Complementary Serologic Investigations in Children with Celiac Disease Is Unnecessary during Follow-Up

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

Study design Between 2009 and 2014, test results of hemoglobin, ferritin, folate, vitamin B12, calcium, vitamin D (25[OH]D), free thyroxin, and thyroid stimulating hormone of children with CD regularly seen at the Leiden University Medical Center were investigated. Laboratory reference ranges were used to define abnormal results. Pearson χ^2 test for trend, unpaired *t* test, and 1-way ANOVA were used for statistical analysis.

Results Of the 182 children evaluated, 119 were newly diagnosed. On average, 17% of results per year were missing because of incomplete blood investigations. Iron deficiency (28%) and iron deficiency anemia (9%) were found at the time of diagnosis of CD. 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, and folate and vitamin D deficiency were observed in 8%, 2%, 3%, and 25% of patients, respectively. Vitamin B12 deficiency, hypocalcemia, and thyroid disease were not found.

Conclusions Complementary blood investigations are relevant at the time of diagnosis of CD 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. *(J Pediatr 2016;169:55-60)*.

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Celiac disease (CD) can be successfully treated with a gluten-free diet (GFD).[1,2] Small bowel mucosal damage in patients with CD can lead to malabsorption and, subsequently, nutritional deficiencies causing osteoporosis, iron deficiency, or iron deficiency anemia (IDA). Because gluten-containing cereals such as 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] 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 children with CD,[10] may aggravate the problem.[11] It is common practice to check the indices of iron deficiencies (ie, 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[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,[1] the National Institute for Health and Care Excellence,[14] or the North American Society for Pediatric Gastroenterology Hepatology and Nutrition[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 primary aims were to assess the frequency of nutritional deficiencies, specifically IDA, calcium, folate, and vitamin B12, and to determine the presence of thyroid dysfunction among children with CD at the time of diagnosis and at follow-up while on a GFD.

AbTPO	Thyroperoxidase antibodies
CD	Celiac disease
FT4	Free thyroxin
GFD	Gluten-free diet
IDA	Iron deficiency anemia
TSH	Thyroid stimulating hormone

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Methods

We analyzed the blood testing results of all children with CD who had medical checks between January 2009 and January 2015 at the Leiden University Medical Center. No approval from a Medical Ethical Committee was needed for this study because the blood tests were standard of care and analysis was done anonymously. CD was diagnosed according to the European Society for Pediatric Gastroenterology Hepatology and Nutrition criteria.[1] After diagnosis, these children were then seen regularly according to national guidelines. These visits included blood investigations,[1,13] particularly CD-specific antibodies, hemoglobin (determined by Sysmex XE-2100; Sysmex Corporation, Kobe, Japan), ferritin, folate, vitamin B12 (all measured by electrochemiluminescence immunoassay using Roche Modular E170; Roche Diagnostics, Basel, Switzerland), free thyroxin (FT4), and thyroid stimulating hormone (TSH) (both determined by colorimetric assay with standardization according to the International Federation of Clinical Chemistry). Calcium levels (measured by Roche Modular P800; Roche Diagnostics) and vitamin D (25[OH]D) (determined by electrochemiluminescence immunoassay using Roche Modular E170; Roche Diagnostics) were only recorded beginning in 2012 because our department had only started doing these routine investigations in patients with CD after 2011. Laboratory reference ranges per blood variable are shown in **Table I**.[17] IDA was defined as iron deficiency plus anemia. Hypothyroidism was defined as an FT4 <10 pmol/L and TSH >4.8 mU/L, and hyperthyroidism was defined following an FT4 >24 pmol/L and TSH <0.3 mU/L. We registered the following patient data: sex, date of birth, age at CD diagnosis, celiac antibodies, HLA-typing, Marsh histologic classifications of the diagnostic small bowel biopsies, and date of blood extraction. The time of diagnosis of CD 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 anti-endomysial antibodies and anti-tissue transglutaminase type 2 antibodies 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.

Table I. Laboratory reference range used to define abnormal results

Biochemical variables	Limit of abnormal value
Hemoglobin, age <7 y	<6.9 mmol/L (<11.0 g/dL)
Hemoglobin, age 7-15 y	<6.5 mmol/L (<10.4 g/dL)
Hemoglobin, age >15 y	<6 mmol/L (<9.6 g/dL)
Ferritin, age <5 y	<12 ug/L
Ferritin, age ≥5 y	<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]D)	<50 nmol/L (<20.8 ng/mL)
TSH	<0.3 mU/L
	>4.8 mU/L
FT4	<10 pmol/L (<0.78 ng/dL)
	>24 pmol/L (<1.86 ng/dL)

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 postdiagnosis, and the second year follow-up tests were done within 1.5-2.5 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 1 variable were available at 1 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 Analyses

Where appropriate, Pearson χ^2 test for trend, unpaired t test, and 1-way ANOVA were used. A 2-tailed probability of $P < .05$ was considered significant. Statistical analysis was performed using SPSS IBM v 20 (SPSS Inc, Chicago, Illinois).

Results

There were a total of 182 children evaluated, wherein 119 children were newly diagnosed during the study period

Table II. Characteristics of 182 children with CD having medical checks between January 2009 and December 2014

Characteristic	Outcome
Sex, % female	65
Ethnicity, %	
European	93
(North) African and Turkish	4
Asian	2
Unknown	1
Age at diagnosis, mean in y (SD)	6.3 (±4.3)
Duration of follow-up, mean in y (SD)	3.1 (±3.1)
Diagnosis without biopsies (ESPGHAN criteria), number	28
Biopsies confirmed CD, number	154
Histology small bowel biopsies at diagnosis, %	
Biopsies performed in another center without report available	1
Marsh 2	4*
Marsh 3a	25
Marsh 3b	49
Marsh 3c	21
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

EMA, anti-endomysial antibodies; *ESPGHAN*, European Society for Pediatric Gastroenterology Hepatology and Nutrition; *TG2A*, anti-tissue transglutaminase type 2 antibodies.
*All with high levels of EMA and/or TG2A.
†Diagnosis at age 16 mo presenting with malabsorption and failure to thrive, small bowel biopsies Marsh 3a and (very) good response to a GFD.
‡CD diagnosed in another hospital, all Marsh 3 proven at biopsy.