsh 2-3) n(%)	52 (83)	35 (88)	17 (74)	0.31

Analysis of cut-off value

ROC-curve analysis showed that the optimal TG2A cut-off value for performing a biopsy in children with T1DM, as calculated by ROC-curve analysis, is 11xULN. This cut-off value provides a sensitivity of 87% and a specificity of 73%. The cut-off value of 3xULN, described and advised in the latest ESPGHAN guideline, results in our study in a sensitivity of 96% and a specificity of 36%. **Figure 2** shows the ROC-curve, **Table 2** shows the coordinates of the curve, with sensitivity and 1-specificity.

Figure 2 *Receiver operating characteristics curve to determine the optimal tissue transglutaminase antibodies cut-off value for performing diagnostic biopsies for coeliac disease in type 1 diabetes mellitus.*

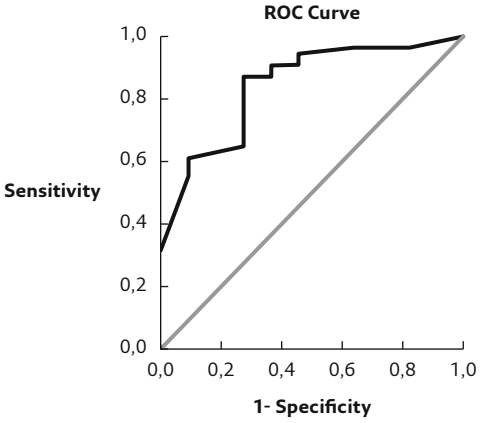


Table 2 *Coordinates of the receiver operating characteristics curve to determine the optimal tissue transglutaminase antibodies cut-off value for performing duodenal biopsies.*

Cut-off value (xULN)	Sensitivity	1-Specificity
1,0	1,000	1,000
2,0	0,963	0,818
3,0	0,963	0,636
5,0	0,926	0,455
7,0	0,907	0,455
9,0	0,907	0,364
10,0	0,870	0,364
11,0	0,870	0,273
13,0	0,852	0,273
15,0	0,648	0,273

Changing the cut-off value of TG2A: consequences for clinical practice

Table 3 illustrates the different positive predictive values (PPV) for small bowel mucosal atrophy, depending on different cut-off values for normality (CoN), from 3xULN to 11xULN.

PPV for CoN increases from a minimal of 89% at 3xULN to a maximum of 94% at 11xULN with NPV of 67% and 53% respectively. Raising the CoN from 3xULN to 11xULN results in a decrease in sensitivity from 96 to 87% (p=0.06) and an substantial increase in specificity from 36 to 73% (p=0.13). Overall, a CoN of respectively 3 and 11xULN resulted in 12% (7/59) and 6% (3/50) normal (false positive) duodenal biopsies.

EMA as additional test

EMA was negative in only 3 cases, who all had Marsh 0-1 histology. Adding EMA positive results did not improve the PPV of increased TG2A levels for villous atrophy, since 50% of EMA positive patients had normal duodenal histology.

Table 3 *Diagnostic performance of different cut-off values of normality of tissue transglutaminase antibodies*

TG2AxULN	Number of children biopsied	Diagnosed with CD	Sensitivity (%)	Specificity (%)	Positive predictive value for CD (95% CI interval)	Negative predictive value for CD (95% CI interval)
>1.0	65	54	100	18	85.7 (82-89)	100
>3.0	59	52	96	36	88.7 (83-92)	66.7 (29-91)
>5.0	55	50	93	55	90.9 (84-95)	60 (34-82)
>10.0	51	47	87	64	92.2 (84-96)	50 (31-70)
>11.0	50	47	87	73	94.0 (86-98)	53.3 (34-71)
>13.0	49	46	85	73	93.9 (85-98)	50 (32-68)
>15.0	38	35	65	73	92.1 (81-97)	29.6 (20-41)

Discussion

With this study in T1DM children, we have shown that it is justified to increase the specificity and PPV by increasing the current TG2A cut-off value for performing diagnostic biopsies for CD. In order to have the highest NPV, we believe it is best raised to 11xULN and not to 10xULN even though the latter is commonplace for pediatricians vice versa not to perform duodenal biopsies in symptomatic children. Our data underline the fact that the choice of the cut-off of >3xULN for performing diagnostic biopsies in children with T1DM detected by screening, as part of the diagnostic algorithm for asymptomatic risk groups in the 2012 ESPGHAN guidelines for CD[10], was not based on evidence, but on

consensus aiming to avoid unnecessary biopsies. We decided not to exclude the symptomatic children, who were also found in our study population, since the TG2A screening was done during regular, planned visits rather than due to symptoms. In our cohort, 55% of the children with Marsh 0-1 histology were also "symptomatic", which may reflect the fact that the symptoms are non-specific for CD. Our results are in line with those of several recent studies in children with T1DM showing that TG2A levels varying from 5-8 to 10xULN were stronger predictive of villous atrophy than >3xULN[7,8,13,14]. While screening tests usually optimize sensitivity to find new patients[15], specificity is the parameter that needs optimization to avoid false-positive results. The latter is in our opinion crucial in T1DM children suspected of CD. First because endoscopy is an important burden for children who already have a chronic disease and omitting biopsies means avoiding the risks of anaesthesia in these children. Second, unnecessary biopsies should be prevented from a cost-effective point of view[15].

In our opinion, the decrease in sensitivity by increasing the CoN of TTGA to >11xULN is acceptable, since normalization of elevated TG2A can occur in up to a third of the asymptomatic T1DM patients on a gluten containing diet[16], allowing clinicians to wait and see first in these patients. In this recent retrospective study in Israel of newly diagnosed children with T1DM, high TG2A levels (>10x ULN) at diagnosis and three months thereafter, were predictive of CD[16]. Furthermore, if normalization does not occur and CD diagnosis is established with duodenal biopsies after all, this does not seem to have short term adverse effect either on diabetes regulation or on bone mineral density[17,18]. In our cohort, EMA did not contribute to the question whether or not to perform diagnostic biopsies. EMA negativity seemed to point to normal histology, but we were not able to draw a definite conclusion due to the small number of EMA negative patients, so future (prospective) studies are needed to sort this out.

Since children with T1DM with positive CD serology but normal duodenal histology can be considered potential CD patients, regular monitoring of CD serology is warranted. Recommendations on treatment and frequency of follow-up in potential CD are lacking, but since development of active CD is described in a third of the patients on a gluten containing diet[19], follow-up is important. In patients with T1DM and potential CD, young age and persistent positive TG2A over time seem to increase the risk of transfer into active CD[16]. In our cohort, we also found a tendency towards younger age in the group that was diagnosed with CD (p=0.06). Our result indicate that withholding biopsies is acceptable in children with T1DM and low TG2A titers if serology is being followed over time. Other studies, that show spontaneous normalisation[8,20] or fluctuation[13,17] of CD specific antibodies support our finding that low TG2A titers should be followed over time without performing immediate duodenal biopsies. Since children with T1DM need medical checks on a regular basis, assessment of TG2A every 6-12 months is in our opinion feasible. Performing CD

specific serology only in symptomatic children is not advised, since we have found an even higher prevalence of CD in asymptomatic patients when compared to symptomatic children, 88% and 74% respectively. Other studies report varying results. In one study, 71% of children with both T1DM and CD reported no gastrointestinal symptoms[21], while in another study, 76% of children with both conditions had at least 1 gastrointestinal symptom[22].

One of the strengths of our study is that this is the first to perform ROC-curve analysis to determine the TG2A cut-off value for performing biopsies in children with T1DM. Furthermore, biopsies were revised by a single pathologist specialized in Marsh typing. For this reason, histological examination was not affected by interobserver variability[23]. In addition, this is as far as we know, the first and largest study in type 1 diabetic children revising the performance of the revised ESPGHAN diagnostic criteria for CD[10].

The limitations of our study relate to its retrospective character and the sample size. Furthermore, we have compared TG2A measured with different types of assays. In the absence of an international standard, expressing the outcome in multiples of the ULN is currently the best option which has been used in several studies. Hopefully, in the near future an international standard will be introduced. It has been stated that comparison based on multiples of the ULN are valid[24], because studies have shown acceptable agreement between most second-generation kits[25,26]. Several other studies[26,27] have used multiples of ULN to compare data from different manufacturers. ROC-curve analysis in which only data from Phadia, Aesku and Euroimmun (chosen because they showed good agreement in xULN in Table A from the ESPGHAN guidelines[10]) were included, resulted in the same ROC-curve (data not shown).

In conclusion, we have shown that raising the current cut-off level of TG2A to 11xULN in children with T1DM to perform duodenal biopsies results in a 50% decrease of false positive and thus unnecessary biopsies. In diabetic children with TG2A levels lower than 11xULN, no diagnostic biopsies should be performed, but serological follow-up on a gluten containing diet should be done.

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