Biochemical abnormalities among patients referred for celiac disease antibody blood testing in a primary health care setting

Kårhus, Line Lund; Kriegbaum, Margit; Grand, Mia Klinten; Lind, Bent Struer; Møllehave, Line Tang; Rumessen, Jüri J.; Andersen, Christen Lykkegaard; Linneberg, Allan

Published in: Scientific Reports

DOI: 10.1038/s41598-022-10492-6

Publication date: 2022

Document version Publisher's PDF, also known as Version of record

Document license: CC BY

Citation for published version (APA): Kårhus, L. L., Kriegbaum, M., Grand, M. K., Lind, B. S., Møllehave, L. T., Rumessen, J. J., Andersen, C. L., & Linneberg, A. (2022). Biochemical abnormalities among patients referred for celiac disease antibody blood testing in a primary health care setting. Scientific Reports, 12(1), [6407]. https://doi.org/10.1038/s41598-022-10492-6
Biochemical abnormalities among patients referred for celiac disease antibody blood testing in a primary health care setting

Line Lund Kårhus1*, Margit Kriegbaum2, Mia Klinten Grand2, Bent Struer Lind3, Line Tang Møllehave1, Jüri J. Rumessen4, Christen Lykkegaard Andersen2,5 & Allan Linneberg1,6

To investigate possible biochemical abnormalities associated with celiac disease (CD) antibody positivity in a primary health care setting and thereby identify predictors that could potentially reduce diagnostic delay and underdiagnosis of CD. This observational cohort study included measurements of CD antibodies in the Copenhagen Primary Care Laboratory (CopLab) database from 2000 to 2015; CD antibody positivity was defined as tissue transglutaminase antibody IgA or IgG ≥ 7 kU/L and/or deamidated gliadin peptide antibody IgG ≥ 10 kU/L. Individuals with a prior diagnosis of CD were excluded. We examined differences between individuals with positive and negative CD antibody tests regarding the results of biochemical tests performed six months before and one month after the date of the CD antibody test. We identified 76,265 measurements of CD antibodies during 2000–2015, and 57,061 individuals met the inclusion criteria (706 antibody-positive and 56,355 antibody-negative). We found lower ferritin, hemoglobin, cobalamin and folic acid levels and higher levels of transferrin, ALAT (alanine transaminase), and alkaline phosphate among individuals with a positive CD antibody test. Furthermore, we illustrated more measurements below the sex-specific reference intervals for hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), ferritin, cobalamin and folic acid among individuals with a positive CD antibody test. This study identified several biochemical abnormalities associated with CD antibody positivity among individuals referred to CD antibody testing. The pattern of abnormalities suggested that micronutrient deficiencies were prevalent among CD antibody-positive individuals, confirming malabsorption as a sign of CD. These findings illustrate the possibility of reducing diagnostic delay and underdiagnosis of CD.

Abbreviations
ALAT  Alanine transaminase
CD  Celiac disease
CI  Confidence interval
CGPL  Copenhagen General Practitioners’ Laboratory
CopLab  Copenhagen Primary Care Laboratory
CRP  C-reactive protein
DKK  Danish crowns
DGP  Deamidated gliadin peptide
RDW  Erythrocyte volume, relative distribution width

1Center for Clinical Research and Prevention, Copenhagen University Hospital – Bispebjerg and Frederiksberg, Frederiksberg, Copenhagen, Denmark. 2Copenhagen Primary Care Laboratory (CopLab) Database, Research Unit for General Practice and Section of General Practice, Department of Public Health, University of Copenhagen, Copenhagen, Denmark. 3Department of Clinical Biochemistry, Copenhagen University Hospital Hvidovre, Hvidovre, Denmark. 4Department of Gastroenterology, Copenhagen University Hospital - Herlev and Gentofte, Copenhagen, Denmark. 5Department of Hematology, Rigshospitalet, Copenhagen, Denmark. 6Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark. *email: line.lund.kaarhus@regionh.dk
Celiac disease (CD) is a chronic disease occurring in all age groups and affecting approximately 1% of the population\(^1\), although many cases of CD remain undiagnosed\(^2\)–\(^8\). This condition is caused by an abnormal immune response in genetically susceptible individuals triggered by the ingestion of gluten proteins from wheat, rye and barley\(^2\)–\(^4\).

Celiac disease primarily affects the small intestine, often leading to malabsorption and micronutrient deficiencies. A small intestinal biopsy with recognition of villus atrophy and inflammation has been the gold standard for diagnosis; however, serological testing is increasingly used in the diagnostic process and screening for CD, mainly by the detection of CD-specific antibodies, primarily immunoglobulin (Ig) A against tissue transglutaminase (TTG), the autoantigen in CD\(^5\)–\(^9\). Management consists of a life-long gluten-free diet. Screening for CD among individuals without typical symptoms—or even in the general population—remains controversial because many screen-detected cases have few or no symptoms, and little is known about the prognosis of undiagnosed CD. Nevertheless, studies suggest that asymptomatic patients with serological biomarkers indicative of CD may also benefit from a gluten-free diet\(^11\),\(^12\),\(^1\), and we have recently reported that undiagnosed CD may have an increased risk of adverse long-term consequences such as cancer and cardiovascular diseases\(^13\).

In Denmark, the prevalence of diagnosed CD is lower than in other European countries, despite recent increases in the prevalence of diagnosed cases as recorded in national registries\(^14\)–\(^15\). Furthermore, we have previously shown that CD is markedly underdiagnosed in the general population: the prevalence found by screening (screening prevalence) was up to ten times the registered prevalence of diagnosed CD\(^16\). In line with other studies, we found no differences in symptoms between participants with and without screen-detected disease\(^17\)–\(^19\). This suggests that screening for symptoms may not be an effective strategy for the detection of undiagnosed CD in a population setting. However, there is evidence that patients with CD often have hematological and other biochemical abnormalities at the time of diagnosis\(^20\). Furthermore, CD is known to be associated with malabsorption, especially before diagnosis, as evidenced by various deficiencies in nutrients, vitamins, minerals and trace elements, resulting in iron-deficiency anemia as well as calcium and vitamin B and D deficiencies\(^21\)–\(^2\).

Diagnostic delay is a challenge in CD, and an average time to diagnosis of up to 10 years has been reported\(^2\)–\(^2\). A Swedish study found that the mean delay to diagnosis was ten years from the first symptoms and six years from the first doctor’s visit\(^23\). The long diagnostic delay is a potential burden for both the patient and society, resulting in more health care contacts and costs, as well as costs for sick leave and decreased productivity. Casuistic reports indicate that the diagnostic delay is also significant in Denmark\(^24\), although we do not yet have survey data investigating the delay from first symptom/health care contact to a diagnosis of CD.

The overall aim of the present study was to describe the pattern of hematological and other biochemical test results among individuals referred for CD antibody blood testing in a primary health care setting in Denmark. In a primary health care setting, we aimed to investigate whether there were distinct hematological and/or biochemical abnormalities associated with being CD antibody test positive and/or diagnosed with CD. Identification of hematological and/or biochemical abnormalities that predict CD will potentially enable us to reduce diagnostic delay and underdiagnosis of CD. This could, in turn, lead to early treatment and prevention of complications in patients with CD.

### Methods

#### Data

The Copenhagen primary care laboratory (CopLab) database. This observational cohort study included results from primary care in the Copenhagen area of Denmark. In Denmark, citizens have direct access to primary care and hospital care at no cost. Approximately 98% of Danish citizens are listed with a general practitioner (GP), and Danish GPs are gatekeepers to more specialized patient care by specialized consultants as well as most in- and outpatient hospital care\(^25\). In the Copenhagen area (the Copenhagen Municipality and the former Copenhagen County), with its approximately 1.2 million inhabitants, only one laboratory served general practitioners and practicing specialists until 2015, the Copenhagen General Practitioners’ Laboratory (CGPL, Københavns Praktiserende Lægers Laboratorium). The CGPL provided a broad range of blood and urine biochemical tests, clinical physiological tests, and various cardiac tests and was accredited for International Organization for Standardization (ISO) standards ISO17025 and ISO15189. All data regarding the analyses performed since July 2000 have been saved in The Copenhagen Primary Care Laboratory (CopLab) database containing all results (n = 176,000,000) from July 1, 2000 to December 31, 2015 from the CGPL. Materials concerning the CopLab database have also been described elsewhere\(^26\)–\(^2\).

For the present study, we used data from the CopLab database. The CopLab database contains information concerning the date of blood testing and thereby the age at antibody measurement. Celiac antibody tests performed prior to 2006 were externally analyzed, and since the database does not include numerical results from
these tests, they were not included in the study population. However, information concerning the number of tests was used for Table 2, providing an overview of the distribution of tests in the study period.

**National registers.** In Denmark, all citizens are registered with a unique civil registration number, which enables person-level linkages across nationwide registers. The study population was linked to The Danish Civil Registration System, which provides information on vital status; Statistics Denmark, with information on income; Danish Education Registers, which provides information on highest attained education; Danish National Health Register, which provides information on the number of contacts with the health care system; and The Danish National Patient Register (NPR), which contains recorded information on all hospital contacts in Denmark since 1977, with individual diagnoses registered according to the World Health Organization’s (WHO) International Classification of Diseases (ICD-system)37.

For the present study, we used data from NPR from 1978 to 2018 and The Danish Civil Registration System until 2018. The ICD codes used for this study were the diagnosis codes for CD: ICD-8 269.00 and ICD-10: K90.0 to exclude individuals with a prior known diagnosis of CD from the study population. The age at antibody measurement and sex were derived from the unique civil registration number in the CopLab database. Education, income and country of origin were registered on January 1 in the year of CD measurement.

The *household equivalized income* takes into consideration the total income of the household as well as the composition of the household (number of adults and children). Hence, household equivalized income accurately reflects purchasing power. Information on equivalized household income was obtained from income registers at Statistics Denmark and was adjusted for inflation and related to the consumer price index in 2015. Income was recorded in Danish crowns (DKK) but displayed in Euros using the conversion rate from 2015 (100€ = 744 DKK).

**Educational attainment** was retrieved from the education registry at the year of the CD test and classified into three categories according to the International Standard Classification of Education (ISCED)-system (UNESCO 1997): up to 10 years of education = primary or lower secondary education (ISCED level 0–2), 11–12 years of education = upper secondary education (ISCED level 3) and 13 and more years of education = post-secondary and tertiary education (ISCED level 4–6). For individuals aged 24 or younger, information on the educational level of both parents was categorized as described above, and the individual was assigned the highest value of one’s own, the father’s and mother’s education.

**Country of origin** was derived from the population registry and grouped into Danish versus non-Danish (comprising migrants and descendants of migrants).

**Biochemical blood tests.** Celiac disease antibody tests. Tissue transglutaminase antibody (IgA) (TTG-IgA), tissue transglutaminase antibody (IgG) (TTG-IgG), deamidated gliadin peptide antibody (IgA) (DGP-IgA) and deamidated gliadin peptide antibody (IgG) (DGP-IgG) were measured in serum by fluorescence enzyme immunoassay (EIA) on the UniCAP 100 and ImmunoCAP 250 platforms (Phadia Laboratory Systems, Thermo Fisher Scientific, Hvidovre Denmark) according to the instructions of the manufacturer. For all four assays, the results were reported as negative (< 7 kU/L), equivocal (7–10 kU/L) or positive (> 10 kU/L). The results are valid from CGPL from July 13, 2006 (TTG-IgA) and July 1, 2013 (TTG-IgG, DGP-IgA and DGP-IgG). Before these dates, the CD antibodies were analyzed by the same method by an external collaborator, Phadia/Thermo Fisher, and the results were saved at CGPL. The interserial (day to day) coefficient of variation percentage determined on patient samples was estimated at 5–10% (at levels 7–10 kU/L), which indicates that the probability of a test result changing from negative to positive or vice versa due to random variation is highly unlikely (p < 0.01). The reportable ranges were 0.1–128 kU/L (TTG-IgA), 0.6–600 kU/L (TTG-IgG), 0.1–142 kU/L (DGP-IgA) and 0.4–302 kU/L (DGP-IgG). The four assays were subject to external quality control through participation in the UK NEQAS for COELIAC DISEASE external quality assessment service (Sheffield, United Kingdom). The assessment scheme included 6 distributions annually. Each distribution comprised 1 sample classified as either positive, negative or equivocal. The results from the UK NEQAS confirmed the reliability of the assay, and the samples were correctly classified in all cases for TTG-IgA (n = 54), TTG-IgG (n = 14) and DGP-IgA (n = 14). For DGP-IgG disease, 13 of 14 samples were correctly classified, and 1 equivocal sample was classified as positive.

In the present study, we defined, as in earlier studies13,38, CD antibody positivity as IgA TTG ≥ 7 kU/L, IgG TTG ≥ 7 kU/L and/or IgG DGP ≥ 10 kU/L.

Other hematological and biochemical tests. Other variables used from the CopLab database were hemoglobin, MCV (erythrocytes, mean corpuscular volume), MCHC (mean corpuscular hemoglobin concentration), transferrin, hematocrit, ferritin, ALAT (alanine transaminase), alkaline phosphate, 25-OH vitamin D, folic acid, cobalamin, CRP (C-reactive protein), ReticMCH (reticulocyte, mean corpuscular hemoglobin), RDW (erythrocyte volume, relative distribution width), and immunoglobulin A. A basic characterization of other blood tests is summarized in Additional Table S1 (Additional file 1). Reference intervals used to define abnormal values are presented in Additional Table S2 (Additional file 1).

**Study population.** The flow chart in Fig. 1 depicts the study population, which comprised all individuals referred to CGPL during 2000–2015, both from GPs and practicing specialists, for measurement of CD antibodies: IgA- and IgG-TTG and IgG and IgG deamidated gliadin (DGP). The CopLab database included a total of
76,265 blood sample analyses with at least one celiac antibody analysis during 2000–2015. As mentioned under “Data”, CD antibody tests performed prior to 2006 were externally analyzed, and since the database does not include numerical results from these tests, they were not included in the study population. However, the numbers of tests were used for Table 2, giving an overview of the distribution of tests in the study period. The study population ultimately consisted of individuals with a first-time CD antibody measurement who were living in Denmark on January 1 of the year of CD measurement without migrations six months before and one month after the first CD antibody testing. Individuals with a prior diagnosis ICD-8/10 code for CD in the registries were excluded.

**Statistics.** Differences in baseline characteristics between CD antibody-positive and CD antibody-negative patients and trends in requisitions (Tables 1 and 2). The distribution of categorical covariates was summarized using counts and percentages. Continuous covariates with a nonnormal distribution were summarized using the median and interquartile range (IQR).

In Table 1, chi-square tests were used to test differences between categorical variables. For continuous variables (age and income), the Wilcoxon test was used, as none of the included variables were normally distributed.
Two-sided p values were reported for all tests. The p values were adjusted for multiple testing, and values < 0.05 were considered statistically significant.

**Associations with biochemical biomarkers (Tables 3 and 4).** The distribution of categorical covariates was estimated using counts and percentages. Continuous covariates with a log-normal distribution were summarized using the median, calculated as the exponential of the mean of the log-transformed values which is also known as the geometric mean. The 95% confidence intervals were calculated using a robust variance estimator to account for potential outliers.

| Year | Number of tests | IgA-TTG | IgA-DGP | IgG-TTG | IgG-DGP | Number of persons | Age median (IQR) | Percent female |
|------|-----------------|---------|---------|---------|---------|------------------|-----------------|---------------|
| 2000 | 156             | 156     | 25      | .       | .       | .                | 156             | 25            |
| 2001 | 334             | 334     | 96      | .       | .       | 329              | 27             | 61.7          |
| 2002 | 384             | 384     | 133     | .       | .       | 378              | 31     | 63.2          |
| 2003 | 1204            | 1186    | 488     | 18      | <5      | 1171             | 30     | 65.8          |
| 2004 | 1466            | 1435    | 540     | 31      | <5      | 1247             | 30     | 66.0          |
| 2005 | 1883            | 1862    | 749     | 21      | <5      | 1836             | 32     | 64.6          |
| 2006 | 2139            | 2088    | 913     | 51      | 9       | 2035             | 30     | 66.9          |
| 2007 | 2567            | 2524    | 1089    | 43      | 15      | 2384             | 30     | 65.6          |
| 2008 | 3953            | 3877    | 1784    | 76      | 25      | 3744             | 31     | 66.7          |
| 2009 | 4655            | 4583    | 2074    | 72      | 26      | 4457             | 30     | 67.1          |
| 2010 | 5234            | 5171    | 2322    | 63      | 24      | 5029             | 30     | 65.4          |
| 2011 | 5938            | 5864    | 2841    | 74      | 36      | 5676             | 29     | 65.6          |
| 2012 | 7698            | 7587    | 4187    | 111     | 59      | 7338             | 29     | 66.2          |
| 2013 | 10,515          | 9848    | 5687    | 133     | 85      | 9512             | 30     | 67.0          |
| 2014 | 13,440          | 12,101  | 7610    | 214     | 137     | 564              | 29     | 66.7          |
| 2015 | 14,699          | 13,072  | 8442    | 212     | 136     | 689              | 31    | 66.6          |

Table 1. Characteristics of individuals with positive and negative celiac disease antibody test results. IQR Interquartile range. *positive celiac antibody test was defined as: IgA/IgG TTG of at least 7 U/ml or IgG DGP of at least 10 U/ml and a negative test was defined as IgA/IgG TTG under 7 U/ml and IgG DGP under 10 U/ml. Chi square tests were used to test differences between categorical variables. For continuous, (age and income) Wilcoxon test was used (age and income were not normally distributed), 2-sided p-values were reported for all tests. P-values were adjusted for multiple testing. *Origin was derived from the population registry and grouped into Danish vs. non-Danish (comprising migrants and descendants of migrants). dThe household equivalized income in Euros. Income was missing for 361 individuals. The total number of contacts with primary health care, recorded from the reimbursement system the five years before celiac disease antibody measurement.
Table 3. Results of hematological and biochemical measurements during the period six months before to one month after blood drawing and testing among individuals with positive and negative celiac antibody tests.

| Measurement                      | % (95% CI) | 706 CD antibody-positive individuals | 706 CD antibody-negative individuals |
|----------------------------------|------------|--------------------------------------|--------------------------------------|
| **Hemoglobin (mmol/L)**          | 58,883     | 81.7                                 | 7.8 (7.8–7.9)                        | 81 (8.1–8.1)                          |
| MCV (Erythrocytes, mean corpuscular volume) (fl) | 58,883     | 81.7                                 | 86.6 (85.9–87.4)                     | 88.6 (88.6–88.7)                      |
| MCHC (Mean corpuscular hemoglobin concentration) (mmol/L) | 14,044     | 22.4                                 | 20.3 (20.1–20.4)                     | 20.7 (20.7–20.7)                      |
| Transferrin (µmol/L)             | 8,000      | 13.3                                 | 37.5 (35.7–39.3)                     | 34.2 (34.0–34.3)                      |
| Ferritin (µg/L)                  | 13,549     | 21.5                                 | 13.7 (11.3–16.7)                     | 35.9 (35.2–36.6)                      |
| ALAT (Alanine transaminase) (U/L)| 47,751     | 68.8                                 | 22.6 (21.6–23.6)                     | 19.8 (19.7–19.9)                      |
| Alkaline phosphate (U/L)         | 43,964     | 63.9                                 | 87.2 (81.7–93.2)                     | 74.1 (73.6–74.6)                      |
| Vitamin D (nmol/L)               | 28,599     | 43.8                                 | 60.3 (56.8–64.1)                     | 58.9 (58.5–59.3)                      |
| ReticMCH (Reticulocyte, mean corpuscular hemoglobin) (fmol) | 2,472      | 3.9                                  | 1.7 (1.6–1.7)                        | 1.8 (1.8–1.9)                         |
| RDW (Erythrocytes volume, relative distribution width (%)) | 58,882     | 81.7                                 | 13.8 (13.6–13.9)                     | 13.2 (13.1–13.2)                      |
| Immunoglobulin A (g/L)           | 58,125     | 98.7                                 | 1.9 (1.8–2.0)                        | 1.8 (1.8–1.8)                         |
| Cobalamin (pmol/L)               | 19,886     | 31.8                                 | 280.1 (261.2–300.4)                  | 289.1 (287.1–291.2)                   |
| Folic acid (nmol/L)              | 2,839      | 4.7                                  | 12.9 (10.5–16.0)                     | 17.5 (17.0–18.0)                      |
| C-reactive protein (CRP) (mg/L)  | 46,205     | 66.6                                 | 0.9 (0.6–1.3)                        | 1.2 (1.1–1.2)                         |

*Table 3: Results of hematological and biochemical measurements during the period six months before to one month after blood drawing and testing among individuals with positive and negative celiac antibody tests.

A positive celiac antibody test was defined as: IgA/IgG TTG of at least 7 U/ml or IgG DGP of at least 10 U/ml and a negative test was defined as IgA /IgG TTG under 7 U/ml and IgG DGP under 10 U/ml. CRP was reported with < in many cases; Low results were reported as < 5 mg/L (between December 2, 2002 and May 28, 2008) and < 4 (from May 29, 2008).

account for dependence between tests from the same person. Only for CRP was a parametric survival model with Gaussian errors and a robust variance estimator used to account for the left censoring due to lower limits of detection.

**Ethics approval and consent to participate.** Ethical and data handling approval were obtained by the Faculty of Health Science, University of Copenhagen (case no. 514-0460/20-3000). According to Danish legislation, no ethical approval or consent is required for registry-based research projects, in which individuals included in the study are not approached at any time during the conduct of the study.

**Results**

The selection of individuals in the study is shown in Fig. 1, resulting in a population of 706 CD antibody-positive and 56,355 CD antibody-negative individuals.

We compared the characteristics of CD antibody-positive individuals with those of CD antibody-negative individuals. We found that the antibody-positive individuals were younger, and a larger percentage were women. There was no statistically significant difference in the number of contacts with primary health care during the five-year period prior to CD antibody measurement, but we observed differences with regard to country of origin, education, and income (Table 1); however, these differences were small. Trends in the frequency of requisitions for CD antibody tests from primary care per calendar year are illustrated in Table 2. There was a clear and continuously increasing trend in the number of persons tested throughout the observed 15-year period, and more women than men were tested. The median age of persons tested was approximately 30 throughout the study period, varying from a median age of 26 to a median age of 32 when calculated per year.

Table 3 depicts differences in median levels of hematological and other biochemical biomarkers between individuals, referred to the laboratory, with positive and negative CD antibody tests stratified by sex. The most remarkable difference was, for both men and women, the markedly lower ferritin among the CD antibody-positive individuals compared with the CD antibody-negative individuals; for women 13.8 versus 34.3 µg/L and for men 34.3 versus 80.4 µg/L, respectively. We also found a tendency of lower hemoglobin among CD antibody-positive individuals; for women, 7.8 versus 8.1 mmol/L, and for men, 8.5 versus 8.8 mmol/L, respectively.
Furthermore, we observed lower cobalamin and folic acid levels, while higher levels of transferrin, ALAT and alkaline phosphatase were noted among CD antibody-positive men and women.

Table 4 presents, for each hematological and other biochemical biomarker, the proportion of tests with biomarker measurements outside (below or above) sex-specific reference intervals for each measurement among individuals, referred to CD antibody testing, with positive and negative CD antibodies. We found that a greater proportion of tests among the CD antibody-positive individuals compared with negative individuals exhibited hemoglobin (10.2% vs. 2.7%), MCV (7.1% vs. 2.9%), MCHC (6.8% vs. 1.2%), and ferritin (37.6% vs. 7.6%) below, while transferrin (20.7% vs. 9.5%) was above the reference interval. Furthermore, deficiency of cobalamin and folic acid was more common among tests from individuals with positive CD antibodies.

### Discussion

This study has revealed relevant prediagnostic differences in hematological and other biochemical test results between individuals with positive and negative CD antibodies among individuals referred to the laboratory for celiac disease antibody measurements. Thus, individuals with positive CD antibodies were more likely to have blood levels of hemoglobin, MCV, MCHC, ferritin, cobalamin and folic acid below and transferrin above the reference interval. These results suggest that hematological and other biochemical abnormalities may be important markers of undiagnosed CD.

Overall, the pattern of these biomarkers suggests malabsorption, for example iron deficiency anemia, among individuals with positive CD antibodies. CD is known to be associated with malabsorption, especially before diagnosis, as exemplified by various deficiencies of micronutrients, resulting in iron-deficiency anemia and vitamin B and D deficiencies, among others. Even though malabsorption is described as a classic sign of CD, studies have found malabsorption to be associated with an increased risk of a long delay of diagnosis. This study confirms the association of CD and malabsorption, as we observed more measurements below the reference interval. These results suggest that hematological and other biochemical abnormalities may be important markers of undiagnosed CD.
reference interval for hemoglobin, ferritin, cobalamin and folic acid among individuals referred to CD antibody testing with positive CD antibodies. These results confirm the potential of screening for CD among individuals with specific laboratory signs of malabsorption. However, the results are not adjusted for age and we do not have information on mineral or vitamin supplements, which might affect the results, and the association could likely be stronger if this adjustment had been possible. Furthermore, several measurements could be included form the same individual, e.g. controls for abnormal results, but we used calculated values taking repeated measurements into account in the statistical methods.

The increase in CD antibody measurements during 2000–2015 corresponds to the increase in CD diagnoses seen in the Danish registers14,15, as well as to the increase observed internationally20. From 2013, the DGP tests were introduced, but not all individuals were tested with both TTG and DGP. There were some combined tests that could be requested; from 2003, a ‘CD test’ included both TTG-IgA and total IgA, and an algorithm resulted in a test for TTG-IgG if both TTG-IgA and IgA were low. Starting in 2013, if TTG-IgG was low in children, both DGP-IgA and DGP-IgG were measured. These algorithms might have influenced the measurements, possibly resulting in more awareness among the GPs and an increase in the number of tests per person, but the physicians could also request separate CD antibodies. The number and type of CD antibody measurements are consistent with the acceptance and considerable evidence supporting the use of TTG-IgA assays as the first-line test for the diagnosis of CD29. More women than men were tested for CD, and these numbers are in line with the notion that more women than men are diagnosed with CD13 but also in line with the knowledge of an increased risk for CD among girls and women41.

A strength of this study is that it includes data from primary care, which is the most important setting for identifying persons in need of diagnostic work-up. This is a unique possibility to describe patterns of CD antibody measurements requested from primary health care. It is also a strength that the same methods for CD antibody measurement were used throughout the study period and that we controlled for confounding factors and other biochemical tests even before the CD measurements were performed at CGPL. The possibility of linkage to the Danish national registers is a strength for comparing the groups. Moreover, it is a strength that the CopLab database includes all blood samples from the Copenhagen area for primary care, resulting in a large number of tests and individuals. Nevertheless, as the prevalence of CD is so low, even with this large number of individuals, the number of CD antibody-positive individuals was small. It is also important to note, that the results in Table 4 are presented by number of tests, and the same individual potentially could have several measurements included, e.g. controls for abnormal results. However, the percentages and 95% confidence intervals are calculated taking repeated measurements into account. Furthermore, the population is highly selected, as they are referred to the laboratory for a CD antibody test, and individuals with other biomarker measurements might be a further selected group. Therefore, the results of this highly selected population of seropositive individuals cannot be generalized to all seropositive cases. Moreover, the control group should not be considered healthy controls, as there might be other reasons for their tests, and we do not know the reason for referral to the laboratory. For example, deficiencies could be a symptom/condition causing testing for CD antibodies and may thereby affect the number of tests, especially iron-deficiency anemia, as guidelines recommend screening for CD if iron-deficiency anemia is unexplained14,45. It is important to note that diagnoses in primary care, e.g., privately practicing gastroenterologists, are not listed in the NPR; therefore, not all diagnosed CD cases are known, and some antibody-negative individuals could have a diagnosis of CD.

**Conclusion**

This study identified several biochemical abnormalities associated with CD antibody positivity in a primary care setting, among individuals referred to CD antibody testing. The present study shows more measurements below the reference interval for hemoglobin, MCV, MCHC, ferritin, cobalamin and folic acid among the individuals with a positive CD antibody test. The pattern of the included biomarkers suggested that micronutrient deficiencies were common among CD antibody-positive individuals and confirmed malabsorption as a sign of CD. These findings illustrate the possibility of prospective development of biochemical algorithms to improve guidelines for CD screening to reduce diagnostic delay and underdiagnosis of CD and to lead to early treatment and prevention of comorbidities in patients with CD.

**Data availability**

The dataset supporting the conclusions of this article are based on data from Danish national health registers and restrictions apply to the availability of these data, which were used under license for the current study, and according to Danish law, this information cannot be publicly available. A request for access to the data needs approval from appropriate Danish authorities and are subject to Danish regulations on personal data protection.

Received: 13 October 2021; Accepted: 1 April 2022
Published online: 18 April 2022

**References**

1. Ludvigsson, J. F. et al. Diagnosis and management of adult coeliac disease: Guidelines from the British Society of Gastroenterology. Gut 63, 1210–1228 (2014).
2. Catassi, C. et al. Natural history of celiac disease autoimmunity in a USA cohort followed since 1974, Annals of Medicine, 42, 530–538. Ann. Med. 42, 530–538 (2010).
3. Lebwohl, B., Ludvigsson, J. F. & Green, P. H. Celiac disease and non-celiac gluten sensitivity. BMJ 351, h347 (2015).
4. Lohi, S. et al. Increasing prevalence of coeliac disease over time. Aliment Pharmacol. Ther. 26, 1217–1225 (2007).
5. Lebwohl, B., Sanders, D. S. & Green, P. H. B. Coeliac disease. Lancet 391, 70–81 (2018).
6. HujoeI, A. et al. Natural history and clinical detection of undiagnosed coeliac disease in a North American community. *Aliment Pharmacol. Ther.*, **47**, 1358–1366 (2018).

7. Maki, M. et al. Prevalence of Celiac disease among children in Finland. *N Engl. J. Med.* **348**, 2517–2524 (2003).

8. Rubío-Tapia, A., Ludvigsson, J. F., Brantner, T. L., Murray, I. A. & Everhart, J. E. The prevalence of celiac disease in the United States. *Am. J. Gastroenterol.* **107**, 1538–1544 (2012).

9. Jabri, B. & Sollid, L. M. T cells in celiac disease. *J. Immunol.* **198**, 3005–3014 (2017).

10. Penny, H. A. et al. Accuracy of a no-biopsy approach for the diagnosis of coeliac disease across different adult cohorts. *Gut* **70**, 876–883 (2021).

11. Kurppa, K. et al. Benefits of a gluten-free diet for asymptomatic patients with serological markers of celiac disease. *Gastroenterology* **147**, 610–7 e1 (2014).

12. Vllippula, A. et al. Clinical benefit of gluten-free diet in screen-detected older celiac disease patients. *BMC Gastroenterol.* **11**, 136 (2011).

13. Kärhus, L. L. et al. Long-term consequences of undiagnosed celiac seropositivity. *Am. J. Gastroenterol.* **115**, 1681–1688 (2020).

14. Dydzensborg, S. et al. Increasing prevalence of coeliac disease in Denmark: A linkage study combining national registries. *Acta Paeediatri.* **101**, 179–184 (2012).

15. Grode, L. et al. Prevalence, incidence, and autoimmune comorbidities of celiac disease: A nation-wide, population-based study in Denmark from 1977 to 2016. *Eur. J. Gastroenterol. Hepatol.* **30**, 83–91 (2018).

16. Horwitz, A. et al. Screening for celiac disease in Danish adults. *Scand. J. Gastroenterol.* **50**, 824–831 (2015).

17. Kärhus, L. L., Thuesen, B. H., Rumessen, J. J. & Linneberg, A. Symptoms and biomarkers associated with celiac disease: Evaluation of a population-based screening program in adults. *Eur. J. Gastroenterol. Hepatol.* **28**, 1298–1304 (2016).

18. Katz, K. D. et al. Screening for celiac disease in a North American population: Sequential serology and gastrointestinal symptoms. *Am. J. Gastroenterol.* **106**, 1333–1339 (2011).

19. Rosen, A. et al. Usefulness of symptoms to screen for celiac disease. *Pediatrics* **133**, 211–218 (2014).

20. Lebwohl, B. & Rubío-Tapia, A. Epidemiology, presentation, and diagnosis of celiac disease. *Gastroenterology* **160**, 63–75 (2021).

21. Shannahan, S. & Leffler, D. A. Diagnosis and updates in celiac disease. *Gastrointest. Endosc. Clin. N Am.* **27**, 79–92 (2017).

22. Garcia-Manzanares, A. & Lucendo, A. J. Nutritional and dietary aspects of celiac disease. *Nutr. Clin. Pract.* **26**, 163–173 (2011).

23. Cichewicz, A. B. et al. Diagnosis and treatment patterns in celiac disease. *Dig. Dis. Sci.* **64**, 2095–2106 (2019).

24. Fuchs, V. et al. Factors associated with long diagnostic delay in celiac disease. *Scand. J. Gastroenterol.* **49**, 1304–1310 (2014).

25. Fuchs, V. et al. Delayed celiac disease diagnosis predisposes to reduced quality of life and incremental use of health care services and medicines: A prospective nationwide study. *United Eur. Gastroenterol. J.* **6**, 567–575 (2018).

26. Paiz, M. A., Gremelspacher, A. M., Sinacore, J., Winterfield, L. & Venu, M. Delay in diagnosis of celiac disease in patients without gastrointestinal complaints. *Am. J. Med.* **130**, 1318–1323 (2017).

27. Norstrom, F., Lindholm, L., Sandstrom, O., Nordyke, K. & Ivansson, A. Delay to celiac disease diagnosis and its implications for health-related quality of life. *BMJ Gastroenterol.* **11**, 118 (2011).

28. Kärhus, L. L., Linneberg, A. [Forinskret celiakidignose - eksemplificeret ved kasusitikker]. Mønedschrift For Almen Praksis: 2021Okt.: 706–14.

29. Pedersen, K. M., Andersen, J. S. & Sondergaard, J. General practice and primary health care in Denmark. *J. Am. Board Fam. Med.* **25**(Suppl 1), S34–S38 (2012).

30. Medici, B. B. Changes in prescription routines for treating hypothyroidism between 2001 and 2015: An observational study of 929,684 primary care patients in Copenhagen. *Thyroid* **29**, 910–919 (2019).

31. Borg, R. et al. Interpretation of HbA1c in primary care and potential influence of anaemia and chronic kidney disease: An analysis from the Copenhagen Primary Care Laboratory (CopLab) Database. *Diabet. Med.* **35**, 1700–1706 (2018).

32. Pedersen, C. B. The Danish civil registration system. *Scand. J. Publ. Health* **39**, 22–25 (2011).

33. Baadsgaard, M. & Quitzau, J. Danish registers on personal income and transfer payments. *Scand. J. Public Health* **39**, 103–105 (2011).

34. Jensen, V. M. & Rasmussen, A. W. Danish education registers. *Scand. J. Public Health* **39**, 91–94 (2011).

35. Andersen, J. S., Olivarius Nde, F. & Krasnik, A. The Danish national health service register. *Scand. J. Public Health* **39**, 34–37 (2011).

36. Lyng, E., Sandegaard, J. L. & Rebolj, M. The Danish national patient register. *Scand. J. Public Health* **39**, 30–33 (2011).

37. World Health Organization, International Classification of Diseases, 10th version: https://icd.who.int/brows e10/2016/en. 2020.

38. Karhus, L. L. et al. Symptoms and biomarkers associated with undiagnosed celiac seropositivity. *BMJ Gastroenterol.* **21**, 90 (2021).

39. Holm, S. A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* **6**, 65–70 (1979).

40. Kirkwood, B. R., Sterne, J. Essential Medical Statistics 2003.

41. Zeger, S. L. & Liang, K. Y. Longitudinal data analysis for discrete and continuous outcomes.

42. Kalbfleisch, J. D., Prentice, R. L. The statistical analysis of failure time data: Wiley; 2011.

43. Jansson-Knodell, C. L.

44. Guideline from Danish Society for Gastroenterology and Hepatology for unexplained iron deficiency anaemia: https://www.dsghe. dk/index.php/andre/ajernmangel-anemia [January 2021].

45. Ko, C. W.

46. Benefits of a gluten-free diet for asymptomatic patients with serologic markers of celiac disease. *Gastroenterology* **17**, 1358–1366 (2018).

47. Sex Difference in celiac disease in undiagnosed populations: A systematic review and meta-analysis. *Clin Gastroenterol Hepatol* **17**, 954–68 e13 (2019).

48. Guideline from Danish Society for Gastroenterology and Hepatology for unexplained iron deficiency anaemia: https://www.dsghe. dk/index.php/andre/ajernmangel-anemia [January 2021].

49. Ko, C. W. et al. AGA clinical practice guidelines on the gastrointestinal evaluation of iron deficiency anemia. *Gastroenterology* **159**, 1085–1094 (2020).

**Author contributions**

Conceptualization: L.L.K. and A.L. Visualization and methodology: L.L.K., M.K., M.K.G., B.S.L., L.T.M., J.J.R., C.B.L.A. and A.L. Data management and statistical analyses: M.K. and M.K.G. B.S.L. contributed with information on CopLab methods and material, and C.B.L.A. led the CopLab approval process. Writing—Original Draft: L.L.K., supervised by A.L. All authors critically revised the manuscript, contributed to the interpretation of the data and approved the final manuscript.

**Funding**

This study was funded by the Toyota foundation, The Internal Research Foundation at Bispebjerg and Frederiksberg Hospital and The Lundbeck Foundation (R322-2019–2530).

**Competing interests**

LKL has received honoraria for a lecture from ThermoFisher Scientific, the payment was made to Center for Clinical Research and Prevention. All other authors have no conflict of interest.
Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-022-10492-6.

Correspondence and requests for materials should be addressed to L.L.K.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022