COELIAC DISEASE

PREVENTION AND IMPROVEMENT OF CARE

Sabine Lisa Vriezinga

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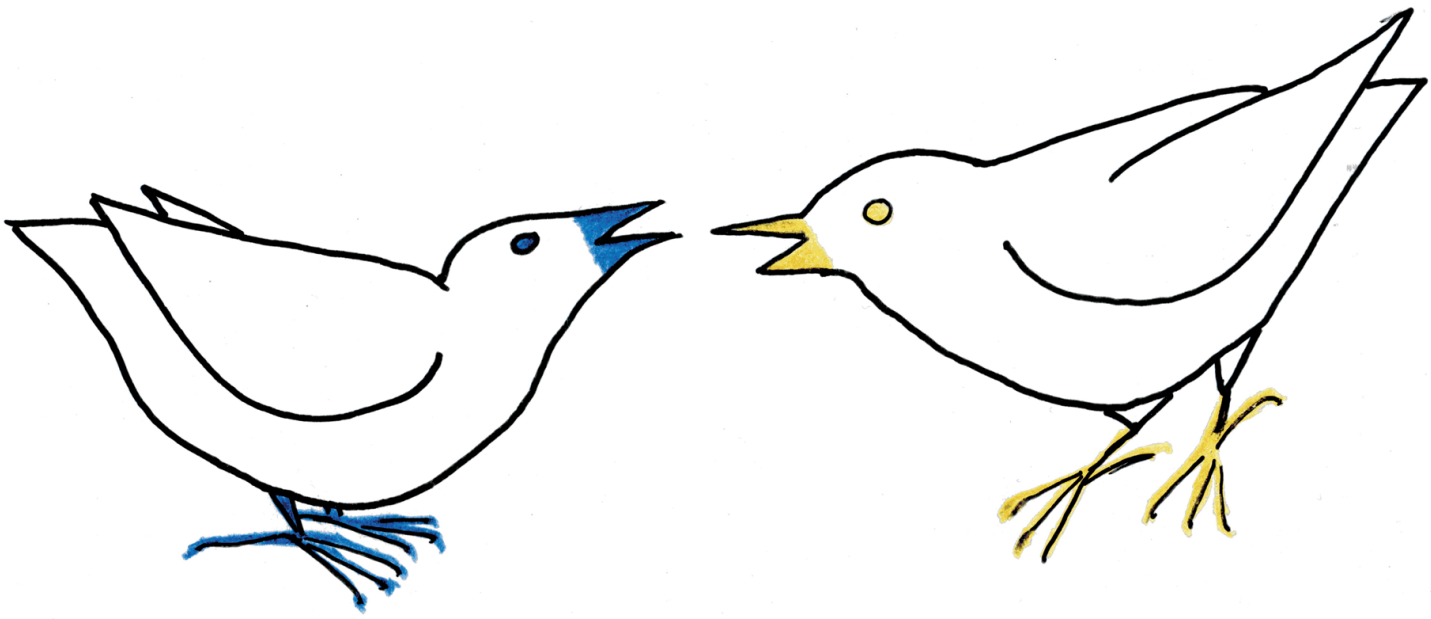

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GENERAL INTRODUCTION AND OUTLINE

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GENERAL INTRODUCTION

Coeliac disease is an immune-mediated systemic disorder elicited by gluten in genetically susceptible individuals, characterized by the presence of a variable combination of gluten-dependent clinical manifestations, coeliac-disease-specific antibodies, HLA-DQ2 or HLA-DQ8 haplotypes and enteropathy.[1] In coeliac disease, gluten peptides activate T cells that mediate a self-perpetuating inflammatory process. This process leads to mucosal damage of the small bowel and other organs, producing symptoms ranging from malabsorption with diarrhoea, abdominal distension and weight loss, to nonspecific signs and symptoms such as fatigue, osteoporosis or iron deficiency anaemia (Box 1).[1]

Childhood coeliac disease is a common disorder, with a 1–3% prevalence in the general Western population that includes the USA, corresponding to about 5 million people in the European community, the highest frequency of which resides in Sweden.[2] Therefore, coeliac disease might be considered a public health problem in both Europe and the USA.[2, 3] Coeliac disease is also frequent in South America,[4, 5] the Middle East, North Africa and India, where wheat has been the major staple food for centuries, but rare among native Africans, Japanese and Chinese people.[6-8] A high index of suspicion for coeliac disease should be maintained in all developing countries in children who present with chronic

Box 1. Symptoms of childhood coeliac disease.

Gastrointestinal	**Extraintestinal**
Diarrhoea	Chronic fatigue
Anorexia	Iron deficiency anaemia
Vomiting	Macrocytic anaema (folic acid and/or vitamin B_{12} deficiency)
Growth retardation, weight loss	Dermatitis herpetiformis
Chronic abdominal pain	Dental enamel hypoplasia
Chronic constipation	Recurrent aphthous mouth ulceration
Distended abdomen	Arthritis
	Arthralgia
	Osteopenia or osteoporosis
	Bone fractures
	Mildly elevated levels of AST and ALT
	Short stature
	Late puberty
	Cerebellar ataxia
	Recurring headaches
	Peripheral neuropathy
	Seizures
	Anxiety
	Depression

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

diarrhoea and malnutrition.[9] Despite the increasing numbers of positive diagnoses for coeliac disease, the condition is frequently unrecognized, possibly due to its variable clinical presentation and symptoms,[10, 11] such that for every one child diagnosed with coeliac disease, there are seven who remain undiagnosed.[12-14] Coeliac disease can also affect extraintestinal organs. In fact, nongastrointestinal manifestations are now more common in children than before, possibly because of a greater awareness of symptom diversity.[1, 15] Coeliac disease can occur at any age. Patients with other autoimmune diseases, including type 1 diabetes mellitus, autoimmune thyroid disease, or patients with selective IgA deficiency, as well as those with Down syndrome, Turner syndrome and Williams syndrome, have an increased risk of developing coeliac disease (Box 2).[1]

Box 2. Conditions associated with childhood coeliac disease.

Type I diabetes mellitus: 3-12%
Selective IgA deficiency: 2-8%
Autoimmune thyroiditis: ≤7%
Down, Turner, Williams syndrome: 2-12%
First-degree relative with coeliac disease: 2-20%

% prevalence listed for each condition.[1]

PATHOGENESIS

Virtually all patients with coeliac disease express the HLA-class II molecules HLA-DQ2 and/or HLA-DQ8, and gluten-specific HLA-DQ2/8-restricted CD4+ T cells can be isolated from their small bowel mucosa.[16] Wheat gluten is composed of different gliadins and glutenins; immunogenic epitopes have been identified in all these proteins.[17-25] Some of these epitopes found in the α-gliadins and ω-gliadins, barley hordeins and rye secalins, are more immunodominant as they trigger T cell responses in almost all patients.[17-19, 21, 22] Typically, these epitopes are proline-rich, which render them resistant to enzymatic degradation.[19] Moreover, they contain an amino acid sequence wherein the glutamine (Q) can be modified into glutamic acid (E) by the enzyme transglutaminase type 2 (TG2), thereby introducing a negative charge required for high-affinity binding to HLA-DQ2 and recognition by CD4+ T cells (Figure 1).[26-28]

In coeliac disease, there is a strong HLA-DQ gene-dose effect: HLA-DQ2 homozygous individuals have a much higher risk of developing coeliac disease than those who are heterozygous.[29] This effect correlates with stronger T cell responses to gluten peptides when presented by HLA-DQ2 homozygous cells, indicating that the level of gluten presentation influences the risk of disease development.[30] Interestingly, there are no indications for an HLA-DQ2 gene-dose effect once the disease has developed because the symptoms

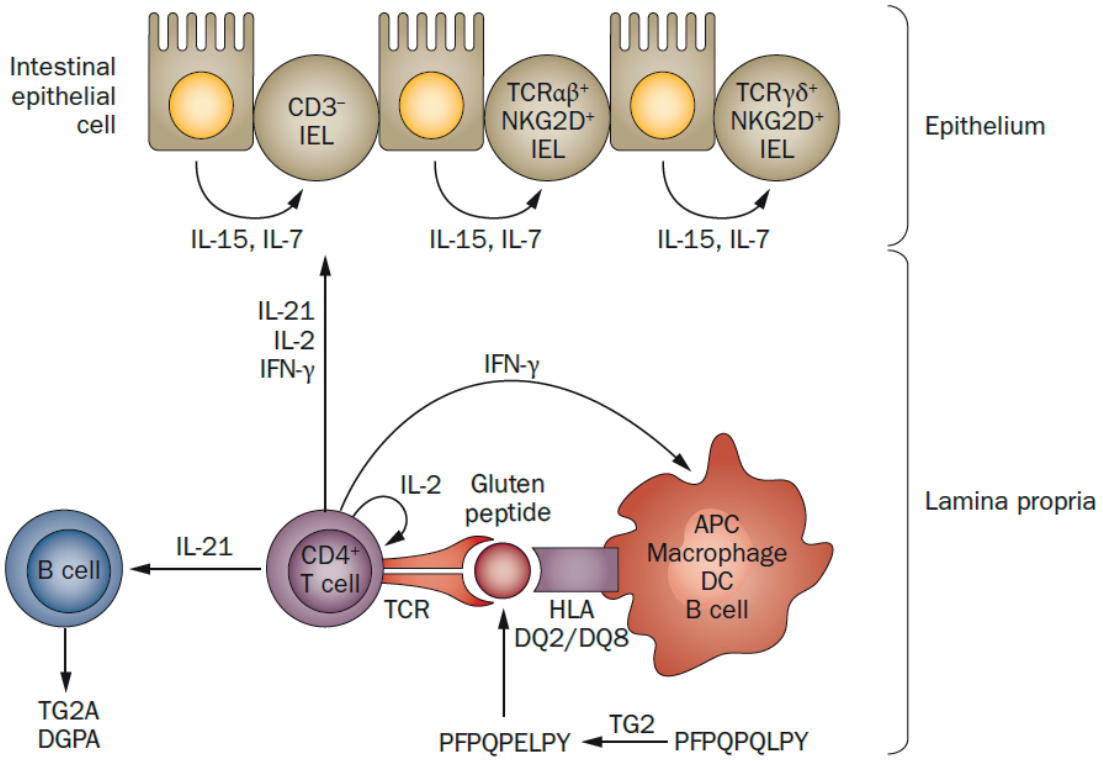

Figure 1 Schematic representation of the immune response to gluten peptides in the small bowel mucosa of patients with coeliac disease. Abbreviations: APC, antigen-presenting cell; DC, dendritic cell; DGPA, anti-deamidated gliadin peptide antibody; IEL, intraepithelial lymphocyte; NKG2D, NKG2-D type II integral membrane protein; TCR, T-cell receptor; TG2, transglutaminase 2; TG2A, anti-transglutaminase type 2 antibody.

and severity of intestinal lesions in childhood coeliac disease are similar in HLA-DQ2 homozygous and heterozygous individuals.[31] Apparently, once tolerance is lost, the level of antigen presentation in the intestine is sufficient to sustain the inflammatory gluten-specific $CD4^+$ T cell response.[30, 32] This process might relate to the local production of IFN-γ by these $CD4^+$ T cells, widely known to enhance HLA expression on antigen-presenting cells.[30] After the disease-causing gluten-specific T cell response in the lamina propria, major changes occur in the composition, size and activation state of the intraepithelial lymphocyte (IEL) compartment in patients with coeliac disease.[33] Normally, IELs are found scattered throughout the intestinal epithelium and are located at the basolateral side of the epithelial cell layer. Although the majority of IELs are $CD8^+$αβ T cell receptor $(TCR)^+$ T cells, higher numbers of both $CD8^+$αβTCR^+ and TCRγδ$^+$ T cells are found in patients with coeliac disease than in healthy individuals (Figure 1).[32, 34] Moreover, IELs are found at the tip of the villi in coeliac disease, indicating a redistribution of the IELs in the epithelium, not observed in healthy individuals.[35] Although the importance of the increased number of TCRγδ$^+$ T cells in coeliac disease remains unclear, $CD8^+$αβTCR^+ T cells gain a natural-killer-like phenotype, suggesting that they might be involved in the epithelial cell killing and remodelling observed in active coeliac disease.[36] IL-15 has a key role in coeliac disease as it is overexpressed by the epithelial cells and can directly activate adjacent IELs.[36, 37] In addition, it is feasible

that cytokines released by adaptive T cells in the lamina propria, such as IL-2 and IL-21, can reach the epithelial compartment and contribute to the activation of IELs. Thus, the changes in the epithelial compartment could be secondary to the activation of $CD4^+$ gluten-reactive T cells in the lamina propria. Alternatively, it is possible that intrinsic aberrations in the epithelial layer cause the observed characteristic changes. Strikingly, the number of $CD8^+\alpha\alpha TCR^+$ T cells normalizes but the numbers of $TCR\alpha\alpha^+$ T cells remain elevated. The $TCR\alpha\alpha^+$ T cells do not seem to have a pathogenic role upon initiation of a gluten-free diet (GFD) but, rather, might be required to maintain epithelial homeostasis.[38]

Next to adaptive IELs, the epithelium also has at least four subsets of innate lymphocytes.[38] Little is known about the function of these innate lymphoid subsets that are present in high numbers in children, especially in young children, but far less so in healthy adults and in adults with coeliac disease. One of these subsets bears a resemblance to the IFN-γ-secreting type 1 innate lymphoid cell (ILC1), the innate homologue of $CD4^+$ T_H1 helper cells whereas another, the lineage-negative IEL, has a distinct phenotype responsive to IL-15.[38] The latter is the likely precursor to the aberrant monoclonally expanded cells in patients with refractory coeliac disease type 2: a premalignant condition unresponsive to a GFD that is very rare in children.[38] In addition to these genetic and immunological factors, environmental factors including elective Caesarean section, perinatal and childhood infections, the use of antibiotics and PPIs, and changes in the microbiota might have a role in the pathogenesis of coeliac disease.[39, 40]

DIAGNOSIS

The key to the diagnosis of coeliac disease in children is a high degree of awareness of its wide spectrum of symptoms (Box 1). Coeliac disease is thereby diagnosed through a combination of techniques: detection of coeliac-disease-specific autoantibodies, HLA-DQ typing and small bowel biopsies that are performed while the patient is on a gluten-containing diet.[1]

Clinical presentation

The clinical presentation of childhood coeliac disease is partially age-dependent. Very young children (<3 years) present more commonly with chronic diarrhoea, abdominal distension and growth retardation whereas older children and adolescents (≤18 years) present with milder gastrointestinal symptoms such as recurrent abdominal pain, vomiting or constipation. Extraintestinal symptoms such as arthritis, neurological symptoms and anaemia are also frequent.[1, 41] In addition, coeliac disease can be asymptomatic.[1]

Autoantibodies

In the serum, specific coeliac disease autoantibodies are detected against TG2 (TG2A), endomysium (EMA), and deamidated gliadin peptides (DGPA).[1] In the case of severe histological small bowel alterations, IgA TG2A and EMA have high sensitivities (98% and 90%, respectively) and specificities (97% and 98%, respectively).[42] In those with less severe intestinal damage, these specificity and sensitivity values are lower.[42] Total IgA measurement is also important because coeliac disease is associated with selective IgA deficiency.[43] In IgA deficiency, IgG coeliac disease antibodies, among which IgG DGPA is most suitable, should be determined. IgG DGPA has diagnostic values comparable to those of IgA TG2A.[42]

HLA-typing

HLA-typing is not advised in the routine diagnosis of coeliac disease because 40% of the general European and American population carry either one or both of these genes.[44] However, HLA-typing is useful to exclude coeliac disease because of its very high negative predictive value, for example in children who have already started a GFD without prior diagnostic tests. HLA-typing is also useful in selecting individuals at risk of coeliac disease that need to undergo serological coeliac disease screening. Parents of affected children support HLA-typing of their other children to assess the risk of the disease.[45]

Histology

The characteristic histological alterations of the small bowel mucosa in coeliac disease are partial to total villous atrophy with crypt hyperplasia and IEL infiltration.[46, 47] These alterations are rated according to the Marsh–Oberhuber classification depending on the severity of the lesion: ranging from type 0 (normal) to 4, wherein type 4 describes hypoplastic lesions.[46, 47] When interpreting the histological alterations one should take the patient's serology, HLA-typing, and clinical manifestations into account. A Marsh–Oberhuber classification type 3 (a, b or c), or type 2 if accompanied by specific coeliac disease antibodies, support the diagnosis of coeliac disease. The severity of the clinical symptoms does not correlate with the severity of the histological alterations. Patients with Marsh–Oberhuber type 3c can be asymptomatic.[3, 13, 48] Up until the past few years, the histological examination of small bowel biopsies was the gold standard for the diagnosis of coeliac disease. However, in 2012, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) introduced an exception for a specific group of children (Figure 2).[1]

Small bowel biopsies can be omitted in children with clear gastrointestinal symptoms, high titres of TG2A (>10 upper limit of normal), positive EMA and HLA-DQ2 and/or HLA-DQ8. In all other cases, small bowel biopsies are still mandatory for diagnosis. The results of the ongoing prospective study ProCeDe investigating the performance of the ESPGHAN

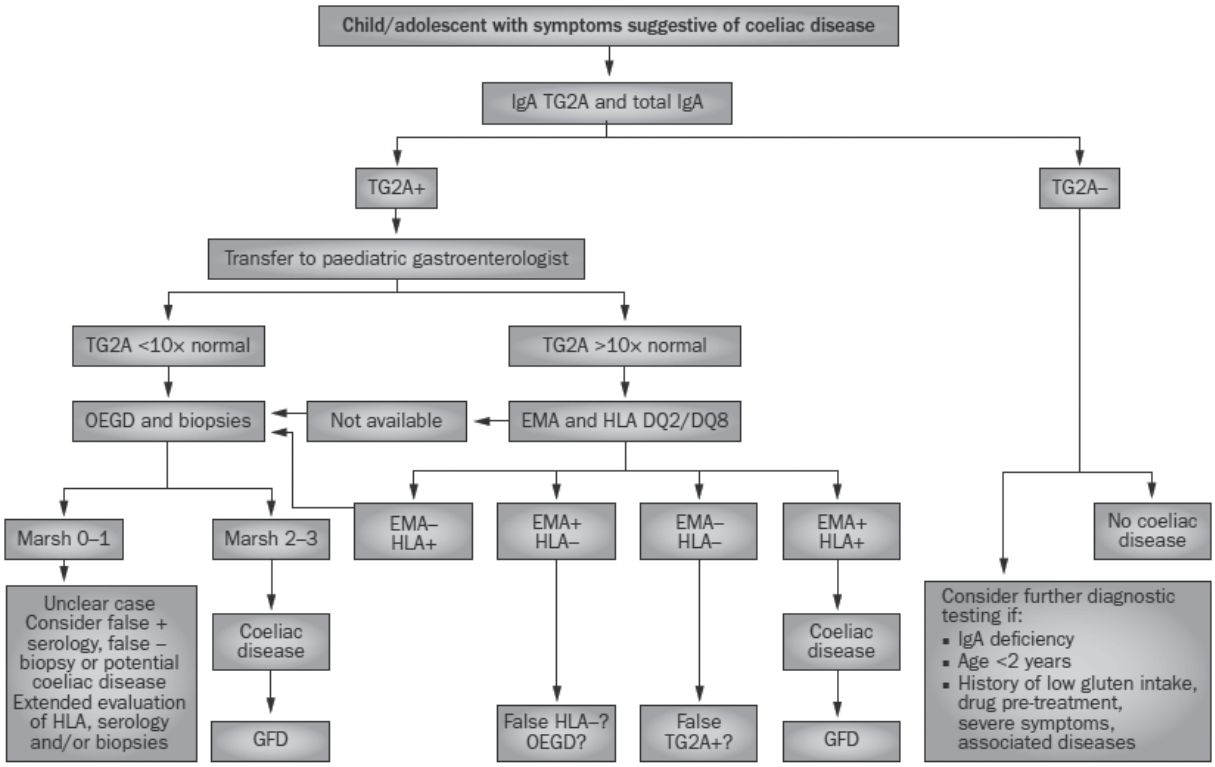

Figure 2 ESPGHAN algorithm for the diagnosis of coeliac disease in children and adolescents with symptoms. Abbreviations: +, positive; –, negative; EMA; anti-endomysium antibody; ESPGHAN, European Society of Paediatric Gastroenterology, Hepatology and Nutrition; GFD, gluten-free diet; OEGD, oesophagogastroduodenoscopy; TG2A, anti-transglutaminase type 2 antibody.

guidelines will be important to further define situations in which coeliac disease might be diagnosed without biopsies.[49]

MANAGEMENT

Coeliac disease can be successfully treated with a GFD, which restores small bowel histology and improves clinical complaints in the majority of patients.[43] Adhering to a GFD might seem simple, but the abundance of gluten-containing food in the Western diet can be challenging, and treatment can considerably affect the child's quality of life.[50, 51] Once diagnosis is confirmed, the child should be referred to a paediatric dietician for in-depth information about the necessary dietary treatment. The GFD can have negative nutritional consequences. For instance, it has been reported that Italian adolescents with coeliac disease consumed an unbalanced diet rich in fat and protein, poor in carbohydrate and deficient in calcium, iron and fibre as a result of a GFD.[52] Gluten-containing cereals such as wheat, barley and rye are important sources of dietary iron, fibre, calcium, folate and vitamin B12, and treatment with a GFD can lead to micronutrient deficiencies.[53, 54] Gluten-free buckwheat or quinoa

are naturally rich in group B vitamins,[55] but commercially available gluten-free products frequently do not contain the same amount of micronutrients as the often enriched wheat flour products that they aim to replace.[56] Non contaminated oats are generally well tolerated by the majority of children with coeliac disease. However, a randomized double-blind study published in 2014 showed that oats prevent normalization of the intestinal mucosa immune status in a substantial fraction of paediatric patients with coeliac disease.[57]

The usual care for children with coeliac disease consists of hospital visits to monitor the patient's response to the diet. Subsequent follow-up is dedicated to assess the child's dietary adherence, well-being and adequacy of growth. Determination of coeliac-disease-specific antibodies in the serum should be done periodically to monitor regression and remission; their levels usually returning to normal within 9–12 months after dietary intervention.[58] Testing for anaemia, iron status and calcium, folic acid, vitamins D and B12 levels at diagnosis and at the follow-up visits of patients undergoing treatment is common practice. However, evidence is weak for the efficacy and adequacy of this practice as there is limited information on the incidence of nutritional deficiencies in patients with treated coeliac disease. The evidence-based British and Dutch guidelines recommend annual visits whereas other evidence-based guidelines, such as the ones from the NIH, ESPGHAN, and the North American Society for Paediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) do not provide guidance on the matter.[1, 59-62]

NOVEL THERAPIES

Knowledge of the molecular mechanisms underlying coeliac disease offers opportunities to develop alternative treatments to the GFD.[63] The use of enzymes as oral supplements to enhance gluten degradation has been extensively studied and could help reduce gluten exposure.[64, 65] Alternatively, the generation of blockers to prevent gluten peptide binding to HLA-DQ2 has been explored.[66, 67] Similarly, blockade of TG2 would prevent gluten modification and the development of a full-blown T cell response to gluten.[68] In addition, gluten peptide vaccination to re-introduce gluten tolerance has been proposed,[69] whereas other studies aim to improve barrier function in the small intestine to prevent the entry of gluten peptides into the lamina propria.[70] So far, none of these approaches has proven capable of replacing the GFD.

PREVENTION

Previous retrospective studies suggested a 'window of opportunity' for primary prevention by introducing gluten between 4–6 months of age.[71, 72] Based on the results of these studies, ESPGHAN recommended that gluten should not be introduced before 17 weeks and not later than 26 weeks of age, preferably concurrent with the period of breastfeeding.[73, 74] However, at time of giving this recommendation, prospective studies and randomized controlled trials investigating this 'window of opportunity' were lacking. Most studies were retrospective, associated with parental recall bias, and none included quantities of gluten administered or randomization.[72, 74-77] At time of initiating this thesis, the true influence of early feeding on the development of coeliac disease was controversial.

IMPROVEMENT OF CARE

Traditional medical care for coeliac patients consists of regular physician visits to evaluate patient's health, weight, height (in children), GFD adherence and coeliac-specific serum antibodies.[62, 78] Although important, these measures can be time-consuming. Moreover, many patients with coeliac disease do not visit their physician for regular follow-up.[79] The limited time allotted for outpatient follow-up also typically restricts comprehensive assessment of a patient's health-related quality of life and dietary adherence. Previous studies in adults with other chronic diseases suggest that e-health can encourage patients to improve health care participation and the decision-making process.[80] At time of initiating this thesis, studies investigating e-health for follow-up of coeliac disease were lacking.

OUTLINE

In **chapter 2**, the results of the European multi-centre randomized controlled trial 'PreventCD' are presented. PreventCD studied the influence of infant feeding on the development of childhood coeliac disease and explored the possibility of inducing tolerance to gluten.

In the following three chapters, new strategies for the improvement of care for children and young adults with treated coeliac disease are presented. **Chapter 3** studies whether patients' and doctors' reports on coeliac disease-specific health-related quality of life agree. **Chapter 4** features the results of the CoelKids study, a multi-centre randomized controlled trial evaluating a self-management e-health system for coeliac children and young adults. **Chapter 5** evaluates the performance of three different commercially available point-of-

care tests for anti-tissuetransglutaminase in children with treated coeliac disease and compares the results against those of serum anti-tissuetransglutaminase measured with conventional ELISA.

In **chapter 6**, the major findings of this thesis are discussed in the light of the current literature and suggestions for future policy and research are made. The English and Dutch summaries are presented in **chapter 7**.

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I

PREVENTION

2

RANDOMIZED FEEDING INTERVENTION IN INFANTS AT HIGH RISK FOR COELIAC DISEASE

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

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ABSTRACT

Background

A window of opportunity has been suggested for reducing the risk of coeliac disease by introducing gluten to infants at 4 to 6 months of age.

Methods

We performed a multicenter, randomized, double-blind, placebo-controlled dietary intervention study involving 944 children who were positive for HLA-DQ2 or HLA-DQ8 and had at least one first-degree relative with coeliac disease. From 16 to 24 weeks of age, 475 participants received 100 mg of immunologically active gluten daily, and 469 received placebo. Anti–transglutaminase type 2 and antigliadin antibodies were periodically measured. The primary outcome was the frequency of biopsy-confirmed coeliac disease at 3 years of age.

Results

Coeliac disease was confirmed by means of biopsies in 77 children. To avoid underestimation of the frequency of coeliac disease, 3 additional children who received a diagnosis of coeliac disease according to the 2012 European Society for Pediatric Gastroenterology, Hepatology, and Nutrition diagnostic criteria (without having undergone biopsies) were included in the analyses (80 children; median age, 2.8 years; 59% were girls). The cumulative incidence of coeliac disease among patients 3 years of age was 5.2% (95% confidence interval [CI], 3.6 to 6.8), with similar rates in the gluten group and the placebo group (5.9% [95% CI, 3.7 to 8.1] and 4.5% [95% CI, 2.5 to 6.5], respectively; hazard ratio in the gluten group, 1.23; 95% CI, 0.79 to 1.91). Rates of elevated levels of anti–transglutaminase type 2 and antigliadin antibodies were also similar in the two study groups (7.0% [95% CI, 4.7 to 9.4] in the gluten group and 5.7% [95% CI, 3.5 to 7.9] in the placebo group; hazard ratio, 1.14; 95% CI, 0.76 to 1.73). Breast-feeding, regardless of whether it was exclusive or whether it was ongoing during gluten introduction, did not significantly influence the development of coeliac disease or the effect of the intervention.

Conclusions

As compared with placebo, the introduction of small quantities of gluten at 16 to 24 weeks of age did not reduce the risk of coeliac disease by 3 years of age in this group of high-risk children. (Funded by the European Commission and others; PreventCD Current Controlled Trials number, ISRCTN74582487)

INTRODUCTION

Coeliac disease (CD), an immune-mediated systemic disorder elicited by gluten in genetically susceptible persons, is characterized by anti-transglutaminase type 2 antibodies (TG2A) and enteropathy.[1] The prevalence of CD is 1-3% in the general population and approximately 10% among first-degree family members of patients with CD.[2-10] CD is treated with a gluten-free diet. More than 95% of patients have the HLA-DQ2 heterodimer, either in the *cis* or *trans* configuration. Most of the remaining patients have the HLA-DQ8 heterodimer or half of the DQ2 heterodimer (DQB1*02).[1, 8, 11-14] However, more than 25% of the general population carries these haplotypes,[8, 13] indicating that additional factors are involved in disease development. CD increases overall mortality risk,[15] reduces quality of life,[16] and has extensive negative economic consequences.[17, 18] The health and quality of life of patients improves with a gluten-free diet, but primary prevention would be more beneficial.[19, 20] Results from observational studies indicate that the development of oral tolerance for gluten is initiated early in life, and that the mode of introducing gluten to infants may influence the risk of CD in predisposed persons.[21-25] The results of these studies suggest that there is a "window of opportunity" at 4 to 6 months of age, when the first exposure to gluten should occur in order to decrease the risk of CD.[24, 25] The results of studies evaluating breastfeeding and the risk for CD are inconclusive, since most of these studies were retrospective and associated with parental recall bias, and none included randomization or specified the quantities of gluten consumed.[23-27] At present, the true influence of early feeding on the development of CD remains controversial.

To investigate the possible primary prevention of CD, the European multicenter project "Prevent Coeliac Disease" (PreventCD; www.preventcd.com) was initiated.[19] It was hypothesized that the frequency of CD at 3 years of age could be reduced by exposing genetically predisposed infants to small quantities of gluten at 16 to 24 weeks of age, preferably while they were still being breastfed.

METHODS

Study design and participants

We performed a prospective, randomized, double-blind, placebo-controlled, dietary-intervention study. The first child was included on May 26, 2007, and the follow-up for this analysis closed on September 10, 2013, when the youngest study participant turned 3 years of age; the oldest participants were up to 6 years of age.

Infants 0 to 3 months of age were recruited consecutively through CD organizations from Croatia, Germany, Hungary, Israel, Italy, the Netherlands, Poland, and Spain. Infants were required to have the HLA-DQ2, HLA-DQ8, or HLA-DQB1*02 heterodimer (centrally typed) and to have at least one first-degree family member with CD confirmed by means of small-bowel biopsies. We excluded premature infants and those with trisomy 21 and Turner's syndrome (online supplementary appendix, available at nejm.org).

Intervention

We randomly assigned participants to receive either 200 mg of vital wheat gluten mixed with 1.8 g lactose (equivalent to 100 mg of immunologically active gluten), or to placebo (2 g lactose), given daily for 8 weeks starting at 16 weeks of age (online supplementary appendix). Previous assessment of the vital wheat gluten by means of ELISA and Western blot analysis had shown the presence of gluten proteins typically found in wheat gluten. Randomization, stratified by participating country, was performed with the use of variable block sizes ranging from 4 to 8 and with SPSS software (version 18.0). The investigators and the parents of the participants were unaware of the intervention assignments. Adherence to the study assignment was assessed by means of frequent interviews with the parents (online table S1). Participants were considered to have adhered to the intervention assignment if at least 75% of the material was ingested and no additional gluten was consumed. After the intervention, parents were advised to introduce gluten gradually, using regular products and standardized recommendations (online supplementary appendix).

Outcomes

The primary outcome was the frequency of CD at 3 years of age. The diagnosis of CD was based on the histologic findings of small-bowel biopsies, according to the 1990 criteria of the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN).[28] Secondary end points were the occurrence of symptoms and the immune response to gluten as indicated by elevated serum antibodies associated with CD (anti-gliadin antibodies and TG2A) (online supplementary appendix).

Follow-up and assessment of CD

We periodically monitored health status, anthropometric variables, and feeding habits (i.e. breastfeeding and formula feeding), and we quantified gluten consumption[29] using standardized questionnaires (online table S1). Measurement of serum antigliadin and TG2A were performed centrally at least seven times during the first 3 years of age and then annually thereafter. The parents of children with elevated CD-associated antibodies or with symptoms suggesting CD were offered small-bowel biopsies to confirm the diagnosis in their child (online supplementary appendix). The biopsy specimens were histologically assessed at the study sites and were also reviewed by an author who is a pathologist.[30] The

age of the patient at which the diagnostic biopsies were performed was considered to be the age at the diagnosis of CD.

Study oversight

The study was approved by the medical ethics committee at each participating center and complied with the Good Clinical Practices regulations (online supplementary appendix). The authors vouch for the veracity and completeness of the data and analyses reported and for the adherence of the study to the protocol, available at nejm.org.

From 2007 to 2011, the study did not have commercial support. After 2011, Thermo Fisher Scientific performed antibody assessments without charge, and together with Eurospital and Fria Bröd, Thermo Fisher Scientific partly funded the project progress meetings. The funding organizations had no role in the conception, design, or conduct of the study, in the analysis or interpretation of the data, or in the writing of the manuscript of the decision to submit it for publication.

Statistical analysis

To detect a 50% reduction in the development of CD in the gluten group at 3 years of age (5%, versus 10% with placebo) with a two-sided significance level of 5% and with 80% power, we calculated that 474 children would be required in each group.[19]

All the data were entered into a Web-based data management application with the use of a central structured-query-language server database (NEN 7510 certified). A statistical analysis plan was published online before the randomization codes were opened (http://preventcd.com/images/stories/Publications/PreventCD_SAP_1_0.pdf, online supplementary appendix). For estimating the cumulative incidence of CD, Kaplan-Meier curves were calculated, with time defined as the patient's age at diagnosis of CD or at the last assessment or withdrawal from the study (when data were censored). For comparison, a log-rank test (two-sided) was used, stratified according to participating country. The hazard ratio for CD in the gluten group, as compared with the placebo group (with 95% confidence intervals), is provided, on the basis of a Cox proportional-hazards regression analysis. The primary analysis was performed according to the intention-to-treat principle. Differences in cumulative incidence of CD were assessed according to the baseline variables by means of Cox proportional-hazards regression (multivariate) analysis and according to the duration of breast-feeding, daily gluten intake, and occurrence of infection by means of a landmark analysis (online supplementary appendix). Different intervention effects were assessed in subgroups by including an interaction term between intervention and subgroup in the Cox proportional-hazards regression analysis. Analyses were performed with SPSS software (version 20.0).

RESULTS

Characteristics of the participants

The parents of 1343 children provided written informed consent for the study. A total of 963 children were randomly assigned to receive gluten (483 participants) or placebo (480) (Figure 1 and online supplementary appendix). After randomization, the number of children was reduced to 944 because 19 children did not fulfill the inclusion criteria. A total of 99 children (10.5%) did not adhere to the intervention assignment (59 children in the gluten group and 40 in the placebo group). A total of 141 children stopped participating before 3 years of age (withdrawal rate 14.9%, 69 participants in the gluten group and 72 in the placebo group). A total of 59 children withdrew during the first year (6.2%), 49 during the second year (5.2%), and 33 during the third year (3.5%); the median follow-up was 4 years (range, 22 days to 6.30 years). The reasons for withdrawal were unknown for 57% of the children, were related to practical issues for 39% (e.g. blood sampling or travel distance to center), and were related to adverse events for 4% (online supplementary appendix).
The baseline characteristics of the children were similarly distributed between the intervention groups, with the exception of homozygosity for HLA-DQ2 (Table 1). Data on breast-feeding were available for 943 children: 882 started breast-feeding; at 6 months of age, 527 (55.8%) were breast-fed, and 265 (28.1%) were breast-fed without complementary feeding except for the intervention product. Of the 455 mothers with CD, 431 were consuming a gluten-free diet during pregnancy and lactation. Rotavirus vaccination was performed in 211 children (22.4%), either before the intervention (176 children) or during the intervention (35).

Diagnosis of CD

The numbers of children who met the criteria to undergo small-bowel biopsies are shown in Figure 1. A total of 101 small-bowel biopsies were performed in 94 children (Table 2, and online supplementary appendix). CD was confirmed by means of biopsies in 77 children. To avoid underestimation of the frequency of CD, 3 additional children, whose parents declined biopsies on behalf of their children but who complied with the 2012 ESPGHAN diagnostic criteria,[1] were considered to have CD in all analyses (Figure 1).

The median age of the 80 children at diagnosis was 2.8 years (range, 1.1 to 5.6), and all the children had an elevated level of TG2A; 59% were girls. The most frequent symptoms were abdominal distension (in 20 children) and diarrhea (in 19). The cumulative incidence of CD at 3, 4 and 5 years of age was 5.2% (95% confidence interval [CI] 3.6 to 6.8), 8.8% (95% CI 6.6 to 11.0) and 12.1% (95% CI 9.2 to 15.0) respectively (online table S2, online figure S1). CD was significantly more frequent in girls; at 3 years of age, the cumulative incidence among girls and boys was 7.2% and 3.4%, respectively; at 4 years of age, 11.8% and 6.1%, and at 5 years of age, 14.5% and 9.9% (p=0.04 by the log-rank test, p=0.02 by multivariate analysis) (online table S2).

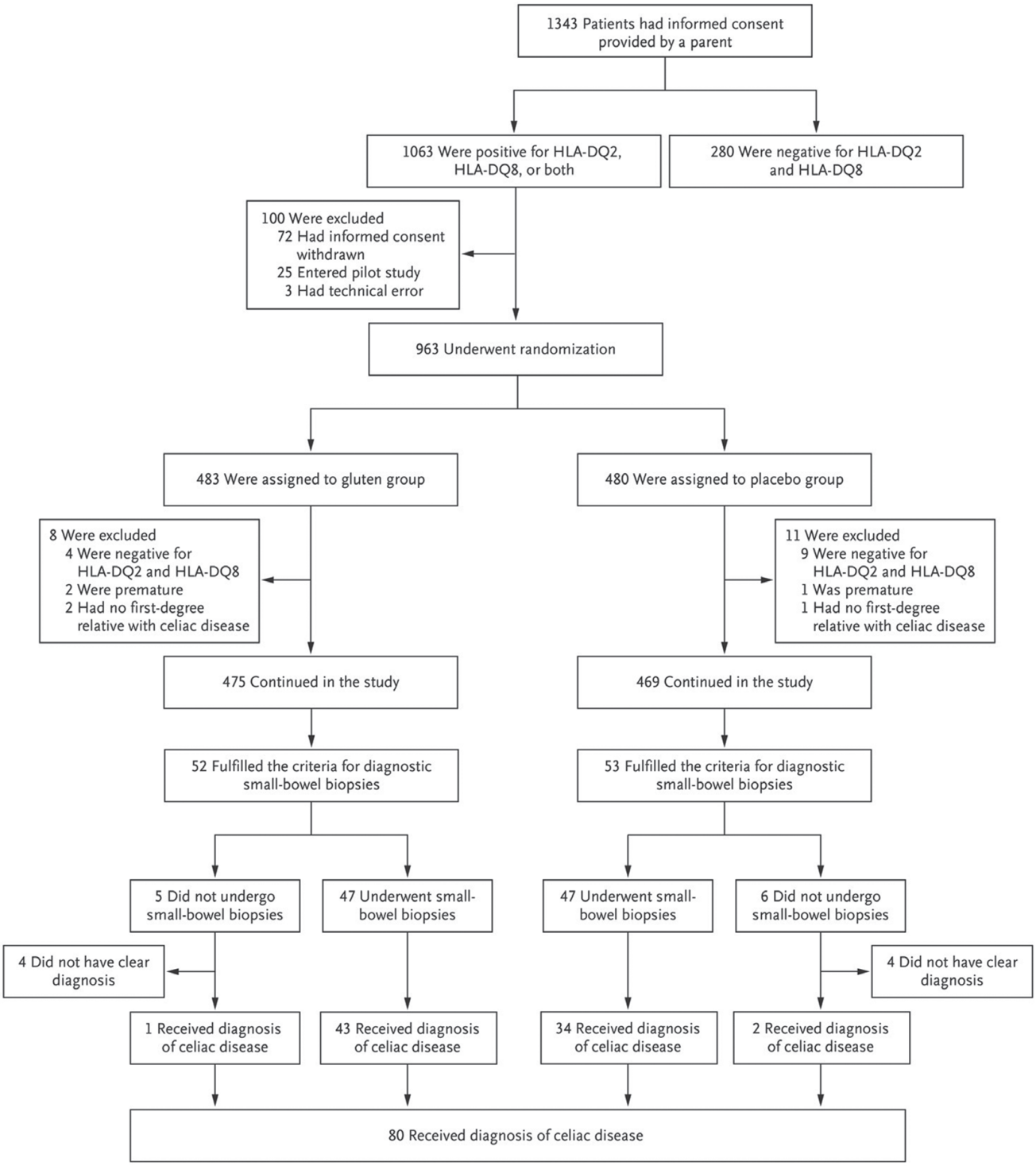

Figure 1 Randomization and diagnosed cases of coeliac disease. A total of 25 children were included in a pilot study to test the infrastructure of the study and were not included in the primary analysis. A total of 19 children underwent randomization in error and were excluded from the study. On the basis of histologic results of small-bowel biopsies, active CD was ruled out in 17 children, although 3 of the 17 had potential CD. There was no clear diagnosis in 8 asymptomatic children whose parents declined small-bowel biopsies on their behalf and who had transient levels of CD–associated antibodies. CD was diagnosed in 3 children according to the 2012 European Society for Pediatric Gastroenterology, Hepatology, and Nutrition diagnostic criteria (without having undergone biopsies).[1]

Table 1 Characteristics of the participating children.[a]

		Gluten (N=475)	Placebo (N=469)
Age (in years) at end of follow-up for this analysis, mean (min-max)		4.9 (3.1-6.5)	5.0 (3.1-6.6)
Female sex, no. (%)		228 (48.0)	226 (48.2)
Gestational age (in weeks), mean (min-max)		39.1 (34-43)	39.2 (35-42)
Birth weight (in grams), mean (min-max)		3316 (1730[b]-5000)	3346 (2000-4740)
Country, no. (%)	Spain	130 (27.4)	119 (25.4)
	Italy	70 (14.7)	69 (14.7)
	Hungary	70 (14.7)	68 (14.5)
	The Netherlands	67 (14.1)	66 (14.1)
	Germany	55 (11.6)	58 (12.4)
	Israel	47 (9.9)	48 (10.2)
	Poland	30 (6.3)	34 (7.2)
	Croatia	6 (1.3)	7 (1.5)
HLA-risk group[c], no./total no. (%)	1	80/462 (17.3)	49/449 (10.9)
	2	46/462 (10.0)	42/449 (9.4)
	3	199/462 (43.1)	218/449 (48.6)
	4	29/462 (6.3)	37/449 (8.2)
	5	108/462 (23.4)	103/449 (22.9)
First degree relatives with CD, no. (%)	1	431 (90.7)	432 (92.1)
	2	42 (8.8)	32 (6.8)
	3 or more	2 (0.4)	5 (1.1)
Type of first degree relative with CD, no. (%)	Mother only	200 (42.1)	207 (44.1)
	1 sibling	183 (38.5)	184 (39.2)
	Father only	48 (10.1)	41 (8.7)
	Mother and ≥1 sibling	23 (4.8)	23 (4.9)
	>1 sibling, but neither parent	12 (2.5)	7 (1.5)
	Father and ≥1 sibling	9 (1.9)	5 (1.1)
	Mother + father	0	2 (0.4)

[a] The characteristics of the children were similarly distributed between the intervention groups ($P < 0.05$), with the exception of homozygosity for HLA-DQ2 ($P = 0.05$).

[b] Data included a pair of healthy twins.

[c] Data on the HLA-risk groups were available for 911 of 944 children, with HLA typing performed by means of single-nucleotide polymorphisms (SNPs) on the basis of the tag-SNP approach.[8] The HLA risk groups were defined as follows: group 1 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 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. For the remaining 33 children, the status with regard to HLA-DQ2 and HLA-DQ8 positivity was determined by means of the EU-Gen Risk test (Eurospital), with no information provided regarding the HLA risk group.

Table 2 Distribution of symptoms and coeliac disease (CD) associated antibodies in 94 children with suspected CD, who underwent 101 diagnostic small-bowel biopsies.[a]

Variable		Eventual diagnosis				
		CD (77 biopsies)	Potential CD[b] (5 biopsies)	Unclear diagnosis (2 biopsies)	No CD (17 biopsies)	Total (101 biopsies)
Symptoms as indication for biopsy (no.)		52	0	2	13	67
Elevated TG2A level as indication for biopsy (no.)[c]		77	5	0	0	82
Elevated antigliadin antibodies as indication for biopsy (no.)***		12	0	0	6	18
Marsh classification of findings in small bowel biopsies (no.)[d]	0	0	4	0	13	17
	1	0	1	0	1	2
	2	3[e]	0	2	3	8
	3A	18	0	0	0	18
	3B	24	0	0	0	24
	3C	32	0	0	0	32
	4	0	0	0	0	0

[a] One child with an elevated anti-transglutaminase type 2 antibodies (TG2A) level underwent biopsy three times: the histologic findings were normal the first two times but compatible with CD the last time. Five children underwent small-bowel biopsies twice. The first time, all had normal histologic findings; the second time, two children had normal histologic findings (none had potential CD), and three received a diagnosis of CD.

[b] Potential CD was defined as an elevated level of TG2A and histologic findings in the small bowel.

[c] An elevated serum level of IgA TG2A was defined as a level of 6 U/ml or more (or in the case of IgA deficiency, an IgG TG2A level of ≥ 10 U/ml). An elevated anti-gliadin antibody level was defined as a level of more than 50 U/ml (or in the case of IgA deficiency, an IgG anti-gliadin level of ≥ 17 U/ml) on three occasions during a 3-month period, or a level of more than 17 U/ml that was clearly increasing in two tests performed during a 3-month period.

[d] Findings of small-bowel biopsies were assessed according to the Marsh classification,[30] on a scale from 0 to 4, with classes 0 and 1 being not characteristic of CD, class 2 being compatible with CD only with a concomitant elevated TG2A level, classes 3A to 3C being characteristic of CD (with higher letter grades indicating more villous atrophy), and class 4 being characteristic of refractory CD.

[e] Three children had a concomitant elevated TG2A level, as compared with the other five children with a Marsh classification of 2.[1]

The disease developed significantly more frequently and earlier in the group of children who were homozygous for HLA-DQ2 (DR3-DQ2/DR3-DQ2 or DR3-DQ2/DR7-DQ2) than in other HLA-risk groups,[4] with cumulative incidence at 3, 4, and 5 years of age of 14.9%, 23.9% and 26.9% respectively ($p<0.001$) (online table S2, online figure S2).

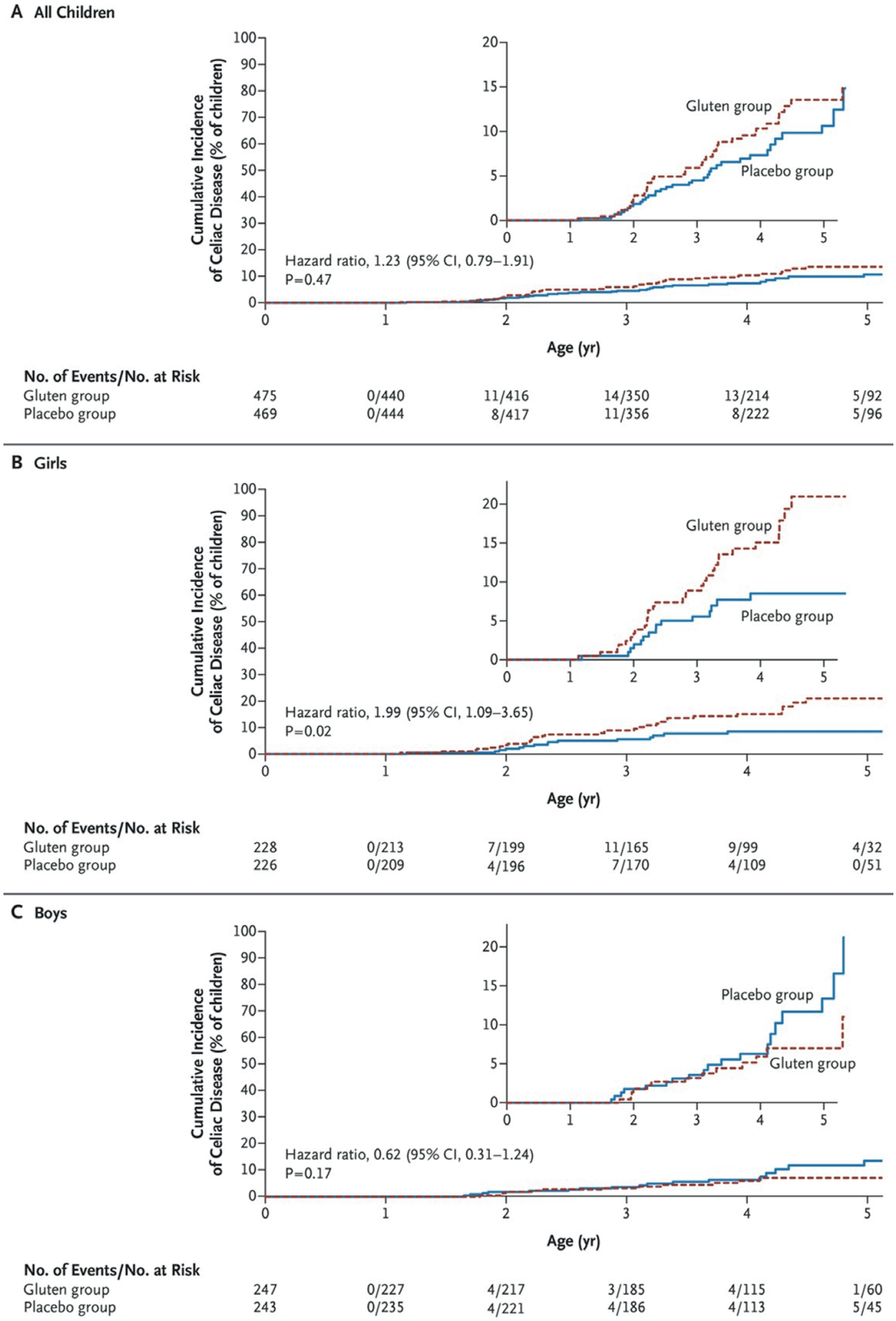

Figure 2 Cumulative incidence of coeliac disease (CD). A total of 75 of 80 children received a diagnosis of CD before 5 years of age. The cumulative incidence of CD in the gluten group versus the placebo group at 3, 4, and 5 years of age was as follows: 5.9% versus 4.5%, 10.3% versus 7.3%, and 13.5% versus 10.6%, respectively (Panel A). The cumulative incidence among 454 girls in the gluten group and the placebo group was as follows: 8.9% versus 5.5%, 15.1% versus 8.5%, and 21.0% versus 8.5%, respectively (Panel B). The cumulative incidence among 490 boys was as follows: 3.2% versus 3.6%, 5.9% versus 3.6%, and 7.0% versus 13.4%, respectively (Panel C). The data in Panels B and C show a significant interaction between sex and intervention (P=0.01). The insets show the same data on an expanded y axis.

Breast-feeding did not influence the development of CD. The cumulative incidence at 3 years of age among children who were not breast-fed, were breastfed for 3 or fewer months, were breast-fed for 4 or 5 months, or were breast-fed for 6 or more months were 7.3%, 4.4%, 8.2% and 4.4%, respectively (p=0.28). Similar cumulative incidences at 3 years of age were observed among children who were never exclusively breast-fed or were breast-fed exclusively for 3 months or less, for 4 or 5 months, and for 6 months or more (5.0%, 9.1%, 5.3% and 2.7%, respectively; p=0.45). Country of origin and the number and type of affected family members were also not related to the development of disease (online table S2), nor were rotavirus vaccination, gastrointestinal or respiratory tract infection, and mean daily gluten intake (online supplementary appendix).

Development of CD in relation to the intervention

The intervention with gluten, as compared to placebo, did not have a significant effect on the frequency of CD development, with cumulative incidences at 3 years of age of 5.9% (95% CI 3.7 to 8.1) and 4.5% (95% CI 2.5 to 6.5), respectively (p=0.47 by a stratified log-rank test; hazard ratio, 1.23; 95% CI 0.79 to 1.9) (Figure 2A). The duration of breast-feeding, whether exclusive or not, did not significantly influence the effect of the intervention on the development of CD (p=0.70 [for exclusive breast-feeding] and p=0.83 [for nonexclusive breast-feeding] for interaction; hazard ratios are provided in online table S3).

The cumulative incidence of CD was significantly higher in girls randomly assigned to gluten than among those randomly assigned to placebo: at 3 years of age, the incidence was 8.9% in the gluten group versus 5.5% in the placebo group (hazard ratio 1.99; 95% CI 1.09 to 3.65; p=0.02) (Figure 2B). This difference was not seen among boys, with frequencies of 3.2% in the gluten group and 3.6% in the placebo group (hazard ratio 0.62; 95% CI 0.31 to1.24; p=0.17; P=0.01 for interaction of sex and intervention) (Figure 2C). No other factors than sex were found to significantly influence the effect of the intervention on the development of CD (Figure 3, online table S3).

The results of the primary per-protocol analysis were similar to those of the intention-to-treat analysis (online supplementary appendix). The cumulative incidence of CD seropositivity (positive TG2A, positive anti-gliadin antibodies, or both on two occasions during a 3-month period) did not differ significantly between the gluten group and the placebo group (7.0% [95% CI, 4.7 to 9.4] and 5.7% [95% CI, 3.5 to 7.9], respectively; hazard ratio, 1.14 95% CI, 0.76 to 1.73; p=0.53) (Table 3, online figure S3). Although elevated levels of TG2A were not found in any of the participants at 6 months of age, transient anti-gliadin antibody levels of more than 17 U/ml were observed in 59 children in the gluten group and 2 in the placebo group. This elevation was not predictive of CD, which developed in only 8 of these children, all in the gluten group.

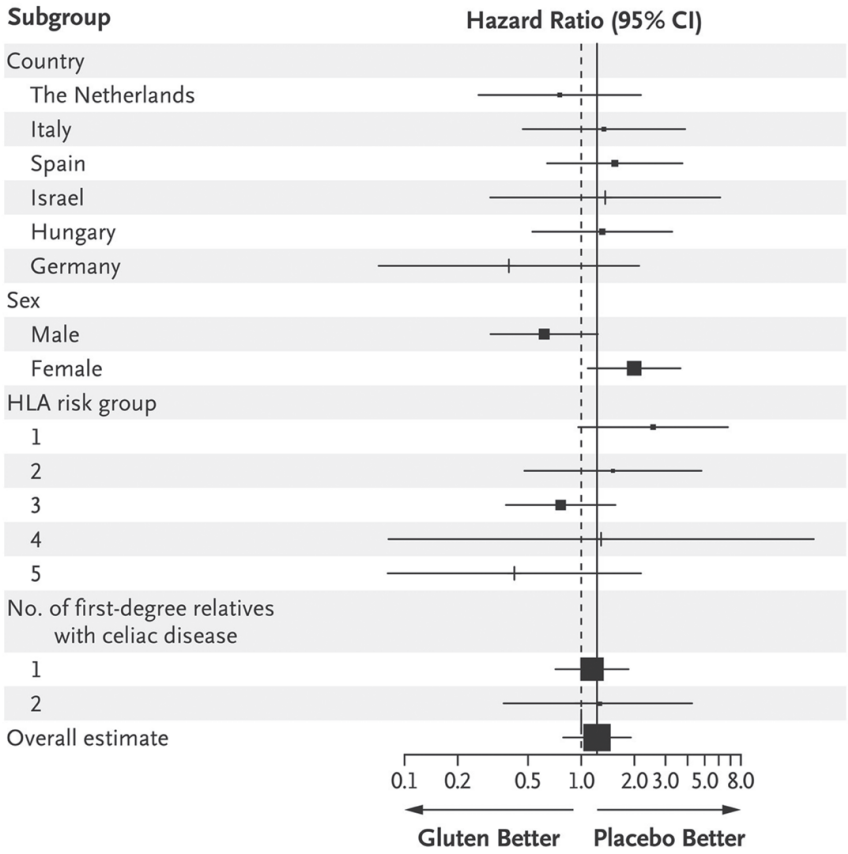

Figure 3 Effect of intervention assignment at 16 to 24 weeks of age on the development of coeliac disease (CD) in 944 children from high-risk families. Female sex was the only factor to significantly favor placebo (P=0.02). The HLA risk groups were defined as follows: group 1 included DR3–DQ2/DR3–DQ2 (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 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. No statistics were computed for children from Poland (64 children) and Croatia (13), or for children with three or more first-degree relatives with CD (7) because of the low number of children with CD in these groups. The black boxes represent the hazard ratio with 95% confidence intervals (horizontal lines); the size of each box is proportional to the size of the corresponding subgroup. The overall estimate is represented by the solid vertical line; a dashed vertical line representing no effect is also shown.

DISCUSSION

Our results indicate that the early introduction (at 16 weeks of age) of small quantities of gluten did not reduce the risk of CD at 3 years of age in genetically predisposed children from high-risk families; therefore, our results do not support the protective effect that we had hypothesized. In addition, we show that breast-feeding, whether exclusive or not, did not have a significant effect on the frequency of CD among these children. In prespecified secondary analyses, we observed an association between the early gluten intervention

Table 3 Antibody elevations and diagnosis of coeliac disease (CD) according to intervention assignment.[a]

Variable		Cumulative incidence		P value[b]	Hazard ratio (95% CI)
		Gluten (N=475)	Placebo (N=469)		
		%			
Elevated anti-gliadin at age 6 months of age		12.4	0.4	<0.001	
Elevated TG2A at 6 months of age		0.0 (0)	0.0 (0)	NA	
Elevated level of TG2A or anti-gliadin antibody	At 1 yr of age	0.9	0.0	0.53	1.14 (0.76-1.73)
	At 2 yr of age	3.2	2.1		
	At 3 yr of age	7.0	5.7		
	At 4 yr of age	11.5	9.5		
	At 5 yr of age	14.0	12.1		
CD	At 1 yr of age	0.0	0.0	0.47	1.23 (0.79-1.91)
	At 2 yr of age	2.6	1.9		
	At 3 yr of age	5.9	4.5		
	At 4 yr of age	10.3	7.3		
	At 5 yr of age	13.5	10.6		

[a] NA denotes not applicable

[b] The p-value for the elevated antibody level at 6 months of age was calculated by means of a Fisher's exact test, and the other p-values were calculated by means of the log rank test.

and CD in girls but not in boys. We did not find significant effects in the other subgroups examined, and the significant finding in girls may be due to chance or to the larger number of girls with HLA-DQ2 homozygosity who were randomly assigned to gluten rather than to placebo (online table S4). Owing to the small number of children in the different HLA risk groups stratified according to sex, we cannot resolve this issue.

The higher frequency of CD among girls than among boys after early exposure to gluten may be related to the well-known increased risk of CD among women[13, 31] , but it appears too early in life to be related to the protective effect of androgens for autoimmunity.[32] The gut microbiota may also play a role in this sexual dimorphism, as was shown recently for type 1 diabetes in rodents, in which hormones and microbes together trigger protective pathways.[32, 33] Our results also show prospectively the effect of HLA-DQ2 homozygosity on the risk of CD in early childhood.

In general, we found no association between the early development of CD and the presence of the disease in one or both parents, but this finding should be interpreted with caution,

given the small number of fathers with CD in our cohort (105 of 944). Possible explanations for the small number of affected fathers are the tendency for mothers to be more involved in research projects[34] and the higher frequency of CD among women.[13]

Contrary to previous reports,[35, 36] our data show that determining the TG2A level, but not the level of anti-gliadin antibodies, is useful in the assessment of the presence of CD in very young children. In fact, we found that symptoms were not prognostic for CD (Table 2), indicating that the early determination of the TG2A level in genetically predisposed children may offer an opportunity for early diagnosis.[37]

The strength of our study lies in its design as a randomized, double-blind, placebo-controlled trial evaluating a food intervention in a high-risk birth cohort, with comprehensive follow-up. The cases of CD were assessed in an identical way, minimizing the risk of bias. Nonetheless, our study has some limitations. It may be argued that we introduced gluten in a rather artificial way, since 100 mg is approximately 2% of the amount normally introduced at weaning.[29] Nevertheless, this quantity has been shown previously to cause histologic damage in the intestines of CD patients.[38] After our gluten intervention, levels of anti-gliadin antibodies were transiently elevated in 59 children at 6 months of age, showing that 100 mg of gluten can indeed be immunogenic. Our power calculation was based on the assumption of a cumulative incidence of CD of 10% by 3 years of age. We found that the actual mean frequency at this age was half the assumed frequency and that it strongly depended on sex and HLA haplotype. The confidence intervals for the hazard ratio for the effect of the intervention on CD ranged from 0.79 to 1.91, indicating that we were not able to rule out a protective effect smaller than 21% or a harmful effect as large as 91%.

Our findings contrast with those from observational studies suggesting that the introduction of gluten between the ages of 4 to 6 months represents a window of opportunity for preventing CD.[23, 24] Much of the information on infant feeding and the risk of CD has been obtained from the Swedish CD epidemic, which started in the mid-1980s[21] and was related to the introduction of an increased amount of gluten after the age of 6 months, when breast-feeding became less common.[9, 22, 23, 39] However, data regarding the timing of gluten introduction in relation to breast-feeding, as well as the amount of gluten, were obtained retrospectively. Our results also contrast with recent findings in a prospective cohort of young children from the general population in Norway.[25] However, that study investigated only clinically diagnosed CD, with probable under-reporting of CD, since most cases are not clinically recognized. Whereas the observations in the Swedish and Norwegian cohorts are based on the general population from single countries, our results are derived from a study population comprising children from high-risk families in 7 European countries and Israel. Observational studies involving children with an increased risk for type

1 diabetes (positive for HLA-DQ2 or HLA-DQ8) have had controversial results. Although the results of a study conducted in the United States support the early introduction of gluten at 4 to 6 months of age,[24] the age at gluten introduction did not influence the risk of CD autoimmunity in a prospective German birth cohort.[40]

In conclusion, this randomized trial did not show the hypothesized benefit of early exposure to small quantities of gluten with regard to reducing the incidence of CD in children from high-risk families. In addition, we did not observe a reduced risk of CD associated with the maintenance of breast-feeding at the time of gluten introduction. The present European guidelines recommend the introduction of small amounts of gluten gradually while the child is breast-fed and the avoidance of both the early (<4 months) or late (>7 months) introduction of gluten.[41] Our results do not provide evidence to support these guidelines or any specific feeding recommendation with respect to the timing of gluten introduction for infants at risk for CD.

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II

IMPROVEMENT OF CARE

3 |

A COMPARISON OF PATIENTS' AND DOCTORS' REPORTS ON HEALTH RELATED QUALITY OF LIFE IN COELIAC DISEASE

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ABSTRACT

Objective

To investigate whether implementation of a coeliac disease (CD)-specific health related quality of life (HRQOL) questionnaire would add value to CD follow-up visits; we compared patients' self-reported CD-specific HRQOL with the physician's report provided during a regular CD follow-up visit in children and young adults.

Methods

A cross-sectional study in the control group of a study on self-management in CD (Coel-Kids). Eligible patients had CD for ≥1year and were ≤25years old. They completed a CD-specific HRQOL questionnaire (CDDUX) after their regular follow-up visit. Their physicians were unaware of the current study's objectives or self-reported HRQOL. Primary outcome: agreement between physician-reported and self-reported HRQOL. Secondary outcomes: patient variables predicting a discrepancy between reports, or a lower HRQOL.

Results

Physician-reported HRQOL was available in 70/78 enrolled patients. The self-reported and physician-reported HRQOL were concordant in 30/70 (κ=0.093), 6 of them had a poor self-reported HRQOL. Reports were discrepant in 40/70; all 40 self-reported a poor HRQOL. Discrepancies occurred more frequently in patients with a disease duration <9years (32/40 with discrepant reports were diagnosed <9years ago versus 17/30 with no discrepancy, $p<0.001$) and in females (35/40 with discrepant reports were girls versus 16/30 with no discrepancy, $p=0.001$). Both factors were predictors of a poorer HRQOL.

Conclusions

During regular CD follow-up visits, physicians did not report a poor HRQOL in 40/46 children and young adults with a poor self-reported HRQOL. This is consistent with previous studies examining other chronic diseases and supports the implementation of self-reported CD-specific HRQOL measurements in CD follow-up visits.

INTRODUCTION

Coeliac disease (CD) is a chronic immune-mediated systemic disorder elicited by gluten in genetically susceptible individuals, affecting 1-3% of the general population.[1, 2] The disease is treated with a life-long gluten-free diet (GFD).[1, 3] Standard medical care for CD patients consists of an evaluation of health status, weight, height (in children), GFD adherence, and CD-specific antibodies in serum.[4] Health-related quality of life (HRQOL) is a more subjective and multidimensional concept containing physical, emotional, social, and cognitive domains that may vary over time and place.[5, 6] Physicians need to be aware of their patients' HRQOL in order to intervene and facilitate improvement. In previous studies, untreated CD was associated with a reduced HRQOL, typically followed by an improvement after initiation of treatment.[7-11] Others found that the HRQOL in treated CD patients was still lower compared to that of the general population,[12, 13] or significantly less in women with CD.[11, 14-16] The generic HRQOL instruments used in these studies allowed for comparison with normative data and across disease populations. Disease-specific instruments may be more discriminating and sensitive to small differences and changes.[17] In 2008, our research group designed and validated a CD-specific HRQOL questionnaire for children and young adults (CDDUX) which has then been used in research settings in different countries.[17-19]

While CD-specific HRQOL is an accepted outcome within a research environment, it is often not specifically measured during actual follow-up visits in clinical practice. Nevertheless, it has been established that in patients with other chronic diseases, the physician overestimates the patient's HRQOL.[20-22] This has not yet been examined in CD. To investigate whether implementation of a CD-specific HRQOL questionnaire would add value to the follow-up visits for CD, we compared the self-reported CD-specific HRQOL of a group of children and young adults with the physician's report provided during a regular outpatient follow-up visit for CD.

METHODS

In this cross-sectional study, we took advantage of the existing control group of the ongoing multicenter research project in the Netherlands, called "CoelKids" (chapter 4).

CoelKids

CoelKids aims to evaluate a multidisciplinary, internet-based, self-management system (SMS) for CD-affected children and young adults (≤25 years) to monitor their disease. CD was diagnosed according to the ESPGHAN criteria.[3] The date of diagnosis was either the date

when small bowel biopsies were performed and/or when positive CD-specific antibodies were determined. Patients were included in the study only if CD had been diagnosed for at least 1 year, and after informed consent was obtained. Exclusion criteria: IgA deficiency, lack of internet access and/or difficulty in comprehending the Dutch questionnaires. Participants were randomized into two groups; the SMS group with online evaluations of their health, or the control group, wherein patients receive regular outpatient care for CD. The control group completed CD-specific HRQOL and GFD adherence questionnaires after their outpatient consultation. The study protocol was approved by the Medical Ethical Committee of the Leiden University Medical Center (LUMC).

Present study

We analysed the HRQOL in the control group of CoelKids participants attending the outpatient clinic of the LUMC. The recruitment period was from May 2012-August 2013. In order to accurately reflect the attention given to HRQOL by the physicians during a regular outpatient follow-up visit for CD, the physicians received no instructions about how to conduct the HRQOL measurement. Furthermore, they were unaware of the study's objective and the patient's self-reported HRQOL. In "real life" physicians do not use a standardized method to assess HRQOL in CD patients. Therefore, the physician-reported HRQOL was extracted from the clinical notes in the patient's Electronic Patient Record (EPR) by a well-instructed author who was blinded to the self-reported HRQOL. The physician-reported HRQOL was classified as: "1=Good" if the physician reported there were no CD-associated problems (e.g. "all is well, GFD accurately followed without problems"); "2=Bad" if CD-associated problems were reported; or "0=not recorded" if the physician did not refer to the quality of life.

The self-reported HRQOL was measured online using the CDDUX, validated in Dutch children and adolescents.[17] A proxy-version for parents was also available. Thus, the self-reported HRQOL was either the patient's own response or his/her parent's. The CDDUX consists of 12 questions divided into 3 subscales: *communication* (3 items on feelings about explaining his/her disease), *having CD* (3 items on feelings when (s)he is offered gluten-containing food), and *diet* (6 items on feelings about dietary restrictions, lifelong aspects).[17] Response options were given on a 5-point Likert Scale (5=very bad; 1=very good).[17] The mean score was calculated to represent an overall evaluation.[17] To compare the self-reported HRQOL with the physician's report, the self-reported HRQOL was dichotomized into "good" ≤3.00; and "bad" >3.00. Reports were considered discrepant when the dichotomized self-reported HRQOL and the physician-reported HRQOL mismatched.

The patients' GFD adherence was assessed using the adapted Dutch version of a previously validated questionnaire, with scores ranging from 0-3.[23] Patients with scores of 0-1 were considered non-compliant. The remaining patients followed a GFD (either with errors [score

2] or without errors [score 3]). Patients not adhering to the GFD (score 0-1) were compared with the others (score 2-3).

Outcomes

The primary outcome was the agreement between the self- and physician-reported HRQOL. Secondary outcomes: (1) the association between a discrepant self- and physician-reported HRQOL and the following patient variables: gender, patient age, age at diagnosis, years since diagnosis, GFD adherence, and whether or not HRQOL was parent-reported; and (2) the relationship between the self-reported HRQOL and the aforementioned patient variables.

Data-management and statistical analysis

The participants entered their CDDUX responses into a web-based data management application (NEN7510 certified). Data collected from the EPR were added manually. A kappa value (κ) was computed to assess the agreement between the self- and physician-reported HRQOL. The *t*-test or chi-square test was used to determine the association between the aforementioned patient variables and a discrepant self- and physician-reported HRQOL. A univariate regression analysis was used to screen for patient variables that potentially influenced the self-reported HRQOL. Variables reaching borderline significance ($p<0.10$) were selected for further evaluation in a multivariate model with a backward elimination approach. The P-value criteria for inclusion and exclusion were set at 0.05 and 0.10 respectively. This method was repeated for the analysis of the HRQOL scores on the 3 subscales of the CDDUX (*communication*, *having CD*, and *diet*). Analyses were performed with SPSS software (version 20.0).

RESULTS

Patients

The characteristics of the 78 patients are presented in Table 1. Twelve patients self-reported to be non-adherent to the GFD: 6 of them consumed normal quantities of gluten (score 0), the others made small dietary transgressions (5 regularly [score 0], 1 rarely [score 1]). Out of the 66 GFD adherent patients, 1 self-reported committing a dietary error (score 2). The parent-proxy version of the CDDUX questionnaire was used by 24/78 patients (mean age 7.8 years, range 2.2-13.3 years). The outpatient consultations were performed by 6 different physicians.

Table 1 Characteristics of the 78 patients with self-reported and physician-reported health related quality of life.

Age (yrs), mean (min-max)	12.5 (2.2-24.5)
Age ≥18 years, N (%)	13 (16.6)
Female, N (%)	57 (73.1)
Age at diagnosis of CD (years), mean (min-max)	5.3 (1.0-23.4)
Duration of CD (years), mean (min-max)	7.2 (1.0-20.6)
Adherent to the GFD[a], N (%)	66 (84.6)

CD=Coeliac disease

[a] Gluten free diet (GFD) score 3 (n=65) or 2 (n=1) measured with the adapted Dutch version of a validated questionnaire.[23]

HRQOL

Self-reported – In general, the HRQOL of the 78 patients ranged from "neutral" to "bad", with a mean score of 3.29 on a scale of 1-5 (1=very good; 5=very bad). Significantly better (lower) scores for the subscale *communication* than for *having CD* or *diet* (Table 2) were observed. A "bad" or "very bad" HRQOL was reported by 42 patients. A "good" HRQOL was reported by 10 patients, while a "very good" HRQOL was reported by 1. Male gender, older age, and longer disease duration were identified as predictors of a better HRQOL (univariate analysis, p=0.007; 0.042 and 0.010 respectively). Gender and disease duration together explained 16.7% of the variance in HRQOL (Table 3). The other patient variables did not influence the HRQOL. Predictors of a better score on the *communication* subscale were the male gender and using the parent-proxy questionnaire (B=0.561, standard error [SE]=0.224, 95% confidence interval [CI]=0.115 to 1.007, p=0.014; and B=0.430, SE=0.215, 95% CI=0.002 to 0.859, p=0.049), explaining 12.4% of the variance in the *communication* score. The only predictor of a better score on the *having CD* subscale was a longer disease duration (B=-0.046, SE=0.015, 95%

Table 2 Self-reported health related quality of life (HRQOL)[a] in 78 children and young adults with coeliac disease (CD) using the CD-specific CDDUX questionnaire.

HRQOL reported by	Total Mean (SD)	Communication Mean (SD)	Having CD Mean (SD)	Diet Mean (SD)
All participants	3.29 (0.68)	2.59[c] (0.92)	3.62 (0.74)	3.48 (0.87)
Patient (N=54)	3.29 (0.73)	2.46[c] (0.92)	3.64 (0.79)	3.52 (0.92)
Parent-proxy (N=24)	3.31 (0.57)[b]	2.90[b,c] (0.88)	3.57[b] (0.63)	3.39[b] (0.74)

[a] A lower score indicating a better HRQOL.

[b] No significant difference between the total mean HRQOL score reported by patients and parents, nor for the scores on the "having CD" and "diet" subscales (Independent samples t-test p=0.872, 0.694, 0.536 respectively) borderline significance for the scores on the "communication" subscale (p=0.048).

[c] Significant difference between the mean score for "communication" and "having CD" and "communication" and "diet" (Related-Samples Wilcoxon Signed Rank Test p≤0.02).

Table 3 Predictors of the health related quality of life (HRQOL) in 78 children and young adults with coeliac disease (CD).

	Univariate analysis[a]				Multivariate analysis			
	B	SE	95% CI	p-value	B	SE	95% CI	p-value
Intercept	NA[b]	NA	NA	NA	2.787	0.308	2.173 –3.401	<0.001
Age (years)	-0.028	0.014	-0.056 – -0.001	0.042				
Age at diagnosis	0.008	0.017	-0.027 – 0.043	0.655				
Duration of CD	-0.038	0.014	-0.066 – -0.009	0.010	-0.036	0.014	-0.063 – -0.008	0.012
Female gender [c]	0.464	0.166	0.133 – 0.795	0.007	0.442	0.161	0.122 – 0.762	0.007
Voluntary gluten intake	-0.141	0.214	-0.567 – 0.285	0.513				
HRQOL reported by parent-proxy	0.027	0.168	-0.307 – 0.361	0.872				

[a] By univariate analysis, patient's age, duration of CD and gender were selected as potential predictors for the HRQOL. Using a multivariate model with a backward elimination approach, the age of the participant was eliminated as a factor influencing HRQOL.

[b] NA=not applicable because each univariate model has different intercepts.

[c] male=0, female=1.

Comparison of self-reported and physician-reported HRQOL

CI= -0.077 to -0.015, p=0.004), explaining 10.5% of the variance in scores on this subscale. A better score on the *diet* subscale was predicted by a longer disease duration and the male gender (B=-0.038, SE=0.018, 95% CI=-0.074 to -0.002, p=0.039; and B=0.461, SE=0.211, 95% CI= 0.040 to 0.882, p=0.032), explaining 11.4% of the variance in the score.

Physician-reported – The EPR of 70/78 patients contained information based on which the physician-reported HRQOL could be obtained. 6/70 reports on HRQOL were interpreted as "bad" because the physician detected problems resulting from the GFD. In the remaining 64 patients, no CD-related problems were identified, thus, the HRQOL was interpreted as "good". Patient age, age at diagnosis, duration of disease, gender, and adherence to the GFD did not influence the physician-reported HRQOL (p=0.137; 0.701; 0.302; 0.930; 0.444 respectively).

The self-reported HRQOL was dichotomized into "good" for 24 patients and into "bad" for 46 patients. The physician-reported HRQOL matched the self-reported HRQOL in 30/70 cases (κ=0.093, Table 4). In the remaining 40 cases, a self-reported "bad" HRQOL was incongruously reported by the physician as being "good". Discrepant reports of HRQOL were significantly more frequent in patients who had been diagnosed within the past 9 years (32/40 patients with discrepant reports diagnosed within past 9 years versus 17/30

Table 4. Comparison of the self-reported and physician-reported health related quality of life (HRQOL) of 70[a] children and young adults with coeliac disease.

	HRQOL	Self-reported		
		Good	Bad	Total
Physician-reported	Good	24	40	64
	Bad	0	6	6
	Total	24	46	70

[a] In 8/78 participants, the physician did not assess the HRQOL, self-reported HRQOL was good in n=5 and bad in n=3.

in whom the physician-reported HRQOL was correct, p<0.001) and these patients were significantly more often female (35/40 patients with discrepant reports were girls versus 16/30 in whom the physician-reported HRQOL was correct, p=0.001). Age, age at diagnosis of CD, adherence to the GFD, and whether or not the HRQOL was assessed by parent-proxy was similarly distributed among both groups (p=0.197; 0.899; 0.394 and 0.766 respectively).

DISCUSSION

To the best of our knowledge, this is the first study comparing the CD patients' self-reported HRQOL against the physician's HRQOL reports during a regular outpatient follow-up visit for CD. Our results indicate that there is an important discrepancy between these reports since in 57% of the patients, the physician had a different perception of the patients' HRQOL than the patient him/herself. What raises concern is that this occurred among patients considered to be especially vulnerable: those with a "bad" self-reported HRQOL. Our data show that this problem occurs significantly more frequently in those who received the diagnosis within the past 9 years and in female patients, possibly due to their significantly poorer self-reported HRQOL, especially for the "*communication*" subscale, compared to their male counterparts (p=0.014). On the other hand, in patients with a "good" self-reported HRQOL, physicians correctly recognized it as being "good".

The female preponderance in our cohort (73.1%) is a well-known phenomenon in CD.[1] Moreover, the neutral to bad self-reported HRQOL in our cohort is comparable with the results from previous studies in the Netherlands.[8, 17] Therefore, our cohort may be considered as representative of the HRQOL of children and young adults with CD in our country. In addition, the improvement of the HRQOL as the disease duration becomes longer is supported by a previous study.[24] The lower HRQOL found in females has been previously described in adults,[11, 14-16] but not in children and/or adolescents.[8, 24, 25] These studies

did not use a validated CD-specific HRQOL questionnaire but a generic questionnaire with [14, 25] or without [11, 15, 16, 24] added CD-specific questions. In our cohort, compliance with the GFD did not influence the self-assessed HRQOL, as was previously described in Dutch children whose HRQOL was evaluated in a research setting.[7] Nevertheless, this should be interpreted with caution given that a relatively small fraction of patients in our cohort were non-compliant to the GFD (12/78). In contrast to our results, literature indicates that parents rate their child's HRQOL lower than the child does him/herself.[17, 18, 24] Owing to the design of the larger research project this study is part of, we only had either a patient or a parent report, not both. Excluding the 24 participants with parent-reported HRQOL gave results similar to those presented in this paper. Furthermore, a recent study with the CDDUX in 214 Spanish children showed similar parent- and child-reported scores.[19]

To address the discrepancy between the self-reported and physician-reported HRQOL in clinical practice, our results are consistent with previous studies on children affected by other chronic diseases.[20-22] One meta-analysis has, in fact, demonstrated that the discrepancies between the physician- and child-reported quality of life were mainly found in subjective rather than objective domains.[20-22] The CD-specific HRQOL questionnaire CDDUX focuses on the subjective matters (feelings).[17] Previous studies do not report data on discrepancies between the physician- and self-reported HRQOL based on patient variables such as age, disease duration, and gender.

The strength of this study is the use of a previously validated CD-specific HRQOL questionnaire. Moreover, the physicians were not informed about this study's objectives. Thus, a training effect was excluded, accurately reflecting the attention given to the HRQOL at a regular CD follow-up encounter. Indeed, it may be questioned whether each participating physician understood the concept of HRQOL similarly, as they did not use a standardized method to assess HRQOL. The physician's evaluation could have been obtained prospectively in a standardized manner. However, our aim was to compare the patient's self-reported HRQOL with the report provided by the physician during regular follow-up. Because the latter is typically done without using a standardized method, a questionnaire for physician-reported HRQOL would not provide us with an accurate reflection of the attention physicians give to this topic. In addition, assessing whether the outcome of a physician-completed HRQOL questionnaire agrees with the real (patient-reported) HRQOL would not be of much use, as insufficient time during regular follow-up visits would prevent the physician from completing such a questionnaire. Consequently, to investigate whether or not the physician's understanding of the patient's HRQOL is accurate we extracted the physician-reported HRQOL from his/her clinical notes. One may argue that clinical notes do not always reflect the physician's understanding of a patient's HRQOL. Even if this were true, the lack of "poor" HRQOL documentation makes subsequent evaluations difficult and

means that measures to increase the HRQOL are not being initiated. Our study included all physicians from our institution taking care of coeliac children and young adults. This may have introduced inter-observer variability. However, this is a good reflection of real life: some physicians may see more coeliac patients than others and some may be more focused on HRQOL than others. Nevertheless, we found that the number of discrepancies was in fact not influenced by whether the participant was assessed by a paediatric or adult gastroenterologist (physician- and self-assessed HRQOL matched in 26/63 participants assessed by a paediatrician versus in 4/7 participants assessed by an adult gastroenterologist, p=0.42) nor by which physician assessed the patient (p=0.67).

Our decision to include a self-reported CD-specific HRQOL score of exactly 3.00 in the category with a "good" score may be somewhat artificial. However, including a score of 3.00 (N=2) into the "bad" category gave results similar to those presented in this paper, as well as when all participants with a "neutral" score (2.61-3.40, N=24) were excluded from our analysis.[17] Measuring the HRQOL in a clinical setting may generate the expectation that the physician can improve the HRQOL.[6] To achieve this in CD is challenging, firstly because the GFD is the only effective treatment for CD, and secondly because of the variety of factors which impact the generic HRQOL in chronic illness. However, to minimize the negative impact of a GFD and maximize dietary adherence, it is important that the physician is aware of the HRQOL of his/her patient. Patients may benefit from a consultation with a nutritionist and/or psychologist, or associating with peer-groups. It is possible that the results on the self-reported HRQOL vary in different countries. For example, Dutch children and adolescents with CD who both have a high general HRQOL, experienced a low to neutral CD-specific HRQOL.[17] In contrast, similarly affected Argentine and Spanish patients had good and neutral CD-specific HRQOL scores respectively.[18, 19] Nevertheless, the Argentine patients indicated that the CDDUX questionnaire helped them express difficulties during the physician visit that otherwise would not have been discussed. Furthermore, their physicians indicated that the CDDUX helped them detect aspects that required action, for example, the need to refer to a nutritionist or psychologist.[18]

In conclusion, there is a clinically significant discrepancy between the self-reported and physician-reported HRQOL in CD-affected children and young adults with a poor self-assessed HRQOL. Female patients and patients with a more recent diagnosis more often had these discrepant reports. Our study supports the implementation of a self-reported CD-specific HRQOL measurement in the clinical follow-up of the patients. As the standard consultation time allotted for follow-up CD visits is limited, we suggest using a validated CD-specific HRQOL questionnaire prior to physician appointments. Sharing the results of the questionnaire may improve the patient/parent-doctor communication and the physicians' understanding of the needs and priorities of children and young adults with CD.

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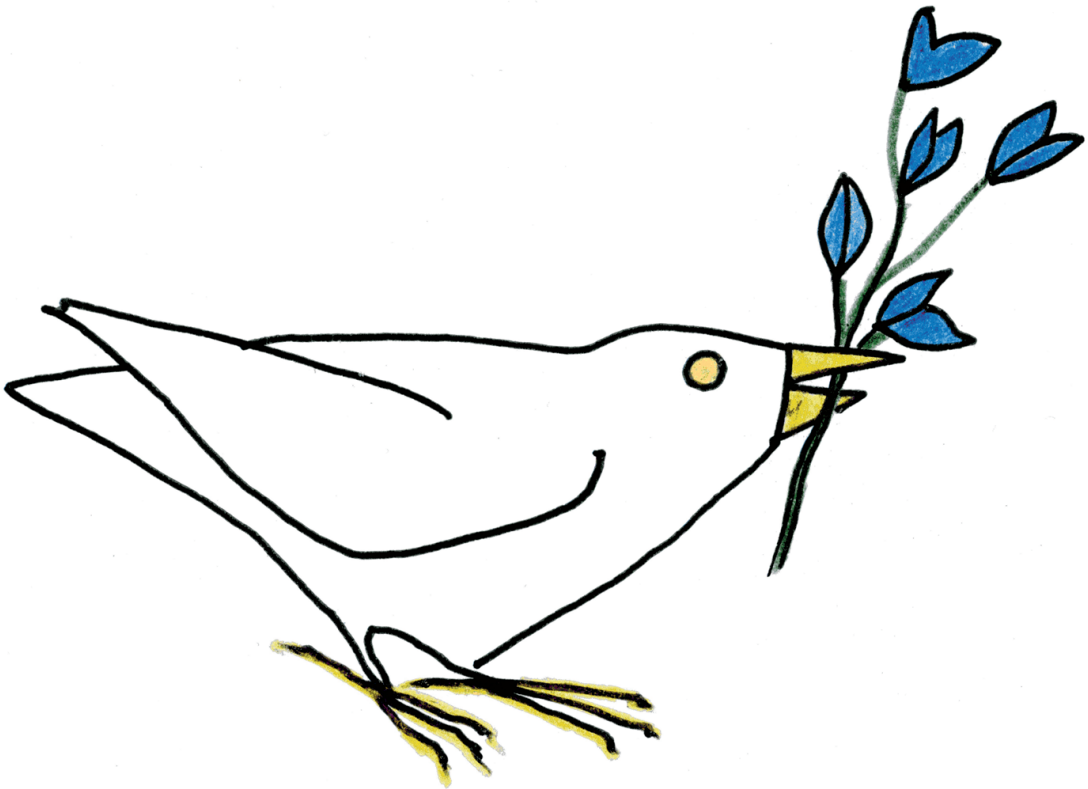

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A MULTICENTER RANDOMIZED CONTROLLED TRIAL EVALUATING E-HEALTH FOR CHILDREN AND YOUNG ADULTS WITH COELIAC DISEASE – THE COELKIDS STUDY

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Submitted

ABSTRACT

Objective

To evaluate the effectiveness of online consultations for follow-up of children and young adults with coeliac disease (CD).

Design

Multicentre randomized controlled trial involving 304 patients aged ≤25 years with CD ≥ 1 year, receiving online (N=156) or traditional consultation (N=148). Online consultations included symptom questionnaires and home measurements of growth and anti-transglutaminase-type-2 antibodies (TG2A) using a point-of-care (POC) self-test. Both groups completed questionnaires concerning CD-specific health-related quality of life (HRQOL), gluten free diet adherence and patient-satisfaction. After 6 months, they performed the POC self-test and repeated HRQOL and patient-satisfaction questionnaires. Primary outcome: disease control, defined as negative TG2A. Secondary outcomes: CD-specific HRQOL, patient-satisfaction, costs.

Results

Abdominal pain, lassitude and increased appetite were detected significantly more frequently in the online group than in controls. Growth problems were detected similarly in both groups. TG2A was positive in 2 online participants and 13 controls (POC versus laboratory, p=0.003). CD-specific HRQOL (1=good;5=poor) was similar in both groups, but improved after online consultation (3.25 to 3.16, p=0.013; versus controls 3.10 to 3.23, p=0.810). Patient-satisfaction (1=low;10=high) was 7.6 in the online group and 8.0 in controls (p=0.001). Mean costs in the online group were €202 less than in the control group (p<0.001).

Conclusion

Online consultations for children and young adults with CD are cost-saving and increase CD-specific HRQOL. Additionally, patients find these to be satisfactory. The discrepancy between the POC test and laboratory results suggests that the used POC test is not sensitive enough to detect low antibody levels and thereby unsuitable to monitor treated CD.

INTRODUCTION

Coeliac disease (CD) is an immune-mediated systemic disorder occurring in genetically susceptible individuals. It is elicited by gluten ingestion.[1] CD may be considered a public health problem, with a prevalence ranging from 1-3% that corresponds to about 5 million affected people in the European community.[1-3] Patients are treated with a gluten free diet (GFD). This restores small bowel histology and improves clinical complaints in the majority.[1, 4] Traditional medical care for CD patients consists of regular physician visits to evaluate their health, weight, height (in children), GFD adherence and CD-specific serum antibodies.[5, 6] Although important, these measures can be time-consuming. Moreover, many patients do not visit their physician for regular CD follow-up.[7] Time constraints during outpatient follow-up also typically restrict comprehensive assessments of a patient's health-related quality of life (HRQOL) and dietary adherence. Previous studies in adults with other chronic diseases suggest that an online self-management system (SMS) can encourage patients to improve health care participation and decision-making process.[8] Patients are able to deal with their symptoms, treatment, physical and psychosocial consequences and lifestyle changes that are inherent in living with a chronic condition through successful disease self-management.[9] We developed an online SMS as a substitute for outpatient consultations in the follow-up of CD in children and young adults (CoelKids, www.coelkids.nl). We hypothesized that disease control in the course of the study would be similar in patients using the online SMS and traditional outpatient follow-up.

METHODS

Study design and participants

Children and young adults ≤25 years with diagnosed CD ≥1 year prior to recruitment received an invitation to participate in this multicentre randomized clinical trial. They were recruited between May 2012 and July 2014 from 3 academic and 4 non-academic hospitals in the Netherlands. Exclusion criteria were IgA deficiency, no internet access and insufficient comprehension of Dutch.

Intervention

After written informed consent was obtained, participants were randomized to the online or control group, stratified by age at inclusion and gender (Figure 1).

The online group used the SMS to replace one traditional outpatient consultation. The patients (or parents) were asked to complete a symptom questionnaire (i.e. abdominal pain, appetite, lassitude and defecation). They were also instructed to measure their height and

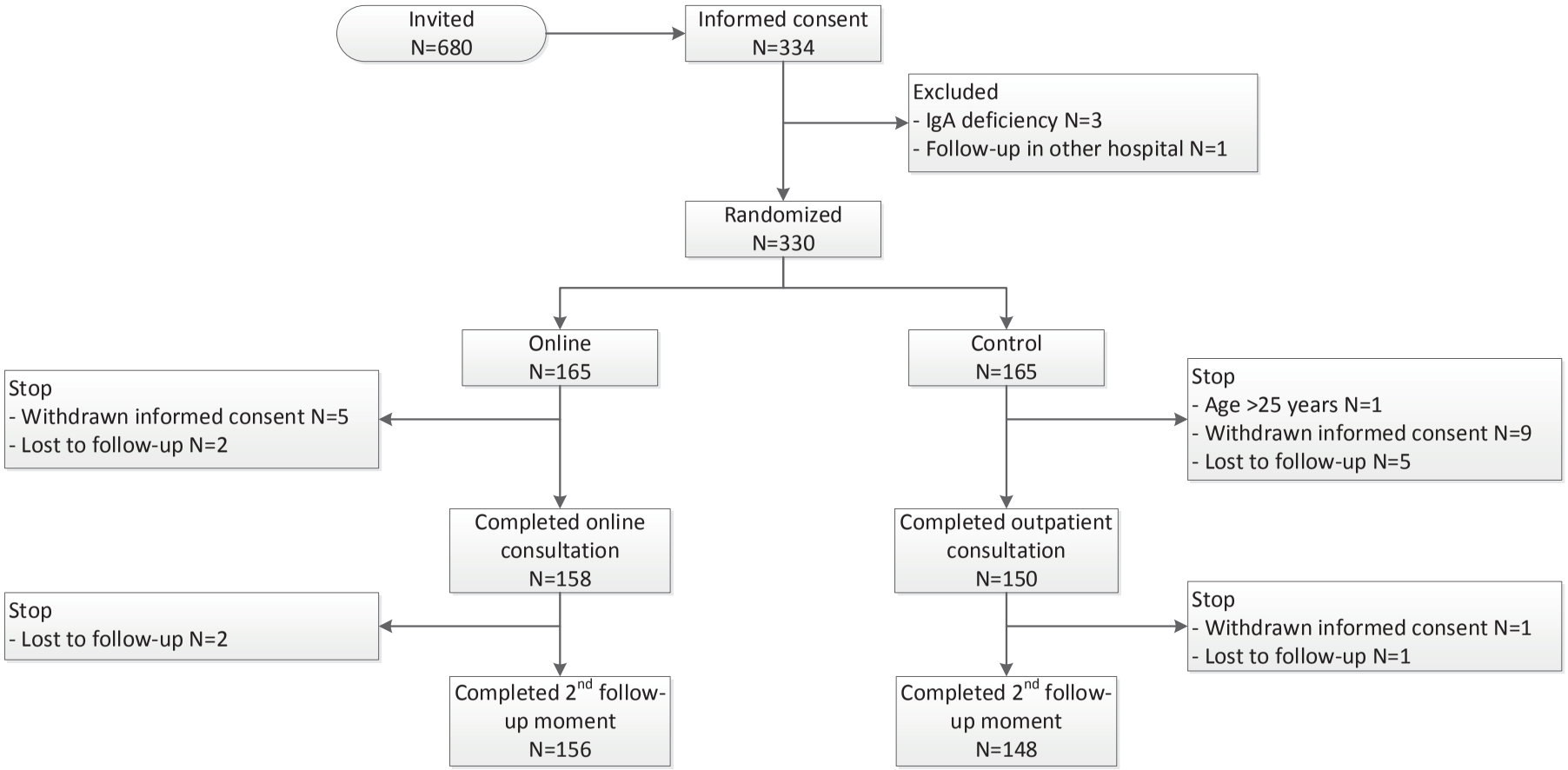

Figure 1 Randomization of 330 participants and follow-up of 304 participants.

weight themselves, which were subsequently plotted on their growth charts and compared with previous in-hospital measurements.[10, 11] CD-specific IgA anti-transglutaminase-type-2 antibodies (TG2A) were self-measured using a commercially available point-of-care (POC) test (Biocard Celiac Test, AniBiotech, Vantaa, Finland), validated for detection of IgA TG2A.[12-15] It requires 1 drop of fresh blood, obtained by finger-prick. The result (positive/negative) should be interpreted after 10 minutes in a well-lit place. Written instructions were sent along with the POC self-test and a video tutorial was available on the study website. Participants were requested to e-mail us a picture of the result and to return this test by mail. In addition, they completed online questionnaires on GFD adherence, CD-specific HRQOL and parents' and/or patients' (in the case of young adults) satisfaction with the consultation. The researcher discussed the results with the participants (or parents) over the telephone, and sent a copy of the results to their physician. In case of abnormalities, an outpatient consultation was scheduled.

The control group received traditional care at the outpatient clinic, with their own physician. A standardized summary of the consultation's narrative (symptoms, growth) was used for data analysis. TG2A in serum was measured with conventional ELISA (EliA™ Celikey™ IgA test; ThermoFisher Scientific, Freiburg, Germany) in all participating hospital laboratories. Units per millilitre (U/ml) <7 were considered negative, and U/ml ≥7 was positive (measuring range 0.1-≥128 U/ml). After the consultation, participants completed the questionnaires concerning GFD adherence, CD-specific HRQOL and patient satisfaction. Their physician was blinded to the outcomes of these questionnaires to prevent interference in a traditional outpatient consultation.

Six months after baseline, all participants were asked to measure their TG2A levels at home using the POC self-test and to respond again to the same CD-specific HRQOL and patient satisfaction questionnaires.

Study outcomes

The primary outcome was disease control 6 months after the online or outpatient consultation, defined as negative TG2A results. Secondary outcomes were CD-specific HRQOL, patient-satisfaction and costs.

Measures

Abnormal growth was defined as a deviation from the previous annual measurement of at least 1 standard deviation (SD) for height/age or for weight/height.

GFD adherence was assessed using the Dutch adaptation of a previously validated questionnaire.[16] The score ranged from 0-3 (0-1=GFD not followed; 2=GFD followed but with

errors; 3=strict GFD). Additionally, participants were asked to agree or disagree with the statement, "I follow a strict gluten free diet".

To assess the CD-specific HRQOL, we used the validated CDDUX questionnaire, consisting of 12 questions divided into 3 subscales: *communication*, *diet* and *having CD*.[17] The response options were depicted on a 5-point Likert-scale (1=very good; 5=very bad). Usage of the parent-proxy version was not determined by patient age. The scores for each subscale and the mean overall score were calculated.

Participants rated their satisfaction with the online or outpatient consultation on a scale of 1-10 (i.e. 1=lowest; 10=highest). We used a combination of items from the Dutch translations of the validated Ware's Patient Satisfaction Questionnaire III (PSQ III, 18 items), Telemedicine Satisfaction and Usefulness Questionnaire (TSUQ, 1 item), Parent Satisfaction Survey (PSS, 3 items) and Dick et al. 1999 (2 items).[18-20] Only items that were applicable to the study situation were used. Items were slightly modified to specifically address online or outpatient consultations (supplementary appendix, available from the author upon request). Responses were given on a 5-point Likert-scale (1=strongly disagree; 5=strongly agree). Six months after baseline, they retrospectively assessed their satisfaction (online table S1B), graded on a scale of 1-10.

A cost-minimization analysis was performed from a societal perspective, i.e. factoring in the costs of medical care (including physician and patient/parent-initiated consultations during the study period) and non-medical costs (e.g. parents' and/or participants' work absence, travel time/costs). The cost of a resource was valued using standard prices established by the Dutch Healthcare Authority (laboratory determinations) and by Hakkaart et al (personnel costs).[21] Shipping costs for the online group's equipment were also included. The time spent by the physician during regular consultations, and by the physician-researcher during online consultations, was prospectively measured. Participants also kept track of consultation times.

Ethical consideration

The study protocol was approved by the medical ethics committee of the LUMC and the respective boards of participating centres. It complied with Good Clinical Practice guidelines. Written informed consent was obtained from participants or their parents/ guardians, whenever appropriate. This trial is registered under the Dutch Trial Register NTR3688 (www.trialregister.nl).

Data management and statistical analysis

Assuming that an increase in inadequate disease control (positive TG2A) from 20 to 33% (equivalence margin 13%) is acceptable in the care of CD patients, 298 patients needed to be evaluated with a one-sided alpha of 0.05 and a power of 80%. Taking into account a 7.5% loss to follow-up rate, 316 patients had to be enrolled. Participants completed the online questionnaires using a data management application (NEN7510 certified). For comparison of disease control, HRQOL and satisfaction, the chi-square, Mann-Whitney U and Armitage's trend test were used as appropriate. Changes over time within both groups were detected with the McNemar and Wilcoxon Signed Rank Test as needed. Generalized estimating equations were used to compare costs between both groups. Analyses were performed with SPSS software (version 23.0).

RESULTS

Out of the 680 invited patients, (parents of) 334 (49%) provided written informed consent (Figure 1). We did not inquire their reasons for refusal. Prior to randomization, 4 patients were excluded because they did not meet the inclusion criteria. Consequently, 330 participants were randomly assigned to the online (N=165) or control group (N=165). Post-randomization, 1 participant was excluded because she exceeded the age limit (25.7 years). During the project, the number of participants dropped to 304 (online N=156; control N=148) because 15 participants withdrew their informed consent and another 10 participants became lost to follow-up (Figure 1). Characteristics of the participants were similarly distributed between the online and control group (Table 1). The mean duration between consultation and follow-up was 6.8 months (standard deviation [SD] 2.5) in the online group and 7.6 months (SD 3.3) in the controls (p=0.001).

Table 1 Characteristics of the 304 participants with coeliac disease (CD) randomized to the online or control group.

		Online (n=156)	Control (n=148)
Female – n. (%)		107 (68.6)	97 (65.5)
Age in years – mean (min-max)		11.0 (2.6-24.1)	11.4 (2.1-24.5)
Age at CD diagnosis in years – mean (min-max)		4.3 (0.9-17.9)	4.9 (1.0-23.4)
Disease duration in years – mean (min-max)		6.9 (1.0-20.3)	6.7 (1.0-22.9)
Gluten free diet score[a] – n (%)	0-1	12 (7.7)	19 (12.8)
	2	2 (1.3)	1 (0.7)
	3-4	142 (91.0)	128 (86.5)

[a] Scores 0-1 = gluten free diet not followed; 2 = gluten free diet followed but with errors; 3-4 = strict gluten free diet followed.[16]

Table 2 Frequency of symptoms in 304 participants with self-reported complaints (online group) or reported complaints during the consultation with their physician (control group).

		Online (N=156), N (%)	Control (N=148), N (%)	p-value[c]
Abdominal pain	No	71 (45.5)	107 (72.3)	<0.001
	Incidentally[a]	72 (46.2)	38 (25.7)	
	Frequent[a]	13 (8.3)	2 (1.4)	
	Unknown	0 (0.0)	1 (0.7)	
Appetite	Decreased	10 (6.4)	5 (3.4)	0.036
	Normal	123 (78.8)	135 (91.2)	
	Increased	23 (14.7)	4 (2.7)	
	Unknown	0 (0.0)	4 (2.7)	
Lassitude	No	90 (57.7)	123 (83.1)	<0.001
	Incidentally	45 (28.8)	9 (6.1)	
	Frequent	18 (11.5)	4 (2.7)	
	Unknown	3 (1.9)	12 (8.1)	
Defecation	Constipation[b]	13 (8.3)	9 (6.1)	0.943
	Normal	134 (85.9)	132 (89.2)	
	Diarrhoea[b]	7 (4.5)	3 (2.0)	
	Unknown	2 (1.3)	4 (2.7)	

[a] Incidentally = once a week or less; frequent = multiple times per week.
[b] Constipation = 3 or less stools per week; Diarrhoea = 3 or more stools per day.
[c] Using Armitage's trend test, omitting "unknown" as an answer.

Abdominal pain, lassitude and increased appetite were more frequently and significantly reported by the online participants than by controls (Table 2). Abnormal growth (weight/height deviated ≥1SD) was found in 4 online participants and 2 controls. Other growth problems were detected in 6 participants in the online group: -2SD weight/height growth with a deviation of 0.5SD (N=3); -2SD height/age without catch-up growth after CD diagnosis (N=2); obesity (N=1); and in 1 control: acceleration in height/age above 2SD (N=1). In general, the detection of growth problems was similar in both groups (10 online; 3 controls; p=0.059).

Baseline **TG2A results** were available in 298/304 participants (online N=153; controls N=145) (Table 3). There were significantly more participants with positive TG2A in the control group than in the online group: 13/145 (mean titre 21.5 U/ml; range 8-56 U/ml) versus 2/153 (POC self-test) (p=0.003). In 3/13 controls with positive TG2A, TG2A continued to decrease since the time of CD diagnosis. This was considered normal. Approximately 6 months later, TG2A was reassessed with the POC self-test in 279/298 participants with available baseline TG2A results (online N=148; control N=131) (Table 3). The number of positive POC self-tests in

Table 3 Correlation of IgA transglutaminase type 2 antibody (TG2A) results in 304 participants with coeliac disease during the baseline consultation (randomized to measurement with point of care [POC] self-test or in hospital laboratory) with results upon reassessment approximately 6 months later with the POC self-test.

			TG2A reassessed with POC self-test[a]			
			Positive – n.	Negative – n.	Not available – n.	Total – n. (% baseline)
TG2A at baseline consultation	POC self-test (n=156)	Positive – n.	2	0	0	2 (1.3)
		Negative – n.	3	143	5[b]	151 (96.8)
		Not available – n.	0	0	3[c]	3 (1.9)
		Total – n. (% reassessed)	5 (3.2)	143 (91.7)	8 (5.1)	156 (100)
	Hospital laboratory (n=148)	Positive – n.	0	10	3[d]	13 (8.8)
		Negative – n.	1	120	11[e]	132 (89.2)
		Not available – n.	0	3[f]	0	3 (2.0)
		Total – n. (% reassessed)	1 (0.7)	133 (89.9)	14 (9.4)	148 (100)

[a]Reassessment on average 6.8 months after baseline POC self-test and 7.6 months after hospital laboratory measurement.
Reasons for test results not being available: [b]Anxiety (n=3); technical failure POC self-test (n=1); unwilling to repeat test (n=1); [c]Anxiety (n=2); unclear instructions (n=1); not enough blood obtained with finger prick (n=1); [d]Anxiety (n=2); unclear instructions and interpretation results (n=1) ; [e]Anxiety (n=6); unclear instructions (n=3); not enough blood obtained with finger prick (n=2); technical failure POC self-test (n=1); unclear interpretation result (n=1); [f]Blood was not withdrawn (n=2); only anti endomysium antibodies were assessed (n=1).

the online group was similar to baseline (5/148 versus 2/153, p=0.25). In the control group, significantly less POC self-tests were positive than laboratory tests at baseline (1/134 versus 13/145, p=0.012). The single positive POC self-test corresponded to a participant with negative TG2A at baseline (6 U/ml).

The **self-reported dietary adherence** assessed with the questionnaire was described as "strict" by 142/156 online participants and in 128/148 controls (91% versus 87%, p=0.297) (Table 1). Out of the 34 patients who were non-adherent to a GFD according to the questionnaire, 20 patients (8 online; 12 controls) denied gluten consumption by agreeing with the statement "I follow a strict gluten free diet" (K=0.50). Positive POC self-tests and serum TG2A did not correlate with self-reported gluten consumption as assessed from the questionnaire (K=0.001 and K=-0.024 respectively), nor did these correlate with their answers to the statement on strict GFD adherence (K=0.001 and K=-0.008, respectively).

The **self-reported CD-specific HRQOL** scores are presented in Table 4. Usage of the parent-proxy or patient version of the questionnaire was similarly distributed in the online

Table 4 Mean self-assessed coeliac disease-(CD)-specific Health Related Quality of Life (HRQOL) of 304 participants with CD randomly assigned to an online or outpatient consultation, assessed at time of the consultation (baseline) and upon reassessment.[a] Scale 1-5, lower scores indicate a better HRQOL.

				Subscale								
	Overall			Communication			Having CD			Diet		
	Baseline	*Reassessed*	*p*[b]	*Baseline*	*Reassessed*	*p*[b]	*Baseline*	*Reassessed*	*p*[b]	*Baseline*	*Reassessed*	*p*[b]
Online group (n=156) – mean score (min-max)	3.25 (1.25-4.92)	3.16 (1.25-4.83)	0.013	2.50 (1.00-5.00)	2.35 (1.00-5.00)	0.031	3.66 (1.00-5.00)	3.63 (1.33-5.00)	0.393	3.42 (1.33-5.00)	3.34 (1.33-4.83)	0.040
Control group (n=148) – mean score (min-max)	3.20 (1.08-4.75)	3.23 (1.33-4.50)	0.810	2.48 (1.00-5.00)	2.48 (1.00-5.00)	0.840	3.64 (1.33-5.00)	3.68 (1.33-5.00)	0.812	3.34 (1.00-5.00)	3.38 (1.33-5.00)	0.732
p[c]	0.572	0.432		0.737	0.224		0.646	0.565		0.535	0.659	

[a] On average 6.8 months after online consultation and 7.6 months after outpatient consultation.

[b] Related-samples Wilcoxon Signed Ranked Test was used for analysis of differences between the scores at baseline and reassessment within the online or control group.

[c] Scores of the online and control groups were compared with the Independent-samples Mann-Whitney U Test.

and control groups, both at baseline (p=0.41) and at reassessment (p=0.60). During baseline consultation, participants' overall CD-specific HRQOL was similar in both groups (neutral to bad) (Table 4). Upon reassessment 6 months later, a statistically significant improvement in the overall score was observed in the online group (p=0.013) but not in the controls (p=0.810). The improvement concerned the subscales "Communication" and "Diet" (Table 4).

Responses to **satisfaction** with the consultation (grade from 1-10) were available in all online participants, in 146/148 of controls at baseline, and 147/148 of controls 6 months later. The mean satisfaction was significantly higher in the controls than in the online group at baseline (mean grade: 8.16 [range 5-10] versus 7.65 [range 2-10], p<0.001) as well as 6 months later (mean grade: 8.01 [range 4-10] versus 7.58 [range 3-10], p=0.001). Participants' baseline satisfaction and 6 months later remained uninfluenced by participant age (Spearman's rho online: p=0.362 and p=0.635; controls: p=0.666 and p=0.831) or the duration of CD (Spearman's rho online: p=0.887 and p=0.290; controls: p=0.270 and p=0.437). In comparison with online participants, the controls agreed more often to the idea that everything necessary to provide complete medical care was available. They had also reported more often that they felt free to discuss anything they found important, while the online group thought that the consultation was more impersonal (p<0.001; supplementary table S1). On the other hand, online participants found the timing and location of the consultation to be more convenient than did the controls (p=0.018 and 0.001 respectively; supplementary table S1). Furthermore, 48% (N=75) of the online group regarded the online consultation to be as good as outpatient care (disagree N=47; not agree or disagree N=34). In fact, 58% (N=90) wanted to continue with online consultations (disagree N=32; not agree or disagree N=34) (supplementary table S1B). A traditional consultation was preferred by 41% (N=64) of the online group (disagree N=61; not agree or disagree N=31). Technical problems were experienced by 31% (N=48, no problems N=91; no opinion N=17; supplementary table S1B). The POC self-test was preferred to the conventional venepuncture by 80% of the online participants and 81% of the controls (supplementary table S2). A conventional venepuncture was preferred because of a nurse's expertise, hospital environment, or the possibility of doing additional blood tests (supplementary table S2).

In 29 online participants and 17 controls, abnormalities were detected and further investigated during an **extra follow-up consultation** (p=0.061). This was because of CD-related symptoms in 6 online participants and 2 controls, and growth problems in 10 online participants (1 also had symptoms) and 3 controls. The 2 online participants with a positive POC self-test and the 3 participants with unsuccessful self-tests were referred to the hospital for assessment of serum TG2A. Positive serum TG2A was confirmed in 1 participant with a positive POC self-test. The other participant was already known to have positive serum TG2A and did not give consent for reassessment. Out of the 10 controls with abnormal TG2A, 7 were

referred to a dietician for dietary assessment. In the other 3 participants, TG2A was only slightly positive (8-11 U/ml) and expectant management was provided. A consultation with a dietician and/or physician was scheduled for 3/6 online participants with self-assessed non-adherence. The other 3 participants declined the offer. In the control group, 3 patients

Table 5 Mean costs per participant made during the study period in the group that was randomly assigned to an online consultation for follow-up of coeliac disease (CD) compared with the group assigned to a regular outpatient consultation (price level 2015).

		Mean costs during the study period			
Cost categories		Online group (n=156)	Control group (n=148)	p-value[a]	Difference
Medical costs	Staff costs physician	€51	€65	n.a.	€ -14
	IgA TG2A	€6	€15	n.a.	€ -9
	Extra follow-up consultations after baseline consultation until reassessment, mean (SD)	€33 (60)	€42 (86)	0.28	€ -9
	Extra follow-up blood work after baseline consultation until reassessment, mean (SD)	€40 (90)	€37 (88)	0.37	€ 3
Subtotal medical costs, mean (SD)		*€130 (144)*	*€159 (162)*	0.096	€ *-29*
Non-medical costs	Travel to consultation and back, mean (SD)	n.a.	€14 (19)	n.a.	€ -14
	Duration of consultation for participant or parent, mean (SD)	€13 (10)	€63 (68)	<0.001	€ -50
Subtotal non-medical costs, mean (SD)		*€13 (10)*	*€77 (80)*	<0.001	€ *-64*
Total, mean (SD)		**€143 (144)**	**€236 (189)**	<0.001	**€ -93**

n.a. = not applicable.

[a] Using generalized estimating equations.

Details underlying calculation of unit costs incl. overhead en utility costs in Euros:

- Staff costs physician: €163.12 per hour (including housing and overhead costs). Mean duration consultation: outpatient 0.40 hours (range 0.15-0.78), online 0.31 hours (range 0.10-1.22).
- Point of care test for IgA transglutaminase type 2 antibodies (TG2A) €3.50 per unit; preparation €2.22 per unit (based on 5 min of work per test for a medical secretary); and sending €0.43 per unit.
- Follow-up consultations (physician's or patient/parent's initiative) after the outpatient or online consultation and before the end of participating (~6 months) were considered and included: consultations with the physician (outpatient or telephonic), dietician, psychologist/pedagogue, or endocrinologist for problems related to growth or thyroid function.
- Follow-up blood work (physician's or patient/parent's initiative) after the outpatient or online consultation and before the end of participating (~6 months) was considered if related to follow-up for CD.
- Mean travel distance to the outpatient consultation and back was 26.72 (0-320) km. Costs per km: by car: €0.22 + €3.33 parking-costs (n=104); by public transport: €0.22 (n=15).
- Costs in case leave from work was taken in control group (n=53, mean hours: 3.96 range 1-12 [both parents took a day off]): €32.41 per hour. Otherwise in control group (n=95, mean hours 1.87 range 0.17-6.0) and in the entire online group (mean hours 0.95 range 0.17-8 [experienced technical problems]): €13.87 per hour.

were referred to a dietician because of physician-assessed non-adherence to the GFD (2 of them also had positive TG2A). Furthermore, 3 online participants with previously low vitamin D or folic acid or previously high thyroid stimulating hormone and anti-thyroid peroxidase antibodies were referred to the outpatient clinic. In the same online group, a gluten challenge was requested by 2 participants (parents doubted the diagnosis) and 1 participant was referred to the outpatient clinic because of premature pubarche. Furthermore, extra follow-up consultations were scheduled in 4 controls: slightly positive anti-endomysium antibodies despite negative TG2A (N=1); failure of TG2A determination (N=1); repeating iron determination (N=1); consultation with a social worker (N=1).

In addition, 6 online participants had an extra follow-up consultation because of dissatisfaction with the POC self-test (N=5) or the online consultation (N=1).

Mean **costs** in the online group were €93 lower than in the controls (total costs €143 versus €236 [p<0.001]) (Table 5). The non-medical costs of e-health were €64 lower than those of the outpatient consultation (p<0.001). The medical costs of the consultations (including follow-up visits during the study period) were not significantly different from each other (p<0.096) (Table 5). For this calculation, only the TG2A determination (€15.10) determines the costs of blood work in the controls. However, complete blood counts and others tests, e.g. folic acid, vitamin B12, calcium, alkaline phosphatase and iron status, were also determined in the outpatient group, as recommended by the Dutch evidence based CD guidelines. [6] When taking into account the complete blood work performed during the outpatient consultation (on average, €124 per consultation; SD€58), the difference between medical costs in the online and outpatient group reached statistical significance (€130 versus €268 [p<0.001]). In total, mean costs in the online group are €202 lower per participant during the study period (medical savings €138; non-medical savings €64).

DISCUSSION

Our results indicate that the online consultation for children and young adults with CD is an effective and satisfactory instrument for self-management of their disease. To the best of our knowledge, this is the first study investigating a self-management intervention in this specific population on a physical, psychological, nutritional and economic level. Symptoms were recognized significantly more often in the online than the outpatient consultations. Abnormal growth was similarly recognized through both approaches. Moreover, online consultations increased the CD-specific HRQOL while its mean costs were lower compared with the costs of traditional care.

The online participants had reported significantly more abdominal pain and lassitude than the controls. It is unlikely that such difference occurred by chance but may be partly explained by different methods to assess symptoms in both groups. The online group responded to a multiple-choice questionnaire while the outpatient participants responded to the physician's verbal questioning. In paediatrics, it has been reported that parents are most likely to present the child's problem to the paediatrician instead of the child him/herself, and that paediatricians tend to listen more to the parents than the child.[22] In a study among asthmatic children, there was a large discrepancy between physician assessment and the child's description of disease control.[23] In 42% of the children who described their asthma as uncontrolled, physicians assessed the asthma to be well-controlled, just as 73% of the parents reported good control, while their children disagreed.[23] In our study, parents accompanied all the (paediatric) controls during outpatient clinic visits. In the online group however, 36% of the parents specifically indicated that they had completed the consultation together with the child, and 8% of the children had completed it without their parents. In the open narratives the parents described the online questionnaire frequently initiated a conversation with their child, and that their children told them about symptoms parents were not always aware of. This may account for the higher symptom frequency in this group.

Results from the POC self-test for TG2A assessment show the used test is unfit for the population under study. The used self-test has been validated for CD screening, with reported sensitivities and specificities of ≥94% and ≥93%, respectively.[24, 25] However, its efficiency or that of the other POC self-tests for monitoring treated CD has not yet been prospectively evaluated when this study commenced in 2011. The results of 2 studies published in 2013, comparing conventional laboratory results with POC tests other than the one used in our study, showed sensitivities of 63% and 84% in treated CD.[26, 27] The false-negative POC tests concerned samples with antibody titres near the cut-off of normality.[26, 27] Since these low titres are expected in CD patients occasionally making dietary transgressions, this group requires a very sensitive POC self-test. The preliminary data of ongoing comparative studies using different POC tests to monitor children with treated CD indicate that some of them are suitable for this population. The vast majority of our participants favoured the POC self-test rather than the conventional venepuncture (80%), implying that these patients would welcome the implementation of a properly functioning self-test.

The lack of correlation between TG2A results and self-reported dietary adherence are in agreement with those previously reported: in a group of 15 adult CD patients from the Netherlands (9 untreated, 6 making dietary transgressions), only 1 patient had a positive serum IgA TG2A.[28] Moreover, serum TG2A was positive in <50% of a group of Italian patients who reported dietary transgressions,[27] suggesting that patient interviews are better to evaluate

compliance. Nevertheless, a positive serum TG2A is common in patients with self-reported 'strict adherence'. They should be referred to a dietician to evaluate their dietary gluten content.[6] Moreover, our results show that only a moderate agreement exists between the results of the validated questionnaire to assess GFD adherence and the participants' responses to: "I follow a strict gluten free diet". We did not investigate the reason for this discrepancy.

The improvement in CD-specific HRQOL observed after the online consultation, but not after the outpatient consultation, is consistent with the previously described positive effect of SMSs on patients' coping strategies in relation to their chronic disease.[8, 9] The online consultation had a positive effect on the participant's attitudes towards the GFD and communicating their thoughts about their CD. Participant satisfaction with the online consultation indicates that the optimal combination of online and outpatient care will be different for every patient. In studies with chronically ill adults, age and disease duration have been associated with patient activation for e-health.[29, 30] It may be hypothesized that in our cohort, young adults who have been diagnosed earlier would use the online consultation with ease to manage their disease while those who were recently diagnosed (or their parents) would be less enthusiastic. However, these factors were not associated with their satisfaction. Information about other factors that may have been associated with patient motivation for e-health, such as (parental) educational level or place of residence, was unavailable in our study.[29, 30]

Medical and non-medical costs of CD follow-up were lower in the online group than in the controls. In other chronic diseases, the impact of various SMSs on costs has been less conclusive. Some reported positive impacts but others reported increased costs or healthcare usage.[8, 31-33] In our study, we found that costs for healthcare usage after online consultations were not increased when compared with controls (Table 5). It may be argued that the costs of the development and maintenance of the ICT-system for the online consultation should have been included in our analysis.[32] However, these costs should be distributed over a larger group of users during a longer time period. Moreover, our online consultation is not designed to supplement, but substitute, the traditional outpatient consultation. In order to incorporate SMS into the healthcare system for CD patients, rendered online services must be reimbursed. In the Netherlands, for example, it is possible to register screen-to-screen contact as part of a treatment plan but payment has not yet been resolved. By showing that an online consultation is cost-saving, our results suggest a return on investment, thereby supporting its reimbursement.

Strengths of our study include its randomized design, comparing an online consultation with traditional care in a multicentric setting, including academic and non-academic hospitals.

While a previous online intervention successfully targeted increasing the GFD adherence and knowledge in Australian CD patients,[34] our study is the first to evaluate an online SMS for CD follow-up, taking into account the participant's physical condition, CD-specific HRQOL, dietary adherence and satisfaction, and providing a cost analysis. HRQOL, dietary adherence and satisfaction were assessed using validated questionnaires. In addition, we did not only consider the expenses incurred by the healthcare sector but also that of the participants. National and international guidelines recommend annual testing for anaemia and determinations of calcium, folic acid, vitamins D and B12 levels.[1, 6, 35-37] One may argue that these parameters were not evaluated in the online consultation. However, evidence for the practice of checking such parameters is weak since there is limited information on the incidence of nutritional deficiencies in patients with treated CD.[38] Despite this finding, some patients (or their parents) refused to participate in the study because of the possibility that they (or their child) would be randomized to the group without the aforementioned blood tests.

Patients who chose to participate in our study may have had a positive attitude towards e-health. This could impact on the generalizability of our results. As we did not inquire patients' reasons for refusal (51%), we remain unaware of their attitude towards e-health. On the other hand, comparing our participation (49%) and drop-out rates (8%) with other studies evaluating online interventions in chronic diseases (participation rates of 13-42%; attrition rates of 12-37%), our study's recruitment and follow-up were quite successful.[34, 39-41] Moreover, the lower percentage of drop-outs in the online group than in the controls (6% versus 11%) stands in contrast with the findings of a systematic review on e-health interventions in gastroenterology, showing higher drop-out rates in the intervention group.[42] Controls returned for their follow up visits significantly later than online participants, possibly because the online group was already familiar with using the POC self-test.

In conclusion, our study showed that online consultations for children and young adults with CD are satisfactory and cost-saving instruments that increased CD-specific HRQOL. Before implementing online consultations in the follow-up of CD patients, a POC self-test that is sensitive enough to detect low positive TG2A levels is required, as these are common in patients with treated CD secondary to dietary transgressions. Furthermore, efforts should be made to arrange reimbursement of online consultations as part of the treatment plan for CD patients.

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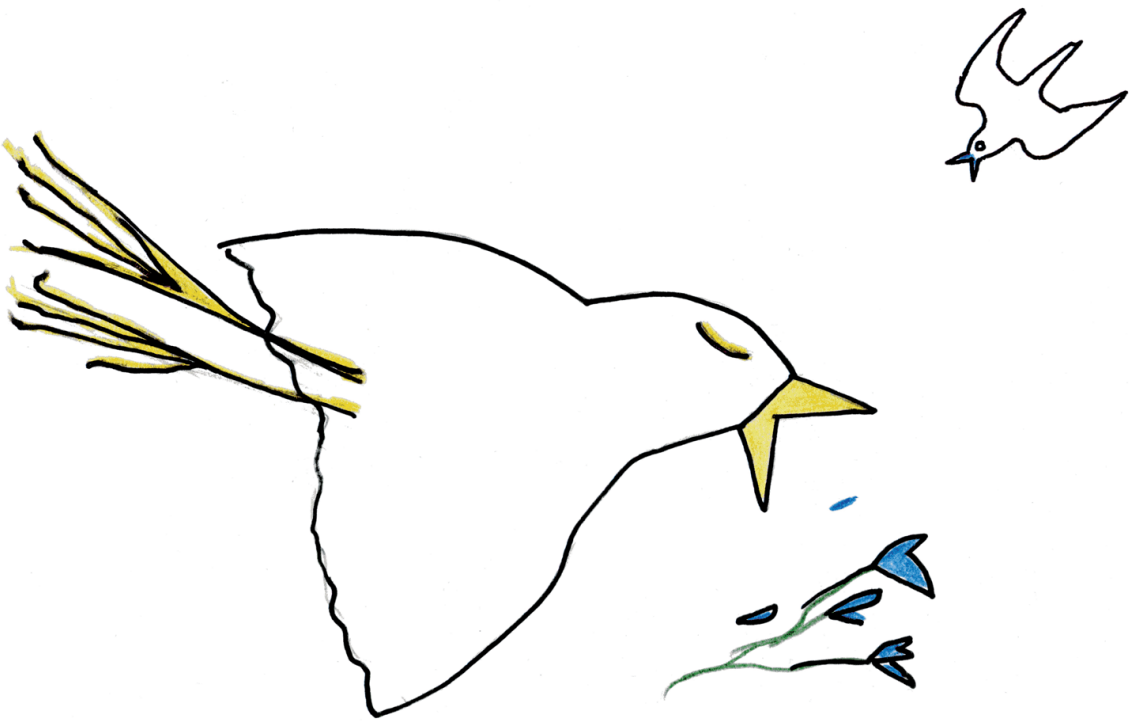

5

ACCURACY OF THREE COMMERCIALLY AVAILABLE POINT-OF-CARE TESTS IN MONITORING COELIAC DISEASE

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Submitted

ABSTRACT

Objective

To evaluate and compare three different, commercially available point-of-care (POC) tests for anti-tissue transglutaminase (TG2A) in children with treated coeliac disease (CD) against results of conventional TG2A at the laboratory using ELISA.

Design

Cross-sectional study, evaluating three different POC tests (X, Y and Z) on 142 blood samples from IgA-competent CD patients aged ≤18 years, attending the paediatric gastroenterology outpatient clinic of Leiden University Medical Center, the Netherlands. Results were evaluated while blinded to the outcome of conventional TG2A assessment (EliA™ Celikey™ IgA test) at 10 and 30 minutes, and 1 day after performing the test (T10, T30 and T1d respectively). We calculated the sensitivity and specificity at 95% confidence intervals (CI) and the negative and positive predictive values. A test was considered acceptable if its sensitivity was ≥90%.

Results

Serum TG2A was positive in 47/142 samples. Test Y* had a greater sensitivity than the other 2 evaluated tests (89% [95% CI 0.81-0.98] versus test X: 34% [95% CI 0.20-0.48] and Z: 55% [95% CI 0.41-0.70], and its sensitivity was 96% [95% CI 0.90-1.0]) when results were read one day after the test was conducted. Prolonging the reading time from T10 to T30 significantly improved the performance of tests X and Z in case of positive serum TG2A (sensitivity test X 62% [95% CI 0.48-0.76], p<0.001; and test Z 70% [95% CI 0.57-0.83], p=0.016) but for test Z this was associated with a drop in specificity.

Conclusions

The studied POC tests have different sensitivities for the relatively low positive TG2A in treated CD patients. Prolonging the reading time may improve a test's performance. To implement POC tests in the follow-up of treated CD patients, we recommend the use of tests that have been validated in this specific group of patients.

* Celiac quick test for IgA, IgG and IgM TG2A (Biohit Oyj, Helsinki, Finland)

INTRODUCTION

Coeliac disease (CD) is a chronic disorder of the small intestine and other organs of genetically susceptible individuals, affecting 1-3% of the Western population.[1] The disease is characterized by the production of autoantibodies against a.o. transglutaminase type 2 (TG2A), among others, during a period of gluten ingestion, which usually disappear 9-12 months after a gluten free diet (GFD) is initiated.[2-5] Serum detection of TG2A is therefore not only useful to screen and diagnose CD but also to monitor a patient's remission *and* dietary adherence.[6-9] TG2A serum testing is part of standard care in the CD follow-up.[9-12] However, TG2A measurement requires specialized laboratories and the results are not immediately available. Readily available TG2A results during consultations with the physician would facilitate doctor-patient communication about dietary adherence. Self-testing can empower patients in the management of their disease (chapter 4). The call for point-of-care (POC) testing, defined as performing a diagnostic procedure outside the laboratory,[13] has resulted in the commercial availability of several POC tests for TG2A. These tests obviate the need for purified or recombinant transglutaminase type 2 (TG2) or for serum separation because TG2 is also found in red blood cells (RBCs). Therefore, the patient's own TG2 can be used in TG2A detection by haemolysing a whole blood sample and liberating the self-TG2 from the RBCs. Tests can be performed at home and results become available within 10 minutes, which may save costs and prove to be more convenient for the patients (Chapter 4).[14-17] Several studies have investigated the accuracy of POC tests based on TG2A for CD screening and diagnosing, and sensitivities and specificities similar to those of determination of TG2A in serum were reported (70.1- 97% and 76-100% respectively).[14, 16-23] There is, however, less consensus over the accuracy of POC testing to monitor CD once treatment has been initiated.[20] These patients usually have less TG2A titers than untreated patients.[24] Subsequently, the aim of this study was to compare the performance of three different, commercially available POC tests against the serum TG2A of CD-affected children treated with a GFD.

METHODS

Study design and patients

In this cross-sectional study, we performed 3 different POC tests on IgA-competent CD patients aged ≤18 years who attended the paediatric gastroenterology outpatient clinic of Leiden University Medical Center (LUMC). The study ran from March 28, 2014 to August 18, 2015. Patients were included if all inclusioncriteria were met: 1) CD diagnosed according to the guidelines of the European Society of Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN); [9] 2) GFD initiated prior to POC testing; and 3) TG2A determined at the

hospital laboratory as part of standard care.[12] Information about a patient's sex, age and disease duration was obtained. All identifying details were encoded.

POC tests

POC testing was performed on-site by using 30uL (10uL per POC test) of fresh waste blood from a venous whole blood sample that remained in the needle system after venipuncture for conventional TG2A determination. The blood was not coagulated or dried. The three POC tests that were compared against the conventional TG2A results are henceforth referred to as tests X, Y and Z, respectively. Using a lateral flow immunochromatographic strip system, the diluted blood sample migrates through the test strip. The principles of the different tests vary. For test X, if TG2A are present in the blood sample, they form complexes with the liberated self TG2. These complexes bind to a solid surface protein coated with TG2-capturing proteins. A red/pink test line becomes visible with the help of labelled anti-human IgA solution. In tests Y and Z, TG2A (IgA, IgG for test Z and IgA, IgG and IgM for test Y) antibodies in a blood sample react with human recombinant TG2 labelled with latex particles. These latex particle-TG2-TG2A complexes reach the reaction zone through a chromatographic process, where immobilised human TG2 captures the complex, forming a red/pink line. In addition, an integrated control system checks proper function of the test by showing a control line. A positive test result shows two lines (test and control line), while only one line (the control line) appears if the test is negative. A test result with no control line is invalid and cannot be used.[25, 26] According to the manufacturers, the test results should be evaluated after 5-10 minutes. Three of this study's authors, after being trained to assess the POC tests, evaluated and photographed the test results on-site in a well-lit room to determine the test result after 10 minutes, but also after 30 minutes and 1 day to assess the stability of the test result. Another trained author who was blinded to the outcome of the on-site result interpretation and without having any clinical information, evaluated the pictures of the POC test results. For assessment of interobserver agreement, the interpretations of the on-site and photographed test results were compared.

Conventional serologic testing

The results of IgA TG2A serum testing at the hospital laboratory were used as the gold standard. Serum IgA-class TGA were determined with the ELISA technique, using EliA™ Celikey™ IgA test (Phadia GmbH, Freiburg, Germany) following the manufacturer's instructions. A value of <7 units per milliliter (U/ml) was considered negative while ≥7 U/ml was deemed positive. The test has a measuring range of 0.1 - ≥128 U/ml. Results were available after approximately 1 week.

Statistics

For the POC tests, a sensitivity of 90% or higher is considered acceptable. The power calculation is made based on the non-inferiority principle, using test Y. Assuming that the real sensitivity of the POC test equals 98% and assuming a required power of 80%, we needed to include a minimum of 45 patients who would test positive on the gold standard for TG2A determination. In our practice, approximately 35% of the treated CD patients have elevated TG2A yearly, including those in their first year after initiating the GFD. This means that the expected number of samples which we had to test was estimated to be 129 ((10/3.5)*45) Results of each POC test after 10 minutes, 30 minutes and 1 day were used to calculate the test's sensitivity and specificity (with 95% confidence intervals [95% CI]) using two different approaches: uninterpretable test results were considered "negative" in the case of a positive gold standard, and as "positive" in the case of a negative gold standard ("worst case" sensitivity and specificity); and when uninterpretable testresults are omitted ("conventional" sensitivity and specificity). In the samples with a positive gold standard, the McNemar test was used to compare the performance of each POC test when reading time was prolonged to 30 minutes or to 1 day, and to compare the performance of the different POC tests' with each other. Inter-observer agreement was evaluated using Cohen's Kappa (K). Analyses were performed with SPSS software (version 23.0).

Ethics

The study protocol was approved by the medical ethical committee of the LUMC. No informed consent was needed because residual blood was used and TG2A serum determination is standard care. This study is performed according to the Standards for the Reporting of Diagnostic Accuracy studies (STARD). The manufacturers of the POC tests used in this study provided the tests free of charge for the purpose of the study. They were neither directly or indirectly involved in the study design or conduct, nor in the analysis of the results or preparation of the manuscript.

RESULTS

There was a total of 144 blood samples obtained. In 2/144 samples, the amount of blood was insufficient to perform all three POC tests. Therefore, 142 blood samples from 122 different patients were analysed (testing on 1, 2 or 3 separate occasions during the study period in N=104, N=16 and N=2, respectively). Patient characteristics at time of blood withdrawal are presented in Table 1. Results of the gold standard for TG2A testing were positive in 47/142 samples (33%). These belonged to patients with significantly shorter disease duration than patients with a sample negative for TG2A (Table 1). The inter-observer agreement between interpretation of on-site and photographed POC test results was substantial (0.80, 0.70, and

Table 1 Characteristics of 122 individual patients with coeliac disease (CD) whose blood samples were withdrawn and tested for anti-transglutaminase type 2 antibodies (TG2A) with the conventional ELISA in the hospital laboratory on 142 occasions, split out for and compared between positive and negative TG2A results in serum (cut-off for normality ≥7 U/ml).

Patient characteristic	All samples (n=142)	Positive TG2A samples (n=47)	Negative TG2A samples (n=95)	*p*-value
Female gender – n (%)	91 (64.1)	30 (63.8)	61 (64.2)	0.97
Age in years – mean (± SD)	9.7 (4.3)	9.8 (4.6)	9.6 (4.1)	0.93
Duration of CD in years – mean (± SD)	4.4 (4.2)	1.9 (3.0)	5.7 (4.1)	<0.001
Serum TG2A titer in U/ml – mean (± SD)	12.5 (24.6)	34.8 (33.1)	1.47 (1.48)	<0.001

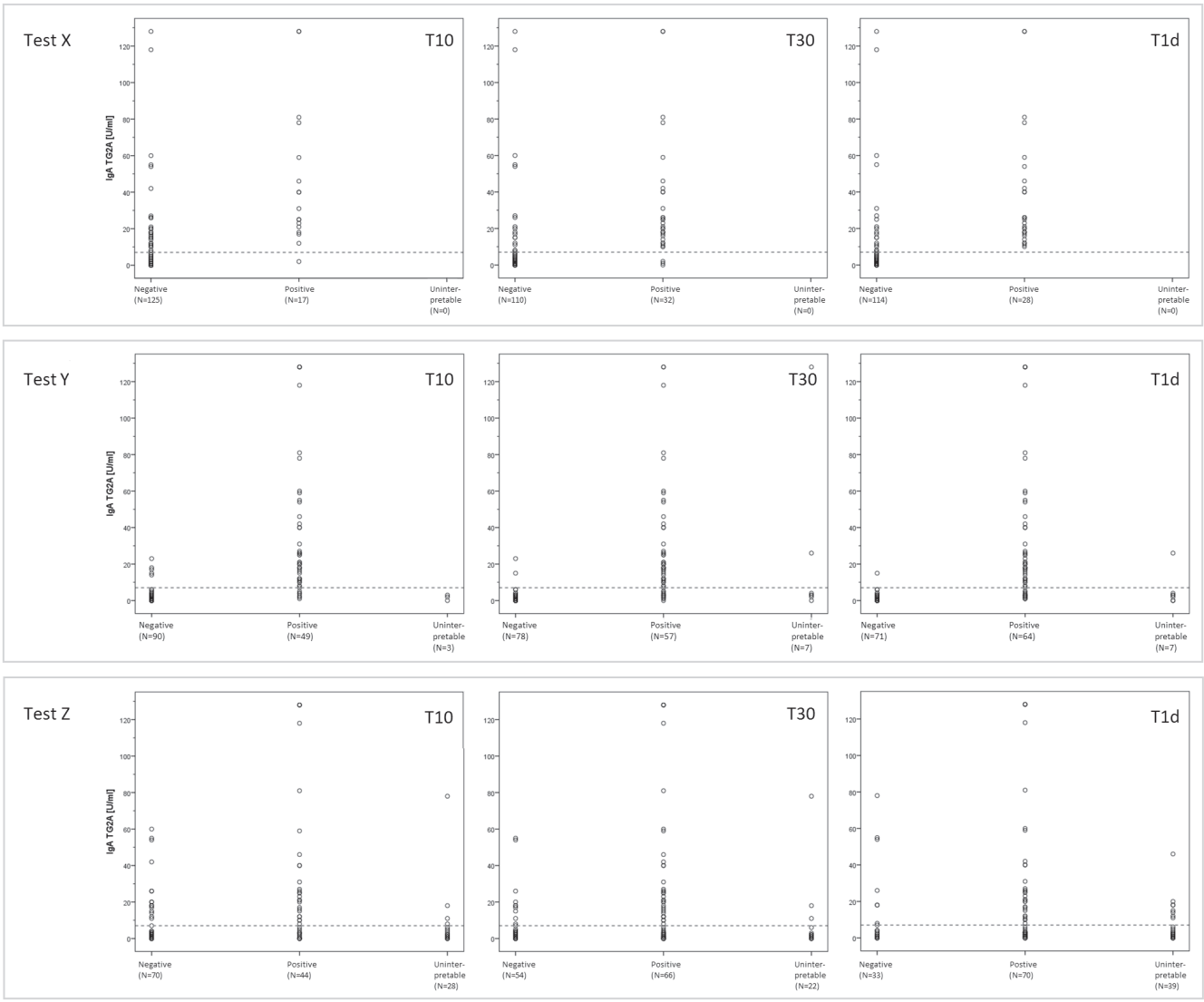

Figure 1 Correlation of the results of IgA anti-transglutaminase type 2 (TG2A) in U/ml measured in the laboratory (y-axis) and results of the point-of-care tests (x-axis) at 10 minutes (T10), 30 minutes (T30) and 1 day (T1d) after applying the blood sample. The horizontal dotted line represents the cut-off for normality of TG2A as measured in the laboratory (7 U/ml).

0.66 for tests X, Y and Z, respectively). The results of the POC tests after 10 minutes (T10), 30 minutes (T30) and 1 day (T1d) compared with the gold standard are presented in Figure 1 and in Table 2. Uninterpretable test results only occurred in tests Y and Z, and were due to the absence of a control line or appearance of a red haze in the test window.

When analysing the results at T10, as recommended by the manufacturer, test X was considered as false-negative in 31 samples, with serum TG2A titers ranging from 7-128 U/ml (mean 27.84 U/ml) (Figure 1). Test Y was false-negative in five tests, correlating with TG2A levels ranging from 14-23 U/ml (mean 17.40 U/ml) (Figure 1). The three uninterpretable tests Y concerned samples with negative serum TG2A (Figure 1). Test Z was false-negative in 17 tests, with serum TG2A ranging from 7-60 U/ml (mean 25.47 U/ml) and the 28 uninterpretable tests occurred in samples with serum TG2A titers ranging from 0-78 U/ml (mean 5.43 U/ml). The outcome of all three tests (false negative or true positive) was not influenced by time interval since CD diagnosis (p=0.572, 0.323, 0.125 for tests X, Y and Z, respectively) or the age of the participant (p=0.492, 0.569, 0.797 for tests X, Y and Z, respectively). In addition, the outcome of tests X and Z (false negative or true positive) was uninfluenced by the TG2A serum titer (p=0.962 and 0.461 respectively). This in contrast to test Y, where serum TG2A levels of tests with a false negative outcome were significantly lower than those of tests with a true positive outcome (17.40 U/ml versus 36.80 U/ml, p=0.027). When considering the "worst case" scenario (i.e. uninterpretable test results considered "negative" in the case of a positive gold standard and as "positive" in the case of a negative gold standard), the sensitivities of all 3 tests were less than 90% at T10, with the best outcome for test Y* (89% [95% CI 0.81-0.98]) and poorest outcome for test X (34.0% [95% CI 0.20-0.48]) (Table 2).

When confining to those samples with a positive gold standard (N=47), performance of test X and Z increased significantly when reading time was prolonged from T10 to T30 (positive tests X: N=16 to N=29, p<0.001; positive tests Z: N=26 to N=33, p=0.016) or from T10 to T1d (positive tests X: N=16 to N=28, p=0.004; positive tests Z: N=26 to N=31, p=0.031). However, their performance at T1d was not significantly improved when compared with T30 (test X p=1.000; test Z p=0.500). The improvement in the performance of test Y when reading time was prolonged to T30 or T1d, was not statistically significant (p=0.250 and p=0.125 respectively). Nevertheless, test Y is the only test whose sensitivity reached a value of over 90% (T30: 91% [95% CI 0.84-0.99] and T1d: 96% [95% CI 0.90-1.0]) (Table 2). Furthermore, test Y performed significantly better than X and Z at all evaluated time points in case of positive serum TG2A (T10: Y versus X p<0.001, Y versus Z p=0.002; T30: Y versus X p<0.001, Y versus Z p=0.021; T1d: Y versus X p<0.001, Y versus Z p=not applicable because all 39 interpretable results of test

* Test Y = Celiac quick test for IgA, IgG and IgM TG2A (Biohit Oyj, Helsinki, Finland)

Table 2 Comparison of 3 point-of-care (POC) tests for anti-transglutaminase type 2 antibodies (TG2A) with the TG2A result in conventional laboratory (gold standard). Sensitivity and specificity (95% confidence intervals [95% CI]) were calculated with or without taking the un-interpretable test results into account (worst case [A] and conventional [B] respectively). Results are given after an evaluation time of 10 minutes, 30 minutes and 1 day.

			TG2A in conventional laboratory						
			Positive (N=47)	Negative (N=95)	Total	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
Test X	10 min	Positive	16	1	17	0.34 (0.20-0.48)	0.99 (0.97-1.0)	0.94 (0.83-1.00)	0.75 (0.68-0.83)
		Un-interpretable	0	0	0				
		Negative	31	94	125				
	30 min	Positive	29	3	32	0.62 (0.48-0.76)	0.97 (0.93-1.0)	0.91 (0.81-1.00)	0.84 (0.77-0.91)
		Un-interpretable	0	0	0				
		Negative	18	92	110				
	1 day	Positive	28	0	28	0.60 (0.46-0.74)	1.00	1.00	0.83 (0.76-0.90)
		Un-interpretable	0	0	0				
		Negative	19	95	114				
Test Y	10 min	Positive	42	7	49	A: 0.89 (0.81-0.98) B: 0.89 (0.81-0.98)	A: 0.92 (0.83-0.96) B: 0.92 (0.87-0.98)	0.86 (0.76-0.96)	0.94 (0.90-0.99)
		Un-interpretable	0	3	3				
		Negative	5	85	90				
	30 min	Positive	43	14	57	A: 0.91 (0.84-0.99) B: 0.96 (0.90-1.0)	A: 0.80 (0.72-0.88) B: 0.84 (0.77-0.91)	0.75 (0.64-0.87)	0.97 (0.94-1.00)
		Un-interpretable	2	5	7				
		Negative	2	76	78				
	1 day	Positive	45	19	64	A: 0.96 (0.90-1.0) B: 0.98 (0.94-1.0)	A: 0.74 (0.65-0.83) B: 0.79 (0.70-0.87)	0.70 (0.59-0.82)	0.99 (0.96-1.00)
		Un-interpretable	1	6	7				
		Negative	1	70	71				

Table 2 (continued)

			TG2A in conventional laboratory						
			Positive (N=47)	Negative (N=95)	Total	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
Test Z	10 min	Positive	26	18	44	A: 0.55 (0.41-0.70) B: 0.60 (0.46-0.75)	A: 0.56 (0.46-0.66) B: 0.75 (0.65-0.85)	0.59 (0.44-0.74)	0.76 (0.66-0.86)
		Un-interpretable	4	24	28				
		Negative	17	53	70				
	30 min	Positive	33	33	66	A: 0.70 (0.57-0.83) B: 0.77 (0.64-0.89)	A: 0.45 (0.35-0.55) B: 0.57 (0.45-0.68)	0.50 (0.38-0.62)	0.80 (0.69-0.90)
		Un-interpretable	3	19	22				
		Negative	11	43	54				
	1 day	Positive	31	39	70	A: 0.66 (0.53-0.80) B: 0.79 (0.67-0.92)	A: 0.26 (0.17-0.35) B: 0.39 (0.27-0.51)	0.44 (0.33-0.56)	0.76 (0.61-0.90)
		Un-interpretable	8	31	39				
		Negative	8	25	33				

Z were positive with test Y). Test Z performed significantly better than test X at T10 (p=0.007), but both tests performed similarly at T30 and T1d (p=0.180 and 0.096, respectively).

DISCUSSION

This study evaluated and compared three different commercially available TG2A POC tests in children with treated CD against conventional serologic TG2A testing. Our results show that test Y had a greater sensitivity than the other 2 POC tests, and that its sensitivity was acceptable (95% CI excluded values <90%) if reading time was prolonged from 10 minutes to 1 day. Implementation of such a test in a clinical setting may reduce the frequency of venipuncture for conventional TG2A testing in children (and probably also in adults) with treated CD and could improve self-management of their disease. An important advantage of a POC test is its potential to be a more rapid alternative to conventional serologic testing. To accommodate the longer reading time of test Y, well instructed patients could do the test at home on the day prior to their outpatient clinic visit and bring the test for evaluation by their physician. This allows for an on-the-spot management decision in case of a positive result. This may include, for instance, conventional serologic testing, dietetic counselling on GFD adherence, and discussion with the physician on the harmful effects of gluten ingestion. Furthermore, the use of a POC test instead of the conventional TG2A testing may have health cost-saving implications, and a finger-prick is considered less invasive than a venipuncture, particularly for children (Chapter 4).[27]

Our cohort's prevalence of positive TG2A in serum (according to conventional serology) of 33% is slightly higher than what is reported in literature, with a reported prevalence that ranges from 12-32%.[18, 28-32] In contrast to most of these studies, however, we also included children diagnosed with CD less than 1 year and therefore with a high likelihood of having positive serum TG2A.[5]

A high sensitivity and negative predictive value (NPV) are crucial for monitoring CD activity, leading to fewer missed cases of uncontrolled CD. The specificity and positive predictive value (PPV) are considered to be less important in this situation, although they do influence the efficiency of a POC test. Tests X and Y had been validated in CD screening, with reported sensitivities compared to biopsy of 72.2-96.7% for test X,[17, 19, 22, 27] and 77.8% for test Y [27]. The lower sensitivities in our study could be explained by the fact that antibody titers in treated CD are typically lower than in untreated CD cases identified by screening or case-finding. However, in a study with 15 patients treated with a strict GFD for 1 year, test X was used and compared with the outcome of serum TG2A testing.[19] In 13/15 patients, test X was negative, as well as serum TG2A. In the remaining 2 positive tests, serum TG2A values

were considered borderline (4.2 and 4.6 U/mL).[19] In addition, in the same study 91 long-term treated coeliac disease patients (median duration of a strict gluten-free diet = 9 years, range 1–24 years) were tested for TG2A with test X and conventional laboratory: 88 (96.7%) were negative with test X and 90 (98.9%) with the conventional method.[19] As virtually all tests were negative for serum TG2A, this study does not provide enough information about the performance of this test in case of low positive serum TG2A. Another study with test X suggested that prolonging the reading time to 10-15 minutes increased the sensitivity as faint test-lines became clearer.[16] While we also observed this phenomenon in our study, it did not result in an acceptable sensitivity for test X. The results of two studies published in 2013, comparing conventional laboratory results with POC tests other than the ones used in our study, showed sensitivities of 63% and 84% in treated CD.[24, 33] The false-negative POC tests concerned samples with antibody levels near the cut-off of normality.[24, 33] While we also observed this in our study for test Y, tests X and Z yielded false-negative results in samples with serum TG2A up to 128 U/ml and 60 U/ml respectively. Based on these results, we may conclude that all three tests have different cut-offs of normality. Another explanation for their difference in performance may be attributed to the varying principles of each of them. Test X uses self-TG2 while tests Y and Z use recombinant human TG2. Self-TG2 is known to be a sensitive protein. If the protein is damaged, it may be unable to form immunocomplexes with the serum auto-antibodies.[17] It is recommended to avoid using blood that has been frozen and thawed multiple times, or blood that is coagulated or dried.[17] In our study, however, fresh blood was used on-site and directly from the needle system. As the blood was not coagulated or dried, it seemed unlikely that the self-TG2 was damaged. Furthermore, test X only detects IgA TG2A; while test Z also detects IgG TG2A and test Y detects IgA, IgG and IgM TG2A. However, the added value of IgG and IgM antibody measurements in our cohort is questionable since all our patients were IgA sufficient and all 47 patients with positive conventional ELISA had positive serum TG2A of the IgA class. Thus, IgA TG2A was present in all samples that yielded false-negative results.

In a previous study from our hands, evaluating the effectiveness of online consultations for follow-up of children and young adults with CD, we found that test X was not sensitive enough for home measurements of TG2A in the follow-up of children and young adults with treated CD. The results of our present study confirm our previous findings. To the best of our knowledge, we are the first to report that prolonging the reading time of test X improves its performance in case of positive serum TG2A. A possible explanation could be that the haemolysation process, releasing self-TG2 from the erythrocytes, is not completely finished after 10 minutes. However, at its peak performance (T30), test X still failed to recognize almost 40% of the positive samples, indicating that prolonging the reading time from 10 to 30 minutes or 1 day will not make the test suitable for utilization in patients with treated CD. For test Z, we do not recommend prolonging reading time to 30 minutes or 1 day due to

the associated significant drop in specificity. Furthermore, test Z showed a high number of uninterpretable test results (N=28 after 10 minutes) caused by the absence of a control line in 27 of them. As these tests' expiration dates have not yet passed and this event occurred in tests that were all from the same batch, this was almost certainly a manufacturing defect. Nevertheless, when these uninterpretable results were disregarded, the sensitivity of test Z at T10 was still unacceptable for follow-up of treated CD (conventional sensitivity 0.60; 95% CI 0.46-0.75).

The strengths of our study include a design wherein the same trained personnel carried out three different commercially available POC tests on the same blood sample and evaluated the outcome, blinded to the results of the conventional ELISA that was performed in the same laboratory throughout the study. Additionally, we used the widely implemented EliA™ Celikey™ IgA test (Phadia GmbH, Freiburg, Germany) as the gold standard to measure serum TG2A, thereby increasing the generalizability of our results. To the best of our knowledge, we are the first to report the results of three different commercially available POC tests in monitoring treated CD. One may argue that we should have included adherence to the GFD as a variable in our analysis. However, as an objective tool to assess dietary adherence is not available, serum TG2A and dietary adherence do not always agree.[24, 34] Furthermore, it may be argued that we assessed inter-observer variability by comparing the on-the-spot evaluation with the interpretation of a photograph of the result. While the agreement between these methods was acceptable for all three tests, the positive tests with a faint test line were difficult to capture on a photograph. However, our results suggest that photographs of POC test results have the potential to be used as an alternative for on the spot evaluation of the results. This could be useful in self-management programmes, wherein CD patients could send a photograph of the test result to their physician. Alternatively, a reader could be developed that will interpret the photographed test result, minimizing the risk of observer bias.

In conclusion, we found that out of the 3 studied POC tests for TG2A, the Celiac Quick Test for IgA, IgG and IgM TG2A (Biohit Oyj, Helsinki, Finland) had the highest sensitivity for TG2A in children with treated CD. We recommend reading this test's results a day after performing it and that further validation studies be carried out in patients with treated CD.

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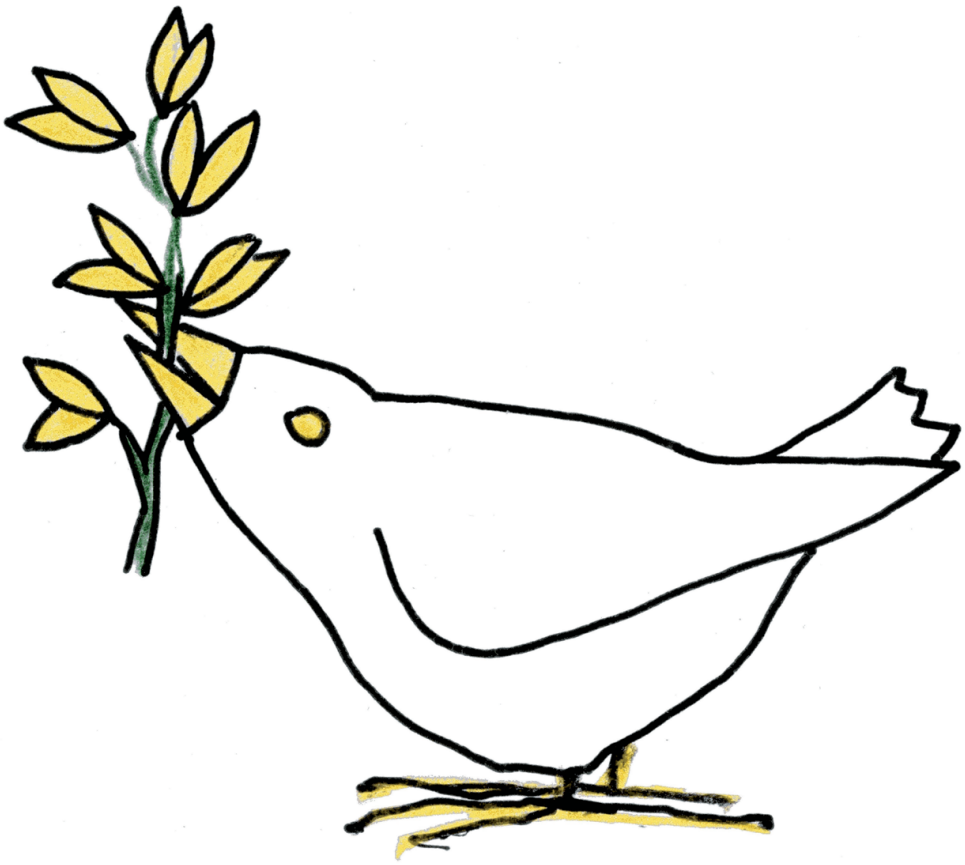

6 | GENERAL DISCUSSION AND CONCLUSION

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Is it time for mass screening for celiac disease?
Ned Tijdschr Geneeskd. 2015 159:A9110.

Coeliac disease is a common condition with a variable presentation, and is frequently not recognized by physicians. Although a gluten-free diet has a positive effect on the health of the coeliac patient, prevention would be even more beneficial. Accordingly, the first aim of this thesis was to investigate the influence of infant feeding on the prevention and development of childhood coeliac disease.

Primary prevention of coeliac disease means that tolerance for gluten needs to be achieved or maintained. As mentioned in the introduction of this thesis, results from previous retrospective studies suggested that the introduction of gluten between the age of 4 months and 6 months represents a window of opportunity for preventing coeliac disease.[1, 2] Based on these results both Dutch and European guidelines recommended introducing gluten not before the age of 17 weeks and not after the age of 26 weeks, preferably during a period of breastfeeding. However, the results of the PreventCD study, which are presented in **chapter 2**, did not show the hypothesized benefit of early exposure to small quantities of gluten with regard to reducing the incidence of coeliac disease among children from high risk families. In addition, maintenance of breastfeeding at the time of gluten introduction, and breastfeeding in general, did not reduce the risk of this disorder. Although we want to underscore the overall importance of breastfeeding for child health, our results may alleviate the stress that is placed on mothers, especially those from coeliac families, who are unable to breastfeed their baby: it will not increase their baby's risk for coeliac disease.

Our results are in agreement with those of other recent prospective studies (CELIPREV, Generation R, MoBa, and TEDDY).[3-6] CELIPREV is a prospective multicenter intervention study with Italian children who have a familial risk for coeliac disease, followed from birth and randomized to gluten introduction at the age of 6 months or 12 months.[5] Delaying gluten introduction and breastfeeding did not modify the risk of coeliac disease, although gluten introduction at 12 months was associated with a delay in disease onset. The HLA genotype was an important predictor of disease. Furthermore, the results of the Dutch 'GenerationR Study', a Rotterdam population-based prospective cohort study from foetal life until young adulthood, showed that the risk of coeliac disease autoimmunity is not influenced by introducing gluten after the age of 6 months, or by breastfeeding during the first 6 months of life.[4] The Norwegian Mother and Child Cohort Study (MoBa) is a prospective population-based pregnancy cohort study, conducted by the Norwegian Institute of Public Health.[6] Introducing gluten after the age of 6 months and breastfeeding past the age of 1 year were associated with a modest increase in the clinical diagnosis of coeliac disease. Gluten introduction during the period of breastfeeding was not protective. The Environmental Determinants of Diabetes in the Young (TEDDY) is a multinational study with children at high risk for type 1 diabetes and a genetic predisposition of coeliac disease (HLA-DQ2/DQ8).[3] Gluten introduction before the age of 17 weeks or after the age of 26 weeks was

not associated with an increased risk of coeliac disease. Finally, a systematic review that included the aforementioned prospective studies concluded that breastfeeding and timing of gluten introduction have no effect on the risk of developing coeliac disease during childhood, necessitating for an update of the current European guidelines.[7] These guidelines were been prepared by the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) in collaboration with the PreventCD group, and recommend that gluten can be introduced into the infant's diet between the ages of 4 and 12 months, but state that the age of gluten introduction in infants in this age range does not seem to influence the absolute risk of developing coeliac disease during childhood.[8] Furthermore, according to the guideline, introducing gluten while the infant is being breastfed cannot be recommended as a means of reducing the risk of developing coeliac disease.[8]

While prevention of coeliac disease is not possible at this moment, diagnosing the disease in its earliest stage – secondary prevention – may be a realistic alternative. One way to pursue this is by case-finding: actively seeking for symptoms associated with coeliac disease (see box 1 in **chapter 1**) and a low threshold use of further examinations. Because of the variable presentation of coeliac disease, this will not resolve its underdiagnosis. Therefore, the current evidence-based guidelines recommend screening for coeliac disease in high-risk groups (see box 2 in **chapter 1**). This screening involves determination of coeliac disease specific antibodies against tissue transglutaminase (TG2A) or endomysium (EMA) in serum.[9] As presented in **chapter 2**, TG2A are reliable predictors of coeliac disease, also in very young children.[10] First-degree relatives of coeliac patients have an elevated risk for coeliac disease of 2-20%, depending on sex and HLA-genotype.[10] Furthermore, coeliac disease appears at a very young age (<3 years old) in children with a first-degree relative with coeliac disease: In children diagnosed with coeliac disease and a positive family history, 50% had developed the disease by age 3 years, implying that one should start with screening prior to this age.[10] In addition, coeliac disease has a female preponderance of 2-3:1 by 3 years of age, with in an incidence of 7.2% in girls with a positive family history versus 3.4% in boys.[10] Children homozygous for HLA-DQ2 clearly have a higher risk of coeliac disease (14.9%) than HLA-DQ2 heterozygous (3.9%) and HLA-DQ8 positive (hetero- and homozygous) children (0.9%), ($p<0.001$).[10]

Would mass screening for coeliac disease be a sensible alternative to case-finding? Ten years ago, when Wilson and Junger's criteria for mass screening were applied to coeliac disease, there was controversy over whether the general population would accept mass screening and whether the health status of minimally symptomatic or asymptomatic patients identified by mass screening improved after treatment.[11, 12] Furthermore, the natural course of the disease and cost-effectiveness of mass screening were unclear.[11, 12] Results of recent prospective studies allow for re-opening of this discussion. The Swed-

ish ETICS-PreventCD study has shown that mass screening for coeliac disease in 13.279 children aged 12-year old was feasible and well-accepted.[1] Results from the 'GenerationR Study' among 6-year old children from the general population provided information about the natural course of coeliac disease. Undiagnosed coeliac disease was found in 1.3% (57/4442 screened children was positive for TG2A) and was associated with important health problems, such as a reduced bone mineral density and a delayed growth in weight.[13] In addition, children of women with undiagnosed and thus untreated coeliac disease had a reduced fetal growth and a lower birth weight.[14] Concerning the costs of mass screening, the results of a study concerning coeliac disease screening in adults showed that screening has a cost-effectiveness ratio of 48.960 USD per QALY. Based on this outcome, the authors suggest that mass screening for coeliac disease is indeed cost-effective.[15] Confirming the diagnosis in asymptomatic patients still requires obtaining small bowel biopsies according to the current evidence-based guideline of the ESPGHAN.[9] This in contrast to patients with coeliac disease associated symptoms, TG2A titres of more than 10 times the upper limit of normal, a positive EMA in a new blood sample and HLA-DQ2/DQ8 positivity. Results from the Swedish ETICS screening study show that this also applies to children diagnosed after screening.[16] The prospective and international PRoCeDe study will provide information about this as well.[17] It would make the process of diagnosing coeliac disease in children identified by screening simpler, less invasive and cheaper. An argument that is frequently used against mass screening is that minimally symptomatic or asymptomatic patients will not adhere to the gluten free diet. However, long-term follow-up of young Dutch children and the results of the study with 12-year old Swedish children disprove this argument: the children were compliant with their diet.[18, 19] Furthermore, a prospective study showed that treatment of coeliac disease diagnosed based on mass screening yields health gains, both for adults and children.[18] In Dutch children with coeliac disease identified by mass screening, chronic symptoms of diarrhoea, abdominal pain, constipation, fatigue, irritability, oral aphthous ulcers and growth failure, improved after diagnosis and treatment.[18] In adult Finnish coeliac patients who were diagnosed after screening, chronic health problems as indigestion, gastro-oesophageal reflux and anxiety improved significantly after treatment with a gluten-free diet.[20] Furthermore, dietary adherence was excellent after screening and there were no significant differences between screening-detected and symptom-detected patients with regard to coeliac disease-health related quality of life.[18, 20, 21] Consequently, secondary prevention may only be achieved on large scale by mass screening of the general population.

Traditional medical care for patients with treated coeliac disease consists of regular physician visits to evaluate their health, weight, height (in children), gluten-free diet adherence and coeliac-specific serum antibodies.[22, 23] Although important, these measures can be time-consuming. Moreover, many patients do not visit their physician for regular follow-

up.[24] Time constraints during outpatient follow-up also typically restrict comprehensive assessments of a patient's health-related quality of life (HRQOL) and dietary adherence. Accordingly, the second aim of this thesis was to explore new strategies for the improvement of care for children and young adults with coeliac disease.

While coeliac disease-specific HRQOL is an accepted outcome within a research environment, insufficient time during actual follow-up visits may prevent the physician from specifically measuring it, and therefore only general assessments can be provided. It has been established that in patients with other chronic diseases, the physician overestimates the patient's HRQOL [25-27]. We are the first to have compared the self-reported coeliac disease-specific HRQOL in a group of children and young adults with the physician's report provided during a regular outpatient follow-up visit for coeliac disease (**chapter 3**). Our results indicate that there is an important discrepancy between these reports since in 57% of the patients, the physician had a different perception of the patients' HRQOL than the patient him/herself. What raises concern is that this occurred among patients considered to be especially vulnerable: those with a "bad" self-reported HRQOL. Our data show that this problem occurs significantly more frequently in those who received the diagnosis within the past 9 years and in female patients, possibly due to their significantly poorer self-reported HRQOL, especially for the "communication" subscale, compared to their male counterparts (p=0.014). Our results concerning the discrepancy between the self-reported and physician-reported HRQOL are consistent with previous studies with children affected by other chronic diseases [25-27]. Our study supports the implementation of a self-reported coeliac disease-specific HRQOL measurement in the clinical follow-up of the patients. Sharing the results of the questionnaire may improve the patient/parent-doctor communication and the physicians' understanding of the needs and priorities of children and young adults with coeliac disease. As the standard consultation time allotted for follow-up visits is limited, the outpatient clinic of the department of paediatric gastroenterology of Leiden University Medical Centre will ask the patient to complete the CDDUX questionnaire prior to physician appointments. If our results are successful, we will advise other hospitals caring for patients with coeliac disease to follow-up on our example.

Previous studies in adults with other chronic diseases suggest that an online self-management e-health system can encourage patients to improve health care participation and the decision-making process.[28] Patients are able to deal with their symptoms, treatment, physical and psychosocial consequences and lifestyle changes that are inherent in living with a chronic condition through successful disease self-management.[29] In **chapter 4**, we presented the results of an online consultation replacing outpatient consultations in the follow-up of coeliac children and young adults (CoelKids). We hypothesized that disease control in the course of the study would be similar in patients using the online consultation

and traditional outpatient follow-up. To the best of our knowledge, this is the first study investigating a self-management intervention in this specific population on a physical, psychological, nutritional and economic level. Our results indicate that the online consultation for children and young adults with coeliac disease is an effective and satisfactory instrument for self-management of their disease. Symptoms were recognized significantly more often in the online than the outpatient consultations, possibly because the online consultation initiated a conversation about symptoms between parent and child that was not restricted by the limited time available for an outpatient consultation. Abnormal growth was similarly recognized through both approaches. Additionally, online consultations increased the coeliac disease-specific HRQOL while its mean costs were lower compared with the costs of traditional care. Results from the POC self-test for TG2A assessment showed the used test is unfit for the population under study. The used self-test has been validated for coeliac disease screening, with reported sensitivities and specificities of ≥94% and ≥93%, respectively. [30, 31] However, its efficiency or that of the other POC self-tests for monitoring treated coeliac disease had not yet been prospectively evaluated when this study commenced in 2011. These patients usually have less high titers of TG2A than untreated patients.[32] Before implementing online consultations in the follow-up of coeliac patients, a POC self-test that is sensitive enough to detect low positive TG2A levels is required, as these are common in patients with treated CD secondary to dietary transgressions.

Triggered by the inadequate performance of the POC self-test used in the CoelKids study (**chapter 4**), we compared the performance of three commercially available POC tests against the serum TG2A of coeliac disease-affected children treated with a gluten free diet (**chapter 5**). Our results show that the sensitivity of one of these three tests was acceptable (95% confidence interval excluded values <90%) if the reading time was prolonged from 10 minutes to 1 day. For all three POC tests, we find lower sensitivities than previous studies using these tests in screening for coeliac disease. This could be explained by the fact that antibody titres in treated CD are typically lower than in untreated CD cases identified by screening or case-finding. Another explanation for their difference in performance may be attributed to the varying principles of each of them; one only tests for IgA TG2A, one for IgA and IgG TG2A and the other for IgA, IgG and IgM TG2A. However, the added value of IgG and IgM antibody measurement in our cohort is questionable since IgA TG2A was present in all samples that yielded false-negative results. Implementation of a POC test for TG2A in a clinical setting may reduce the frequency of venepuncture for conventional TG2A testing in children (and probably also in adults) with treated coeliac disease and could improve self-management of their disease. An important advantage of a POC test is the potential of being a more rapid alternative to conventional serologic testing. To accommodate the longer reading time that is required to obtain an acceptable sensitivity, well instructed patients could do the test at home on the day prior to their outpatient clinic visit and bring the

test for evaluation by their physician. This allows for an on-the-spot management decision in case of a positive result, for example: conventional serologic testing, dietetic counselling regarding the adherence to the gluten free diet and discussion with the physician on the harmful effects of gluten ingestion. Furthermore, as shown in **chapter 4**, the use of a POC test instead of conventional TG2A testing may have health cost-saving implications, [33] and a finger prick is experienced to be less invasive than a venepuncture, particularly for children. For implementation of POC tests in the follow-up of treated coeliac patients we recommend to use tests that have been validated in this specific group of patients. To rule out the risk of observer variability, a 'reader' that automatically interprets the result of the POC self-test should be developed and prospectively tested in the follow-up of patients with treated coeliac disease. This could, for example, be a smartphone application that interprets a photograph of the test result.

In conclusion, new developments with respect to prevention and management of coeliac disease are taking place at a fast pace. The results presented in this thesis have altered the conceptual landscape of coeliac disease[34] and increased our understanding of the risk factors associated with this disease (**chapter 2**). Furthermore, they call for an update of the current national and international guidelines on infant feeding. A recommendation for improvement of patient/parent-doctor communication on patients' HRQOL is presented in **chapter 3** and a self-management e-health system for coeliac patients is evaluated in **chapter 4**. Finally, we presented a POC test that may be suitable for follow-up of treated coeliac disease in **chapter 5**.

Future perspectives

The lack of an association between the timing of gluten introduction, the duration and presence of breastfeeding, and the development of coeliac disease keeps us guessing for an explanation for the striking increased incidence of this disease in the Western world during the recent decades. It remains likely that other environmental factors play an important role, such as the gut microbiome. It has been suggested that intestinal dysbiosis is associated with coeliac disease.[35] It is however unknown whether the alterations are cause or consequence of the disease. To answer questions such as "Who will develop coeliac disease?" and "When will this happen?" it is crucial to prospectively collect and study the blood, faeces and duodenal biopsies ('biobanking') from patients at high-risk for developing coeliac disease (see the appendix for a letter to the medical ethical committee of the Leiden University Medical Centre concerning biobanking). In a subproject of the PreventCD study, we plan to annually collect faeces of the participating children to establish the relationship between the composition of the gut microbiome and coeliac disease.

Is it time to start with mass screening for coeliac disease instead of only screening people from coeliac families? Perhaps we are nearly there, but a couple of questions remain unanswered. Such as: what is the optimal screening method and frequency and at what age should we screen? It also needs to be clarified whether the costs of screening and treatment are acceptable when compared with the total costs of healthcare. Recent advances in the diagnostics of coeliac disease, such as the POC tests for TG2A, will lower the costs of mass screening and will increase cost-effectiveness. Future studies should take these features into account but also focus on the ethical aspects of mass screening with the different available diagnostic tools in different age categories.

In the coming years, e-health is envisaged to play an increasing role in the care for patients with a chronic disease. This thesis (**chapters 4** and **5**) showed that also in treated coeliac disease, an online consultation is a satisfactory alternative for an outpatient follow-up visit. Before implementing online consultations in these patients' health care, it is necessary to validate the POC self-test for TG2A in this specific population. Recently, a new option for the assessment of gluten free diet adherence has been described: detection of gluten immunogenic peptides in urine.[36] This may be a less invasive, cheaper and possibly even more reliable alternative to conventional blood testing or POC testing for TG2A. However, studies that validate the relevance of this method in clinical practice are required. To maintain participant engagement with e-health, we suggest adjusting to the increased popularity of medical applications on smartphones and tablets. Furthermore, efforts should be made to arrange reimbursement of online consultations as part of the treatment plan for coeliac patients.

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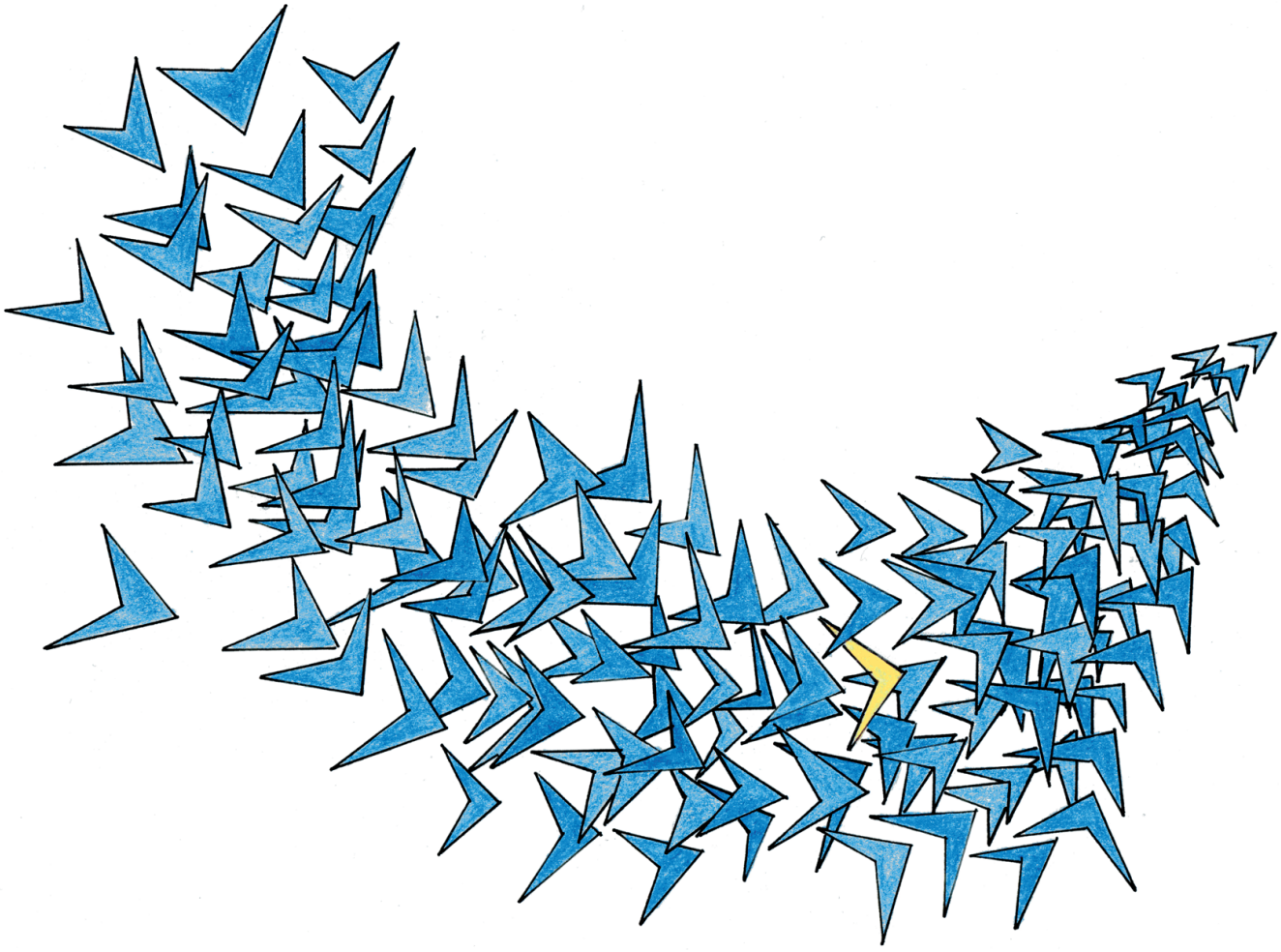

7 | ENGLISH AND DUTCH SUMMARIES

ENGLISH SUMMARY

This thesis aims to investigate the influence of infant feeding on the development and primary prevention of childhood coeliac disease. It explores new strategies for the improvement of care for children and young adults with this disorder.

Chapter 1 provides a general introduction and an overview of the literature on the pathogenesis, diagnosis, management and primary prevention of childhood coeliac disease.

Chapter 2 presents the results of a multicentre, randomized, double-blind placebo controlled dietary intervention study involving 944 children, all positive for HLA-DQ2 and/or HLA-DQ8 and with at least one first-degree relative with coeliac disease. During 16 to 24 weeks of age, participating children received 100 mg of immunologically active gluten per day, or placebo. Anti-transglutaminase type 2 (TG2A) and antigliadin antibodies were measured regularly. When all children had reached the age of 3 years, 80 children were diagnosed with coeliac disease. The median age at diagnosis was 2.8 years and 59% were girls. The cumulative incidence of coeliac disease among 3-year-old children was 5.2% (95% confidence interval [CI], 3.6-6.8), with similar rates in the gluten group and the placebo group (5.9% [95% CI, 3.7-8.1] and 4.5% [95% CI, 2.5-6.5], respectively). The hazard ratio in the gluten group was 1.23 (95% CI, 0.79-1.91). Breastfeeding, regardless of whether it was exclusive or whether it was ongoing during gluten introduction, did not significantly influence the development of coeliac disease or the effect of the intervention. In conclusion, the introduction of small quantities of gluten at 16 to 24 weeks of age, as compared with placebo, did not reduce the risk of coeliac disease by 3 years of age in this group of high-risk children. The present European guidelines recommend introducing small amounts of gluten gradually while the child is breastfed and avoiding both early (<4 months) or late (>7months) introduction of gluten. Our results have contributed to the development of new European guidelines for the introduction of gluten in the diet of young children.

Chapter 3 reports the results of a cross-sectional study investigating whether implementation of a coeliac disease-specific health related quality of life (HRQOL) questionnaire would add value to the follow-up visits for coeliac disease; we compared patients' self-reported coeliac disease-specific HRQOL with the physician's report provided during a regular follow-up visit for coeliac disease in children and young adults. Eligible patients were diagnosed with coeliac disease for at least 1 year, and were up to 25 years old. They completed a coeliac disease-specific HRQOL questionnaire, the CDDUX, after their regular follow-up visit for coeliac disease. Their physicians were unaware of the current study's objectives or the self-reported HRQOL. Physician-reported HRQOL was available in 70/78 enrolled patients. The self-reported and physician-reported HRQOL were concordant in 30/70 cases

(κ=0.093), 6 of these patients had a poor self-reported HRQOL. The reports were discrepant in 40/70 cases, all 40 patients reported a poor HRQOL. We found that discrepancies occurred more frequently in patients with a disease duration shorter than 9 years (32/40 children with discrepant assessments were diagnosed <9 years ago versus 17/30 with no discrepancy, p<0.001) and in females (35/40 children with discrepant assessments were girls versus 16/30 with no discrepancy, p=0.001). Both factors were predictors of a poorer HRQOL. In conclusion, we found that during regular follow-up visits for coeliac disease, physicians did not report a poor HRQOL in 40/46 children and young adults with a poor self-reported HRQOL. This is consistent with previous studies examining other chronic diseases and supports the implementation of self-reported coeliac disease-specific HRQOL measurements in follow-up visits of coeliac patients.

The objective of the study presented in **chapter 4** was to evaluate the effectiveness of online consultations for follow-up of children and young adults with coeliac disease. They were included in the CoelKids study, a multicentre randomized controlled trial involving patients diagnosed with coeliac disease for at least 1 year and aged up to 25 years old. They received an online consultation or a traditional outpatient consultation. Online consultations included symptom questionnaires and home measurements of growth and TG2A using a point-of-care (POC) self-test. Both groups completed questionnaires concerning coeliac disease-specific HRQOL, gluten free diet adherence and patient-satisfaction. After 6 months, they performed the POC self-test and repeated HRQOL and patient-satisfaction questionnaires. We found that with the online consultation, abdominal pain, lassitude and increased appetite were detected significantly more frequently than in controls with the traditional consultation. Growth problems were detected similarly in both groups. TG2A was positive in 2 online participants and 13 controls (POC versus laboratory, p=0.003). CD-specific HRQOL (1=good; 5=poor) was similar in both groups, but improved after online consultation (3.25 to 3.16, p=0.013; versus controls 3.10 to 3.23, p=0.810). Patient-satisfaction (1=low; 10=high) was 7.6 in the online group and 8.0 in controls (p=0.001). Mean costs per participant of the online group were €202 less than in the control group (p<0.001). In conclusion, we found that online consultations for children and young adults with coeliac disease are cost-saving and increase coeliac disease-specific HRQOL. Additionally, patients find these to be satisfactory. The discrepancy between the POC test and laboratory results suggests that the used POC test is not sensitive enough to detect low antibody levels and thereby unsuitable to monitor treated coeliac disease.

Chapter 5 present the result of a cross-sectional study evaluating and comparing 3 different commercially available POC tests for TG2A in children with treated coeliac disease against results of conventional TG2A at the laboratory with ELISA. Tests X, Y and Z were performed on 142 blood samples from IgA competent coeliac patients aged up to 18 years, attending

the paediatric gastroenterology outpatient clinic of Leiden University Medical Center, the Netherlands. Results were evaluated blinded to the outcome of conventional TG2A assessment (EliA™ Celikey™ IgA test) 10 and 30 minutes and 1 day after performing the test (T10, T30 and T1d respectively). Performance of tests was acceptable if the sensitivity was ≥90%. The serum TG2A was positive in 47/142 samples. Test Y had a greater sensitivity than the other 2 evaluated tests (89% [95% CI 0.81-0.98] versus test X: 34% [95% CI 0.20-0.48] and Z: 55% [95% CI 0.41-0.70]), and its sensitivity was 96% [95% CI 0.90-1.0]) when results were read 1 day after the test was carried out. Prolonging the reading time from T10 to T30 significantly improved the performance of tests X and Z in case of positive serum TG2A (sensitivity test X 62% [95% CI 0.48-0.76], p<0.001; and test Z 70% [95% CI 0.57-0.83], p=0.016) but for test Z this was associated with a drop in specificity. In conclusion, our results showed that the studied POC tests have different sensitivities for the relatively low positive TG2A in treated coeliac disease patients. Performance of these tests may improve when reading time is prolonged. Before implementing POC tests in the follow-up of treated coeliac patients, we recommend to use tests that have been validated in this specific group of patients.

Chapter 6 contains the general discussion and the conclusion of this thesis. We set out future perspectives on the prevention of coeliac disease and the care for patients with this disorder.

NEDERLANDSE SAMENVATTING

Dit proefschrift heeft als doel het onderzoeken van de invloed van vroege voeding op de primaire preventie en ontwikkeling van coeliakie. Daarnaast richt het zich op nieuwe strategieën voor verbetering van zorg voor kinderen en jong volwassenen met deze aandoening.

Hoofdstuk 1 geeft een overzicht van de huidige literatuur over de pathogenese, diagnose, behandeling en primaire preventie van coeliakie.

Hoofdstuk 2 beschrijft de resultaten van een multi-centrum, gerandomiseerde, dubbelblinde placebogecontroleerde voedings-interventie studie. Alle 944 deelnemende kinderen zijn positief voor HLA-DQ2 en/of HLA-DQ8 en hebben ten minste één eerstegraads familielid met coeliakie. Deelnemers kregen gedurende de leeftijd van 16 tot en met 24 weken dagelijks 100 mg immunologisch actief gluten, of placebo. Hun bloed werd regelmatig onderzocht op anti-transglutaminase type 2 (TG2A) en antigliadine antilichamen. Toen alle kinderen drie jaar oud waren, was bij 80 kinderen de diagnose coeliakie gesteld. De mediane leeftijd waarop dit gebeurde was 2.8 jaar, meisjes waren in de meerderheid (59%). Op de leeftijd van drie jaar was de cumulatieve incidentie van coeliakie 5.2% (95% betrouwbaarheidsinterval [BI], 3.6-6.8), met gelijke frequenties in de gluten en placebo groep (respectievelijk 5.9% [95% BI, 3.7-8.1] en 4.5% [95% BI, 2.5-6.5]). De hazard ratio in de gluten groep was 1.23 (95% BI, 0.79-1.91). Hoe lang borstvoeding werd gegeven, en of borstvoeding nog werd gegeven toen gluten werden geïntroduceerd, had geen invloed op het risico op coeliakie en ook niet op het effect van de gluten-interventie. Samenvattend: in vergelijking met placebo had de introductie van kleine hoeveelheden gluten gedurende de leeftijd van 16 tot en met 24 weken geen effect op het risico op coeliakie bij drie jaar oude kinderen uit hoog-risico gezinnen. De huidige Europese richtlijnen adviseren om kleine hoeveelheden gluten geleidelijk te introduceren terwijl het kind nog borstvoeding krijgt. Daarnaast adviseren zij om gluten niet voor de leeftijd van vier maanden en niet na de leeftijd van zeven maanden te introduceren. Onze resultaten hebben bijgedragen aan de ontwikkeling van nieuwe Europese richtlijnen voor de introductie van gluten in het dieet van jonge kinderen.

Hoofdstuk 3 rapporteert de resultaten van een onderzoek naar de toegevoegde waarde van het gebruiken van een vragenlijst over de coeliakie-specifieke gezondheids-gerelateerde kwaliteit van leven bij het volgen van kinderen en jongvolwassenen met coeliakie. Daarvoor hebben wij een vergelijking gemaakt tussen de coeliakie-specifieke gezondheids-gerelateerde kwaliteit van leven zoals de patiënt het zelf rapporteert met behulp van een vragenlijst, en zoals de arts het rapporteert tijdens een standaard vervolgconsult voor coeliakie. Patiënten kwamen in aanmerking voor de studie als zij ten minste 1 jaar coeliakie hadden en maximaal 25 jaar oud waren. Na hun standaard vervolgconsult voor coeliakie vulden ze een

coeliakie-specifieke gezondheids-gerelateerde kwaliteit van leven vragenlijst in, de CD-DUX. De deelnemende artsen waren niet op de hoogte van het doel van deze studie, noch van de uitkomst van de vragenlijst. Uit het patiëntendossier van 70/78 deelnemers kon de kwaliteit van leven van de patiënt zoals beoordeeld door de arts worden geëxtraheerd. De zelf-gerapporteerde en arts-gerapporteerde kwaliteit van leven kwam overeen in 30/70 patiënten (K=0.093), 6 van deze patiënten had een lage zelf-gerapporteerde kwaliteit van leven. In 40/70 deelnemers kwamen de metingen niet overeen, alle 40 patiënten rapporteerden een lage kwaliteit van leven. Deze discrepantie kwam vaker voor bij patiënten met een ziekteduur korter dan 9 jaar (32/40 patiënten met niet-overeenkomende metingen waren minder dan 9 jaar geleden gediagnosticeerd, versus 17/30 met wel-overeenkomende metingen, p<0.001), en in vrouwen (35/40 deelnemers met niet-overeenkomende metingen waren vrouwelijk, versus 16/30 met wel-overeenkomende metingen, p=0.001). Beide factoren waren indicatief voor een lage zelf-gerapporteerde kwaliteit van leven. In de conclusie wordt tijdens een standaard vervolgconsult voor coeliakie een lage coeliakie-specifieke gezondheids-gerelateerde kwaliteit van leven gemist bij 40/46 kinderen en jong volwassenen. Dit komt overeen met eerdere studies naar andere chronische ziekten en ondersteunt de implementatie van een vragenlijst voor het meten van de coeliakie-specifieke gezondheids-gerelateerde kwaliteit van leven tijdens (of voorafgaande aan) een vervolgconsult voor coeliakie.

Het doel van de studie in **hoofdstuk 4** is het evalueren van de effectiviteit van een online consult als vervanging van een vervolgconsult voor kinderen en jongvolwassenen met coeliakie. Zij doen mee aan de CoelKids studie, een multicentrum en gerandomiseerde studie met coeliakiepatiënten die ten minste 1 jaar geleden gediagnosticeerd zijn en maximaal 25 jaar oud zijn. Ze werden gerandomiseerd naar de online groep of de controle groep. De online groep voltooide een online consult, de controle groep een traditioneel poliklinisch consult. Tijdens het online consult werden symptomen uitgelicht aan de hand van een vragenlijst en werd TG2A thuis gemeten met behulp van een point-of-care (POC) test. Beide groepen vulden vragenlijsten in over coeliakie-specifieke gezondheid-gerelateerde kwaliteit van leven, het volgen van het glutenvrij dieet en patiënttevredenheid. Na 6 maanden deden alle deelnemers de POC test en herhaalden ze de vragenlijsten over kwaliteit van leven en patiënttevredenheid. De online groep gaf, vaker dan de controle groep, aan last te hebben van buikpijn, moeheid en een toegenomen eetlust. Groeiproblemen werden in beide groepen even vaak gevonden. TG2A was positief in 2 online deelnemers en in 13 controles (POC versus standaard laboratorium test, p=0.003). De coeliakie-specifieke kwaliteit van leven (1=hoog, 5=laag) was gelijk in beide groepen, maar nam 6 maanden na een online consult significant toe (3.25 naar 3.16, p=0.013; versus controlegroep 3.10 naar 3.23, p=0.810). Patiënttevredenheid (1=laag; 10=hoog) was 7.6 in de onlinegroep en 8.0 in de controlegroep (p=0.001). De gemiddelde kosten per persoon in de onlinegroep waren

€202 lager dan in de poligroep (p<0.001). Wij concluderen dat een online consult voor kinderen en jong volwassenen met coeliakie kostenbesparend is en de coeliakie-specifieke gezondheids-gerelateerde kwaliteit van leven verhoogt. Tevens blijken patiënten tevreden over het online consult. De discrepantie tussen de uitslagen van de POC en laboratorium testen suggereert dat de gebruikte POC test niet sensitief genoeg is om laag-verhoogd TG2A aan te tonen en is daarmee ongeschikt voor het volgen van patiënten met behandelde coeliakie.

Hoofdstuk 5 presenteert de resultaten van een studie waarin 3 verschillende commercieel beschikbare POC testen (test X, Y en Z) voor TG2A worden toegepast in de follow-up van kinderen met behandelde coeliakie. De uitslagen van de testen werden onderling vergeleken, en met de 'gouden standaard': de uitslag van conventionele TG2A bepaling in het laboratorium met ELISA (EliA™ Celikey™ IgA test). Deze drie testen werden uitgevoerd op 142 bloedsamples van IgA competente kinderen met behandelde coeliakie (maximaal 18 jaar oud), die de polikliniek van het Leids Universitair Medisch Centrum bezochten voor follow-up van hun ziekte. De uitslag van de POC test werd geëvalueerd na 10 en 30 minuten, en na 1 dag (T10, T30 en T1d). De uitslag van de conventionele ELISA bepaling was toen nog niet bekend. De POC test werd geschikt bevonden als de sensitiviteit ≥90% was. Volgens de conventionele ELISA was het serum TG2A positief in 47/142 bloedsamples. De sensitiviteit van test Y was groter dan van de andere 2 testen (89% [95% BI 0.81-0.98] versus test X: 34% [95% BI 0.20-0.48] en Z: 55% [95% BI 0.41-0.70]). Op T1d was de sensitiviteit van test Y 96% [95% BI 0.90-1.0]. Voor testen X en Z was het significant gunstiger om de uitslag op T30 in plaats van T10 af te lezen (sensitiviteit test X 62% [95% BI 0.48-0.76], p<0.001; en test Z 70% [95% BI 0.57-0.83], p=0.016). Voor test Z ging dit gepaard met een daling in de specificiteit. Tenslotte laten onze resultaten zien dat de bestudeerde POC testen een verschillende sensitiviteit hebben voor het relatief laag-positieve TG2A in patiënten met behandelde coeliakie. De prestaties van de testen nemen mogelijk toe als de afleestijd van het resultaat verlengd wordt. Voordat POC testen toegepast worden in de follow-up van patiënten met behandelde coeliakie, raden wij aan een test te gebruiken die gevalideerd is voor deze specifieke patiëntenpopulatie.

Hoofdstuk 6 bevat de algemene discussie en de conclusie van dit proefschrift. Tevens zetten wij de toekomstperspectieven voor de preventie van coeliakie en de zorg voor patiënten met deze ziekte uiteen.

APPENDICES

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aan Commissie Medische Ethiek LUMC
T.a.v.: Mw. Mr. S.Y.M. van der Heijden
H1-Q

Geachte mevrouw Van Der Heijden,

Middels deze brief willen wij reageren op het besluit van de Commissie Medische Ethiek om ons verzoek tot biobanking bij eerstegraadsfamilieleden van coeliakiepatiënten af te wijzen (29-07-2013, uw ref MDL28/SH/sh).
In het LUMC is het op dit moment alleen toegestaan om materiaal van patiënten met (verdenking op) coeliakie op te slaan in de biobank. Eerstegraads familieleden van een coeliakiepatiënt hebben echter een verhoogd risico op coeliakie. De huidige nationale en internationale e*vidence based* richtlijnen adviseren dan ook eerstegraadsfamilieleden van een coeliakiepatiënt te screenen op coeliakie door de coeliakie-specifieke en -sensitieve antistoffen tegen weefseltransglutaminase en/of endomysium in het serum te bepalen (Husby et al. 2012; Richtlijn Coeliakie en Dermatitis Herpetiformis 2008). Op de coeliakiepoli van de afdeling kindergeneeskunde volgen wij dit advies op en komen deze kinderen (twee)jaarlijks naar onze poli.

Tot voor kort waren er geen prospectieve gegevens beschikbaar over het risico op coeliakie bij kinderen met een eerstegraadsfamilielid met de ziekte. In oktober 2014 hebben wij de resultaten van een Europese studie in een dergelijke groep kinderen (*PreventCD*, gecoördineerd vanuit het LUMC) gepubliceerd in de *New England Journal of Medicine* (Vriezinga et al. 2014). De resultaten lieten o.a. het volgende zien:

1. Coeliakie ontstaat al op jonge leeftijd (gemiddelde leeftijd 2.8 jaar, range 1.1-5.6 jaar);
2. Meisjes hebben op de leeftijd van drie jaar al een tweemaal zo hoog risico als jongens (7.2% versus 3.4%);
3. Kinderen met twee HLA-DQ2 genen hebben op de leeftijd van drie jaar al een duidelijk hoger risico op coeliakie (14.9%) dan kinderen met één HLA-DQ2 gen (3.9%) of dan kinderen met HLA-DQ8 (0.9%).

Daarnaast lieten de resultaten zien dat een aanpassing aan de manier waarop gluten in de voeding van jonge kinderen werd geïntroduceerd coeliakie niet kon voorkomen. In tegenstelling

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tot wat tot altijd werd gedacht, heeft het geven van borstvoeding ook geen beschermend effect op het risico op coeliakie. Onze resultaten worden bevestigd door een andere prospectieve studie (Lionetti et al. 2014). Samen vormen deze studies de basis voor de nieuwe Europese richtlijnen over glutenintroductie in de voeding van kinderen (Szajewska et al. 2016).
Aangezien primaire preventie niet mogelijk is ligt een belangrijke kans bij het zo snel mogelijk opsporen en behandelen van de ziekte, zoals wordt gedaan op onze coeliakie poli in het LUMC. Dit is belangrijk want coeliakie kan gepaard gaan met uiteenlopende symptomen zoals malabsorptie met chronische diarree, een bolle buik, gewichtsverlies en – bij kinderen – slechte groei, tot aspecifieke signalen als moeheid, osteoporose en ijzergebreksanemie. Daarnaast is gebleken dat kinderen waarbij de diagnose is gesteld op basis van screening (zij hadden geen klachten) op de leeftijd van zes jaar al een significant kleinere lengte en vaker osteoporose hadden dan de kinderen in de algemene populatie (Jansen et al. 2014).

Waarom willen wij materiaal van eerstegraadsfamilieleden van een coeliakiepatiënt verzamelen in een biobank?
De resultaten van de Europese PreventCD studie onderstrepen het belang van het verzamelen van prospectieve data en biomateriaal van goed omschreven groepen jonge kinderen. Het doel is het natuurlijk beloop van coeliakie te onderzoeken en mogelijke andere preventieve strategieën te verkennen. **De data en het biomateriaal van kinderen met eerstegraads-familieleden van coeliakiepatiënten voor en na het ontwikkelen van coeliakie vormen dan ook een unieke en een zeer waardevolle bron van informatie over het natuurlijk beloop van de ziekte:** wie ontwikkelt er coeliakie, wanneer gebeurt dit, en waarom ontwikkelt iemand anders juist geen coeliakie? Door middel van het prospectief verzamelen van data en biomateriaal (alleen indien medisch geïndiceerd: bloed, ontlasting en duodenumbiopten) in deze groep kinderen hopen wij het antwoord op deze vragen te vinden.

Wij willen de Commissie Medische Ethiek dan ook vragen om ons verzoek tot biobanking bij kinderen met eerstegraadsfamilieleden van patiënten met coeliakie nogmaals in overweging te nemen. Uiteraard zijn wij bereid meer informatie te verstrekken indien gewenst.

In afwachting van uw reactie en met vriendelijke groeten,

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CURRICULUM VITAE

Sabine Vriezinga was born March 13th 1987 in Amsterdam. Having completed secondary school in Huizen she enrolled medical school at the University of Leiden in 2005. For a clinical internship, she spent the summer holidays of 2008 working in a small family hospital in Akum, Cameroon. During a research internship that was supervised by dr. Mearin (department of paediatric gastroenterology, Leiden University Medical Centre), she studied the parental impact of genotyping in healthy children from coeliac families. Sabine Vriezinga obtained her medical degree in 2012. After graduation, she started her PhD trajectory under guidance of dr. Mearin and prof. dr. Rings (department of paediatrics, Leiden University Medical Centre). She focused on the prevention of coeliac disease and the improvement of care for coeliac patients. In December 2015, she started as an attending physician at the Willem Alexander Children's Hospital of Leiden University Medical Centre. Since May 2016, she works as a physician in psychiatry for children and young adults in Amsterdam (GGZ inGeest). She finished her PhD project December 7th 2016.