Enhanced monitoring and screening in pediatric coeliac disease

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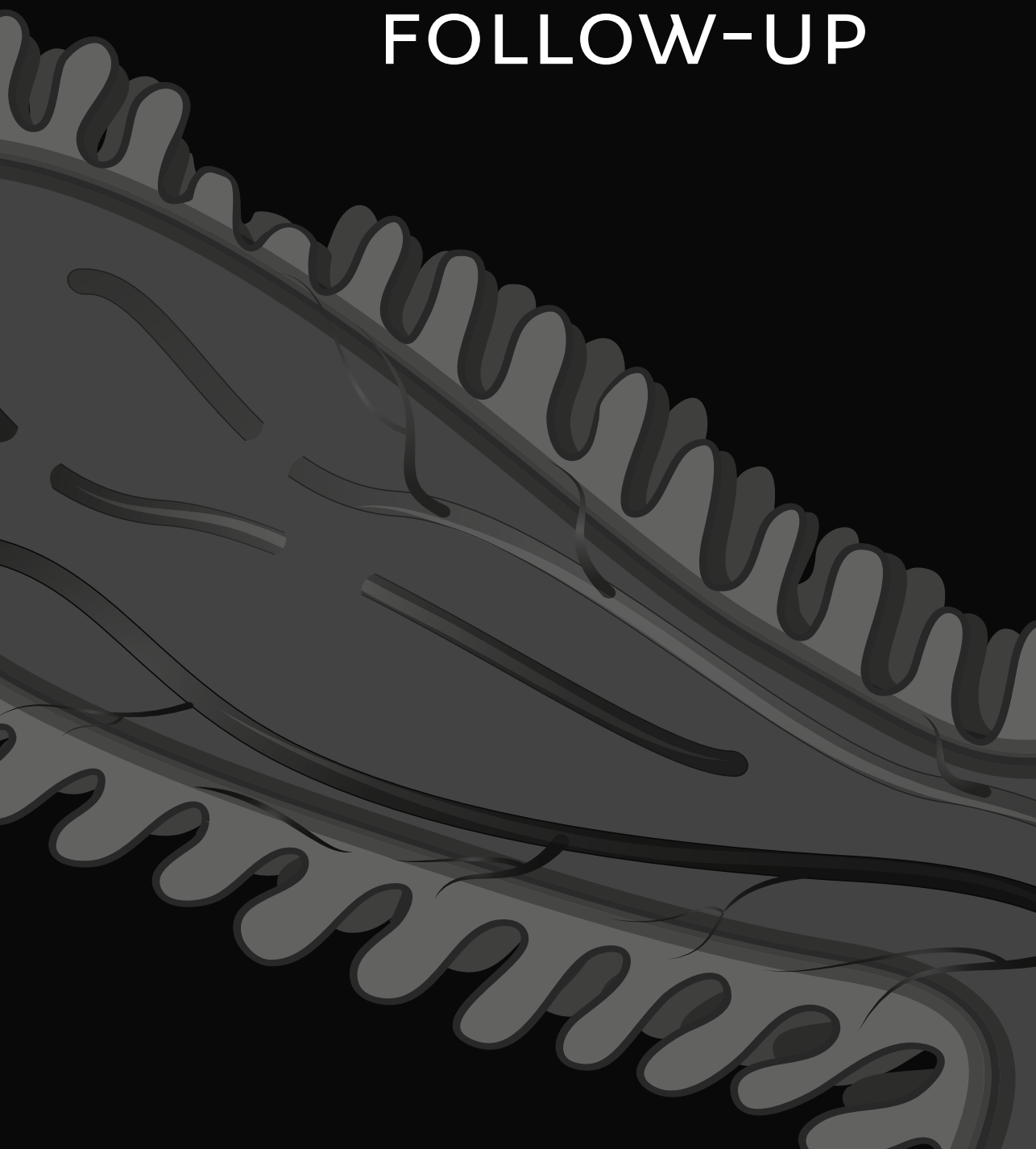
PART I

FOLLOW-UP



CHAPTER 2

Complementary serologic investigations in children with coeliac disease is unnecessary during follow-up

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Abstract

OBJECTIVES To determine the frequency of nutritional deficiencies and thyroid dysfunction in children with coeliac disease (CD) at diagnosis and during follow-up after initiation of a gluten-free diet, since laboratory investigations of hemoglobin, ferritin, calcium, folate, vitamin B12, vitamin D and thyroid function are regularly ordered in CD children despite sufficient evidence for these.

METHODS Between 2009 and 2014, test results of hemoglobin, ferritin, folate, vitamin B12, calcium, vitamin D-25-OH, FT4 and TSH of CD children regularly seen at the Leiden University Medical Center were investigated. Laboratory reference ranges were used to define abnormal results. Pearson's chi-square test for trend, unpaired t-test and one-way ANOVA were used for statistical analysis.

RESULTS 182 children were evaluated, wherein 119 were new diagnoses. On average, 17% of results per year were missing due to incomplete blood investigations. Iron deficiency (28%) and iron deficiency anemia (9%) were found upon CD diagnosis. Folate (14%), vitamin B12 (1%) and vitamin D deficiencies (27%) were also seen. No hypocalcemia or thyroid dysfunction was found. At follow-up, iron deficiency, iron deficiency anemia, folate and vitamin D deficiency were respectively observed in 8%, 2%, 3% and 25% of patients. No vitamin B12 deficiency, hypocalcemia or thyroid disease was found.

CONCLUSION Complementary blood investigations are relevant at time of CD diagnosis but have little diagnostic yield during follow-up visits once the patient is placed on a gluten-free diet. Thus, we recommend that these variables only be assessed on indication, such as fatigue or abnormal growth.

Introduction

Coeliac disease (CD) is an immune-mediated systemic disorder elicited by gluten in genetically susceptible individuals. It is characterized by anti-tissue transglutaminase type 2 antibodies (TG2A) and enteropathy[1]. The disease can be successfully treated with a gluten-free diet (GFD)[2]. Small bowel mucosal damage in CD patients can lead to malabsorption and, subsequently, nutritional deficiencies causing osteoporosis, iron deficiency (ID) or iron deficiency anemia (IDA). Since gluten-containing cereals like wheat, barley and rye are important sources of dietary iron, calcium, folate and vitamin B12, the treatment of CD with a GFD can also lead to nutritional deficiencies[3-6]. Gluten-free grains such as buckwheat or quinoa are naturally rich in group B vitamins[7] but commercially available gluten-free products do not contain the same amount of iron, vitamin B12 and folate as the wheat flour products that they aim to replace[8,9]. A lack of variation in food choices, often seen in CD children[10], may aggravate the problem[11]. It is common practice to check the CD patients' ID/IDA indices (i.e. a complete blood count, including mean corpuscular volume, red cell distribution width, serum ferritin), calcium, folate and vitamin B12 levels, both at diagnosis and at follow-up. However, there is limited information on the incidence of nutritional deficiencies in patients with treated CD. Some evidence based CD guidelines such as the one from the National Institutes of Health (NIH)[12] and the Dutch Society for Gastroenterology[13] recommend that all aforementioned blood tests continue to be performed in patients who already receive ongoing medical treatment for their CD. Other CD guidelines such as those by the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN)[1], the National Institute for Health and Care Excellence (NICE)[14] or the North American Society for Pediatric Gastroenterology Hepatology and Nutrition (NASPGHAN)[15] provide no guidance on the matter. In addition, several guidelines recommend testing for thyroid autoimmunity at various intervals but give no information on how frequently this should be done[13,16].

Our study's primary aims were to assess the frequency of nutritional deficiencies, specifically iron (and the iron deficiency anemia that may follow), calcium, folate and vitamin B12, and to determine the presence of thyroid dysfunction among CD children at the time of diagnosis and at follow-up while on a GFD. The secondary aim was to determine whether these investigations were necessary in the routine follow-up of children with treated CD.

Methods

We analyzed the blood testing results of all CD children who had medical checks between January 2009 and January 2015 at the Leiden University Medical Center (LUMC).

CD was diagnosed according to the ESPGHAN criteria[1]. After diagnosis, these children were then seen regularly according to (inter-)national guidelines. These visits included blood investigations[1,13], particularly CD-specific antibodies, hemoglobin (determined by Sysmex XE-2100), ferritin, folate, vitamin B12 (all measured by ECLIA using Roche Modular E170), free thyroxin (FT4) and thyroid stimulating hormone (TSH) (both determined by colorimetric assay IFCC). Calcium levels (measured by Roche Modular P800) and vitamin D-25-OH (determined by ECLIA using Roche Modular E170) were only recorded beginning in 2012 because our department had only started doing these routine investigations in CD patients after 2011. Laboratory reference ranges per blood parameter are shown in **Table 1**. IDA was defined as ID plus anemia[17]. Hypothyroidism was defined as an FT4 < 10 pmol/L and TSH > 4.8 mU/L while hyperthyroidism was defined following an FT4 > 24 pmol/L and TSH < 0.3 mU/L.

Table 1 *Laboratory reference range used to define abnormal results.*

Biochemical parameter	Limit of abnormal value
Hemoglobin, age <7 years	< 6.9 mmol/L (< 11.0 g/dL)
Hemoglobin, age 7-15 years	< 6.5 mmol/L (< 10.4 g/dL)
Hemoglobin, age >15 years	< 6 mmol/L (<9.6 g/dL)
Ferritin, age <5 years	< 12 ug/L
Ferritin, age ≥5 years	< 15 ug/L
Folate	< 10 nmol/L (< 4.45 ng/mL)
Vitamin B12	< 150 pmol/L (203 pg/mL)
Calcium	< 2.15 mmol/L
Vitamin D-25-OH	< 50 nmol/L (< 20.8 ng/mL)
Thyroid Stimulating Hormone	< 0.3 mU/L > 4.8 mU/L
Free Thyroxin	< 10 pmol/L (< 0.78 ng/dL) > 24 pmol/L (< 1.86 ng/dL

We registered the following patient data: sex, date of birth, age at CD diagnosis, coeliac antibodies, HLA-typing, Marsh histologic classifications of the diagnostic small bowel biopsies, and date of blood extraction. The time of CD diagnosis was defined as the date of diagnostic small bowel biopsies or, if there was no indication for a diagnostic biopsy, the date when high titers of endomysial antibodies (EMA) and TG2A in the serum were

confirmed[1]. Furthermore, we recorded the presence of hypo- or hyperthyroidism at the time of diagnosis or its subsequent development during follow up. Prescribed supplementation therapy for hypothyroidism and deficiencies was also noted.

Laboratory investigations performed from 6 months prior to and 3 months after the diagnosis were considered as blood tests "at time of diagnosis". The first year follow up blood tests were taken between 9 and 18 months post-diagnosis while the second year follow up tests were done within 1.5 to 2.5 years of CD diagnosis, the third year follow up between 2.5 and 3.5 years from diagnosis, and so on. If multiple samples for one parameter were available at one time period, the most abnormal result was used for analysis. If laboratory results were unavailable, they were recorded as missing values. Blood samples taken more than 5.5 years after diagnosis were not used for analysis. Blood tests done after supplementation of iron or vitamins in order to evaluate treatment effects were not considered in the analysis.

Data analysis

Where appropriate, Pearson's chi-square test for trend, unpaired t-test and one-way ANOVA were used. A two-tailed probability of $p < 0.05$ was considered significant. Statistical analysis was performed using Statistical Package for the Social Sciences (IBM, version 20; SPSS Inc., Chicago, IL, USA). No approval from a Medical Ethical Committee was needed for this study since the blood tests were standard of care and analysis was done anonymously.

Results

Patient characteristics are shown in **Table 2**. There were a total of 182 children evaluated, wherein 119 children were newly diagnosed during the study period. The other children were diagnosed prior to 2009 or only had follow-up investigations because CD was previously diagnosed in another hospital. The distribution of age, age at CD diagnosis, mean follow-up duration, Marsh classification and HLA-typing were similar in girls and in boys (data not shown).

Laboratory results

In all participants, 436 blood investigations were performed: 119 times at the time of CD diagnosis and 317 times during follow-up visits. On average, 17% of the children had incomplete annual blood investigations, where it can further be observed that 58% of this group did not have vitamin D tests done.

Table 2 *Characteristics of 182 children with coeliac disease (CD) having medical checks between January 2009 and December 2014.*

Sex, % female	65
Ethnicity, %	
European	93
(North) African and Turkish	4
Asian	2
Unknown	1
Age at diagnosis, mean in years (SD)	6.3 (± 4.3)
Duration of follow-up, mean in years (SD)	3.1 (± 3.1)
Diagnosis without biopsies (ESPGHAN criteria), nr	28
Biopsies confirmed CD, nr	154
Histology small bowel biopsies at diagnosis, %	
Biopsies performed in another center without report available	1 4^
Marsh 2	25
Marsh 3a	49
Marsh 3b	21
Marsh 3c	
HLA-typing result, %	
DQ2 or DQ8 positive	94
Unknown	6
IgA level, %	
>0.2 g/l	96
<0.2 g/l	4
CD specific antibodies at diagnosis, %	
EMA and/or TG2A positive	97
EMA and TG2A negative*	1
EMA and TG2A unknown†	2

^ All with high levels of anti-endomysial antibodies (EMA) and/or anti-tissue transglutaminase type 2 antibodies (TG2A).

* Diagnosis at age 16 months presenting with malabsorption and failure to thrive, small bowel biopsies Marsh 3a and (very) good response to a gluten-free diet.

† CD diagnosed in another hospital, all Marsh 3 proven at biopsy.

At diagnosis

The results of the laboratory tests are shown in **Table 3**. The mean hemoglobin value in the children with ID and IDA was 6.6 mmol/L (SD 0.2). The ten children with IDA were

significantly younger than the others (mean 2.64 years SD 1.1; 6.5 years SD 4.3 respectively, p < 0.001). All children showed normalization of their hemoglobin without any prescribed iron supplementation a year after a GFD, except for a 3 year old girl whose hemoglobin level remained low (6.7 mmol/L) despite supplementation. The mean folate level in children with folate deficiency was 7.7 nmol/L (SD 1.4). The age at diagnosis was similar among the children with and without folate deficiency (mean age 7.6 years SD 6.4; 6.2 years SD 4.1 respectively, p = 0.23). Normalization of folate occurred within one year after starting the GFD in all folate-deficient children regardless of supplementation status. Of note, 40% of the children with folate deficiency were prescribed supplements. One child with vitamin B12 deficiency (64 pmol/L) and abnormal homocysteine and

Table 3 *Frequency of deficiencies and thyroid dysfunction in children with coeliac disease at the time of diagnosis and during follow-up.*

Variable assessed between January 2009 and December 2014	**Diagnosis n=119* (%)**	**1st Year n=83* (%)**	**2nd Year n=79* (%)**	**3rd Year n=57* (%)**	**4th Year n=50* (%)**	**5th Year n=48* (%)**
Iron deficiency#	29/104 (28)	4/79 (5)	4/77 (5)	4/57 (7)	4/48 (8)	2/48 (4)
Iron deficiency anemia##	10/110 (9)	2/81 (2)	1/78 (1)	1/57 (2)	0/49	0/47
Folate deficiency^	12/84 (14)	0/73	2/71 (3)	0/55	0/40	0/44
Vitamin B12 deficiency^^	1/85 (1)	1**/73 (1)	1**/71 (1)	0/55	0/40	0/44
Elevated Thyroid Stimulating Hormone (TSH)‡	12/99 (12)	10/76 (13)	7/71 (10)	3/55 (5)	3/46 (7)	9/47 (19)
Hypo‡ ‡/hyperthyroidism‡ ‡ ‡	0/99	0/79	0/73	0/54	0/46	0/47
Variable assessed between January 2012 and December 2014	**Diagnosis n=71* (%)**	**1st Year n=50* (%)**	**2nd Year n=43* (%)**	**3rd Year n=36* (%)**	**4th Year n=26* (%)**	**5th Year n=31* (%)**
Hypocalcemia±	0/65	0/37	0/34	0/25	0/14	0/31
Vitamin D deficiency±±	8/30 (27)	9/48 (19)	7/42 (17)	4/34 (12)	3/22 (14)	7/28 (25)

* Total number of children at different time points.

Ferritin < 12 µg/L in children < 5 years of age or Ferritin < 15 µg/L in older children; ## Iron deficiency plus anemia (Hemoglobin < 6.9 mmol/L if age < 7 years, < 6.5 mmol/L if age 7-15 years, < 6.0 mmol/L older children); ^ Folate < 10 nmol/L; ^^ Vitamin B12 < 150 pmol/L; ‡ TSH > 4.8 mU/L; ‡ ‡ Free Thyroxin 4 < 10 pmol/L and TSH > 4.8 mU/L; ‡ ‡ ‡ Free Thyroxin 4 > 24 pmol/L and TSH < 0.3 mU/L.

** 1 girl with normal homocysteine and methylmalonic acid ruling out true vitamin B12 deficiency. ± Calcium < 2.15 mmol/L; ±± Vitamin D-25-OH < 50 nmol/L.

methylmalonic acid levels had folate deficiency as well (these tests were performed in a referring hospital, thus, the exact data could not be retrieved). Both folate and vitamin B12 had normalized six months after their respective supplementations.

Anthropometric evaluation of the children with iron, folate and vitamin B12 deficiencies, done in order to see whether these relatively young children had a classic presentation of CD with malabsorption, showed stunting (defined as height < -2.0 SDS) and underweight (defined as weight for height < -1/5 SDS) in 30% and 15% of the children. In all children, recovery of height and weight was seen while on a GFD.

The mean level of vitamin D-25-OH in deficient children was 38 nmol/L (SD 6.8). Only 25% of these children were prescribed with vitamin D supplements (i.e. calcium carbonate/ vitamin D 500 mg/400 IU, for 3-6 months), yet normalization of values occurred in all of these children after one year, except for two adolescents who did not receive these prescriptions. The mean age of the children with vitamin D deficiency at diagnosis was significantly higher compared to the children with normal vitamin D levels (mean 7.6 ± SD 4.6 and mean 5.9 ± SD 4.1 respectively, p=0.03). No child had hyper- or hypothyroidism. Prior to 2009, Graves' disease and Hashimoto's thyroiditis were diagnosed in 1 and 3 patients respectively, both prior to the development of CD. The male-female ratio was similar among the children with and without thyroid deficiencies, and with and without elevated TSH levels (data not shown).

During follow up

The results of the laboratory tests are shown in **Tables 3 and 4**. In the first 3 years after diagnosis, 3 girls developed IDA (mean hemoglobin 6.6 mmol/L SD 0.2) and 1 girl had persistent IDA (hemoglobin 6.7 mmol/L; She had existing IDA at time of CD diagnosis and it continued on despite prescribed iron supplementation post-diagnosis). These girls were significantly younger compared to the children without IDA (mean 3.4 years SD 1.4, and 6.5 years SD 4.3, at time of IDA respectively, p = 0.02).The hemoglobin and ferritin levels normalized in the rest of the girls within 1 year of CD diagnosis. Iron supplementation was only given to one of these patients.

Two patients (3%) developed mild folate deficiency (folate levels 8.7 and 9.5 nmol/L) during the second follow-up year. Supplementation was given to one of them, a 12-year old boy, with normalization of folate after 6 months. Supplementation was withheld in the other patient, a 17 year old asymptomatic girl, since her folate level was only marginally low (9.5 nmol/L). Her follow-up measurements could no longer be obtained after she transferred to the gastroenterology department of another hospital. One adolescent had low vitamin B12 at the first and second year visits, but since normal levels of homocysteine and methylmalonic acid were found, true vitamin B12 deficiency was ruled out and no supplementation was thus prescribed.

Table 4 *Summary of the literature on the prevalence of iron, vitamin B12 and folate deficiency in coeliac disease patients*.*

Study and year published	Study population	No. of patients	Nutrient deficiency** at diagnosis	Nutrient deficiency during follow-up
Bonamico M[39] 1987	Children	80	Iron deficiency (56%)	Not available
Dahele A[40] 2001	Adults	39	Iron deficiency (49%) Vitamin B12 deficiency (41%)	Vitamin B12 deficiency resolved after one year gluten-free diet
Kemppainen T[41] 1998	Adults	40	Folate deficiency (35%) Iron deficiency (32.5%)	Folate and iron deficiency 8% and 22.5% after one year gluten-free diet respectively
Dickey W[42] 2002	Adults	159	Vitamin B12 deficiency (12%)	Not available
Haapalahti M[18] 2005	Adolescents and young adults	26	Iron deficiency (28%) Folate deficiency (31%) Vitamin B12 deficiency (12%)	Not available
Bergamaschi G[19] 2008	Adults	132	Iron deficiency (34%)	30% "*some degree*" of iron deficiency after one year with GFD
Fernandez A[43] 2010	Adults	68	Iron deficiency (49%) Folate deficiency (24%)	Not available
Botero-Lopez JE[20] 2011	Children and adults	73	Iron deficiency (45%)	Not available
Wierdsma NJ[21] 2013	Adults	80	Iron deficiency (46%) Folate deficiency (20%) Vitamin B12 deficiency (19%)	Not available
Gokce S[44] 2014	Children	191	Iron deficiency (8%)	Not available

* By means of Medline search from 1980 until December 2014 using coeliac disease, anemia, iron deficiency, folate deficiency, vitamin B12 deficiency, nutritional deficiencies and nutritional status as Mesh terms.

** Defined as levels of hemoglobin, ferritin, folate and vitamin B12 below reference values.

Vitamin D deficiency (mean vitamin D-25-OH 38.5 nmol/L, SD 7.7) was present in up to 25% of the patients. Calcium carbonate/ vitamin D, once daily 500 mg/400 IE, was prescribed in 40% of deficient children, with the levels returning to normal in a third of these children.

No hyper- or hypothyroidism was found during our follow-up period. However, a 10 year old asymptomatic girl, whose mother was known to have hypothyroidism due to a rare TSHR-gene mutation (C.1631G>A), was diagnosed with subclinical hypothyroidism secondary to the same genetic defect (FT4 11.8 pmol/L; TSH 12.8 mU/L). Elevated TSH levels (mean 6.2, range 4.8-13.6, SD 1.6) were seen in 33 patients. It was noted to occur once

in 48% of them and repeatedly in 39% . The high TSH values only normalized in 17% of these patients. However, all children with repeatedly elevated TSH levels had negative thyroperoxidase antibodies (AbTPO). Two patients developed hypothyroidism after the 5th year of CD follow-up. Both children complained of fatigue and showed decelerating growth. By accounting for thyroid dysfunction prior to CD diagnosis and beyond our follow-up period (after 5.5 years of follow-up), the prevalence of hypothyroidism in our cohort was 3.2% (n=6, 4 female) and hyperthyroidism, 0.5% (n=1, male).

Discussion

As far as we know, this is the first study on the outcome and relevance of complementary blood investigations in the follow-up of children with CD. The results indicate that these investigations are relevant at the time of diagnosis because up to 28% of the children presented then with varying iron, folate and vitamin B12 deficiencies. However, ordering these tests at patient follow-up visits may be questionable since only mild deficiencies occurred in a minority of the children (5-10%). This outcome has implications in the organization of care for CD children because blood tests are time-consuming and expensive. As of 2014, this costs approximately €150-200 per patient, merely for extracting and handling blood samples in our laboratory, and exclusive of coeliac serology charges.

There is limited information on the incidence of nutritional deficiencies in patients with treated CD. Published data vary widely, most probably because they have evaluated small and heterogeneous patient groups focusing on certain nutritional deficiencies, only at time of diagnosis (**Table 4**). In general, the nutritional deficiencies at diagnosis found in our study were noted to occur similarly or even less frequently than earlier studies, except for vitamin B12 deficiency. This deficiency was seen to be much lower in our cohort (2% versus 12-41% found in adolescents and adults)[18-21]. The absence of IDA, folate and vitamin B12 deficiency during follow-up may be explained in two ways. First, adherence to the GFD leads to recovery of the intestinal mucosa, thereby normalizing nutrient absorption. Second, dietary counselling is offered to the patients after diagnosis. This includes daily nutritional intake of iron, folate and vitamin B12[5]. Interestingly, a recent study in adult CD patients showed an increased use of over the counter supplements simultaneous with a GFD treatment[22], something that we did not investigate in our cohort. In the Netherlands, over the counter use of supplements in children is uncommon. Dietitians, whose role is to provide advice on GFDs, likewise do not promote its use. However, the fact that we only recorded prescribed supplements may have underestimated the prevalence of deficiencies at follow-up.

ID and IDA were infrequently seen during follow-up visits. These values were considerably less compared to the rest of the children from the general Dutch population aged 6-36 months. In the latter group, the frequency of ID was 18.8% and IDA, 8.5%[23]. Moreover, the frequency of ID in our patients is lower than the reported 17% among healthy Finnish adolescents[18]. Therefore, it may be questioned whether these deficiencies are related to CD or merely reflect its presence in the general child population .

Our findings on the frequency of thyroid dysfunction (3.7%) are similar to the ones from previous studies, with the prevalence of thyroid autoimmunity (elevated TSH or presence of AbTPO), hypothyroidism and hyperthyroidism varying from 10-26%[24,25], 2-6%[24,25] and 1%, respectively[24,25]. The rationale behind thyroid function testing as part of a CD patient's follow up rests on the fact that there exists a high frequency of thyroid autoimmunity in CD[24,26]. In addition, there is conflicting evidence on the GFD's protective effect in the development of auto-immune thyroid disease[27,29]. However, the clinical relevance of elevated TSH is debatable since elevated TSH levels can fluctuate or normalize, as was seen in our patients. They were also observed to occur or persist, in the absence of AbTPO and without the development of clinical hypothyroidism. Furthermore, thyroid disease was only diagnosed in symptomatic children whose family history and clinical presentation were suggestive of hypo-/ hyperthyroidism.

The strength of our study is the relatively large patient group with well-documented CD, most likely representative of the West European pediatric CD population. The long follow-up period allowed us to demonstrate the natural course of nutritional deficiencies after treatment with a GFD.

One limitation of our study is an incomplete annual laboratory measurement, despite its availability in the majority of cases. Most missing laboratory investigations occurred due to insufficient blood obtained at venipuncture. We believe that since the analysis of calcium and vitamin D took place in a large group of patients within a short follow-up period, the values obtained represent the general population of coeliac children.

One could argue that deficiencies during follow-up might reflect non-compliance to the GFD and therefore, a degree of malabsorption. We have thus retrospectively examined the TG2A levels in children with IDA and folate deficiency and found them all to be normal, thus confirming the patient compliance.

It is known that CD can lead to a diminished bone mineral density in 40-66% of the coeliac patients at diagnosis[30,31], with low calcium levels in 18-24%[31,32]. In coeliac children and adults, a GFD has proven to be effective in ameliorating bone mineralization[31,33]. Vitamin D deficiency has been found to equally occur at diagnosis and during follow-up[32,34]; this

is similar to our results. It is known that vitamin D deficiency occurs in up to 20-70% of children, regardless of age, sex, socio-economic status and dietary supplementation. The main variation in its occurrence may easily be explained by race or ethnicity and seasonal influences, i.e. it is more commonly observed among darker individuals owing to differing skin pigmentation and in the winter due to reduced sun exposure[35-38]. Our analysis indicates that vitamin D status depends on more than a gluten-free diet and supplementation, considering that 2/3 of patients who were prescribed supplements to correct vitamin D deficiency still did not achieve normal levels. Therefore, it seems that vitamin D deficiency may not be directly linked to CD, but merely represents its frequency in the general population. However, assessment of vitamin D status and correcting the deficiency or ensuring its spontaneous resolution can be generally considered as good patient care because of the known effects of untreated CD on bone health.

Conclusion

We have shown that at the time of pediatric CD diagnosis, iron deficiency, iron deficiency anemia, and folate deficiency occur frequently. However, the vast majority of these values normalize after a GFD treatment, even without the prescribed supplementations. The low frequency of deficiencies at follow-up may not even be related to CD, since they are also found to the same degree in the general pediatric population. Furthermore, we have shown that vitamin B12 deficiency only sporadically occurred at the time of CD diagnosis, whereas hypocalcemia did not occur at all. Presence of thyroid disease in our cohort was low and occurred only in symptomatic children. We therefore recommend that these variables only be evaluated on indication in follow up CD visits, for example, after specific complaints such as fatigue or abnormal growth.

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