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Serum levels of selected micronutrients in patients with inflammatory bowel disease in clinical remission

Short title: Serum trace elements in patients with IBD in clinical remission

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Key words: copper, inflammatory bowel disease, iron, selenium, zinc
**What’s new?**

Micronutrient deficiency affects the immune system and occurs in a significant proportion of patients with inflammatory bowel disease (IBD), even during remission. Deficiency of some trace elements may be associated with disease-related morbidity, interfere with the achievement of deep remission, and cause exacerbations earlier during disease course. We evaluated the micronutrient status in patients with IBD in clinical remission, with involvement of the colon alone, and without malabsorption syndrome, receiving immunosuppressive treatment. The study showed lower zinc concentrations in patients with IBD compared with controls. We concluded that patients with IBD, despite maintaining clinical remission, should undergo systematic laboratory test for anemia or micronutrient deficiencies.
ABSTRACT

Introduction  Micronutrient deficiency can occur in patients with inflammatory bowel disease (IBD) regardless of the disease activity and extent.

Objectives  We aimed to evaluate the serum concentrations of selected trace elements in adult patients with IBD in clinical remission, with involvement limited to the colon, and receiving immunosuppressive treatment.

Patients and methods  We enrolled 32 patients with IBD (mean [SD] age, 41.0 [15.2] years) and 30 healthy controls (mean [SD] age, 39.1 [11.8] years). Serum selenium (Se), iron (Fe), copper (Cu), and zinc (Zn) levels as well as complete blood count were measured in both groups.

Results  Patients with IBD had lower Zn concentrations than controls (mean [SD], 0.76 [0.13] mg/l vs 0.83 [0.13] mg/l, P = 0.047). No significant differences were observed for Se (mean [SD], 0.90 [0.24] μmol/l vs 0.93 [0.19] μmol/l) and Cu levels (mean [SD], 1.03 [0.27] mg/l vs 0.97 [0.22] mg/l). Compared with controls, patients with IBD had lower red blood cell count (mean [SD], 4.4 [0.6] 10⁶/μl vs 4.7 [0.4] 10⁶/μl, P = 0.03), hemoglobin (mean [SD], 12.7 [2.2] g/dl vs 14.3 [0.8] g/dl, P = 0.001), and Fe levels (mean [SD], 14.2 [9.4] μmol/l vs 23.4 [2.7] μmol/l, P=0.0001). Patients with IBD showed a positive correlation between Se and Fe (R = 0.499; P = 0.004) as well as Se and hemoglobin levels (R = 0.579; P = 0.001).

Conclusions  Patients with IBD, despite maintaining clinical remission, should undergo systematic laboratory test for anemia or micronutrient deficiencies.
**Introduction**

Inflammatory bowel disease (IBD) is a group of chronic and relapsing diseases that affect the gastrointestinal tract, especially the intestines [1]. While ulcerative colitis (UC) is restricted to the colon, Crohn disease (CD) may affect any portion of the gastrointestinal tract [2]. The etiology of IBD is multifaceted. The disease may result from dysregulated immune responses to the intestinal microbiota due to genetic predispositions [2,3]. Patients with IBD experience relapses and remissions and present with bothersome symptoms such as abdominal pain, diarrhea, or rectal bleeding [4]. The disease is incurable. The primary goal of therapy is to achieve clinical remission, followed by endoscopic remission. Current medical treatment involves the use of 5-aminosalicylic acid, corticosteroids, immunomodulators, or biologic drugs.

Approximately 85% of patients with IBD present with signs of malnutrition [5]. Malnutrition can be classified as macronutrient or micronutrient deficiency [6]. Macronutrient deficiency is determined by protein–energy malnutrition and usually affects patients with active and severe disease [6]. Patients with IBD in remission usually have normal macronutrient intake and thus show a lower incidence of macronutrient deficiency [7]. However, patients with a mild course of IBD or those in remission may develop micronutrient deficiency. Various types of micronutrient deficiency have been described [6-10]. Malnutrition in IBD may be caused by restrictive diet, malabsorption, inflammatory process, or drugs used for treatment [11]. Nutritional deficiencies are associated with prolonged hospitalization, a complicated perioperative course, and higher mortality rates [12,13]. Micronutrient deficiency may influence the immune system and predispose an individual to the onset and progression of IBD [14].

The micronutrients involved in inflammatory signaling pathways include selenium (Se), zinc (Zn), iron (Fe), and copper (Cu) [11]. Selenium shows antioxidant activity and protects cells
against the harmful effects of free radicals. It regulates growth processes and is a component of numerous essential enzymes [15]. Zinc is a micronutrient that plays an important role in cellular metabolism, namely, it supports the catalytic activity of numerous enzymes, facilitates protein and DNA synthesis, modulates the immune function, and improves intestinal barrier function [16]. Copper, on the other hand, is an essential cofactor for enzymes and electron transport proteins required for antioxidant, neurotransmitter, and histamine metabolism, oxidative phosphorylation, as well as Fe transport [17]. Iron deficiency, the most common systemic complication and extra intestinal manifestation in IBD leads to defective T-cell proliferative response and impaired cytokine production by lymphocytes [18].

Unfortunately, there is little evidence on the importance of micronutrients and their routine evaluation in patients with IBD [9]. Previous studies on micronutrients in this population are limited by the inclusion of nonhomogeneous populations (patients with CD and UC), inclusion of patients with different extent and activity of the disease, and the use of different therapy regimens.

The aim of this study was to determine the serum concentrations of selected trace elements, such as Se, Fe, Cu, and Zn, in adult patients with IBD in clinical remission, with the involvement limited to the colon, and receiving immunosuppressive treatment, in comparison with healthy individuals, selecting among these micronutrients a potential sensitive marker of disease activity.

**Patients and methods**

**Study population**

A total of 62 participants were enrolled in the study, including 32 patients with IBD (51.6%; 20 women and 12 men) and 30 healthy controls (48.4%; 14 women and 16 men). The mean (SD) age of IBD patients was 41.0 (15.2) years, and of controls, 39.1 (11.8) years. All patients
with IBD were recruited from the Outpatient Gastroenterology and Hepatology Clinic at Jagiellonian University Medical College in Kraków, Poland. In the IBD group, 15 patients had CD and 17 had UC. Patients with CD presented with isolated colitis. Patients with UC presented at least left-sided colon involvement. All patients with IBD were in clinical remission (Harvey Bradshaw Index ≤4 in CD and Total Partial Mayo Index Score <2 in UC) and received treatment with mesalazine and azathioprine. The mean duration of IBD ranged from 2 to 10 years on enrollment to the study.

The exclusion criteria were as follows: pregnancy, malignancy, diabetes mellitus, obesity (body mass index ≥30 kg/m²), cardiovascular disease (hypertension, coronary artery disease, valvular heart disease, or cardiac arrhythmias), tobacco smoking, body mass index < 18.5 kg/m², intake of medications potentially affecting serum Fe, Se, Zn, or Cu concentrations and diet supplements containing the tested microelements, malabsorption syndromes (eg, celiac disease, short bowel syndrome), as well as total or partial parenteral nutrition.

**Biochemical studies**

The biochemical analyses, including complete blood count and the measurement of serum Fe concentrations, were conducted at the Diagnostics Unit of the University Hospital in Krakow. Venous blood samples were obtained in a fasting state. The levels of Fe and complete blood count (hemoglobin [Hb], mean corpuscular volume [MCV], white blood cells [WBCs], red blood cells [RBCs], platelets, and red blood cell distribution width [RDW]) were evaluated on the same day. For Se, Zn, and Cu assays, blood samples were centrifuged at 1000 g for 15 minutes at a temperature of 4°C, and the serum was collected and stored at a temperature of –80°C until further analysis.

Serum Cu, Zn, and Se concentrations were measured in the laboratory of the Department of Food Chemistry and Nutrition at Jagiellonian University Medical College. The serum levels
of Cu and Zn were determined with a Perkin-Elmer 5100 ZL atomic absorption spectrometer (PerkinElmer, Inc., Norwalk, Connecticut, United States) using the flame technique. The calibration curves for Cu and Zn were performed using 0.00-, 0.25-, 0.50-, 1.00-, 2.00-, and 3.00-mg·l⁻¹ standards. These solutions were prepared by diluting certified standard solutions (1000 mg·l⁻¹, Merck, Darmstadt, Germany): Cu – 1.19786.0500; and Zn – 1.19806.0500. Defrosted serum (0.5 ml) was mixed with 2 ml of demineralized water (Millipore RQ10, Vienna, Austria) and analyzed as described above. Each sample was measured 3 times. If the relative standard deviation of the 3 measurements exceeded 5%, then the measurement was repeated. The correctness of the results was checked with Seronorm™ (200805) and Pathonorm™ (100705) control serum (Sero, Billingstad, Norway). The parameters of the Cu and Zn analysis were as follows: 1) for Cu – wavelength, 324.8 nm; lamp current, 15 mA; slit width, 0.7 nm; air flow, 9.0 l·min⁻¹; and acetylene, 2 l·min⁻¹; and 2) for Zn – wavelength, 213.9 nm; lamp current, 18 mA; slit width, 0.7 nm; air flow, 10.8 l·min⁻¹; and acetylene 2 l·min⁻¹.

The Se level was determined using an Agilent Technologies 240Z AA atomic absorption spectrometer (Agilent Technologies, Santa Clara, California, United States) with graphite furnace atomization and Zeeman background correction. The calibration curve was prepared using 3 serum standards containing 0.35, 0.61, and 1.77 µmol·l⁻¹ Se. The accuracy of the results was confirmed with Seronorm™ reference material (Trace Elements Serum L-1, Sero). As a light source, a hollow-cathode lamp with enhanced light intensity (“ultra-lamp”) was used, and as an inert gas, high-purity argon (99.998%). Serum samples were mixed with 0.1% Triton solution (150 µl + 100 µl) to improve homogeneity. Owing to the presence of complex organic matrix in the samples, a chemical modifier was used and air-assisted ashing was included in the temperature program of the graphite furnace. The samples were measured in duplicate unless the obtained precision was
not satisfactory (in such cases, the measurement was repeated). The parameters of the analysis were as follows: wavelength, 196.0 nm; lamp current, 12 mA; slit, 1.0 nm; chemical modifier, 5% HNO$_3$ containing 1500 mg·L$^{-1}$ Pallad and 6000 mg·L$^{-1}$ Mg(NO$_3$)$_2$; air-assisted ashing temperature, 400°C; inert-gas ashing temperature, 1150°C; and atomization temperature, 2600°C.

**Ethical approval**

The study protocol was approved by the Local Bioethics Committee at Jagiellonian University (decision no. KBET 122.6120.86.2015; as of May 24, 2015). All participants gave their written informed consent to be included in the study.

**Statistical analysis**

Descriptive statistics were calculated for all parameters in the IBD and control groups. The differences between groups were tested using the $t$ test, the Welch test or Mann-Whitney test, as appropriate. The Levene test was used to assess the equality of variances in the compared groups. The 2-tailed Fisher test was used to assess the significance of the difference between 2 Pearson correlation coefficients. A probability level of $P$ less than 0.05 was considered to be significant. The Pearson correlation coefficients were calculated for pairs of parameters. For parameters with non-normal distribution Spearman correlation coefficients were calculated. The principal component analysis (PCA) model was used to further describe the correlation structure between parameters in the studied group (using component weight analysis). The parameters with large weights (>0.3) in the PCA model were assumed to be correlated. To express the strength of bivariate associations, for the pairs of correlated parameters the correlation weights were calculated. They are the algebraic products of corresponding weights of these two given parameters and cosine of the corresponding angle. The corresponding angle
means the angle determined by 2 lines connecting the origin with coordinates of both parameters on the PCA weights plot. Statistical analyses were conducted using the STATISTICA v.12 package (StatSoft, Tulsa, Oklahoma, United States), SIMCA-P v.9 package (Umetrics, Umeå, Sweden), IBM SPSS Statistics v.27 and an online calculator (http://faculty.vassar.edu/lowry/rdiff.html) for the Fisher $r$-to-$Z$ transformation. Finally, correlation weights for the pairs of parameters in the PCA were calculated using software delivered by MP System Co. (Chrzanów, Poland).

**Results**

The comparison of analyzed parameters between the IBD and control groups is shown in Table 1. All but three patients in the IBD group had normal CRP values (<5 mg/l), with the maximum CRP in this group being 7.66 mg/l (median 1.95). None of the IBD patients had significantly elevated CRP. Patients with IBD had significantly lower RBC count, Hb levels, and serum Fe concentrations than controls. On the other hand, platelet count was significantly higher in patients with IBD. There was no difference in WBC count and MCV between groups. Serum Zn concentrations were lower in patients with IBD than in controls, but no significant differences were noted for Se and Cu levels.

The PCA model, which fulfilled cross-validation criteria, had 2 significant components with eigenvalues of 3.58 and 1.54, respectively. This model explained 73.2% of variance in the set of original parameters, reduced by 4 parameters that appeared to be uninformative (Cu, platelets, RDW-SD, WBC). In addition, 2 outlying patients were excluded from the model. The loadings for the first 2 principal components are shown in Figure 1. The first principal component in this model had positive loadings predominantly for Hb, Fe, and Se. Therefore, the highest positive correlation weights based on this component were revealed between these parameters. Hb, Fe, and Se created the set of mutually correlated parameters. Se and Hb were
strongly negatively correlated with RDW-CV (Table 2). The second principal component was loaded mainly positively by mutually correlated RBC and Zn, and negatively, by MCV. Thus, MCV was negatively correlated with both RBC and Zn (Figure 1, Table 2). The PCA model confirmed some of the correlations revealed by the Pearson analysis (all in the cluster of parameters: Hb, Fe, Se) as well as negative correlation between RDW-CV and Hb (Table 3, “Whole group of subjects”). Other negative correlations, presented in Table 2 were disclosed only by the by PCA.

Discussion

Patients with IBD are at high risk for nutritional deficiencies because of long-term inflammation in the gut mucosa and reduced oral intake. Chronic inflammation leads to the sequestration of some trace elements in the liver due to proinflammatory cytokine release induced by inflammatory response [11]. This type of deficiency is typical at diagnosis in active disease generally due to impaired absorption but may also persist throughout the course of the disease due to reduced food intake, direct enteric loss of nutrients, or a hypercatabolic state in patients with IBD [11]. Chronic diarrhea and fistula output can lead to Zn, calcium, and potassium wasting [19], while Fe deficiency is the most common nutritional deficiency in colitis due to chronic gastrointestinal bleeding [20]. Malabsorption most commonly occurs in CD with active small bowel disease or small bowel resection. Moreover, numerous medications used for IBD, such as glucocorticoids, can affect normal micronutrient absorption.

Micronutrient deficiencies occur in more than half of patients diagnosed with IBD [5]. Some authors revealed that systemic inflammatory response reduces the serum levels of micronutrients including Zn, Se, and Cu as well as vitamins A, B6, C, and D, which may occur irrespective of the actual nutritional status [21,22]. On the one hand, these deficiencies
result from serious disease, but on the other hand, they also cause morbidity [12,13,23] and are associated with a high risk of poor outcome. Thus, trace elements seem to be an important subject of research on the prevention and control of IBD. Unfortunately, little attention is paid to nutritional support and estimation of micronutrient content in these patients. Moreover, most previous studies on micronutrient deficiency in IBD were performed in patients with active disease with different disease locations and therapy regimens. To our knowledge, this study is the first to assess micronutrient status in IBD patients during disease remission, with involvement limited to the large intestine, and receiving the same treatment with mesalazine and azathioprine.

Selenium and Zn are important trace elements with various functions that influence the immune system. Dietary Zn and Se deficiency was shown to worsen experimental colitis by affecting various signaling pathways involved in inflammation and oxidative stress, as well as by affecting the intestinal microbiota [11,24]. Zinc reduces the number of proinflammatory cells and production of proinflammatory cytokines [5,25]. Furthermore, it was shown to reduce the activity of inducible nitric oxide synthase in activated macrophages, thus protecting against the production of reactive oxygen and nitrogen species and cellular damage [25]. Zinc intake is also believed to reduce the risk of CD and UC [27]. As shown by previous studies, Zn deficiency is common in patients with IBD, with a prevalence ranging from 15% to 40% [28,29]. Our study also demonstrated decreased serum Zn concentrations despite clinical remission and absence of evident malabsorption. This result is in line with the findings reported by MacMaster et al [10], who identified several biochemical micronutrient deficiencies, including that of Zn, among IBD patients in remission.

Zinc is absorbed in the small intestine, which explains its deficiency in patients with chronic diarrhea or malabsorptive disorders. It is noteworthy that this deficiency is not only a consequence of poor Zn intake but is also related to an inflammatory process in IBD. This is
supported by the finding that Zn deficiency is common even among IBD patients who have received sufficient oral supplementation [30]. In our patients, malabsorption syndrome was not observed and the small intestine was not affected by the disease. However, the limitation of this study is the fact that we did not confirm endoscopic remission and did not evaluate the concentration of calprotectin in the stool in our patients. It is possible that reduced Zn concentrations are caused by persistent colonic inflammation.

There is compelling evidence that chronic inflammation in the intestine is related to oxidative and nitrosative stress both in patients with UC and those with CD [31]. The impact of prooxidative status is further reinforced by a reduced antioxidant level (which is also commonly observed during remission), suggesting that oxidative stress plays an important role in disease recurrence [31]. Zinc functions as an antioxidant [32]. It is possible that its reduced levels may be secondary to enhanced production of a Zn-containing enzyme with antioxidant activity. Research revealed lower activity of Cu–Zn superoxide dismutase in patients with IBD, which explains the reduced ability to scavenge free radicals in these patients. This reduction may be partly due to Zn deficiency and impaired antioxidant system[26].

Zinc is an important component for the synthesis of proteins and erythrocytes [15]. This is in line with our observations on the positive correlation between RBC count and Zn concentrations.

Siva et al [33] showed that serum Zn deficiency is correlated with disease-related morbidity, as reflected by increased rates of hospitalizations, surgery interventions, and disease-related complications in patients with CD compared with individuals with normal Zn concentration [33].

Ananthakrishnan et al. [27] suggested that normal Zn concentrations may contribute to longer remission periods. Therefore, it might be hypothesized that lower Zn levels in patients during
remission might predict early exacerbation. This is supported by the study of MacMaster et al [10], who reported a strong relationship between Zn deficiency and time to next relapse, especially in patients with CD. It appears that decreased Zn concentrations could be a sensitive marker for disease activity in patients with subclinical mucosal inflammation.

Selenium is an important micronutrient associated with numerous selenoproteins with important functions. The absorption of Se has been generally poorly studied, although the highest absorption has been reported in the ileum, followed by the jejunum and large intestine [8].

An epidemiological study suggested that Se status is negatively correlated with IBD activity and colon cancer risk [34]. The mechanism behind this correlation has not been elucidated, but it may be hypothesized that the effect is due to the ability of selenium to polarize macrophages from an M1- to M2-like phenotype, hence reducing inflammation and accelerating healing of the epithelium [35].

Some studies reported reduced Se concentrations in patients with IBD as compared with controls, regardless of disease activity and location [36,37-39]; however, the exact prevalence is unknown. Selenium deficiency was revealed in IBD patients even during remission, and reduced Se concentrations were shown to be associated with increased disease severity [40,41]. Regarding the extent of the disease, Castro Aguilar-Tablada et al [41] reported significantly higher serum Se concentrations in patients with proctosigmoiditis than in those with ileal or colonic involvement and significantly lower concentrations in IBD patients with an inflammatory form of IBD as compared with those with a chronic intermittent or chronic continuous form. They also reported a possible relationship between corticosteroid treatment and a reduction of serum Se levels [41]. In addition, Han et al [6] reported that corticosteroid use and inflammation may predispose to Se deficiency. In such a case, Se deficiency may
result from insufficient supply, decreased absorption, or increased mobilization for antioxidant defense [39].

In contrast to the above studies, Sikora et al [41] and Stochel-Gaudyna et al [43] did not find any significant differences in Se concentrations between IBD patients and the control group. Moreover, Sikora et al [42] reported significantly lower serum Fe and Zn levels in children with IBD compared with controls. These results are in line with our observations. We reported no significant differences for Se levels between study groups; however, patients with IBD had significantly lower RBC count, Hb level, and serum Fe concentrations than controls. Moreover, Se concentrations correlated positively with Hb and Fe levels. This is in line with the study of Castro Aguilar-Tablada et al [41], who reported a positive correlation between serum Se levels and biochemical parameters related to the Fe status [41].

Relatively high Se levels in IBD patients in our study can be explained by the fact that the disease did not involve the small intestine, which is the main site of absorption. Whether Se deficiency is the cause or effect of IBD remains the subject of debate. The comparable Se level between healthy individuals and patients with IBD in remission rather suggests that the Se concentrations is affected by inflammatory process and not the other way round. Copper is a trace element involved in biological electron and oxygen transport. Its deficiency is rather rare owing to large stores in the liver, muscle, and bone [8]. Various studies reported discrepant results regarding serum Cu concentrations in patients with IBD as compared with healthy controls [26,39,44]. These differences may be partly due to different degrees of disease severity. Filippi et al [7] showed decreased serum Cu levels in patients with CD in remission compared with controls. However, this finding was not corroborated by other studies [38,45]. As for patients with UC, some studies reported similar Cu levels to controls, while others revealed elevated levels [38,46]. In the study by Sochel-Gaudyn et al [43], the mean serum Cu concentration was significantly higher in IBD patients at diagnosis than in
healthy controls. Mohammadi et al [26] reported that Cu concentrations did not differ between patients with IBD and controls. This result is in line with our study.

Among micronutrients, Zn and Cu have an essential function in inflammation, with an increased Cu-to-Zn ratio in chronic inflammation and free radical overproduction [44]. Systemic inflammation has the opposite effect on serum Zn and Cu concentrations, resulting in an increased Cu-to-Zn ratio [47]. In contrast to Zn, Cu concentrations increase during the acute-phase response [48]. A higher Cu-to-Zn ratio has been described in patients with active colitis with elevated Cu and diminished Zn levels in the serum of patients with IBD compared with healthy controls [45]. These results are in line with our findings. Despite remission, the Cu-to-Zn ratio in our patients seemed to be increased because Zn concentrations were reduced and Cu levels were slightly (although nonsignificantly) increased as compared with controls. This supports the finding of Mohammadi et al [26] that the ratio of Cu to Zn is clinically more important than the concentration of either element alone [26]. Therefore, an increased ratio may be also a potential marker of inflammatory process in clinical remission in patients with IBD. However, this observation, reported previously by Malavolta et al [44], has to be confirmed in future studies assessing correlations of the Cu-to-Zn ratio with endoscopic and histologic disease markers.

Multiple factors in IBD contribute to iron deficiency: intestinal blood loss, reduced dietary iron intake, duodenal disease involvement or surgical resection of the duodenum leading to diminished iron absorption [20]. Iron deficiency is linked to disease activity [20]. Inflammatory cytokines increase the production of liver hepcidin, which blocks ferroportin-1 and prevents iron release from enterocytes, macrophages, and hepatocytes [49]. As shown by previous studies, iron deficiency is common in patients with IBD [42,43]. Our study also reported decreased serum Fe concentrations despite clinical remission and absence of evident malabsorption. Because of the concentrations of some micronutrients, including iron
concentration may change as a result of proton pump inhibitors use [50], no one enrolled in this study took these drugs long-term.

In conclusion, if we looked at patients in clinical remission only, some of nutritional deficiencies persisted. Undoubtedly drawback of the study is a small group of patients studied and an relatively big variety of disease locations and extents, although we tried to minimize these differences by elimination only rectal involvement. Patients with IBD, despite maintaining clinical remission, should undergo systematic laboratory test for anemia or micronutrient deficiencies. Correction of these deficiencies, which impair the immune response, could facilitate the achievement of long and deep remission. This is consistent with the European Society for Clinical Nutrition and Metabolism guidelines published in 2017, which recommend that micronutrients should be assessed in patients in clinical remission to guide the decision on supplementation but also alert to the fact that inflammatory process may influence the results. Further research is needed to confirm whether Zn levels could serve as a predictor of the course of IBD or as a therapeutic target to maintain disease remission.

**Contribution statement:** AP-G and MZ-W conceived the concept of the study. PP contributed to the design of the research. KW, OK and AD-D were involved in data collection. AP-G and MP-F conducted the research. PZ analyzed the data. AP-G wrote the paper. MZ-W coordinated funding for the project. TM and MZ-W revised and edited the manuscript for final submission.

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Table 1. Comparison of study parameters between patients with inflammatory bowel disease and healthy controls

| Parameter          | Patients with IBD | Controls | P value |
|-------------------|-------------------|----------|---------|
|                   | Mean (SD)        | Median   | Min     | Max     | Mean (SD) | Median   | Min     | Max     |
| Age, y            | 41.0 (15.2)      | 36.0     | 23.0    | 75.0    | 39.1 (11.8) | 37.0     | 21.0    | 69.0    | 0.62    |
| RBC, 10⁶/µl       | 4.4 (0.6)        | 4.5      | 2.7     | 5.5     | 4.7 (0.4)  | 4.9      | 3.9     | 5.4     | 0.039   |
| WBC, 10³/µl       | 7.4 (2.3)        | 7.2      | 3.8     | 13.5    | 7.3 (1.2)  | 7.1      | 5.0     | 9.9     | 0.79    |
| Platelets, 10³/µl | 308.3 (92.9)     | 300      | 146     | 601     | 263.3 (56.0) | 276.0   | 195     | 345.0   | 0.027   |
| Hb, g/dl          | 12.7 (2.2)       | 13.1     | 7.1     | 16.0    | 14.3 (0.8) | 14.4     | 12.4    | 15.5    | 0.001   |
| Fe, µmol/l        | 14.2 (9.4)       | 14.5     | 2.4     | 37.9    | 23.4 (2.7) | 23.1     | 19.1    | 28.8    | 0.000   |
| RDW-CV, %         | 14.6 (2.1)       | 14.0     | 12.6    | 19.8    | 13.6 (3.4) | 12.9     | 12.1    | 31.5    | 0.001   |
| RDW-SD, fl        | 45.4 (5.5)       | 44.1     | 40.1    | 64.4    | 40.8 (2.1) | 40.5     | 36.8    | 46.2    | 0.000   |
| MCV, fl           | 86.2 (7.4)       | 86.0     | 65.5    | 98.7    | 84.8 (3.3) | 84.0     | 79.9    | 91.3    | 0.370   |
| Zn, mg/l          | 0.76 (0.13)      | 0.76     | 0.50    | 1.10    | 0.83 (0.13) | 0.81     | 0.61    | 1.12    | 0.047   |
| Cu, mg/l          | 1.03 (0.27)      | 0.98     | 0.57    | 1.62    | 0.97 (0.22) | 0.94     | 0.64    | 1.62    | 0.32    |
| Se, µmol/l        | 0.90 (0.24)      | 0.93     | 0.36    | 1.43    | 0.93 (0.19) | 0.91     | 0.64    | 1.40    | 0.50    |

a Parameters were compared using the t test, the Welch test or Mann-Whitney test.
Abbreviations: Cu, copper; Fe, iron; Hb, hemoglobin; IBD, inflammatory bowel disease; MCV, mean corpuscular volume; NS, nonsignificant; RBC, red blood cells; RDW-CV, red blood cell distribution width; RDW-SD, red blood cell distribution width, standard deviation; Se, selenium; WBC, white blood cells; Zn, zinc

**Table 2.** Correlation weights for the pairs of parameters based on the principal component analysis model (only correlation weights with absolute values higher than 0.100 are shown)

| Pairs of correlated parameters | Correlation weights |
|-------------------------------|---------------------|
| RBC – Zn                      | 0.251               |
| Hb – Se                       | 0.189               |
| Hb – Fe                       | 0.170               |
| Fe – Se                       | 0.145               |
| RBC – RDW-CV                  | –0.113              |
| MCV – Zn                      | –0.134              |
| RDW-CV – Se                   | –0.149              |
| Hb – RDW-CV                   | –0.183              |
| RBC – MCV                     | –0.192              |

Abbreviations: see Table 1
Table 3. Pearson ($r$) or Spearman ($r_s$) correlation coefficients and their significance level for the pairs of parameters in patients with inflammatory bowel disease and controls.

| Pairs of parameters | IBD                      | Controls                  | Whole group of subjects (IBD+Controls) |
|---------------------|--------------------------|---------------------------|---------------------------------------|
| RBC – Hb$^a$        | $r = 0.706; P = 0.000$   | $r = 0.375; P = 0.041$    | $r = 0.694; P = 0.001$                |
| RDW-CV – RDW-SD     | $r = 0.690; P = 0.000$   | $r_S = 0.292; P = 0.11$   | $r_S = 0.604; P = 0.001$              |
| Hb – Se             | $r = 0.579; P = 0.001$   | $r = 0.260; P = 0.17$     | $r_S = 0.370; P = 0.004$              |
| Fe – Se             | $r = 0.499; P = 0.004$   | $r = -0.093; P = 0.63$    | $r = 0.324; P = 0.012$                |
| Hb – Fe             | $r = 0.452; P = 0.012$   | $r = -0.066; P = 0.73$    | $r = 0.539; P = 0.001$                |
| Hb – Zn             | $r = 0.417; P = 0.022$   | $r = 0.085; P = 0.66$     | $r = 0.349; P = 0.007$                |
| RBC – Zn            | $r = 0.413; P = 0.023$   | $r = -0.088; P = 0.65$    | $r = 0.298; P = 0.021$                |
| Fe – MCV            | $r = 0.411; P = 0.024$   | $r = 0.148; P = 0.43$     | $r = 0.243; P = 0.06$                 |
| PLT – Fe            | $r = -0.372; P = 0.047$  | $r = -0.250; P = 0.18$    | $r = -0.470; P = 0.001$               |
| RDW-CV – Se         | $r = -0.378; P = 0.040$  | $r_S = 0.199; P = 0.30$   | $r_S = -0.027; P = 0.84$              |
| RDW-CV – MCV        | $r = -0.492; P = 0.006$  | $r_S = 0.241; P = 0.20$   | $r_S = -0.112; P = 0.39$              |
| Fe – RDW-CV         | $r = -0.521; P = 0.003$  | $r_S = -0.016; P = 0.93$  | $r_S = -0.462; P = 0.001$             |
| Hb – RDW-CV         | $r = -0.624; P = 0.000$  | $r_S = 0.029; P = 0.88$   | $r_S = -0.333; P = 0.009$             |
| MCV – Se            | $r = 0.325; P = 0.08$    | $r = 0.498; P = 0.006$    | $r = 0.332; P = 0.010$                |

$^a$ There is a significant difference ($P = 0.014$) between Pearson correlation coefficients in these 2 groups (IBD and controls).

Abbreviations: see Table 1
Figure 1. Weights of the first and second principal components in the principal component analysis model. Abbreviations: see Table 1.