# Early diagnosis and management of celiac disease in childhood

Meijer, C.R.

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# Association in Clinical Practice between Gluten-Intake and Detection of Gluten Immunogenic Peptides in Celiac Children

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Caroline R. Meijer
Jaap Bakker
Anneloes Boers
Sophie Jansen
Zeliha Mengi
M. Luisa Mearin

## ABSTRACT

**BACKGROUND AND AIM:** The dietary compliance and its assessment in celiac disease (CD) patients on a strict gluten-free diet (GFD) remain a challenge. Two relatively new, validated methods have been proposed to detect occasional gluten ingestion: standardized dietary questionnaire and determination of urinary gluten immunogenic peptides (GIP). Our aim was to prospectively assess dietary compliance via these methods and compare their results with those of tissue-transglutaminase antibodies (TGA).

**METHODS:** Prospective single-centre. Consecutive CD-patients (1-18 years) on a GFD scheduled for regular consultation between March-August 2019 were invited. In addition to standard care, a completed dietary questionnaire and urine sample for GIP were collected. Pearson's chi square test, Fisher's exact test and Mann-Whitney U test were performed.

**RESULTS**: 110 of the 156 eligible children provided informed consent. Completed dietary questionnaire-, GIP- and TGA-results were available from 86 children (median age 12.8 years, median GFD-duration 30 months, 65% female). Adherence to the GFD evaluated by GIP, dietary questionnaire and anti-TGA was 94.2%, 75.6% and 94.2% respectively. No association was found between the TGA results and the detection of GIP as well as between the TGA results and the dietary questionnaires scores ($p = 0.5$ and 0.312 respectively). The participants perceived both the questionnaire and the measurement of GIP as reassuring with regards to correct implementation of the GFD.

**CONCLUSION:** All the three methods have limitations to monitor dietary compliance. The comparison of their performance shows that the best, single method is the use of the validated dietary questionnaire which should therefore be implemented in the regular care for children with CD. The most effective combination of dietary questionnaire and urinary GIP determination should be used in specific clinical situations.

## INTRODUCTION

The only effective treatment for celiac disease (CD) is a strict life-long gluten-free diet (GFD) which usually improves symptoms, restores small bowel histology and avoids long-term complications[1,2]. Nevertheless, dietary adherence is a challenge due to dietary restrictions, poor labelling regulations, sociocultural restrictions, decreased QoL and limited availability and high costs of gluten-free alternatives[3-5]. Dietary compliance in children with CD has been estimated as 25% - 50%[1,5,6]. The golden standard to assess mucosal healing (that is endoscopy and small bowel biopsies) is an invasive procedure that is not performed during regular check-ups. Usually, compliance with the GFD is evaluated by dietary interview with a dietician and/or by serum determination of IgA against tissue transglutaminase (TGA) [4,7]. However, both methods have limitations as TGA testing is insufficiently sensitive for detecting occasional dietary transgressions, and dietary evaluation by a trained dietician is time-consuming and not always available in clinical settings and dependent on patient-reporting [5,8,9].

Other methods to determine gluten ingestion by CD children are a standardized dietary questionnaire reflecting an regular interview by a specialized dietician and the measurement of gluten immunogenic peptides (GIP) in urine or stool [5,6,10,11]. GIP are small fragments of gluten resistant to gastrointestinal digestion causing the immunotoxic T-cells reaction in CD patients. A fraction of the GIP makes it into the circulation and is excreted in urine, being detectable after 4-6 hours and remain detectable for up to 24-36 hours[11]. The test is highly sensitive with a limit of detection of ≥ 50 mg of ingested gluten, taking into account that the maximal gluten ingestion during a strict GFD should not exceed 10-20 mg/day [12].

The aim of this study is to prospectively compare the performance of three methods to assess dietary compliance in clinical practice during the follow-up of CD children: via validated dietary questionnaire, GIP in urine and TGA determination in serum.

## METHODS

### Study population

For this prospective single-centre implementation study, consecutive patients with CD (1-18 years) attending the celiac out-patient clinic of the Leiden University Medical Centre (LUMC) for a regular follow-up visit, were recruited between March 2019 and August 2019. Inclusion criteria were: CD diagnosed according to the guidelines of the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN), following a GFD, parents/child having sufficient knowledge of the Dutch language and

written informed consent[13]. Consent was provided by parent/legal guardians and also by children ≥12years old. Patient characteristics were collected from the electronic medical record. All authors had access to the study data and reviewed and approved the final manuscript.

## Procedure

Invitation to participate in the study was sent by letter to (parents of) the children 2-3 weeks prior to their appointment at the out-patient clinic. It was explained that participation included providing a urine sample for the detection of GIP collected at the day of the consultation. In addition, (parents of) children were asked to complete the Dutch version of the validated dietary questionnaire on the compliance with the GFD [5; Annex 1]. The questionnaire addresses several domains, including compliance with and knowledge of the GFD and the attitude towards the diet. Each answer corresponds with a point score, which were not visible for the (parents of) the children, providing a score between 0-84 which corresponds to: 1. Strict GFD (0-2 points); 2. GFD with important errors (3-20 points); and 3. GFD not followed (21-84 points)[5]. Furthermore, the children received the standard care for CD, including TGA determination (Thermofisher, Germany; ImmunoCAP250; cut-off of normality 7 U/ml).

GIP in urine was determined at the clinical chemistry laboratory of the LUMC, blinded for clinical information and TGA results, using the iVYCHECK GIP Urine kit (Biomedal, Spain), following the manufacturer instructions. The results were expressed as ng GIP per 1 mL of urine, with the limit of detection being 2.2 ng GIP/mL (>50 mg of ingested gluten). If dietary adherence was considered as insufficient by raised tGA titers, positive GIP or dietary questionnaire, a referral to a dietician was offered.

## Statistical analysis

A Shapiro-Wilk-test was used to test for normality of the data. Where applicable, Pearson's chi-square test, Fisher's exact test or Mann-Whitney U test were used for evaluating baseline characteristics. Furthermore, these tests were used to estimate the strength of association between the outcomes reported in the dietary questionnaire, GIP and TGA levels. A two-tailed probability of $p < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS 25.0.

## Ethical considerations

The study was approved by the Medical Ethics Committee of Leiden-Den Haag-Delft (P18.241).

## RESULTS

The flowchart of the eligible children for the study is presented in figure 1. In total, 156 children were eligible for participation and (the parents of) 110 gave informed consent (70.5%): median age: 12.8 years, 71 females (64.5%). Characteristics of the children who gave informed consent and of the children who refused participation were similar, except for age above 13 years, since these children refused participation significantly more (p = 0.03, Table 1). In total 86 children had complete data (filled out dietary questionnaire and measured TGA and GIP) and were included in the analyses (Fig 1).

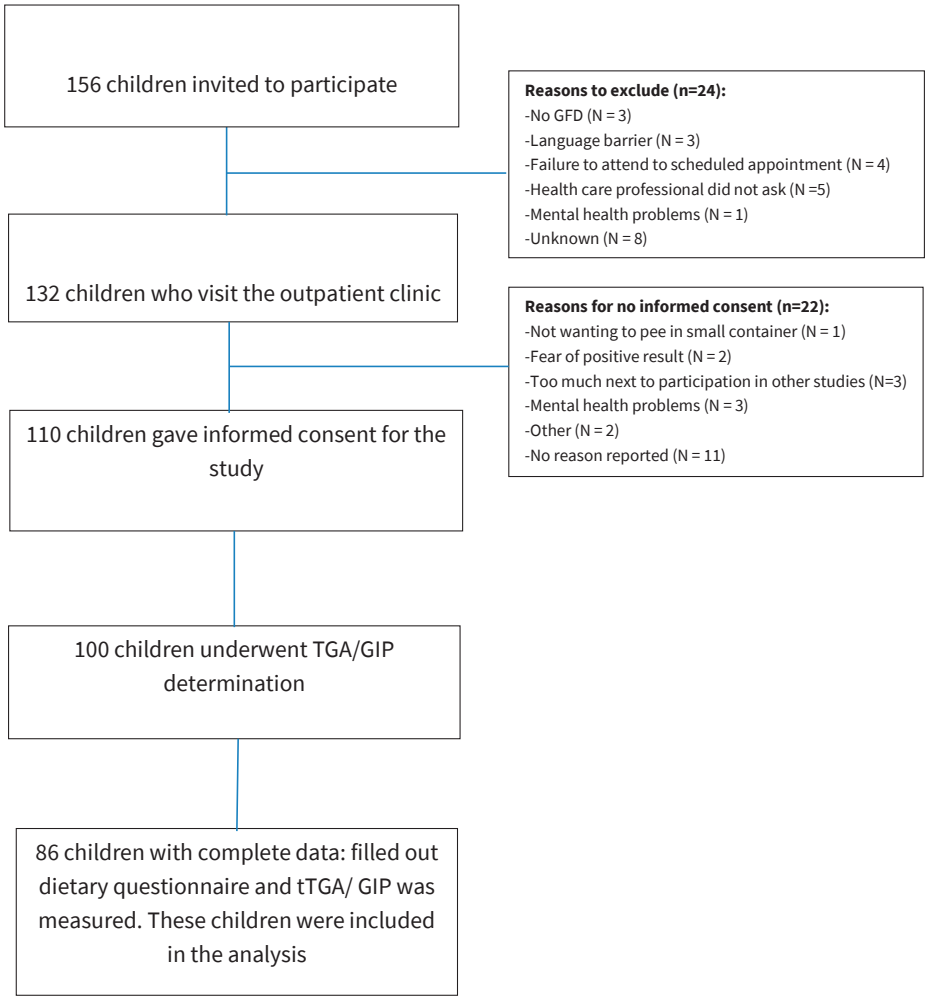


**Figure 1.** flowchart of the eligible children with celiac disease (CD), including the reasons for exclusion and no informed consent.

**Table 1.** Characteristics of the 110 children who gave informed consent for the study and the children who declined informed consent (n=22).

| | Participating CD children (N = 110) | Declined informed consent (N = 22) | P-value |
|---|---|---|---|
| Age (years), median | 12.8 | 11.1 | 0.128 |
| Age groups, no. (%) | | | |
| 0-4 years | 16 (14%) | 4 (18.2%) | 0.06 |
| 5-12 years | 61 (56%) | 6 (27.3%) | 0.13 |
| ≥ 13 years | 33 (30%) | 12 (54.5%) | **0.03** |
| Female, no. (%) | 71(64.5%) | 13 (59.1%) | 0.1 |
| Age at diagnosis of CD (years), median | 4 | 7.1 | 0.158 |
| Duration of GFD (months), median | 30 | 74 | 0.24 |
| 0 – 24 months, no. (%) | 35 (32%) | 6 (27.2%) | 0.31 |
| 25 – 48 months, no. (%) | 21 (19%) | 3 (13.6%) | 0.83 |
| ≥ 49 months, no. (%) | 54 (49%) | 13 (59.1%) | 0.68 |
| Positive family history of CD, no. (%) | 55 (50%)^ | 7 (31.8%) | 0.318 |
| Filled in dietary questionnaires, no. (%) | 95 (86.3%) | - | |
| Score dietary questionnaire, mean (range) | 1.77 (0 – 14) | - | |
| Anti-tTG measured, median (range) | 8.9 (0-104) | 7.7(0.1-44) | 0.655 |
| Elevated anti-tTG*, no. (% of measured anti-tTG) | 22 (20%) | 5 (22.7%) | 0.922 |
| GIP measured in urine, no. (%) | 100 (90.1%) | - | |
| GIP present, no. (% of measured GIP) | 5 (4.5%) | - | |
| GIP levels (ng/mL), median (range) ⍰ | 8.74 (7.88 – 14.27) | - | |

CD=celiac disease, GFD=gluten free diet; GIP= gluten Immunogenic Peptides; tTG=anti-tissue transglutaminase type 2 antibodies; ^ Family history was unknown in 4 children # Score ≥ 3 on dietary questionnaire * Cut-off of normality > 7 U/mL ° Concentrations of > 128 U/mL were considered as a concentration of 128 U/mL, some titers were diluted for standard CD care giving a value above 128. ⍰ Median concentration from positive tests only

Twenty-one children (24.4%) had elevated TGA levels (median age 11.0 years (range 2-15)), median duration GFD 22.9 months (range 3-135). Of them, 16 had a decreasing trend in antibody-titers from the start of their GFD, with 10 children following the diet for less than 1 year. Increased TGA results were observed in 5 children, 4 of which were older than 11 years of age, with a median duration of GFD of 35 months (range 23-138 ). The children with elevated TGA (n=21) followed a GFD significantly shorter than those with negative antibodies (median duration 22.9 vs. 69.3 months; p = 0.02), although median age of both groups was similar (p = 0.937).

Five children (5.8%; 4 females; median age 8 years (range: 4-16)) had detectable urinary GIP with a median level of 8.74 ng/mL (7.88 – 14.27). Their characteristics were similar in terms of age, median duration of the GFD, age at diagnosis and gender to the ones of the GIP negative children (p = 0.382, p = 0.293, p = 0.996 and p = 0.068, respectively; data not shown).

Only the parents of a 7-year-old girl with positive GIP and rising TGA, who followed the GFD for 36 months, agreed to have a consultation with the dietician. The parents of the other 4 children reported 'not seeing an added value in an appointment with a dietician' as they already knew what caused the positive GIP test, including "gluten contamination or a small mistake at a new school", "probable mistake in hand hygiene", "probable mistake at grandparents or school" and "occasional intentional gluten consumption".

On the dietary questionnaire, 21 children (24.4%) reported important errors (median age 11.3 years (range 2-17; median duration GFD 64.4 months (range 4-193). The most frequently errors were "consuming food with a label "may contain traces of gluten or wheat" (n=42), "consuming food with a label "prepared in an environment where gluten/wheat is processed" (n=29) and "consuming naturally gluten-free flour with no gluten-free label or logo" (n=16). The characteristics of the children who reported strictly complied with the GFD (n=65) were similar to those reporting dietary errors (n=21) (Table 2).

**Table 2.** Comparison of the patient characteristics following a strict gluten-free diet or reporting dietary errors according to the dietary questionnaires

| Score dietary questionnaire | Strict GFD* N = 65 (%) | GFD with important errors** N = 21 (%) | p-value |
|---|---|---|---|
| Median age, years | 9.23 | 11.3 | 0.974 |
| Age groups (years) | | | 0.097 |
| 0-4 | 9 (13.8) | 1 (4.7) | 0.08 |
| 5-12 | 43 (66.2) | 10 (47.6) | **0.01** |
| ≥ 13 | 13 (20.0) | 10 (47.6) | **0.01** |
| Median age at diagnosis (years) | 4.97 | 5.29 | 0.398 |
| Female | 43 (68.3) | 14 (60.9) | 0.932 |
| Sympt. after unintentional gluten intake | 51 (81.0) | 18 (78.2) | 0.313 |
| Positive family history for CD | 34 (54.0) | 9 (39.1) | 0.467 |
| Only one person following a GFD at home | 37 (58.7) | 8 (34.7) | 0.374 |
| Other dietary restrictions | 6 (9.5) | 1 (4.3) | 0.184 |
| Median duration of GFD in months (range) | 38.7 (2-184) | 64.4 (4-193) | 0.533 |

CD= celiac disease; DQ=dietary questionnaire; GFD=gluten free diet; *strict GFD is defined as score between 0-2 ** scores between 3-20 on dietary questionnaire on adherence to the gluten-free diet [5]

In total 80 (93.0%) children and/or their parents stated that they had enough knowledge of the GFD. The 6 children/parents reporting insufficient knowledge, followed the GFD significantly shorter than the others (14 months vs 59 months; p=0.008). Two of these children had a positive urinary GIP and scored important errors on the dietary questionnaire.

The absence of association between the TGA results and the detection of urinary GIP as well as between the TGA results and the ones from the dietary questionnaires is presented in Table 3 (p = 0.5 and 0.312 respectively). Likewise, no significant association was found between the scores of the dietary questionnaire and measurement of GIP in the urine (p = 0.08) (Table 4).

**Table 3.** Association between TGA and GIP results and between TGA and dietary questionnaire (DQ) scores in 86 children

| TGA n (%) | GIP n (%) | | p-value | Score DQ | | | p-value |
|---|---|---|---|---|---|---|---|
| | Positive N=5 | Negative N=81 | | Strict GFD N=65 | Errors GFD N=21 | Non adherence | |
| Elevated 21 (24) | 2 (9) | 19 (91) | 0.5 | 17 (81) | 4 (19) | 0 | 0.312 |
| Normal 65 (76) | 3 (5) | 62 (95) | | 48 (74) | 17(26) | 0 | |

**Table 4.** Association of GIP results and dietary questionnaire (DQ) scores in 86 children

| Score DQ / GIP | Strict GFD N=65 | Errors GFD N=21 | p-value |
|---|---|---|---|
| **Positive** (N=5) | 3 | 2 | 0.08 |
| **Negative** (N=81) | 62 | 19 | |

TGA= Anti-tissue transglutaminase antibodies GIP=gluten immunogenic peptides DQ=dietary questionnaire; GFD=gluten free diet; *strict GFD is defined as score between 0-2, ** important errors between 3-20 and *** not following the gluten-free diet on the dietary questionnaire[5]

## DISCUSSION

To the best of our knowledge, this is the first study comparing two relatively new methods to detect (un)intentional non-compliance with the GFD in children with CD, namely a validated dietary questionnaire reflecting a regular dietary interview as performed by an experienced dietician and the measurement of urinary GIP. Our results did not show an association between the results of TGA and the dietary questionnaire, TGA and GIP or the dietary questionnaire and urinary GIP.

By using the dietary questionnaire, we found that 24.4% of our population was not fully compliant to the GFD versus 5.8% as assessed by urinary GIP. Elevated but decreasing TGA was found in 16 out of 21 children (76%) who followed the GFD for a relatively short time of 22.9 months, which is in agreement with the time-frame in which normalisation of TGA usually occurs[7,14,15]. Also, five children showed an increase/stagnation in their TGA titers (5.8%), but only one of them had positive GIP. Our results therefore show that the dietary questionnaire is the best single method to detect occasional gluten intake. The discrepancy between the dietary compliance assessed by the questionnaire and the results of TGA in our study confirms the lack of sensitivity of CD serology to detect occa-

sional transgressions as shown in previous studies[6,10,16-19]. Nevertheless, three out of five children with detectable GIP in their urine did not report dietary transgressions in their questionnaire. This suggests that the combination of the dietary questionnaire and urinary GIP is the most effective method to detect occasional/inadvertent gluten intake. To calculate the diagnostic performance of the evaluated methods in terms of sensitivity, specificity, PPV and NPV is not possible by lacking a gold standard to assess dietary compliance.

The non-compliance with the GFD of almost 25% found in our study using the questionnaire agrees with the previously reported non-compliance of 25%- 50%[5], indicating that our population is representative for children with CD. However, the number of patients with detectable GIP in their urine in our study (5.8%) is surprisingly low compared to other studies performed in CD children in other countries. A systematic review of the literature reported fecal GIP detection in 25% of the children[1]. Four prospective studies among children (two combined with adults) assessing diet adherence by fecal/urinary GIP showed non-compliance in 45%, 29.8%, 16% and 14.5% of patients, respectively[8,16,20,21]. This discrepancy may be explained by the difference in methodology. Our population received information two weeks prior to the consultation describing the aim of the study and the purpose of the use of the urine for detection of excreted gluten peptides. This may have established a time-frame in which the children could re-evaluate and improve their compliance with the diet, Also, urinary GIP as assessed in this study is only detectable for 36 hours after gluten ingestion, in comparison to 4-7 days in the faeces as used in other studies[8,20,21], and we may have missed dietary transgressions made before this time-frame. Another possible limitation of our study is the relatively small number of participating children, although it is comparable to (or even higher) than sample sizes from previous studies performed in children[8,11,16,21].

Most of the previous studies on GIP in faeces and/or urine did not describe the manner in which the included patients were informed[11,16,22]. It is possible that recruitment of patients on short notice could have led to a higher number of positive GIP in those studies, as the 16% reported by one study in which the participants were not specifically aware of the GIP-measurement [8]. Nevertheless, if urinary GIP determination are implemented in the regular consultations on the long-term the CD patients would become aware of it. As such, sending study-information prior to the consultation in our study is comparable with the possible implementation of GIP in the standard of care for CD children.

Another possible explanation of the low frequency of positive GIP in our study may be the high number of children older than 13 years who refused participation in the

age-category with the highest percentage of non-compliance to the GFD [16]. A possible reason for declined consent may have been the fear of the exposure of potential non-compliance with the diet through a positive GIP result.

In addition to detect errors in the GFD, urinary GIP determinations may also be used to guarantee or reassure (parents of) patients that the GFD is correctly adhered to. This was also reported in the interviews which were taken by a randomly selected number of children and/or parents after terminating the study. The majority of (parents of) the patients believed that the test had an added value, especially in children who remained symptomatic or who were still familiarizing themselves with the diet (results not shown).

Our results show that from the three evaluated methods, the dietary questionnaire is the best single one to detect non-compliance with the GFD compared to the other methods and we, therefore, propose it for assessing diet adherence during the regular follow-up of CD children. In addition, the validated dietary questionnaire also identifies sources of non-compliance, facilitating self-correction by the patient. With the increasing use of E-health, partly due to the COVID pandemic, completing and processing the questionnaire will become easier and the implementation in standard healthcare more accessible and less time-consuming.

The combination of the dietary questionnaire and urinary GIP test is the most effective method in detecting (un)intentional gluten transgressions. This combination may be implemented in specific clinical settings to rule out (un)intentional gluten consumption or gluten cross-contamination, namely in children (1) with recently diagnosed with CD as they familiarize themselves with the GFD, (2) reporting symptoms with normal TGA and no errors in the dietary questionary, (3) with (persistent) elevated or very slow normalisation of TGA-levels despite no errors in the dietary questionnaire and, (4) with suspected intentional gluten intake.

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