RESEARCH LETTER

Altered fecal short-chain fatty acid profile as a potential marker of disease activity in patients with ulcerative colitis and Crohn's disease: a pilot study

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Introduction Growing evidence shows that the alteration of microbiota and its metabolites, especially short-chain fatty acids (SCFAs), affects the health and disease of the human body, including gastrointestinal disorders.[1,2] Inflammatory bowel diseases (IBDs), including ulcerative colitis (UC) and Crohn's disease (CD), are characterized by chronic gastrointestinal inflammation and dysbiosis.[3] A reduced amount of SCFA-producing bacteria was reported in patients with IBD when compared with healthy individuals.[4] While a number of studies have shown an altered SCFA profile in patients with IBD, the exact quantitative and qualitative differences in comparison with healthy controls have not been determined so far.[5,6]

The aim of this study was to determine the SCFA profile in patients with CD and UC depending on the disease activity (remission vs active phase). To comprehensively assess the SCFA profile, 8 acids were identified, including butyric, propionic, acetic, valeric, isovaleric, isobutyric, succinic, and lactic. Moreover, the relationship between the SCFA profile and C-reactive protein (CRP) levels was evaluated.

Patients and methods The diagnosis of IBD was based on the European Crohn's and Colitis Organisation guidelines.[7] The patients with UC were divided into groups with inactive and active disease according to the full Mayo score. Disease activity in the patients with CD was determined on the basis of Crohn's Disease Activity Index, colonoscopy (Simple Endoscopic Score for Crohn's Disease), or imaging tests (computed tomography or magnetic resonance enterography). In our population, the patients with UC had at least left-sided colitis and the patients with CD presented only with colon involvement. All patients underwent a clinical and dietary interview. The control group included volunteers without a history of organic gastrointestinal disease or any alarming symptoms, and who did not fulfill the Rome IV criteria for irritable bowel syndrome. The exclusion criteria were as follows: any other organic intestinal disease, acute gastrointestinal infections, lactation, pregnancy, malignancy, severe chronic diseases, malabsorption syndromes, intake of prebiotics, probiotics, or dietary supplements containing sodium butyrate, as well as total or partial parenteral nutrition.

Ethical approval All participants gave their written informed consent to participate in the study. The study protocol was approved by the Bioethics Committee at the Jagiellonian University in Kraków, Poland (1072.6120.18.2018, as of February 23, 2018), and the study was performed in accordance with the Declaration of Helsinki.

Biochemical analysis All participants underwent standard blood work, including complete blood count and the measurement of serum CRP levels. All biochemical tests were performed in the Laboratory of the University Hospital in Kraków, Poland, in accordance with the manufacturer's instructions.

Measurement of fecal short-chain fatty acid levels The organic acids in stool samples were determined by means of capillary electrophoresis, using PA 800 Plus Pharmaceutical Analysis System (Beckman Coulter Inc., Brea, California, United States), furnished with an ultraviolet spectrophotometric detector, in accordance with a previously described method.[8] Median acid levels were expressed as microgram per gram dry weight of feces.

Statistical analysis Descriptive statistics were calculated for all organic acids in the study groups. As the parameters showed non-Gaussian distribution in the Kolmogorov–Smirnov test, the differences between the groups were assessed using the Kruskal–Wallis test with the post hoc Dunn test. The Spearman correlation coefficients were calculated for the pairs of parameters. A probability level below 0.05 was considered significant. The statistical analyses were carried out using Graph Pad Prism v.3.02 (GraphPad Software, San Diego, California, United States). The STATISTICA v. 13.3 package (TIBCO Software Inc., Palo Alto, California, United States) was used for the graphic representation of data.

Results

Study population A total of 77 participants were enrolled in the study, including 43 patients with UC (mean [SD] age, 35.5 [13.2] years), 18 patients with CD (mean [SD] age, 30.6 [7.3] years), and 16 controls (mean [SD] age, 31.7 [16.7] years). The participants were divided into the following subgroups: 1) 14 patients with inactive UC, 2) 29 patients with active UC, 3) 8 patients with inactive CD, 4) 10 patients with active CD, and 5) 16 healthy controls.

Fecal short-chain fatty acid profile in patients with Crohn's disease, ulcerative colitis, and controls The median acetic acid levels were higher in controls than in the patients with CD and those with UC (1827.5 μg/g, 989.7 μg/g, and 1086.6 μg/g, respectively, $P = 0.01$ and $P = 0.02$, respectively). However, the difference was significant only between the patients with CD and the control group ($P = 0.008$). Lactic acid showed the highest median concentrations in the patients with CD. Of all the assessed SCFAs, valeric acid showed the lowest median levels in all study groups. In addition, its median levels were lower in the patients with UC and CD (5.5 μg/g and 5.5 μg/g, respectively) than in the control group (30.2 μg/g; $P = 0.01$, $P = 0.02$, respectively). The patients with CD had lower median acetic and butyric acid levels than controls (acetic acid, 989.7 μg/g vs 1827.5 μg/g, $P = 0.007$; butyric acid, 102.4 μg/g vs 473 μg/g, $P = 0.001$). In the UC group, the median lactic acid level was higher than that in controls (1023.0 μg/g vs 242.0 μg/g; $P = 0.008$).

The median SCFA levels in the patients with inactive and active UC, inactive and active CD, as well as controls are presented in FIGURE 1. In the patients with inactive UC and in controls, the highest median levels were observed for acetic acid, while in the patients with active UC, inactive CD, and active CD, the highest median levels were reported for lactic acid. Median acetic acid levels were lower in the patients with active UC than in controls (891.4 μg/g vs 1827.5 μg/g; $P = 0.004$). In addition, median lactic acid levels were higher in the patients with active UC as compared with controls (1593.3 μg/g vs 242.0 μg/g; $P = 0.001$). Interestingly, median butyric acid levels were 59.3 μg/g in the patients with active UC and 42.5 μg/g in those with active CD, which was lower than in controls (473.0 μg/g; $P = 0.003$ and $P = 0.001$). Finally, median valeric acid levels were lower in the patients with active UC than in controls (5.5 μg/g vs 30.2 μg/g; $P = 0.004$).

Fecal organic acid levels and biochemical parameters The median CRP level positively correlated with lactic acid levels ($R = 0.534$; $P < 0.05$), and inversely correlated with butyric acid levels ($R = -0.573$; $P < 0.05$).

Discussion

This study showed that an altered SCFA profile is associated with the activity of CD and UC. Butyric acid levels were lower in the patients with active UC and those with active CD, as compared with controls, which suggests that butyric acid may serve as a marker of active IBD. Moreover, the serum levels of CRP, a well-established marker of inflammation, inversely correlated with butyric acid levels. Our results are in line with previous studies.[5,9,10]

The most recent research indicates that gut microbiota composition varies depending on the type of IBD and severity of intestinal inflammation.[5,11] Moreover, the depletion of butyrate-producing bacteria, such as *Faecalibacterium prausnitzii* and *Clostridium clusters* IV, XIVa, and XVII, was reported in the course of both UC and CD.[4,5,9,10] This is an important finding, considering the anti-inflammatory properties of butyric acid.[12] It is also consistent with our observations.

In this research, we observed similar changes in SCFA levels in CD and UC patients with colon involvement. We demonstrated that the patients with active UC had reduced levels of valeric acid, which was reported to have numerous beneficial effects on intestinal inflammation.[13] A similar tendency was observed in the patients with active CD. In addition, the UC and CD groups showed different lactic acid levels in comparison with healthy controls. This particularly refers to the patients with CD, because higher lactic acid levels were present irrespective of the disease activity. On the other hand, in the UC group, only the patients with active disease showed the accumulation of lactic acid. Moreover, lactic acid levels correlated with CRP levels. This is in line with previous reports showing that elevated lactic acid levels were associated with severe disease in the patients with UC.[5,14] It seems that low pH and high oxygenation in the intestinal lumen, which occurs in mucosal inflammation, promote the growth of lactate-producing bacteria.[14] In

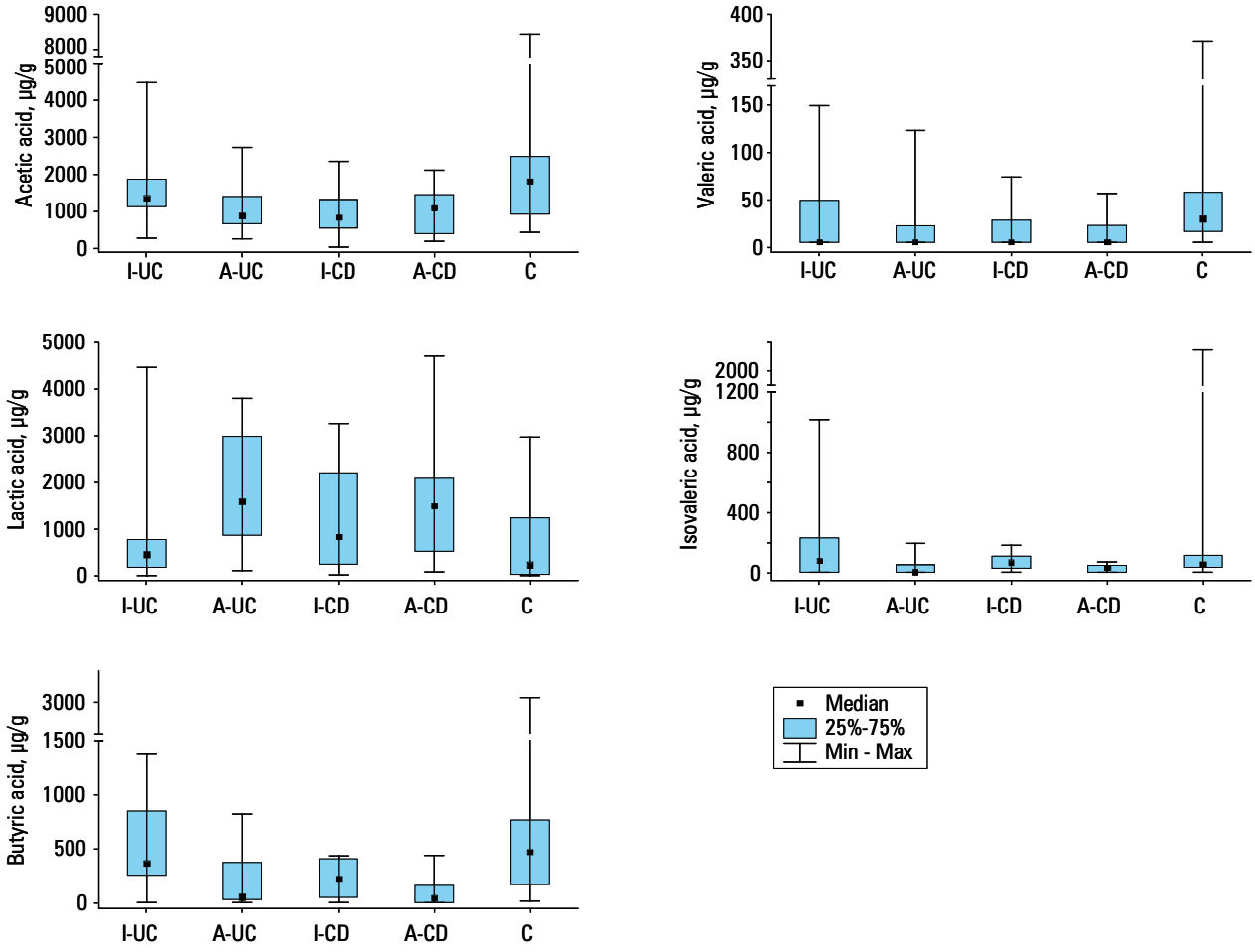

FIGURE 1 Organic acid levels in patients with inactive ulcerative colitis (I-UC), active UC (A-UC), inactive Crohn's disease (I-CD), active CD (A-CD), and controls (C). Significant differences between the groups of patients: acetic acid: A-UC vs C, $P = 0.004$; lactic acid: A-UC vs C, $P = 0.001$; butyric acid: A-UC vs C, $P = 0.003$, A-CD vs C, $P = 0.001$; valeric acid: A-UC vs C, $P = 0.004$; significance levels in the Kruskal–Wallis test for the same groups of patients and controls, and acids given in the same order were as follows: $P = 0.01$; $P = 0.01$; $P = 0.002$; $P = 0.03$; and for isovaleric acid: $P = 0.04$.

addition, the accumulation of lactic acid appears to be a marker of colitis but not of IBD type.[15] As in our study higher lactic acid levels were observed both in inactive and active CD, we hypothesize that this finding may be related to the patients' diet rather than to the type of IBD. However, further studies are needed to confirm this hypothesis. Future research should also compare SCFA levels depending on the site of the inflammatory disease: the small intestine vs the colon.

The main limitations of our study are the small number of participants and heterogeneity of the study population. However, we are currently conducting further research in this area on a larger sample size.

In conclusion, this study demonstrated that the SCFA profile may be a marker of disease severity in the patients with IBD. The assessment of SCFA levels may become a useful tool for monitoring the disease activity and guiding the management of patients with IBD.

ARTICLE INFORMATION

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CONFLICT OF INTEREST None declared.

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