

# Low Yield for Routine Laboratory Checks in Follow-up of Coeliac Disease

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## ABSTRACT

**Aim:** To evaluate the yield of routine laboratory tests and Dual Energy X-ray Absorptiometry (DEXA) scans in coeliac disease.

**Methods:** A retrospective analysis of medical files of all followed-up patients with coeliac disease attending Rijnstate Hospital in 2016 was conducted with respect to blood tests of hemoglobin, vitamin B12, folate acid, iron status, calcium, vitamin D, glucose, thyroid function, DEXA-scans and related symptoms or signs of abnormalities. All patients had positive coeliac serology and/or biopsy-proven coeliac disease and attended regular visits after diagnosis. The chi-square test for trend was used for statistical analysis: a two-tailed probability of  $p < 0.05$  was considered significant.

**Results:** We analyzed 250 patients with a median follow-up of 7.8 (1-22) years. At diagnosis, we found anemia in 24.4%, iron deficiency in 38%, folic acid deficiency in 22.6% and vitamin B12 deficiency in 15.9%. All deficiencies recovered within 1-2 years with or without supplements. Deficiencies or autoimmune diseases occurred in 50 patients (37 possibly coeliac-related) during follow-up. Twelve cases of coeliac-related deficiencies or autoimmune diseases occurred in patients with normal values at diagnosis of whom 10 were asymptomatic (incidence 10/1000 patient years). Osteoporosis and osteopenia were present in 23.3% and 35% at diagnosis. In most patients bone mineral density (BMD) improved or stabilized during follow up ( $p < 0.05$ ), 8% deteriorated.

**Conclusions:** The incidence of asymptomatic coeliac-related deficiencies or autoimmune diseases is low in patients with normal values at diagnosis. Therefore, routine laboratory screening is not necessary in this group: attending regular follow-up visits should be sufficient. DEXA scans are recommended.

**Key words:** Coeliac disease – Gluten-free diet – Autoimmune diseases – DEXA/BMD – Nutritional deficiencies.

**Abbreviations:** BMD: bone mineral density; DEXA: Dual Energy X-ray Absorptiometry; EMA: endomysial autoantibodies; ESPGHAN: European Society for Paediatric Gastroenterology; HbA1c: glycated hemoglobin; MCV: mean corpuscular volume; TSH: thyroid-stimulating hormone.

## INTRODUCTION

In coeliac disease, ingestion of gluten triggers a T-cell mediated autoimmune response that damages small intestinal mucosa in genetically susceptible individuals. Gluten sensitive enteropathy typically leads to symptoms such as abdominal pain, diarrhea, weight loss and nutritional deficiencies causing osteoporosis and anemia [1, 2]. Eliminating gluten from the diet halts the autoimmune response

and allows mucosal healing in the majority of patients [3, 4]. Mucosal healing is expected to normalize absorption in the small intestine and resolve nutritional deficiencies. Therefore, a gluten-free diet is the keystone of coeliac disease treatment.

Gluten-containing cereals such as wheat, barley and rye, however, are important sources of iron, calcium and group B vitamins. Although gluten-free grains such as buckwheat or quinoa are naturally rich of the group B vitamins, commercially available gluten-free products do not contain the same amount as the products that they aim to replace. This puts patients on a gluten-free diet at a possible risk for development of nutritional deficiencies despite mucosal healing [5, 6]. Therefore, it is recommended to screen for deficiencies both at diagnosis and during follow up. The Guidelines from e.g. National Institutes of Health and the Dutch Society for Gastroenterology

recommend annual routine blood checks during follow up in patients adhering to a gluten-free diet [7, 8]. In addition, testing for thyroid dysfunction, type 1 diabetes and osteoporosis is recommended at various intervals in view of their higher incidence in these patients [9, 10]. Data upon the incidence of nutritional deficiencies and associated autoimmune disorders during follow up, however, are scarce and inconclusive [6, 11-13]. Furthermore, routine blood checks are expensive, time consuming and inconvenient for asymptomatic patients. Practice variation is common. While some physicians abide to the guidelines others do not screen mainly because of the low level of evidence as well as the perceived low yield of screening.

We studied patients with coeliac disease adhering to a gluten-free diet attending our outpatient clinic to assess the frequency of nutritional deficiencies, thyroid dysfunction, type 1 diabetes and osteoporosis at time of diagnosis and during follow-up. In addition, we assessed whether these comorbidities were detected because of symptoms that triggered diagnostic procedures or as a result of routine laboratory screening.

## METHODS

This retrospective cohort study included adult patients with coeliac disease visiting the Rijnstate Hospital Arnhem, The Netherlands between January and December 2016. We analyzed all laboratory test results and Dual Energy X-ray Absorptiometry (DEXA)-scans. The regional medical Ethics Committee reviewed the protocol and decided that the study could be conducted without formal CMO approval.

All patients had positive coeliac serology and/ or biopsy-proven coeliac disease, histologically Marsh 2 or higher and self-reported dietary adherence. Patients with Marsh 2 enteropathy without positive serology were only included when the HLA genotype was supportive for coeliac disease and patients had a positive clinical response to the gluten-free diet. If patients were diagnosed during childhood, the ESPGHAN diagnostic criteria were followed. According to the Oslo revision of the European Society for Paediatric Gastroenterology (ESPGHAN) guidelines (2012), diagnosis in children may be made on serology, HLA genotyping and clinical response to a gluten free diet, without histology [14, 15]. Patients diagnosed with refractory coeliac disease and enteropathy associated T-cell lymphoma were excluded from analysis. After diagnosis, patients routinely visited the outpatient clinic in 12-24 month intervals according to the national guidelines [7]. These routine visits included history taking, physical examination upon indication, and blood investigations: hemoglobin (Hb), mean corpuscular volume (MCV) (both determined by XE 2100 Sysmex), calcium, folic acid, vitamin B12, vitamin (25-hydroxy) D, ferritin, iron (all determined by Roche Modular P800), thyroid-stimulating hormone (TSH), free thyroxin (FT4), glucose (fasting or non-fasting), glycated hemoglobin (HbA1c) (determined by Roche Modular P800) and tissue transglutaminase/endomysial antibodies (determined by ImmunoCap 250). DEXA scans were performed at diagnosis with 5-year follow up intervals or on indication (using GE Lunar IDXA).

Laboratory reference ranges per variable are shown in the corresponding table. Thyroid disease and type 1 diabetes were

diagnosed according to international guidelines (Suppl. Table I). Bone mineral density (BMD) cut-off levels for osteoporosis and osteopenia were classified according to the World Health Organization criteria [16].

The following patient data were registered: sex, date of birth, time of diagnosis, coeliac antibodies, small bowel histology according to the Marsh classification and date of blood extraction. Furthermore, we recorded the presence of hypo- or hyperthyroidism, type 1 diabetes and osteopenia or osteoporosis at the time of diagnosis or its subsequent development during follow-up. In addition, prescribed supplements for deficiencies as well as therapy for thyroid disease, type 1 diabetes and osteoporosis were included in the database. Test results in patients with type 1 diabetes and thyroid disease who started taking medication were excluded from that moment onwards. We considered laboratory investigations and DEXA-scans performed from 6 months prior to and 3 months after diagnosis as "at time of diagnosis". All blood tests and DEXA-scans with an interval of at least 9 but no more than 24 months were registered as a follow-up test. For patients with follow-up duration more than 8 years we chose to register data with intervals of 24 months in order to collect data regarding follow-up over a longer period. In cases of multiple tests within a time interval, the most abnormal test result was used. Unavailable results were recorded as missing values. In cases where the number of missing values for a certain parameter was over 50 percent we excluded that parameter from analysis. The medical charts of patients that were identified to have deficiencies or associated diseases were separately reviewed by two authors. Standard care in follow-up includes questions regarding the diet and symptoms as well as signs of any clinical problem, whether or not coeliac-related (e.g. fatigue, abdominal complaints, diarrhea, weight loss, dyspnea, palpitations, polyuria). In the case of abnormal laboratory values and clinical remission, other explanations were investigated and if no other cause was found, we labelled the abnormalities as probably coeliac related.

### Statistical analysis

Descriptive statistics are presented as mean with standard deviation for normally distributed continuous data, median and range for skewed continuous variables and as numbers and percentages for dichotomous and categorical variables. Differences between groups were tested using the Pearson chi-square test. Kruskal-Wallis was used to compare age groups. Incidence is displayed per 1000 patient years. We considered  $p < 0.05$  significant for 2-tailed probability. We performed all analyses with SPSS IBM v 21 (SPSS Inc, Chicago, Illinois).

## RESULTS

We analyzed data of 250 patients with coeliac disease with a median follow-up duration of 7.8 (0.75-22) years: 197 patients were diagnosed in our hospital, 35 were diagnosed in a referring hospital but attended regular follow up visits in our hospital. An additional 18 patients were diagnosed during childhood in our hospital and were now under adult care. Patient characteristics are summarized in Table I.

**Table I.** Patient characteristics

	Patients diagnosed in Rijnstate at age > 18 yrs N= 197	Patients diagnosed in another hospital N= 35	Patients diagnosed in Rijnstate at age < 18 yrs N= 18
Age, years (SD)	45.5 ( $\pm$ 17.1)	42.9 ( $\pm$ 12.0)	9.0 ( $\pm$ 5.1)
Sex (Male/Female)	55 (28%)/142 (72%)	8 (23%)/27 (77%)	6 (33%)/12 (67%)
Marsh			
- 2	16 (8%)	5 (14%)	0
- 3 A or higher	181 (92%)	30 (86%)	15 (83%)
- no biopsy			3 (17%)
CD <sup>1</sup> specific antibodies*			
- EMA <sup>2</sup> and/or TTG <sup>3</sup> positive	168 (85%)	9 (26%)	8 (44%)
- EMA and TTG negative	9 (5%)	5 (14%)	1 (6%)
- EMA and TTG unknown	16 (8%)	18 (51%)	9 (50%)
IgA deficiency <sup>4</sup>			
- Present	2 (1.0%)	1 (3%)	1 (6%)
- Absent	195 (99%)	34 (97%)	17 (94%)
Symptoms at diagnosis			
- Present <sup>5</sup>	149 (76%)	25 (71%)	13 (72%)
- Absent <sup>6</sup>	32 (16%)	1 (3%)	1 (6%)

Ranges: <sup>1</sup>coeliac disease, <sup>2</sup>endomysial autoantibodies [no normal values available], <sup>3</sup>tissue transglutaminase [ $<$  3 mmol/l], <sup>4</sup>immunoglobulin A total [ $>$  0.06 mmol/l]

\*Most specific antibody combinations, other less specific are not displayed; <sup>5</sup>patients detected after diagnosis because of either symptoms of classical or non-classical CD; <sup>6</sup>patients detected after diagnosis because of family screening

### Missing values

The number of patients included and duration of follow-up vary per analyzed parameter due to missing values. Calcium and vitamin D were excluded due to a high number of missing values, because no further tests were performed after supplements were prescribed.

### Anemia and nutritional deficiencies

#### At diagnosis

The frequencies of anemia and nutritional deficiencies at diagnosis are displayed in Table II; 5-16% of patients without anemia used iron, vitamin B12 and/or folic acid supplements.

#### Follow-up

Regardless of the use of supplements, recovery of anemia, iron, vitamin B12 and folic acid deficiency occurred in 90.0% (45/50), 97.0% (66/68), 92.6% (25/27), 100% (36/36) of the patients within the first year after gluten-free diet initiation respectively. After 2 years, all deficiencies were resolved.

We recognized three groups of patients: those with normal values at diagnosis, with abnormal values at diagnosis and with unknown values at diagnosis.

Eight patients developed anemia after normal Hb values at diagnosis. In 4 patients, anemia was possibly related to coeliac disease, and 2 patients reported symptoms (incidence 2/1000 patient years). In both, anemia resolved without supplements. Five patients developed another anemia episode after anemia at diagnosis (incidence 15.4/1000 patient years). Anemia was related to coeliac disease in 3 of these 5 patients. Two of these 3 patients reported symptoms. Six patients with unknown baseline values developed anemia during follow-up (incidence 11.1/1000 patient years). Anemia was related to coeliac disease in 1 patient who reported symptoms.

Four patients developed vitamin B12 deficiency after an initial normal value of whom 3 were coeliac related (incidence 4/1000 patient years). All were asymptomatic and none

developed anemia. Six patients had another episode of vitamin B12 deficiency after initial recovery (incidence 33.1/1000 patient years). Two of these 6 reported symptoms. In patients with unknown baseline value 3 developed possible coeliac related vitamin B12 deficiency (incidence 4.2/1000 patient years), of whom 1 was asymptomatic.

Three patients with normal folic acid at diagnosis developed a deficiency (incidence 4/1000 patient years), while re-deficiencies occurred in 4 (incidence 18/1000 patient years). Three patients had deficiency after unknown values (incidence 4/1000 patient years). No one presented symptoms and all were probably related to coeliac disease.

Iron deficiency developed in 2 patients after a normal value at diagnosis (incidence 3.1/1000 patient years). Both patients were asymptomatic, iron deficiency was related to coeliac disease in 1. Re-deficiency occurred in 7 (incidence 17.2/1000 patient years) of whom 1 was possibly coeliac related. None was symptomatic, none developed anemia.

Overall, we found 9 patients with asymptomatic coeliac related anemia or deficiency after initial normal values (incidence 9/1000 patient years). Characteristics of all patients with deficiencies are displayed in Suppl. Tables II-IV.

### Endocrinological

#### At diagnosis

The frequencies of thyroid disease and type 1 diabetes at diagnosis are displayed in Table III. Subclinical thyroid disease was present in 17 patients, 8 had an impaired glucose tolerance.

#### Follow-up

No patient with normal thyroid function at diagnosis developed manifest thyroid disease during follow-up. Eleven patients developed subclinical hypothyroidism and one subclinical hyperthyroidism. Two patients with abnormal TSH at diagnosis eventually developed thyroid disease (incidence

**Table II.** Frequencies of anemia and nutritional deficiencies during follow up of coeliac patients adhering to gluten-free diet

	Hemoglobin		Vitamin B12		Folic Acid		Iron status					
	Proportion	Incidence /1000 pt yrs	Proportion	Incidence /1000 pt yrs	Proportion	Incidence /1000 pt yrs	Proportion	Incidence /1000 pt yrs				
Deficiency at diagnosis	24.4%	(50/205)	15.9%	(27/170)	22.6%	(36/169)	38%	(68/179)				
Deficiency during follow up after initial normal values	5.2%	(8/155)	8	2.8%	(4/143)	5	2.3%	(3/133)	4	1.8%	(2/111)	3
CD related	2.6%	(4/155)	4	2.1%	(3/143)	4	2.3%	(3/133)	4	0.9%	(1/111)	2
Symptomatic	1.3%	(2/155)	2	0			0			0		
Asymptomatic	1.3%	(2/155)	2	2.1%	(3/143)	4	2.3%	(3/133)	4	0.9%	(1/111)	2
Not CD related	2.6%	(4/155)	4	0.7%	(1/143)	1	0			0.9%	(1/111)	2
Symptomatic	2.6%	(4/155)	4	0.7%	(1/143)	1	0			0		
Asymptomatic	0			0			0			0.9%	(1/111)	2
Median FU (years)	5.4			3.90			4.7			4.9		
Recurrence of deficiency after initial recovery	10%	(5/50)	15	22.2%	(6/27)	33	11.1%	(4/36)	18	10.3%	(7/68)	17
CD related	6%	(3/50)	9	22.2%	(6/27)	33	11.1%	(4/36)	18	1.5%	(1/68)	2
Symptomatic	4%	(2/50)	6	7.4%	(2/27)	11	0			0		
Asymptomatic	2%	(1/50)	3	14.8%	(4/27)	22	11.1%	(4/36)	18	1.5%	(1/68)	2
Not CD related	4%	(2/50)	6	0			0			8.8%	(6/68)	15
Symptomatic	4%	(2/50)	6	0			0			4.4%	(3/68)	7
Asymptomatic	0			0			0			4.4%	(3/68)	7
Median FU (years)	5.0			7.3			5.3			4.9		
Deficiency after unknown data at diagnosis	13.3%	(6/45)	11	3.8%	(3/80)	4	3.7%	(3/81)	4	6.8%	(4/71)	6
CD related	2.2%	(1/45)	2	3.8%	(3/80)	4	3.7%	(3/81)	4	1.4%	(1/71)	2
Symptomatic	2.2%	(1/45)	2	2.5%	(2/80)	3	0			0		
Asymptomatic	0			1.3%	(1/80)	1	3.7%	(3/81)	4	1.4%	(1/71)	2
Not CD related	11.1%	(5/45)	10	0			0			4.2%	(3/71)	5
Symptomatic	8.9%	(4/45)	8	0			0			1.4%	(1/71)	2
Asymptomatic	2.2%	(1/45)	2	0			0			2.8%	(2/71)	4
Median FU (years)	12.3			8.2			8.0			8.0		

Ranges: <sup>1</sup>Hemoglobin male [8.4-10.8 mmol/l], <sup>2</sup>Hemoglobin Female [7.4-9.9 mmol/l], <sup>3</sup>Vitamin B12 [150-640 mmol/l], <sup>4</sup>Folic acid [9-36 mmol/l], <sup>5</sup>Ferritin [20-300 mmol/l], <sup>6</sup>Iron [10-30 mmol/l]. FU: follow-up.

20.3/1000 patient years). One patient presented symptoms. None of them had positive anti-TPO titers.

One patient without data regarding thyroid function at diagnosis developed symptomatic hypothyroidism (incidence 1.1/1000 patient years). Nine patients had subclinical hypothyroidism and 3 patients had subclinical hyperthyroidism.

One patient with normal glucose at diagnosis developed type 1 diabetes, she presented with fatigue and blurry vision without polyuria or polydipsia (incidence 1.4/1000 patient years). No patient with impaired glucose tolerance at diagnosis and no patient with unknown data at diagnosis developed diabetes during a follow up of 5.8 and 7.7 years, respectively.

Overall, we found an incidence of 10/1000 patient years for coeliac-related asymptomatic deficiencies and autoimmune diseases in patients with initial normal values. The incidence of coeliac-related asymptomatic (re)-deficiencies and autoimmune diseases (34/1000 patient years) was significantly higher ( $p < 0.05$ ) in patients with abnormal values at diagnosis.

### Bone density

#### At diagnosis

DEXA-scans were performed in 223 patients. We found osteoporosis in 52 (23.3%), osteopenia in 78 (35.0%) and a normal bone mineral density (BMD) in 93 patients (41.7%). Patients with normal BMD (median age 38.3 years) were

**Table III.** Frequencies of thyroid disease and type 1 diabetes during follow up of coeliac patients adhering to gluten-free diet

	Hypothyroidism <sup>1,2</sup>		Hyperthyroidism <sup>1,2</sup>		Type 1 diabetes <sup>3,4,5</sup>	
	Proportion	Incidence/ 1000 pt yrs	Proportion	Incidence/ 1000 pt yrs	Proportion	Incidence/ 1000 pt yrs
Frequency at diagnosis	2.4% (6/50)		0.4% (1/250)		2.8% (7/250)	
Frequency after normal value at diagnosis	0 (0/141)		0		0.8% (1/126)	1
- Symptomatic						
- Asymptomatic					0.8% (1/126)	1
Median FU (years)	4.0		4.0		4.6	
Frequency after abnormal value at diagnosis	11.8% (2/17)	20	0		0	
- Symptomatic	5.9% (1/17)	10				
- Asymptomatic	5.9% (1/17)	10				
Median FU (years)	5.6		5.6		5.8	
Frequency after unknown value at diagnosis	1.1% (1/90)	1	0		0	
- Symptomatic	1.1% (1/90)	1				
- Asymptomatic						
Median FU (years)	10.0		10.0		7.7	

Ranges: <sup>1</sup>Thyroid stimulating hormone [0.27 – 4.2 mmol/l], <sup>2</sup>thyroxine [12 – 22 mmol/l], <sup>3</sup>fasting glucose [4 – 6 mmol/l], <sup>4</sup>non-fasting glucose [5 – 12.2 mmol/l], <sup>5</sup>glycated hemoglobin [4 – 6 mmol/l]. FU: follow-up; pt yrs: patient years.

younger than patients with osteopenia or osteoporosis (median age 45.3 and 62.3, respectively) (p < 0.05). Twenty patients used vitamin D and/or calcium supplements prior to their first DEXA scan, 11 of them presented with a normal BMD, 6 with osteopenia and 3 with osteoporosis. Calcium and / or vitamin D supplements were prescribed to 82 patients (7 with normal BMD, 41 with osteopenia and 34 with osteoporosis) after baseline DEXA.

**Follow-up**

Follow up DEXA-scans were available in 151 patients (see Table IV). Deterioration of BMD occurred less frequently compared to preservation and improvement of BMD (p < 0.01). In all 61 patients who were prescribed supplements either before or after baseline DEXA no significant deterioration in

BMD was found during follow-up, 13 patients improved. In patients who did not use supplements BMD improved in 12, deteriorated in 6 and was preserved in 60. Overall, we found no difference between the groups of patients with or without supplements with respect to changes in BMD (p > 0.05), however there were more patients with osteoporosis in the group using supplements (p < 0.05).

**DISCUSSION**

In this retrospective cohort study including 250 coeliac disease patients, we found that up to 38% had anemia or nutritional deficiencies at diagnosis that resolved with or without supplements in > 90% within one and in all within

**Table IV.** Osteoporosis, osteopenia and normal BMD after follow up (n = 151)

	Normal BMD <sup>1</sup>	Osteopenia <sup>2</sup>	Osteoporosis <sup>3</sup>	Total number of patients
At diagnosis				
BMD-status	93	78	52	223
Follow up (FU)				
Number of patients with available FU	45 (48%)	58 (74%)	48 (92%)	151 (68%)
Median follow up in years [range]	10.7 [1.3 – 19.5]	6.9 [1 – 20.1]	6.7 [1 – 18.8]	7.5 [1 – 20.1]
Changes in BMD-status during FU	33 (74%) status quo	13 (22%) become normal	2 (4%) become normal	
	11 (24%) develop osteopenia	44 (76%) status quo	28 (58%) develop osteopenia	
	1 (2%) develop osteoporosis	1 (2%) develop osteoporosis	18 (38%) status quo	

FU: follow-up, BMD: bone mineral density

<sup>1</sup>Normal bone mineral density [T-score of -1.0 or higher], <sup>2</sup>osteopenia [T-score between -1.0 and -2.5],

<sup>3</sup>osteoporosis [T-score < -2.5].

two years after gluten-free diet initiation. During a median follow up of over seven years, we found that 50 patients developed incidental anemia, iron, vitamin B12, folic acid deficiency or autoimmune disease associated with either coeliac disease or other conditions. When we focus on patients with normal values at diagnosis, approximately 50 to 60%, and search for coeliac related deficiencies, only 12 cases are left. Two of these patients had symptoms leaving 10 patients with asymptomatic coeliac related deficiency or autoimmune disease, that would not have been found without screening (incidence 10/1000 patient years). In contrast, 16 patients with abnormal values at diagnosis developed coeliac related re-deficiencies or autoimmune diseases, of whom 11 were asymptomatic (incidence 34/1000 patient years). Thus, coeliac related asymptomatic re-deficiencies and autoimmune diseases are significantly ( $p < 0.05$ ) more frequent in patients with abnormal values at diagnosis, which initially fully recovered in the cases of deficiencies.

The prevalence of deficiencies at diagnosis found in our study was in the range of results from other studies and higher compared with healthy controls [17, 18]. Data upon the incidence of deficiencies during long term follow-up in patients with coeliac disease, however, are scarce and recommendations are based upon expert opinion [6, 11-13]. A recent study conducted in children found low frequencies of deficiencies during follow-up and consequently considered additional laboratory investigations during follow up unnecessary [19]. The prevalence of nutritional deficiencies in the general population appears to be close to the values we found during follow up [16, 20-25]. This suggests that patients with coeliac disease adhering to a gluten free diet are not at risk of developing nutritional deficiencies.

We found slightly lower to similar frequencies for autoimmune thyroid disease and type 1 diabetes compared to other studies [26, 27]. More importantly, the majority of these diseases were detected prior to the diagnosis of coeliac disease; only four new cases were found during follow up. Furthermore, all but one patient presented with typical associated symptoms or already had subclinical disease at diagnosis. Literature upon the protective effect of gluten-free diet on the development of autoimmune disease is controversial. It is hypothesized that longstanding gluten exposure in untreated disease may promote the development of related diseases and excluding gluten from the diet may decrease this risk by lowering antibodies [28, 29]. Recent literature, however, suggests that a gluten-free diet does not prevent the occurrence of autoimmune disorders and states that the length of gluten exposure before diagnosis does not determine the future development of these diseases [9, 30].

The frequency of osteopenia and osteoporosis at diagnosis of coeliac disease in our study is in accordance or slightly lower compared to recent studies conducted on this subject [10, 31]. Mean age of the studied population and/or relatively high calcium intake from the Dutch dairy rich diet may partially explain this difference. We also found more patients' BMD to improve, rather than deteriorate after starting gluten-free diet. This is consistent with the reported fact that gluten-free diet improves BMD substantially [10, 31]. However, because this deterioration of BMD still occurred in 8.6% of our cohort and

additional therapy is easily available, we recommend follow up DEXA-scans.

A major strength of our study is that we scrutinized data of a large cohort of patients with a long follow-up. We included both patients diagnosed at our hospital as in other institutes showing similar results, indicating generalizability. Finally, our study bears clinical relevance by decreasing the burden of disease for patients through less blood draws and by lowering the costs of healthcare by 1.5 million dollars per year in the Netherlands.

This study comes with limitations, mainly as a result of its retrospective nature. The missing data might have resulted in the underestimation of deficiencies during follow up. However, intercurrent and resolved deficiencies are probably mild and less clinically relevant. In the majority of patients with low vitamin B12 values, no further diagnostic steps (methylmalonic-acid, parietal and intrinsic-factor auto-antibodies) to prove etiology have been undertaken. This may have led to the overestimation of the frequency and made it impossible to distinguish between coeliac related and other natures of vitamin B12 deficiency.

We only investigated nutritional deficiencies suggested by treatment guidelines [7, 8]. Other deficiencies e.g. zinc and vitamin B6 that seem more prevalent among newly diagnosed patients were not included. The exact numbers, however, vary but more importantly, they resolve and the clinical relevance of zinc deficiency remains ambiguous [12, 13, 17].

The evaluation of BMD over time might be affected by technical changes due to new equipment. The majority of patients, however, has been scanned in recent years using the same machine. We might have underestimated the use of over-the-counter supplements, which may be higher in patients than in healthy individuals. A Finnish study showed that over 50% of patients used significantly more vitamin and micronutrient supplements than before diagnosis [32]. Last, treating physicians were not blinded for laboratory results, which may have influenced their strategy during history taking. However, history taking is the hallmark of treating patients. The frequency of deficiencies was low and absence or presence of symptoms were described in over 80 percent of patients with normal results.

## CONCLUSIONS

The incidence of asymptomatic coeliac-related deficiencies or autoimmune disease is low in patients with normal values at diagnosis, therefore routine laboratory screening seems not necessary in these patients when attending regular follow-up visits. Patients with abnormal test results at diagnosis however are at a significantly increased risk that still warrants routine tests. Follow-up DEXA scans are recommended for all patients.

**Conflicts of interest:** None to declare.

**Authors' contributions:** J.B. and J.v.d.L. conceived the study design, performed data extraction and analysis and drafted the manuscript. J.B. and J.v.d.L. contributed equally to this manuscript. T.J. helped with data extraction and analysis. E.R. helped with the data analysis and

reviewed the manuscript for important intellectual content. P.W., M.W. and J.D. reviewed the manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

**Supplementary material:** To access the supplementary material visit the online version of the *J Gastrointest Liver Dis* at <http://www.jgld.ro/wp/archive/y2018/n3/a7> and <http://dx.doi.org/10.15403/jgld.2014.1121.273.jp>

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**Supplementary Table I. Characteristics of patients with anemia during follow up**

<b>Group</b>	<b>Sex</b>	<b>Value (mmol/l)</b>	<b>Etiology</b>	<b>Symptoms</b>
1	Female	6.9	Pregnancy	Present
1	Female	7.0	Menorrhagia	Present
1	Female	7.0	Possibly CD related	Absent
1	Male	8.3	Possibly CD related	Absent
1	Female	7.0	PMR, Hemorrhoids	Present
1	Female	8.3	Possibly CD related	Present
1	Female	6.7	Pregnancy	Present
1	Female	6.4	Possibly CD related	Present
2	Female	5.4	Menorrhagia, IBD	Present
2	Female	7.3	Possibly CD related	Present
2	Male	8.0	Possibly CD related, Lymphoma	Present
2	Female	7.1	Possibly CD related	Absent
2	Female	7.3	IBD	Present
3	Female	6.7	Possibly CD related	Present
3	Female	7.2	IBD	Present
3	Female	7.0	Pregnancy	Absent
3	Female	7.3	Menorrhagia	Present
3	Female	7.0	Menorrhagia	Present
3	Female	5.3	Menorrhagia	Present

\* Coeliac disease \*\* Inflammatory Bowel Disease

Group 1 = Deficiency after initial normal value

Group 2 = Deficiency after recovery of deficiency at diagnosis

Group 3 = Deficiency after unknown value at diagnosis

**Supplementary Table II. Characteristics of patients with Vitamin B12 deficiency during follow up**

<b>Group</b>	<b>Value (mmol/l)</b>	<b>Etiology</b>	<b>Symptoms</b>
1	139	Possibly CD related	Absent
1	126	Ileoanal pouch due to CU*	Present
1	143	Possibly CD related	Absent
1	135	Possibly CD related	Absent
2	123	Possibly CD related; veganism	Present
2	126	Possibly CD related	Absent
2	148	Possibly CD related	Absent
2	144	Possibly CD related	Present
2	123	Possibly CD related	Absent
2	163	Possibly CD related	Absent
3	134	Possibly CD related	present
3	141	Possibly CD related	present
3	92	Possibly CD related	absent

\*Ulcerative colitis

Group 1 = Deficiency after initial normal value

Group 2 = Deficiency after recovery of deficiency at diagnosis

Group 3 = Deficiency after unknown value at diagnosis

**Supplementary Table III. Characteristics of patients with folic acid deficiency during follow up**

<b>Group</b>	<b>Value (mmol/l)</b>	<b>Etiology</b>	<b>Symptoms</b>
<b>1</b>	7.5	Possibly CD related	Absent
<b>1</b>	6.4	Possibly CD related	Absent
<b>1</b>	8.1	Possibly CD related	Absent
<b>2</b>	8.9	Possibly CD related	Absent
<b>2</b>	7.9	Possibly CD related	Absent
<b>2</b>	4.9	Possibly CD related	Absent
<b>2</b>	7.2	Possibly CD related	Absent
<b>3</b>	8.8	Possibly CD related	Absent
<b>3</b>	8.4	Possibly CD related	Absent
<b>3</b>	8.6	Possibly CD related	Absent

Group 1 = Deficiency after initial normal value

Group 2 = Deficiency after recovery of deficiency at diagnosis

Group 3 = Deficiency after unknown value at diagnosis

**Supplementary Table IV. Characteristics of patients with iron status deficiency during follow up**

<b>Group</b>	<b>Value (mmol/l)</b>	<b>Etiology</b>	<b>Symptoms</b>
1	9	Menorrhagia	Absent
1	10	Possibly CD related	Absent
2	17	Inflammatory bowel disease	Absent
2	9	Inflammatory bowel disease	Present
2	19	Lymphoma	Present
2	12	Angiodysplasia	Absent
2	19	Colon carcinoma	Absent
2	5	Menorrhagia	Present
2	19	Possible CD related	Absent
3	3	Menorrhagia	Present
3	5	Menorrhagia	Present
3	7	Pregnancy	Absent
3	14	Possibly CD related	Absent

Group 1 = Deficiency after initial normal value

Group 2 = Deficiency after recovery of deficiency at diagnosis

Group 3 = Deficiency after unknown value at diagnosis