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Low serum zinc levels predict presence of depression symptoms, but not overall disease outcome, regardless of ATG16L1 genotype in Crohn's disease patients

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Abstract

Background: Zinc deficiency (ZD) in Crohn's disease (CD) is considered a frequent finding and may exacerbate CD activity. ZD is associated with depression in non-CD patients. We aimed to assess the prevalence of ZD in CD patients in clinical remission, its association with mood disturbances and to analyze a potential impact on future disease course.

Methods: Zinc levels from CD patients in clinical remission at baseline and an uncomplicated disease course within the next 3 years ($n = 47$) were compared with those from patients developing complications ($n = 50$). Baseline symptoms of depression and anxiety were measured with the Hospital Anxiety and Depression scale.

Results: Mean zinc level in the 97 patients (40.4 ± 15.7 years, 44.3% males) was 18.0 ± 4.7 µmol/l. While no ZD (<11 µmol/l) was observed, we found low zinc levels (<15.1 µmol/l) in 28 patients (28.9%). Males had higher zinc levels compared with females (19.4 ± 5.7 *versus* 16.8 ± 3.3, $p = 0.006$). Patients with low zinc levels more often reported depression symptoms compared with patients with higher levels (27.3 *versus* 9.4%, $p = 0.047$). In a multivariate analysis, zinc levels were an independent negative predictor for depression symptoms [odds ratio (OR) 0.727, 95% confidence interval (CI) 0.532–0.993, $p = 0.045$]. Zinc levels of patients with a complicated disease course were not different from those of patients without (17.7 ± 4.3 *versus* 18.3 ± 5.1, n.s.). Baseline zinc levels did not predict disease outcome regardless of ATG16L1 genotype.

Conclusion: Low–normal zinc levels were an independent predictor for the presence of depression symptoms in CD patients. Zinc levels at baseline did not predict a complicated disease course, neither in CD patients overall, nor ATG16L1^{T300A} carriers.

Keywords: ATG16L1, anxiety, Crohn's disease, depression, disease course, single nucleotide polymorphism, zinc

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Introduction

While observational studies have shown a role for macronutrients as risk factors for inflammatory bowel disease (IBD), fewer studies have investigated that of micronutrients.[1–4] Nonetheless, it is well recognized that micronutrients have a relevant impact on different biological processes in IBD pathophysiology such as adaptive and innate immunity, and integrity of gastrointestinal barrier.[1] Zinc is a micronutrient, which has been linked to inflammatory diseases such as IBD.

Zinc deficiency (ZD) appears to compromise gastrointestinal barrier function, which can perpetuate different diseases such as celiac disease, chronic diarrhea or IBD.[5] There are several pathways demonstrating how zinc or ZD may interact with these diseases. First, zinc plays a crucial role in the development and function of cells mediating innate immunity,[6] has direct anti-inflammatory effects *via* zinc-finger protein A20,[7] and has a positive effect on intestinal tight junctions[8] and intestinal repair.[9] Furthermore, zinc can act *via* metallothioneins (MTs). MTs are a family of small proteins with a high cysteine content at conserved positions that are rapidly upregulated in response to an inflammatory stimulus such as tumor necrosis factor (TNF).[10] MT function seems to be dependent upon the presence of zinc.[11] Effects of MTs include reduction of apoptosis[12] and antimicrobial activity.[13] Finally, zinc is critical for early and late autophagy.[14] Autophagy is thought to suppress inflammation *via* degradation of inflammasomes and inflammasome-agonists.[14] Genome-wide association studies revealed an association between a single nucleotide polymorphism in ATG16L1 – a key player in autophagy – and Crohn's disease (CD).[15] In view of the latter, ZD might increase the impact of ATG16L1 polymorphisms.

Zinc deficiency is common in CD, with up to one third of all patients presenting with low serum zinc levels,[16] even in patients in clinical remission.[17] ZD in turn may exacerbate CD by increasing mucosal permeability, leading to neutrophil transmigration and luminal antigen permeation.[18] Such increased mucosal permeability has been shown to correlate with both, CD activity[19] and relapse probability.[20] Despite high prevalence of ZD in IBD and its links to inflammation, so far no study investigated the role of serum zinc as a potential predictive serum marker for future disease course and its potential causative role in patients with a low or absent inflammatory disease activity.

Zinc levels have also been implicated in psychiatric disorders, where zinc is involved in several neurobiological and inflammation processes affecting mood.[21–23] Increasing evidence suggest an association between low serum zinc levels and depression;[23–28] however, whether this also applies to patients with IBD is, as yet, unknown. Scrutinizing the relationship of ZD with psychiatric symptomatology in IBD seems particularly important, as 24% and 37% of patients with CD have depression and anxiety, respectively, with these prevalence rates being almost twice as high in patients with active IBD.[29] Both, depression and anxiety predict clinical recurrence in patients with CD[30] and a bi-directional link between depressive mood and anxiety with the inflammatory response and disease course in IBD has been proposed.[31] Particularly in the context of clinical disease remission, low serum zinc levels due to intestinal disease activity might be an important contributor to psychological symptoms in CD patients. Despite the high prevalence of both, ZD and symptoms of depression and anxiety in IBD patients, the impact of zinc levels and symptoms of depression or anxiety in IBD patients has not been explored. Table 1 provides a summary of existing and new knowledge regarding zinc deficiency.

We therefore aimed to assess the prevalence of ZD in CD patients in clinical remission and to analyze both a potential impact on future disease course as well as an interaction with mutations in ATG16L1. We further aimed to examine the association of zinc levels with symptoms of anxiety and depression.

Methods

Patients

The Swiss IBD cohort study (SIBDCS) is a nationwide cohort study from all regions of Switzerland, which has been including patients meeting the diagnostic criteria for IBD in accordance to established guidelines.[32] Enrollment started in 2006. The SIBDCS is supported by the Swiss National Science Foundation and is approved by the local ethics committees of the participating centers (institutional review board no. EK-1316, approved on 5 February 2007). Written informed consent was obtained from all patients before enrollment into the study. Inclusion criteria for the SIBDCS have been published elsewhere.[33] All CD patients currently enrolled in the SIBDCS were screened for eligibility. For this study, only CD patients with a minimum follow-up period of 1 year were selected. In order to analyze the impact of zinc on future disease course, only patients with disease remission at baseline were selected based on a Crohn`s Disease Activity Index (CDAI) $\leqslant$ 150 and absent endoscopic or histological disease activity (if endoscopy and histological examinations were performed). Further inclusion criteria were: (a) age $\geqslant$ 18 years, (b) documented evidence/

Table 1. Summary of existing and new knowledge regarding zinc deficiency.

What is known
ZD is considered a common finding in CD with up to one third of all patients presenting with low serum zinc levels. ZD is found even in patients in clinical remission
ZD may exacerbate CD activity, however long-term observational data are missing
ZD interferes with early and late autophagy, while an ATG16L1 mutation, which is involved in autophagic processes, has been linked to CD
ZD is associated with depression in non-CD patients. However, data on IBD patients are lacking, although up to 25% of CD patients have depression symptoms
What is new
In a cohort of 97 CD patients with disease remission, no single case of ZD has been identified
Nonetheless, low-normal zinc levels were an independent predictor for the presence of depression symptoms in CD patients
Zinc levels at baseline did not predict complicated disease course, neither in CD patients overall nor ATG16L1^{T300A} carriers

ZD, zinc deficiency; CD, Crohn's disease; IBD, inflammatory bowel disease.

presence of CD prior to cohort enrollment, and (c) either complicated or uncomplicated disease course during the follow-up period (1:1 ratio) according to the definition below. Exclusion criteria were: (a) documented evidence/presence of ulcerative colitis (UC), (b) evident inflammatory disease activity (one of the following) [CDAI $>$ 150, active endoscopic disease activity (if applicable), histologic findings of chronic or acute inflammation on biopsies (if applicable), active clinical disease course], (c) any zinc supplementation products at baseline or during the follow-up period. Patients were selected for further analyses and serum zinc testing, aimed at obtaining roughly equal fractions of patients with *versus* without future complicated disease course (as defined below) within the follow-up period (roughly 1:1 ratio). A sample size of 44 patients in each group would detect a difference in serum zinc levels of 1.5 μmol/l between CD without complicated disease course and CD with a future complicated disease course with a power of 80% (alpha error 0.05, standard deviation 2.5 μmol/l). A standard deviation of 2.5 μmol/l was chosen based on a recent publication by Beckett and colleagues.[34] In another study regarding serum zinc levels in CD and UC, standard deviation ranged from 1.9 to 2.1 μmol/l.[16]

Definitions

Future complicated disease course was defined as any one of the following: (a) flare up or (b) need for new anti-TNF, or (c) development of stenosis, abscess, fistula or anal fissure, adapted from previously suggested criteria.[35] Uncomplicated disease course was defined as the absence of all the abovementioned criteria for complication and a CDAI of $<$220. Zinc deficiency was defined as serum levels of less than 11 μmol/l (normal range 11–24 μmol/l).[36] Low–normal zinc values were defined as below the 30th percentile of the normal range. For simplification, this cut-off (14.9) was rounded up in order to consider the range 11–15 μmol/l as low–normal.

Data collection

All clinical data were available from the SIBDCS. Zinc was measured from serum tubes stored at −70°C. Analysis was performed by a commercial laboratory (Unilabs, Coppet VD, Switzerland) using ion-coupled-plasma-mass spectroscopy (ICP-MS Varian 820). To rule out the possibility of factitious zinc contamination of serum samples due to long-term frozen sample storage in conventional serum tubes, a pilot study was conducted with 10 random serum samples from the SIBDCS data center (see results section). The following clinical variables were collected from the SIBDCS: patient demographics (age at enrollment, sex, BMI, smoking status), baseline disease characteristics and activity (stool frequency, CDAI, disease localization, date of first symptoms, date of diagnosis, diagnostic delay, past and

current treatments), follow-up disease characteristics and activity (CDAI, past and current medications, complications, stool frequency, flares) and genetic data regarding single nucleotide polymorphism (SNP) rs2241880 (ATG16L1 gene variant).[15] The ATG16L1 polymorphism occurs in three isoforms: homozygous wild-type (AA), heterozygous (AG) and homozygous variant (GG).[37] Depression and anxiety symptoms were measured in the SIBDCS at baseline and during the follow-up period according to Zigmond and Snaith; The Hospital Anxiety and Depression Scale (HADS) is a self-assessment mood scale developed for outpatients comprising 14 questions graded on a 4-point Likert scale with subscales of anxiety and depression (total score 0–21, while 0–7 were considered normal, 8–10 indicative of mild, 11–14 indicative of moderate, and 15–21 indicative of severe anxiety/depression).[38]

Statistical analysis

For statistical analysis, the IBM Software SPSS Statistics Version 22.0.0 (2013, SPSS Science, Inc., Chicago, IL) was used. Complete data were analyzed in a pooled manner, with further separate consecutive analysis for patients with a future complicated *versus* a future uncomplicated disease course. Categorical data are depicted as percentage of the group total. For comparisons between continuous variables, two-sample *t* test and Mann–Whitney *U* test were used depending on whether data were normally distributed or not. Comparison between categorical data was performed with the Chi-square test. For correlation studies investigating the influence of zinc levels on stool frequency and anxiety/depression symptoms, the Pearson and Spearman tests were used, respectively. Multivariate logistic regression regarding prediction of low zinc levels for future CD outcome and for presence of depression symptoms was performed by first taking into account all covariates with a univariate p value of <0.15, removing insignificant covariates, and then adding remaining covariates one by one, checking the model significance and consistency at each step. For the purposes of this study, a p value of <0.05 was considered statistically significant.

Results

Patient demographics

A total of 97 patients, 50 with a complicated and 47 with an uncomplicated future disease course, were analyzed. Mean age was 40.4 years (±15.7); 43 were males (44.3%). Median duration of CD at baseline was 6.6 years [interquartile range (IQR) 2.6–15.6 years]. All patients were in clinical remission at study enrollment with a median CDAI at baseline of 34.0 (IQR 11.0–53.0). A sum of 26 patients (26.8%) were current smokers. Mean BMI was 23.9 kg/m^2 (±4.2 kg/m^2). According to the Montreal classification, 36 patients had ileal disease (L1, 37.1%), while 23 had colonic (L2, 23.7%) and 31 had ileocolonic (L3, 32.0%) disease at baseline. In four patients (4.1%), isolated upper gastrointestinal tract disease (L4) was reported. Four patients with ileal and colonic disease had additional upper gastrointestinal tract involvement (one patient with L1 and three patients with L2). In three patients, Montreal classification was not applicable. Median diagnostic delay (time from first symptoms to diagnosis) was 7.5 months (IQR 1.0–27.5 months). No prior or current anti-tumor necrosis factor (TNF) treatment was reported. None of the patients received any zinc supplementation products. Median frequency of liquid stools (per week) at baseline was 0 (IQR 0–14). Although all patients were in clinical remission at baseline, patients with a future complicated disease course had higher CDAI scores (but still ⩽150) and reported longer CD duration than patients who did not experience complications in the follow-up period. Otherwise, no differences were seen between the two groups at baseline evaluation, including in psychiatric symptoms. For a detailed synopsis over the whole study population and a comparison between group 1 (complicated future disease course) and group 2 (uncomplicated future disease course), see Table 2.

Zinc pilot analysis

Given the fact that special trace element free tubes are frequently used for zinc analysis, because of possible interference of minimal amounts of trace elements in the serum tube itself, we first conducted a pilot study to evaluate accuracy of zinc analysis from 10 random serum samples stored at the SIBDCS datacenter. Results from those samples are depicted in Supplementary Figure 1. Mean zinc level was 11.8 µmol/l with a median of 11.8 µmol/l and a total range of 6.5–16 µmol/l. In eight samples, zinc level was within the normal range, which is 11–24 µmol/l, while the remaining two samples (20%) showed reduced zinc levels. None of the samples showed an

Table 2. Baseline patient and disease characteristics.

	All patients (n = 97)	Complicated future disease course (n = 50)	Uncomplicated future disease course (n = 47)
Age in years, mean (SD)	40.4 (15.7)	43.2 (15.1)	37.5 (15.9)
Sex			
– Male	43 (44.3%)	22 (44.0%)	21 (44.7%)
– Female	54 (55.7%)	28 (56.0%)	26 (55.3%)
BMI in kg/m2, mean (SD)	23.9 (4.2)	24.2 (4.7)	23.5 (3.5)
Duration of CD at enrolment in years, median (IQR)	6.6 (2.6–15.6)	9.0 (3.7–21.0)	4.2 (1.1–14.3)[a]
Diagnostic delay in months, median (IQR)	7.5 (1.0–27.5)	8.0 (1.0–24.5)	7.0 (2.0–36.0)
Current smoking status			
– Yes	26 (26.8%)	12 (24.0%)	14 (29.8%)
– No	55 (56.7%)	26 (52.0%)	29 (61.7%)
– Missing	16 (16.5%)	12 (24.0%)	6 (8.5%)
Past IM			
– Yes	16 (16.5%)	10 (20.0%)	6 (12.8%)
– No	81 (83.5%)	40 (80.0%)	41 (87.2%)
Anti-TNF naïve			
– Yes	97 (100%)	50 (100%)	47(100%)
– No	0	0	0
Current treatment			
– 5-ASA	20 (20.6%)	9 (18.0%)	11 (23.4%)
– AZA/6-MP	43 (44.3%)	26 (52.0%)	17 (36.2%)
– Topical steroids	27 (27.8%)	14 (28.0%)	13 (27.7%)
– MTX	5 (5.2%)	2 (4.0%)	3 (6.4%)
– None	19 (19.6%)	7 (14.0%)	12 (25.5%)
Disease localization			
– L1	36 (37.1%)	17 (34.0%)	19 (40.4%)
– L2	23 (23.7%)	12 (24.0%)	11 (23.4%)
– L3	31 (32.0%)	18 (36.0%)	13 (27.7%)
– L4	4 (4.1%)	2 (4.0%)	2 (4.3%)
– Missing	3 (3.1%)	1 (2.0%)	2 (4.3%)
CDAI, median (IQR)	34.0 (11.0–53.0)	38.0 (22.3–53.8)[a]	21.0 (6.0–42.5)[b]
Number of liquid stools per week, median (IQR)	0.0 (0.0–14.0)	1.0 (0.0–14.0)	0.0 (0.0–7.0)

(Continued)

Table 2. (Continued)

	All patients (n = 97)	Complicated future disease course (n = 50)	Uncomplicated future disease course (n = 47)
HADS anxiety score, median (IQR)	6 (3–9)	6 (4–9)	5.5 (2.25–8)
HADS depression score, median (IQR)	3 (1–6)	4 (2–6)	2.5 (0–5)
Follow up			
– 1y	12 (12.4%)	2 (4.0%)	10 (21.3%)
– 2y	15 (15.5%)	6 (12.0%)	9 (19.1%)
– 3y or +	70 (72.2%)	42 (84.0%)	28 (59.6%)[c]

[a]Duration of CD at enrollment was significantly longer in group 1 (future complicated disease course) compared with group 2 (uncomplicated disease course), $p = 0.018$.
[b]CDAI at baseline was significantly higher in group 1 compared with group 2, $p = 0.014$.
[c]A 3 years or more follow up was reported more often in group 1 compared with group 2, $p = 0.009$.
SD, standard deviation; CD, Crohn's disease; BMI, body mass index; TNF, tumor necrosis factor; IQR, interquartile range; IM, Immunomodulation; HADS, Hospital Anxiety and Depression Scale; 5-ASA, 5-aminosalicylic acid; AZA, azathioprine; 6-MP, 6-mercaptopurine; MTX, methotrexate; L1, Montreal classification ileal disease; L2, Montreal classification colonic disease; L3, Montreal classification ileocolonic disease; L4, Montreal classification isolated upper gastrointestinal tract disease; CDAI, Crohn's disease activity index.

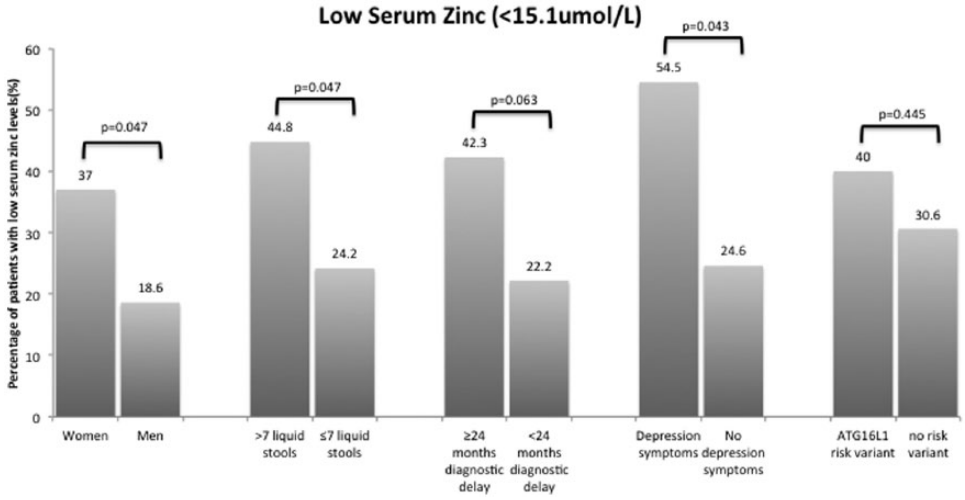

Figure 1. Proportion of patients with low serum zinc level according to sex, stool frequency, diagnostic delay, presence of depression symptoms and presence of ATG16L1 risk variants.

elevated zinc level. Accordingly, measuring zinc from normal serum tubes did not induce false-high results and indeed, reflected adequate levels within an anticipated range.

Zinc levels

Mean zinc level in the study population was 18.0 ± 4.7 μmol/l. No absolute ZD (defined as <11 μmol/l) was observed. Low zinc levels (defined as <15.1 μmol/l) were found in 28 patients (28.9%). Males had significantly higher zinc levels compared with females (19.4 ± 5.7 μmol/l *versus* 16.8 ± 3.3 μmol/l, $p = 0.006$), and the proportion of patients with low zinc levels was higher among females (20/54, 37.0% *versus* 8/43, 18.6%, $p = 0.047$). Patients with a longer diagnostic delay (>23.9 months) showed a trend towards higher proportion of low zinc levels (11/26, 42.3% *versus* 12/42, 22.2%, $p = 0.063$).

Patients with more than seven liquid stools per week ($n = 29$) more frequently had low serum zinc levels (13/29, 44.8%) compared with patients with seven or less liquid stools (15/62, 24.2%, $p = 0.047$). For details, see Figure 1. However, serum zinc levels did not significantly correlate with number of liquid stools at baseline (rho –0.113, $p = 0.285$). In a univariate regression model, female sex, stool frequency, diagnostic delay and immunosuppression at baseline were relevant predictors for low serum zinc levels ($p < 0.15$). When those factors were analyzed in a multivariate regression model, immunosuppression remained the only significant predictor, while stool frequency showed at least a trend towards statistical significance. For univariate and multivariate analyses, see Table 3.

Anxiety and depression symptoms

A total of 27 of the 97 patients (27.8%) reported at least mild anxiety symptoms at baseline evaluation (including 9 patients with moderate and 1 with severe anxiety). Median HADS anxiety score was 6 (IQR 3–9). Depression symptoms were reported by 11 of the 97 patients (11.3%): seven patients had mild, four had moderate, and none had severe depression at baseline. Median HADS depression score was 3 (IQR 1–6). Zinc levels and anxiety score did not show any correlation, nor did zinc levels and classification into normal, mild, moderate and severe anxiety. However, we found a mild, but significant correlation between zinc levels and severity of depression (rho –0.277, $p = 0.016$). Patients with low serum zinc levels significantly more often reported depression symptoms compared with patients with higher serum zinc levels (6/22, 27.3% *versus* 5/53, 9.4%, $p = 0.047$). In a multivariate regression model corrected for stool frequency and CDAI, zinc levels at baseline were an independent negative predictor for the presence of depression symptoms [odds ratio (OR) 0.727, 95% confidence interval (CI) 0.532–0.993, $p = 0.045$]. For details see Table 4.

Single nucleotide polymorphism analysis

ATG16L1 SNP analysis was available from 61 patients (62.9%) with 25 (25/61, 41.0%) being homozygous for the risk variant rs2241880 (T300A), while 23 patients were heterozygotes. Zinc levels of patients with ATG16L1^{T300A} (homozygotes) did not differ from those of patients without or with only one risk allele (16.3 ± 2.7 µmol/l *versus* 17.1 ± 3.2 µmol/l, n.s.). In addition, the proportion of low serum zinc levels did not show any significant differences between the two groups: low zinc levels were found in 10 out of the 25 patients carrying the risk variant and in 11 out of the 36 patients without the risk variant (40.0% *versus* 30.6%, n.s). In a regression model, presence of ATG16L1^{T300A} did not predict low serum zinc levels. For details, see Table 3.

Prediction of future complicated disease course

Patients were followed for 1 (12, 12.4%), 2 (15, 15.5%) or 3 years and longer (70, 72.2%). The following complications occurred: 29 patients (29/50, 58.0%) experienced a flare up, 26 patients (26/50, 52.0%) needed new anti-TNF treatment, and 45 patients (45/50, 90.0%) developed stenosis, abscess, fistula or anal fissure. Zinc levels of patients with a future complicated disease course were not different from those of patients without complications (17.7 ± 4.3 µmol/l *versus* 18.3 ± 5.1 µmol/l, n.s.). Proportions of patients with low serum zinc levels were comparable with 16 out of 50 (32.0%) in the complicated disease group and 12 out of 47 (25.5%) in the uncomplicated disease group (n.s.). We did not observe any differences when females/males were analyzed separately. Looking only at ATG16L1^{T300A} carriers, no difference in zinc levels between patients with *versus* without a complicated disease course was seen (16.0 ± 2.6 µmol/l *versus* 17.3 ± 2.9 µmol/l, n.s). After screening the potential predictors for future disease outcome one by one, the variables age, CDAI at baseline, stool frequency and presence of ATG16L1^{T300A} were retained in the multiple predictor model in addition to serum zinc levels (see Table 5). Presence of ATG16L1^{T300A} seemed to be the only predictive variable, although statistical significance was not achieved ($p = 0.053$). Nevertheless, neither absolute zinc levels at baseline nor presence of low serum zinc levels (<15.1 µmol/l) predicted future complicated disease course.

Discussion

This analysis of prospectively obtained data from a nationwide cohort study in Switzerland tested for an association of serum zinc levels and psychiatric symptoms, stool frequency and future disease course in CD patients in clinical remission at

Table 3. Regression model for prediction of low serum zinc levels.

Variable	Category	$n = 97$	Low serum zinc levels (%)	Single predictor model		Multiple predictor model	
				OR (95% CI)	*p*	OR (95% CI)	*p*
Age				1.001 (0.973–1.029)	0.949		
Sex	Male	43	8 (18.6%)	**0.389 (0.151–1.001)**	**0.05**	0.412 (0.131–1.296)	0.129
	Female	54	20 (37.0%)	1		1	
BMI				1.048 (0.944–1.164)	0.377		
Current smoker	Nonsmoker	55	15 (27.3%)	0.844 (0.303–2.346)	0.745		
	Smoker	26	8 (30.8%)	1			
High stool frequency	7 stools or less	62	15 (24.25)	**0.393 (0.154–1.000)**	**0.05**	0.358 (0.117–1.097)	0.072
	More than 7 stools	29	13 (44.8%)	1		1	
Ileal disease	Not L1	58	16 (27.6%)	0.866 (0.347–2.158)	0.757)		
	L1	36	11 (30.6%)	1			
Diagnostic delay	<24 months	42	12 (22.2%)	**0.390 (0.142–1.068)**	**0.067**	0.420 (0.142-1.249)	0.119
	>23.9 months	26	11 (42.3%)	1		1	
Past IM treatment	No	81	21 (25.9%)	0.450 (0.149–1.360)	0.157		
	Yes	16	7 (43.8%)	1			
Current IM treatment	No	47	9 (19.1%)	**0.386 (0.153–0.974)**	**0.044**	**0.280 (0.089–0.879)**	**0.029**
	Yes	50	19 (38.0%)	1		1	
CDAI				1.003 (0.990–1.017)	0.656		
SNP risk variant	No	36	11 (30.6%)	0.660 (0.227–1.923)	0.446		
	Yes	25	10 (40.0%)	1			

OR, odds ratio; CI, confidence interval; BMI, body mass index; L1, Montreal classification ileal disease; IM, Immunomodulation; CDAI, Crohn's disease activity index; SNP, single nucleotide polymorphism. significant results are in bold font.

Table 4. Regression model for prediction of depression symptoms.

Variable	Category	$n = 75$*	Depression symptoms (%)	Single predictor model		Multiple predictor model	
				OR (95% CI)	*p*	OR (95% CI)	*p*
Age				**1.001 (0.962–1.042)**	**0.952**		
Sex	Male	32	4 (12.5%)	0.735 (0.195–2.761)	0.648		
	Female	43	7 (16.3%)	1			
BMI				1.036 (0.885–1.212	0.661		
Current smoker	Nonsmoker	41	6 (14.6%)	1.457 (0.266–7.992)	0.665		
	Smoker	19	2 (10.5%)	1			
High stool frequency	7 stools or less	48	4 (8.3%)	**0.258 (0.065–1.027)**	**0.055**	0.397 (0.051–3.089)	0.377
	More than 7 stools	23	6 (26.1%)	1		1	
Diagnostic delay	<24 months	43	5 (11.6%)	0.526 (0.125–2.219)	0.382		
	>23.9 months	20	4 (20.0%)	1			
IM treatment at baseline	No	37	4 (10.8%)	0.537 (0.143–2.015)	0.357		
	Yes	38	7 (18.4%)	1			
CDAI				**1.017 (0.998–1.037)**	**0.076**	1.009 (0.978–1.041)	0.559
SNP risk variant	Yes	24	3 (12.5%)	0.607 (0.119–3.092)	0.548		
	No	21	4 (19.0%)	**1**			
Zinc levels				**0.742 (0.562–0.981)**	**0.036**	**0.727 (0.532–0.993)**	**0.045**

*Data on presence or absence of depressive symptoms were available for 75 patients.
OR, odds ratio; CI, confidence interval; BMI, body mass index; IM, Immunomodulation; CDAI, Crohn's disease activity index; SNP, single nucleotide polymorphism. significant results are in bold font.

Table 5. Regression model for prediction of future complicated disease outcome.

Variable	Category	$n = 97$	Complicated disease course (%)	Single predictor model		Multiple predictor model	
				OR (95% CI)	*p*	OR (95% CI)	*p*
Age				**1.025 (0.998–1.052**	**0.076**	1.009 (0.971–1.048)	0.641
Sex	Male	43	22 (51.2%)	0.973 (0.436–2.168)	0.946		
	Female	54	28 (51.9%)	1			
BMI				1.046 (0.946–1.156)	0.382		
Current smoker	Nonsmoker	55	26 (47.3%)	1.046 (0.411–2.665)	0.925		
	Smoker	26	12 (46.2%)	1			
High stool frequency	7 stools or less	62	30 (48.4%)	**0.493 (0.198–1.23)**	**0.130**	0.545 (0.107–2.772)	0.464
	More than 7 stools	29	19 (65.5%)	1		1	
Ileal disease	Not L1	58	32 (55.2%)	1.376 (0.597–3.168)	0.454		
	L1	36	17 (47.2%)	1			
Diagnostic delay	<24 months	54	28 (51.9%)	1.469 (0.572–3.773)	0.425		
	>23.9 months	26	11 (42.3%)	1			
IM treatment at baseline	No	47	21 (44.7%)	0.585 (0.262–1.307)	0.191		
	Yes	50	29 (58.0%)	1			
CDAI				**1.016 (1.002–1.031)**	**0.028**	1.004 (0.978–1.030)	0.776
SNP risk variant	Yes	25	19 (76.0%)	**3.167 (1.026–9.77)**	**0.045**	3.479 (0.983–12.312)	0.053
	No	36	18 (50.0%)	**1**			
Zinc levels				0.968 (0.887–1.058)	0.476	0.956 (0.786–1.162)	0.649
Low serum zinc	No	69	34 (49.3%)	0.729 (0.301–1.765)	0.483		
	Yes	28	16 (57.1%)	1			

OR, odds ratio; CI, confidence interval; BMI, body mass index; L1, Montreal classification ileal disease; IM, Immunomodulation; CDAI, Crohn's disease activity index; SNP, single nucleotide polymorphism. significant results are in bold font.

baseline. In our subset of SIBDCS patients with CD in clinical remission, we did not observe ZD. However, zinc levels revealed to be low–normal in a considerable proportion of patients. Zinc levels were lower among females when compared with their male counterparts as well as among those patients with more than seven liquid stools per week. Intriguingly, lower zinc levels were an independent predictor for depression, but not anxiety symptoms. Zinc levels at baseline did not predict complicated disease course, neither in CD patients overall nor ATG16L1^{T300A} carriers. Yet, in the latter, we observed a clear albeit nonsignificant trend towards an increase in complications in both univariate and multivariate testing.

In contrast to the studies conducted by Vagianos and colleagues[16] and Filippi and colleagues,[17] who reported zinc deficiency in up to 65% even in patients with clinical remission, we did not identify a single case with serum zinc levels below the reference value of 11μmol/l. When we compare our demographic data with that of Filippi and colleagues, our patients had lower CDAI (mean 37.5 *versus* 89.7) and did not report any previous CD-related surgery, while nearly 50% of the patients studied by Filippi and colleagues had undergone ileal, ileocecal or colonic resection.[17] Therefore, presence of ZD may have been overestimated by previous studies not exclusively considering CD patients in remission. Data on the impact of sex on serum zinc levels are conflicting; in contrast to other micronutrient studies, but in accordance to findings from the National Health and Nutrition Examination Survey study, we found significant sex-specific differences, with lower zinc levels among females.[39–42] This is particularly noteworthy, as it has been previously described that daily intake of zinc (assessed with the use of prospective food records for three days) was higher among females compared with males, which may not be the case in our cohort.[17] However, influence of sex on zinc levels may be biased by several confounders such as stool frequency, diagnostic delay and immunosuppressive therapy, as in a multivariate regression model corrected for those variables, sex was not an independent predictor for low serum zinc levels. Accordingly, our results should be replicated in a larger IBD study population.

The proportion of patients with low serum zinc levels tended to be higher among patients with more than seven liquid stools per week. Thus, low serum zinc may be a promotor of diarrhea or higher stool frequency in the presence of clinical and endoscopic disease remission. This finding is supported by prior studies demonstrating increased gut permeability in the context of zinc deficiency and by studies testing zinc supplementation for treatment of acute diarrhea.[18,43–45] Therapeutic effects of zinc might be attributable to reduction of a transmucosal leak and to a K-channel blockage of adenosine 3-5-cyclic monophosphate-mediated chlorine secretion.[43,46] Therefore, even in the absence of absolute zinc deficiency, low zinc levels might be clinically relevant, promoting diarrhea and elevated stool frequency. However, the opposite might also be the case: increased frequency of liquid stools leads to a decrease in serum zinc levels. More clinical data is needed to assess direction of this association and in order to generate clear recommendations regarding zinc supplementation in CD patients with low–normal zinc levels.

In our study, the ATG16L1 risk variant was an independent risk factor for future CD outcome, bordering significance in the multivariate analysis, which contrasts the finding from Cleynen and colleagues which could not show any association of risk gene variants and disease outcome.[47] However, low serum zinc levels did not have a different effect on stool frequency or disease outcome when comparing those patients harboring the risk variant *versus* those that did not. Thus, the hypothesis that zinc deficiency or low zinc levels might increase the impact of ATG16L1 could not be supported. One explanation might be that a mutated ATG16L1 disrupts autophagy to an extent, that any potential additional effect of low zinc becomes negligible.

Several epidemiological and animal studies have shown an association between low serum zinc levels and depression symptoms.[23–28,48,49] In addition, at least some evidence for zinc supplementation can be derived from randomized controlled-trials with patients in clinical depression.[50,51] However, until now, to the best of our knowledge, no study has linked serum zinc levels and depression symptoms in patients with IBD. In a multivariate regression model correcting for relevant confounders such as CDAI or stool frequency, we were able to show that low serum zinc levels are an independent predictor for depression symptoms. This is noteworthy, given a previous study from the Swiss IBD cohort showing that depression is

associated with higher rates of clinical recurrence of IBD.[30] Considering that we evaluated patients in clinical remission only and none of those had absolute zinc deficiency, the inverse effect of zinc on psychiatric symptoms might be even stronger in a larger population with more severe and active disease and further studies are warranted. In fact, the percentage of patients in the current study with clinically relevant (i.e. at least mild) symptoms of depression (11%) or anxiety (28%) was only half of the level expected from recent meta-analytic data.[29] This low level of psychological distress could also be an explanation for the lack of an association between anxiety and zinc level in our patients. To compare, lower serum zinc levels were significantly associated with more severe anxiety symptoms in female students from Iran, of whom 66% had at least mild anxiety measured with the HADS.[52] Also, a series of carefully conducted animal studies imply a role of zinc deficiency in anxiety-like behaviors.[53]

It is advocated that patients with IBD should be screened for psychological comorbidities, including depressive symptoms, and psychological support should be a part of standard care for those in need.[54] However, whether zinc levels in CD patients with relevant depressive symptoms should be tested to start supplementation therapy in case of nutritional deficiency has yet to be determined. Currently available evidence as to whether zinc supplementation alleviates depressive symptoms in humans (without IBD) is scarce and conflicting.[22] Nonetheless, our findings provide a rationale for zinc supplementation in CD patients with depressive symptoms, potentially even in patients with low–normal serum zinc levels.

Our study has several strengths and some limitations. We provide the first study evaluating an association of serum zinc levels and psychiatric symptoms in CD patients. In addition, it is the first analysis that evaluates the effect of zinc levels on future disease course with regards to the presence or absence of the ATG16L1 risk variant, therefore linking epidemiological observations with molecular disease mechanisms. The SIBDCS is a large and well-established cohort with structured annual physician- and patient-based assessments. However, albeit the prospective nature of data acquisition, retrospective analysis with annual follow-up visits only may be associated with underreporting of symptoms in general and psychiatric symptoms in particular. A limitation of our study is that significantly more patients in the future complicated disease group had a follow up of 3+ years compared with those not experiencing any complications, which might lead to underreporting of late complications in the uncomplicated disease course group. However, as we only took into account the first complication (being sufficient to fall into the complication category), we feel rather confident to assume the two groups and their follow up to be comparable. Our study might be underpowered to detect weaker effects of ZD on disease course. The role of zinc may have been underestimated, since all patients were in clinical remission at baseline without frank zinc deficiency. Although all patients were in clinical remission at baseline, patients with a future complicated disease course had higher CDAI values at first visit and reported a longer CD duration. This slightly, but significantly higher clinical disease activity and the longer disease duration might be considerable confounders of our analysis. And finally, zinc levels were only measured at baseline, but not during follow up and during flares, although, presumably, these levels are prone to fluctuations, which should be taken into account in future studies.

Taken together, zinc deficiency in CD patients with clinical remission did not seem to be as relevant as previously thought based on the rather sparse literature. However, low serum zinc levels were an independent predictor for presence of depression symptoms. Whether supplementation can be recommended in those patients in general has to be determined in the future. In the absence of active disease, low zinc levels did not predict complicated disease course regardless of ATG16L1 genotype.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

Supplementary Material

Supplementary material is available for this article online.

References

1. Ananthakrishnan AN, Khalili H, Song M, *et al.* Zinc intake and risk of Crohn's disease and ulcerative colitis: a prospective cohort study. *Int J Epidemiol* 2015; 44: 1995–2005.
2. Ananthakrishnan AN. Epidemiology and risk factors for IBD. *Nat Rev Gastroenterol Hepatol* 2015; 12: 205–217.
3. Chapman-Kiddell CA, Davies PS, Gillen L, *et al.* Role of diet in the development of inflammatory bowel disease. *Inflamm Bowel Dis* 2010; 16: 137–151.
4. Hou JK, Abraham B and El-Serag H. Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. *Am J Gastroenterol* 2011; 106: 563–573.
5. Skrovanek S, DiGuilio K, Bailey R, *et al.* Zinc and gastrointestinal disease. *World J Gastrointest Pathophysiol* 2014; 5: 496–513.
6. Prasad AS. Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. *Exp Gerontol* 2008; 43: 370–377.
7. Jäättelä M, Mouritzen H, Elling F, *et al.* A20 zinc finger protein inhibits TNF and IL-1 signaling. *J Immunol* 1996; 156: 1166–1173.
8. Sturniolo GC, Fries W, Mazzon E, *et al.* Effect of zinc supplementation on intestinal permeability in experimental colitis. *J Lab Clin Med* 2002; 139: 311–315.
9. Sturniolo GC, Mestriner C, Lecis PE, *et al.* Altered plasma and mucosal concentrations of trace elements and antioxidants in active

ulcerative colitis. *Scand J Gastroenterol* 1998; 33: 644–649.

10. Waeytens A, De Vos M and Laukens D. Evidence for a potential role of metallothioneins in inflammatory bowel diseases. *Mediators Inflamm* 2009; 2009: 729172.
11. Coyle P, Philcox JC, Carey LC, *et al.* Metallothionein: the multipurpose protein. *Cell Mol Life Sci* 2002; 59: 627–647.
12. Penkowa M and Hidalgo J. Metallothionein treatment reduces proinflammatory cytokines IL-6 and TNF-alpha and apoptotic cell death during experimental autoimmune encephalomyelitis (EAE). *Exp Neurol* 2001; 170: 1–14.
13. Itoh N, Shibayama H, Kanekiyo M, *et al.* Reduced bactericidal activity and nitric oxide production in metallothionein-deficient macrophages in response to lipopolysaccharide stimulation. *Toxicology* 2005; 216: 188–196.
14. Liuzzi JP, Guo L, Yoo C, *et al.* Zinc and autophagy. *Biometals* 2014; 27: 1087–1096.
15. Rioux JD, Xavier RJ, Taylor KD, *et al.* Genome-wide association study identifies new susceptibility loci for Crohn's disease and implicates autophagy in disease pathogenesis. *Nat Genet* 2007; 39: 596–604.
16. Vagianos K, Bector S, McConnell J, *et al.* Nutrition assessment of patients with inflammatory bowel disease. *JPEN J Parenter Enteral Nutr* 2007; 31: 311–319.
17. Filippi J, Al-Jaouni R, Wiroth JB, *et al.* Nutritional deficiencies in patients with Crohn's disease in remission. *Inflamm Bowel Dis* 2006; 12: 185–191.
18. Finamore A, Massimi M, Conti Devirgiliis L, *et al.* Zinc deficiency induces membrane barrier damage and increases neutrophil transmigration in Caco-2 cells. *J Nutr* 2008; 138: 1664–1670.
19. Ukabam SO, Clamp JR and Cooper BT. Abnormal small intestinal permeability to sugars in patients with Crohn's disease of the terminal ileum and colon. *Digestion* 1983; 27: 70–74.
20. Wyatt J, Vogelsang H, Hübl W, *et al.* Intestinal permeability and the prediction of relapse in Crohn's disease. *Lancet* 1993; 341: 1437–1439.
21. Sarris J, Logan AC, Akbaraly TN, *et al.* Nutritional medicine as mainstream in psychiatry. *Lancet Psychiatry* 2015; 2: 271–274.
22. Sarris J, Murphy J, Mischoulon D, *et al.* Adjunctive nutraceuticals for depression: a systematic review and meta-analyses. *Am J Psychiatry* 2016; 173: 575–587.
23. Maes M, Vandoolaeghe E, Neels H, *et al.* Lower serum zinc in major depression is a sensitive marker of treatment resistance and of the immune/inflammatory response in that illness. *Biol Psychiatry* 1997; 42: 349–358.
24. Grønli O, Kvamme JM, Friborg O, *et al.* Zinc deficiency is common in several psychiatric disorders. *PLoS One* 2013; 8: e82793.
25. Marcellini F, Giuli C, Papa R, *et al.* Zinc status, psychological and nutritional assessment in old people recruited in five European countries: Zincage study. *Biogerontology* 2006; 7: 339–345.
26. Siwek M, Dudek D, Schlegel-Zawadzka M, *et al.* Serum zinc level in depressed patients during zinc supplementation of imipramine treatment. *J Affect Disord* 2010; 126: 447–452.
27. Amani R, Saeidi S, Nazari Z, *et al.* Correlation between dietary zinc intakes and its serum levels with depression scales in young female students. *Biol Trace Elem Res* 2010; 137: 150–158.
28. Swardfager W, Herrmann N, Mazereeuw G, *et al.* Zinc in depression: a meta-analysis. *Biol Psychiatry* 2013; 74: 872–878.
29. Mikocka-Walus A, Knowles SR, Keefer L, *et al.* Controversies revisited: a systematic review of the comorbidity of depression and anxiety with inflammatory bowel diseases. *Inflamm Bowel Dis* 2016; 22: 752–762.
30. Mikocka-Walus A, Pittet V, Rossel JB, *et al.* Symptoms of depression and anxiety are independently associated with clinical recurrence of inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2016; 14: 829–835.e821.
31. Bernstein CN. Psychological stress and depression: risk factors for IBD? *Dig Dis* 2016; 34: 58–63.
32. Gomollón F, Dignass A, Annese V, *et al.* 3rd European evidence-based consensus on the diagnosis and management of Crohn's disease 2016: part 1: diagnosis and medical management. *J Crohns Colitis* 2017; 11: 3–25.
33. Pittet V, Juillerat P, Mottet C, *et al.* Cohort profile: the Swiss Inflammatory Bowel Disease Cohort Study (SIBDCS). *Int J Epidemiol* 2009; 38: 922–931.
34. Beckett JM and Ball MJ. Zinc status of northern Tasmanian adults. *J Nutr Sci* 2015; 4: e15.
35. Cosnes J, Bourrier A, Nion-Larmurier I, *et al.* Factors affecting outcomes in Crohn's disease over 15 years. *Gut* 2012; 61: 1140–1145.

36. Wu A. *Tietz clinical guide to laboratory tests*. 4th ed. St. Louis, Mo: Saunders, 2006.
37. Salem M, Nielsen OH, Nys K, *et al*. Impact of T300A variant of ATG16L1 on antibacterial response, risk of culture positive infections, and clinical course of Crohn's disease. *Clin Transl Gastroenterol* 2015; 6: e122.
38. Zigmond AS and Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983; 67: 361–370.
39. Rükgauer M, Klein J and Kruse-Jarres JD. Reference values for the trace elements copper, manganese, selenium, and zinc in the serum/plasma of children, adolescents, and adults. *J Trace Elem Med Biol* 1997; 11: 92–98.
40. Díaz Romero C, Henríquez Sánchez P, López Blanco F, *et al*. Serum copper and zinc concentrations in a representative sample of the Canarian population. *J Trace Elem Med Biol* 2002; 16: 75–81.
41. Markiewicz-Żukowska R, Gutowska A and Borawska MH. Serum zinc concentrations correlate with mental and physical status of nursing home residents. *PLoS One* 2015; 10: e0117257.
42. Hotz C, Peerson JM and Brown KH. Suggested lower cutoffs of serum zinc concentrations for assessing zinc status: reanalysis of the second National Health and Nutrition Examination Survey data (1976–1980). *Am J Clin Nutr* 2003; 78: 756–764.
43. Hoque KM and Binder HJ. Zinc in the treatment of acute diarrhea: current status and assessment. *Gastroenterology* 2006; 130: 2201–2205.
44. Zou TT, Mou J and Zhan X. Zinc supplementation in acute diarrhea. *Indian J Pediatr* 2015; 82: 415–420.
45. Lamberti LM, Walker CL, Chan KY, *et al*. Oral zinc supplementation for the treatment of acute diarrhea in children: a systematic review and meta-analysis. *Nutrients* 2013; 5: 4715–4740.
46. Sturniolo GC, Di Leo V, Ferronato A, *et al*. Zinc supplementation tightens "leaky gut" in Crohn's disease. *Inflamm Bowel Dis* 2001; 7: 94–98.
47. Cleynen I, Boucher G, Jostins L, *et al*. Inherited determinants of Crohn's disease and ulcerative colitis phenotypes: a genetic association study. *Lancet* 2016; 387: 156–167.
48. Młyniec K and Nowak G. Zinc deficiency induces behavioral alterations in the tail suspension test in mice. Effect of antidepressants. *Pharmacol Rep* 2012; 64: 249–255.
49. Tassabehji NM, Corniola RS, Alshingiti A, *et al*. Zinc deficiency induces depression-like symptoms in adult rats. *Physiol Behav* 2008; 95: 365–369.
50. Nowak G, Siwek M, Dudek D, *et al*. Effect of zinc supplementation on antidepressant therapy in unipolar depression: a preliminary placebo-controlled study. *Pol J Pharmacol* 2003; 55: 1143–1147.
51. Siwek M, Dudek D, Paul IA, *et al*. Zinc supplementation augments efficacy of imipramine in treatment resistant patients: a double blind, placebo-controlled study. *J Affect Disord* 2009; 118: 187–195.
52. Tahmasebi K, Amani R, Nazari Z, *et al*. Association of mood disorders with serum zinc concentrations in adolescent female students. *Biol Trace Elem Res* 2017; 178: 180–188.
53. Młyniec K, Davies CL, de Agüero Sánchez IG, *et al*. Essential elements in depression and anxiety. Part I. *Pharmacol Rep* 2014; 66: 534–544.
54. Mikocka-Walus AA, Andrews JM, von Känel R, *et al*. What are the implications of changing treatment delivery models for patients with inflammatory bowel disease: a discussion paper. *Eur J Gastroenterol Hepatol* 2013; 25: 393–398.