A novel gas chromatography mass spectrometry-based serum diagnostic and assessment approach to ulcerative colitis

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A novel gas chromatography mass spectrometry-based serum diagnostic and assessment approach to ulcerative colitis

ガスクロマトグラフ質量分析計による潰瘍性大腸炎の診断および病勢評価マーカー探索

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Key words:　ulcerative colitis; metabolomics; GC/MS; multiple logistic regression analysis; biomarker

Title:

A novel gas chromatography mass spectrometry-based serum diagnostic and assessment approach to ulcerative colitis

Short Title:

Serum metabolomic analysis of ulcerative colitis

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Abbreviations:

ASCA , anti-*Saccharomyces cerevisiae* antibodies; AUC, area under the ROC curve; CAI,

Clinical activity index; CD, Crohn's disease; CDAI, Crohn's disease activity index; GC/MS, Gas chromatography/mass spectrometry; GSEA, Gene set enrichment analysis; HV, healthy volunteers; IBD, Inflammatory bowel disease; IC, indeterminate colitis; IDO, indoleamine-2,3-dioxygenase; LC/MS, Liquid chromatography/mass spectrometry; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MIAI, multivariate indexes established from plasma aminograms; MSTFA, N-methyl-N-trimethylsilyl-trifluoroacetamide; NMR, nuclear magnetic resonance spectroscopy; OmpC, *Escherichia coli* outer membrane porin; p-ANCA; perinuclear anti-neutrophil cytoplasmic antibodies; ROC, Receiver operating characteristic; SELDI-TOF-MS, surface enhanced laser desorption ionization time-of-flight mass spectrometry; TCA, T tricarboxylic acid cycle; UC, ulcerative colitis; UCa, ulcerative colitis patients in the active phase ; UCr, ulcerative colitis in remission; VIF, variance inflation factors.

Abstract

Background & AIMS: To improve the clinical course of ulcerative colitis (UC), more accurate serum diagnostic and assessment methods are required. We used serum metabolomics to develop diagnostic and assessment methods for UC.

Methods: Sera from UC patients, Crohn's disease (CD) patients, and healthy volunteers (HV) were collected at multiple institutions. The UC and HV were randomly allocated to the training or validation set, and their serum metabolites were analyzed by gas chromatography mass spectrometry (GC/MS). Using the training set, diagnostic and assessment models for UC were established by multiple logistic regression analysis. Then, the models were assessed using the validation set. Additionally, to establish a diagnostic model for discriminating UC from CD, the CD patients' data were used.

Results: The diagnostic model for discriminating UC from HV demonstrated an AUC of 0.988, 93.33% sensitivity, and 95.00% specificity in the training set and 95.00% sensitivity and 98.33% specificity in the validation set. Another model for discriminating UC from CD exhibited an AUC of 0.965, 85.00% sensitivity, and 97.44% specificity in the training set and 83.33% sensitivity in the validation set. The model for assessing UC showed an AUC of 0.967, 84.62% sensitivity, and 88.23% specificity in the training set and 84.62% sensitivity, 91.18% specificity, and a significant correlation with the clinical activity index (r_s=0.7371, P<0.0001) in the validation set.

Conclusions: Our models demonstrated high performance and might lead to the development of a novel treatment selection method based on UC condition.

Keywords: ulcerative colitis; metabolomics; GC/MS; multiple logistic regression analysis; biomarker

Introduction

Inflammatory bowel disease (IBD) involves chronic and recurring inflammation of the gastrointestinal tract and is composed of two major subtypes, ulcerative colitis (UC) and Crohn's disease (CD). The incidence rates of IBD are increasing in several industrialized countries, especially Asian-Pacific countries in which the population's lifestyle has become more westernized, e.g., Japan (1). Previous studies have suggested that these increases in the incidence of IBD are associated with immunological disorders caused by genetic, environmental, and microbiological factors (2,3), and environmental factors are probably the main causes of these increases because it is unlikely that the genetic background of the people in these countries has changed markedly within a few decades (4,5). However, the etiology of IBD remains to be understood, and therefore, disease-specific treatments and diagnostic methods for IBD have yet to be established. At present, comprehensive diagnostic criteria based on clinical, endoscopic, histological, and radiological findings are used to diagnose IBD, but it is difficult to distinguish CD from UC in approximately 2-15% of IBD cases, and these cases are treated as indeterminate colitis (IC) (6-8). Recently, a combination of perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA) and anti-*Saccharomyces cerevisiae* antibodies (ASCA) was demonstrated to be a useful biomarker for discriminating between the different types of IBD (9), but such serological markers are not suitable for clinical application because of their low sensitivity/specificity.

Recently, IBD has started to be evaluated via proteomics and metabolomics-based methods involving nuclear magnetic resonance spectroscopy (NMR), surface enhanced laser desorption ionization time-of-flight mass spectrometry (SELDI-TOF-MS), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), gas chromatography mass spectrometry (GC/MS), and/or liquid chromatography mass spectrometry (LC/MS) (10-14). In this study, metabolomics was used to investigate the alterations in the serum metabolite profiles of IBD patients. Metabolomics is a comprehensive method and is capable of evaluating the characteristics of and interactions between the low molecular weight metabolites of a cell, tissue, organ, or organism. Metabolites are located at the endpoint of the omics cascade; i.e., the last step before the phenotype, and therefore, metabolite profiles might represent the expression patterns of regulatory factors. Thus,

studying such profiles might allow the pathology of IBD to be elucidated in more detail. In our previous reports, it was demonstrated that a serum metabolite profile consisting of amino acids and tricarboxylic acid cycle (TCA)-related metabolites was able to discriminate among UC patients, CD patients, and healthy volunteers (15, 16), although the study only involved a small number of samples. Therefore, in the present study an increased number of samples were used, and both training and validation sets were employed. Using a training set composed of UC patients (N=60) and healthy volunteers (N=60), diagnostic and assessment models for UC were established by selecting candidate metabolites using the volcano plot and stepwise methods and then subjecting them to multiple logistic regression analysis. Then, the validity of the resultant predictive models was assessed using a validation set consisting of UC patients (N=60) and healthy volunteers (N=60). In addition, to establish a diagnostic model for discriminating UC from CD and to validate the diagnostic model for discriminating UC from HV, CD patients' (N=39) data were used as a training set and additional validation set.

Materials and Methods

Patients

This study was approved by the ethics committees at Kobe University Graduate School of Medicine (Hyogo, Japan) and Hyogo Medical University (Hyogo, Japan) and was performed between February 2009 and February 2013. The human samples were used in accordance with the guidelines of each hospital, and written informed consent was obtained from all subjects. The serum samples from the IBD patients (N=159), UC patients (N=120), and CD patients (N=39) were collected at Kobe University Hospital and Hyogo Medical University. UC and CD were diagnosed according to relevant criteria based on clinical symptoms, and radiographic, endoscopic, and pathological findings. Disease activity was assessed using the Crohn's disease activity index (CDAI) (17) and Rachmilewitz index [a clinical activity index (CAI)] (18). Active disease was defined as a CDAI of ≥150 for CD and a CAI of ≥ 6 for UC. Remission was defined as a CDAI of <150 for CD and a CAI of <6 for UC. The serum samples from the HV (N=120) were collected at Aijinkai Chibune General Hospital (Osaka, Japan) and Shinkokai Shinko Hospital Health Examination Center (Hyogo, Japan). No clinical abnormalities were detected in the HV during medical check-ups involving physical, blood, urine, imaging, and/or endoscopic examinations.

Serum collection and preparation

Each whole blood sample was collected in a clean tube and immediately centrifuged at 3,000 x g for 10 min at 4ºC. Then, the serum was transferred to a clean tube and stored at -80ºC until use. The extraction of low molecular weight metabolites was performed according to the method described in our previous report (19). Briefly, 50 µl of serum were mixed with 250 µl of a solvent mixture (MeOH:H_2O:$CHCl_3$=2.5:1:1) containing 10 µl of 0.5 mg/ml 2-isopropylmalic acid (Sigma-Aldrich, Tokyo, Japan) dissolved in distilled water as an internal standard, and then the solution was shaken at 1,200 rpm for 30 min at 37°C, before being centrifuged at 16,000 x g for 3 min at 4°C. Two hundred and twenty-five µl of the resultant supernatant were transferred to a clean tube, and 200 µl of distilled water were added to the tube. After being mixed, the solution was centrifuged at 16,000 x g for 3 min at 4°C, and 250 µl of the resultant supernatant were transferred to a clean tube, before being lyophilized using

a freeze dryer. For oximation, 40 µl of 20 mg/ml methoxyamine hydrochloride (Sigma-Aldrich) dissolved in pyridine were mixed with a lyophilized sample, which was then shaken at 1,200 rpm for 90 min at 30°C. Next, 20 µl of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) (GL Science, Tokyo, Japan) were added for derivatization, and the mixture was incubated at 1,200 rpm for 30 min at 37°C. The mixture was then centrifuged at 16,000 x g for 5 min at 4°C, and the resultant supernatant was subjected to GC/MS measurement.

GC/MS analysis

According to the method described in a previous report (20), the GC/MS analysis was performed using a GCMS-QP2010 Ultra (Shimadzu Co., Kyoto, Japan) with a fused silica capillary column (CP-SIL 8 CB low bleed/MS; inner diameter: 30 m × 0.25 mm, film thickness: 0.25 µm; Agilent Co., Palo Alto, CA). The front inlet temperature was 230°C, and the flow rate of helium gas through the column was 39.0 cm/sec. The column temperature was held at 80°C for 2 min and then raised by 15°C/min to 330°C and held there for 6 min. The transfer line and ion-source temperatures were 250°C and 200°C, respectively. Twenty scans per second were recorded over the mass range 85-500 *m/z* using the Advanced Scanning Speed Protocol (ASSP, Shimadzu Co.).

Data processing

Data processing was performed according to the methods described in previous reports (20,21). Briefly, the MS data were exported in netCDF format. The peak detection and alignment were performed using the MetAlign software (Wageningen UR, The Netherlands). The resultant data were exported in CSV format and then analyzed with in-house analytical software (AIoutput) and an in-house metabolite library for peak identification and semi-quantitative analysis. For the semi-quantitative analysis, the peak height of each ion was calculated and normalized to the peak height of 2-isopropylmalic acid as an internal standard. Names were assigned to each metabolite peak based on the method of a previous report (21).

Statistical analysis

The UC patients were randomly allocated to the training or validation set, and the HV were recruited as age- and sex-matched controls for the UC. In both the training and validation studies, the serum levels of metabolites were compared between the UC patients and HV, between UC patients in the active phase (UCa) and those in remission (UCr), and between the UCa/UCr and HV using the Wilcoxon's rank sum test. Next, volcano plots and the stepwise method were used to select metabolite biomarker candidates for the multivariate analysis according to their significance and fold change values (22). The multiple logistic regression analysis was used to establish a diagnostic model discriminating the UC patients from HV as described previously (19, 23). The multicollinearity of the selected variables was also examined by calculating their variance inflation factors (VIF). Receiver operating characteristic (ROC) analysis was used to calculate area under the ROC curve (AUC), sensitivity, and specificity values for the model in order to evaluate its diagnostic performance, and the optimal cut-off value of the model was determined from its ROC curve. In the validation set study, the accuracy of the diagnostic model was evaluated using the cut-off value obtained in the training set study. Another diagnostic model for discriminating between the UC and CD patients was established by comparing the serum metabolite levels of the UC patients in the training set with those of the CD patients using Wilcoxon's rank sum test and then employing the abovementioned metabolite selection method. In addition, an assessment model for differentiating between the UCa and UCr patients was prepared by comparing the serum metabolite levels of the UCr and UCa patients in the training set using Wilcoxon's rank sum test and then employing the abovementioned metabolite selection method. Next, the correlation between the assessment model and the CAI score was evaluated using Spearman's rank-correlation coefficient.

In our study, 7 UC patients, including 4 UCa patients and 3 UCr patients, who were included in the abovementioned analyses were prospectively evaluated using the assessment model until remission or flare-up occurred, and the utility of the model as a tool for monitoring UC was examined. In addition, we also examined whether various factors such as body weight, body mass index (BMI), serum level of total protein (TP), albumin (Alb) and total cholesterol (T-chol), and medical treatment including medication, enteral nutrition and food restriction therapy influenced the alternations of metabolite biomarker candidates which

we selected in diagnostic and assessment models for UC. At first, the correlation between the metabolite alternations and the following factors; body weight, BMI and serum level of TP, Alb and T-chol in the sera of HV was evaluated using Spearman's rank-correlation coefficient. Next, the correlation between the metabolite alternations and medical treatments; current dose of each salicylate; Pentasa, Asacol and sulfasalazine (SASP), azathioprine, and current dose and total dose within 1, 3, 6 months and 1 year of prednisolone in the sera of UCr was evaluated using Spearman's rank-correlation coefficient. The correlation between the metabolite alternations and current total calorie of enteral nutrition; Elental and amino acid production in the sera of CDr was also evaluated using Spearman's rank-correlation coefficient. As far infliximab, the number of CD patient's samples was sufficient for statistical analysis, so we used CD patients' data. Samples of CD patients were picked up and divided into two groups; administration group and non-administration group which various points such as age, gender ratio, BMI, medicated condition and clinical activity were matched between, and the alternations of metabolites were compared between two groups using the Wilcoxon rank sum test. Similarly, samples of UC patients were divided into two groups; food restriction therapy group and non-food restriction therapy group which various points such as age, gender ratio, BMI, medicated condition and clinical activity were matched between, and the alternations of metabolites were compared between two groups using Wilcoxon rank sum test. As far BMI and serum levels of TP and Alb in HV, we could not obtain data on all the subjects from reports of medical check-ups, and deficient data existed. Therefore, the abovementioned analysis for the correlation between the metabolite alternations and these data was performed using the obtained data only. The details were also demonstrated in the legend of Supplemental Table 10, 11. P-values of less than 0.05 were considered to indicate a significant difference. These analyses were carried out using the default conditions of JMP10.0.2 (SAS Institute Inc., Cary, NC).

Results

The background information of the IBD patients and HV is summarized in Table 1 and Supplementary table 10. In our metabolomic GC/MS-based study, which mainly targeted water-soluble metabolites, 114 metabolites were detected in the sera of IBD patients and HV, and the levels of these metabolites were compared between the UC patients and HV, between the UCa and UCr, and between the UCa/UCr and HV. In both the training and validation sets, 77 metabolites exhibited significant alterations between the UC patients and HV; 48 of the 77 metabolites displayed significantly decreased levels in the UC patients whereas 29 metabolites demonstrated significantly increased levels (Supplemental Table 1). Among these metabolites, the components of the TCA cycle; i.e., succinic acid, fumaric acid, and malic acid, and urea cycle intermediates; i.e., ornithine, citrulline, and urea, exhibited some of the most significant alterations, and all of these metabolites except urea displayed significantly decreased serum levels in the UC patients. Several amino acids such as histidine, isoleucine, leucine, lysine, and methionine, etc., also demonstrated significantly decreased levels in the UC patients. In addition, combining the results from the training and validation sets a total of 24 metabolites exhibited characteristic alterations between the UCa and UCr and/or between the UCa/UCr and HV (Fig. 1A, 1B, Supplemental Table 2), and all of these metabolites except pantothenate demonstrated significant correlations with the CAI in the UC patients (Supplemental Table 3).

In the training set study, to establish a diagnostic model for differentiating the UC patients from the HV, 22 of the 114 metabolites were selected as metabolite candidates from volcano plots using the following criteria: the serum concentration of the metabolite had to differ significantly at the $P<0.05$ level between the UC patients and HV according to Wilcoxon's rank sum test and exhibit a fold change of greater than 2.0 (Supplemental Fig. 1, Supplemental Table 4). Of these 22 metabolites, 4; i.e., taurine, S-benzyl-L-cysteine, maleic acid, and N-acetyl-L-glutamine, were selected using the stepwise method. On the basis of their VIF, it was confirmed that these 4 metabolites did not exhibit multicollinearity (Supplemental Table 5). Next, a diagnostic model consisting of the 4 metabolites was established using multiple logistic regression analysis as follows (Table 2):

$p=1/[1+e-\{0.83-116.3(\text{taurine})-335.3(\text{S-benzyl-L-cysteine})+10801.1(\text{maleic}$

acid)+2220.8(N-acetyl-L-glutamine)}].

The ROC curve obtained for this diagnostic model in the training set study is shown in Figure 2. The AUC of this model was 0.988, and the optimal cut-off value was 0.659. The model's sensitivity and specificity values were 93.33% and 95.00%, respectively, whereas in the validation set study, its sensitivity and specificity values were 95.00% and 98.33%, respectively. Furthermore, as an additional validation set study, the metabolite data of the 39 CD patients were subjected to this diagnostic model. When this set was used, the model was found to have a specificity value of 0.26% (Supplemental Fig. 2, Table 3). Therefore, although this diagnostic model was able to discriminate between UC and HV with high sensitivity and specificity, it could not distinguish between UC and CD.

Next, a diagnostic model for discriminating between UC and CD was investigated using the abovementioned method. In the training set study, 9 of the 114 metabolites were selected as metabolite candidates from a volcano plot according to the same criteria as were used to develop the previous model (Supplemental Fig. 3, Supplemental Table 6). Of these, 4 metabolites; i.e., oxalate, 3-hydroxy-butyrate, ribulose, and 1,6-anhydroglucose, were selected via the stepwise method, and none of them exhibited multicollinearity (Supplemental Table 7). The following diagnostic model was established using multiple logistic regression analysis (Table 4):

p=1/[1+e-{1.36-2281.82(oxalate)-35.00(3-hydroxy-butyrate)-570.81(ribulose)+279.26(1,6-anhydroglucose)}].

The ROC curve obtained for this diagnostic model in the training set study is shown in Fig. 3. The AUC of the model was 0.965, and its optimal cut-off value was 0.653. Its sensitivity and specificity values were 85.00% and 97.44%, respectively**.** In the validation set study, the metabolite data of the UC patients were subjected to this diagnostic model, which demonstrated that it displayed 83.33% sensitivity (Table 5). In addition, we examined whether disease location affected the ability of the model to discriminate between UC and CD. As a result, it was demonstrated that the model was able to discriminate between UC and CD regardless of the location of the disease (Supplemental Fig. 4).

Finally, we investigated whether an assessment model for UC could be established. In the training set study, 12 of the 114 metabolites were selected as metabolite candidates from a volcano plot according to the same criteria as were used for the previous two models (Supplemental Table 8, Supplemental Fig. 5), and 2 metabolites; i.e., histidine and p-hydroxybenzoic acid, were selected from the 12 metabolites via the stepwise method, neither of which displayed multicollinearity (Supplemental Table 9). An assessment model consisting of these 2 metabolites was established using multiple logistic regression analysis as follows (Table 6): p=1/[1+e-{-1.64+380.17(histidine)-2559.38(p-hydroxybenzoic acid)}].

Based on the ROC curve obtained for this assessment model in the training set study (Fig. 4), its AUC was calculated as 0.967, and its optimal cut-off value was 0.421. In addition, its sensitivity and specificity values were 84.62% and 88.23%, respectively, whereas in the validation set study, its sensitivity and specificity values were 84.62% and 91.18%, respectively (Fig. 4, Table 7). Moreover, the values assigned to the patients by this assessment model exhibited a positive correlation with the CAI (Fig 5). The ability of this model to monitor disease activity was also evaluated. In this study, 7 UC patients were prospectively monitored (Fig 6). In the 4 active UC patients, the value of this model decreased when their CAI fell below 4 after treatment. In the 3 UC patients that were in remission, the model's value increased when their condition flared-up to such an extent that their CAI were elevated by more than 6 points, suggesting that our UC assessment model is useful for monitoring disease activity.

In addition, whether various factors such as body weight, BMI, serum levels of TP, Alb and T-chol, and medical treatment including medication, enteral nutrition and food restriction therapy influenced the alternations of selected metabolite biomarker candidates were examined using Spearman's rank-correlation coefficient and Wilcoxon rank sum test (Supplemental Table 11, 12, 13, 15, 17, 18) The background information of the IBD patients who we picked up was summarized in Supplementary table 14 and 16. As a result, regarding 10 metabolites biomarker candidates ; i.e., Oxalate, 3-Hydrobutyrate, Maleic acid, p-Hydroxybenzoic acid, Ribulose, Taurine, 1,6-Anhydroglucose, N-Acetyl-L-glutamate, Histidine, S-Benzyl-L cysteine_1, the significantly high correlation based on the following

statistical criteria; P value < 0.05 and r_s > 0.7, or the significant alternation between 2 groups could not be confirmed except SASP. p-Hydroxybenzoic acid exhibited a high correlation with dose of SASP.

Discussion

The diagnosis of IBD is dependent on comprehensive criteria based on the results of clinical, endoscopic, histological, and radiological examinations; however, performing all of these examinations is expensive and takes a long time, making it difficult to achieve an early diagnosis. Therefore, rapid, low cost, and non-invasive diagnostic tools for IBD are required. In addition, it is also difficult to discriminate UC from CD in spite of the comprehensive criteria that are currently used to diagnose IBD. Therefore, a diagnostic tool for discriminating between the different types of IBD, which would enable clinicians to select an appropriate treatment at an earlier point, is also required. Previously, several serological biomarkers such as perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA), anti-*Saccharomyces cerevisiae* antibodies (ASCA), *Escherichia coli* outer membrane porin (OmpC), *Pseudomonas fluorescens*-associated sequence (I2), and flagellin (CBir1) were suggested as diagnostic tools for IBD (9, 24-30). OmpC, I2, and CBir1 display higher prevalences among CD patients (24-55%) than in UC patients (2-11%) (24-30) and so might be useful for discriminating between UC and CD. The combination of p-ANCA and ASCA is also considered to be a useful serological marker for discriminating between the different types of IBD, and pANCA+/ASCA- displays sensitivity and specificity values of 51% and 94%, respectively, for detecting UC (9). Recently, Hisamatsu T et al. reported a new biomarker for IBD, multivariate indexes established from plasma aminograms (MIAI), which was constructed based on the plasma amino acid profiles of each type of IBD (31). It displayed superior diagnostic utility compared with the abovementioned serological markers and was able to discriminate UC from CD in both the active (AUC for ROC = 0.879) and remission phases (AUC for ROC = 0.744). Our diagnostic model based on metabolite profiling was able to distinguish UC from CD with high sensitivity (85.00%) and specificity (97.44%) and displayed a high AUC value (0.965) (Fig. 3, Table 5).

To select appropriate treatments according to IBD status, a tool for assessing IBD that enables clinicians to evaluate disease activity is also required. The abovementioned serological biomarkers including ASCA and p-ANCA are not available for assessing disease activity, but fecal biomarkers, especially calprotectin, are suggested to be useful for monitoring UC (32-35). These biomarkers exhibit strong correlations with not only mucosal

activity according to endoscopy but also clinical activity based on UC symptoms in both adults (r_s = 0.67) and children (r_s = 0.68) (32,33). The MIAI has also been found to be a useful tool for assessing UC and is able to discriminate between UCr and UCa (AUC for ROC = 0.849). It also displayed a positive correlation with the CAI (r_s = 0.598). Our assessment model displayed a superior ability to evaluate the clinical activity of UC (r_s = 0.737; AUC for ROC = 0.967) (Fig. 4, 5A, Table 7). Therefore, both our diagnostic and assessment models are useful clinical tools for UC.

The IBD patients who we recruited in our study were administrated various medications such as salicylates, immunomoduratory agents and biological agents. The evaluation about influence of each medication on metabolomic profiles is required for improving the accuracy of our model. In addition, it is also important to evaluate whether food intake or food restriction therapy including enteral nutrition therapy influenced the metabolite alternations or not. In our study, metabolite biomarker candidates which consisted of our diagnostic and assessment models did not have the significantly high correlation or significant alternations for abovementioned factors except SASP, although the effects of tacrolimus, 6-mercaptopurine and adalimumab could not be evaluated due to the small number of the patients medicated with tacrolimus, 6-mercaptopurine and adalimumab (Supplemental Table 11, 12, 13, 14, 15, 16, 17, 18). Only the dose of SASP displayed the significantly high correlation with the serum level of p-Hydroxybenzoic acid. Regarding other medications, their sample numbers were small, although their evaluations were performed in this study, and therefore the more detailed evaluations about these issues may be required in the future. Then, in present study, the number of CD patients' sample was smaller than that of UC, so the further research adding more CD patients' samples may be also required to improve accuracy of our diagnostic models in the future.

The metabolite profile of UC seems to reflect the pathogenesis of the condition in more detail. Our metabolomic study detected significant alterations in the levels of 77 metabolites between HV and UC patients, and among these metabolites, 48, including several amino acids, TCA-related metabolites, and urea cycle-related metabolites, exhibited significantly decreased levels in the UC patients (Supplemental Table 1). Previously, it was demonstrated that stressors such as injury and infection enhanced glycolytic activity by

increasing cellular glucose uptake, adenosine triphosphate turnover, and adenosine monophosphate production, which stimulate phosphofructokinase production (36,37); however, a study of the global gene expression profile of the inflamed colonic tissues of UC patients using DNA microarrays detected the widespread downregulation of genes associated with protein metabolism, the urea cycle, the citric acid cycle, and oxidative electron transport (38). Gene set enrichment analysis (GSEA) also demonstrated the downregulation of ribosomal and Krebs cycle proteins in the colons of experimental colitis mice (39), and these reports seem to be in line with our findings.

Our metabolomic study identified several metabolites that showed characteristic alterations between UCa and UCr and/or between UCa/UCr and HV (Fig. 1A, 1B, Supplemental Table 2) and whose levels were significantly correlated with the CAI in UC patients (Supplemental Table 3, Fig. 1A, 1B). IBD is regarded as the multifactorial disorder, and the complexity raises the necessary to take a careful consideration about IBD. However, these metabolites might reflect the pathogenesis of UC more closely. Of these metabolites, glutamine, histidine, and tryptophan have been well documented to have beneficial effects against inflammation. For example, the oral supplementation of an experimental colitis model with glutamine, histidine, or tryptophan exerted anti-inflammatory effects (15, 40, 41). In addition, glutamine was demonstrated to play a key role in the maintenance of gut functions; i.e., it was shown that glutamine has gut-protecting effects and inhibits apoptosis by downregulating Sp3 expression in intestinal epithelial cells (42). Glutamine also has anti-oxidative effects, and the glutamine supplementation of protein-depleted rats during inflammatory shock restored their jejunal glutathione concentrations (43). In addition, significantly increased levels of kynurenine and decreased levels of tryptophan were detected in the UC patients in the present study (Supplemental Table 2, Fig. 1A, 1B), and these results might reflect the pathogenesis of UC. Tryptophan is metabolized into kynurenine by indoleamine-2,3-dioxygenase (IDO). This leads to immunomodulatory effects (44-46), and it was reported that IDO is overexpressed in the intestinal lesions of UC patients (47). Homocysteine and citrulline also exhibited significant alterations in their levels between the different patient groups in our study (Fig. 1A, 1B, Supplemental Table 2), and these metabolites are known to be associated with IBD. Homocysteine, an intermediate product in

methionine metabolism, exhibited significantly increased serum levels in the UC patients (Fig. 1A, 1B, Supplemental Table 2). Hyperhomocysteinemia displays a prevalence of 30% in UC patients, which is higher than that seen in healthy controls (48). Homocysteine promotes oxidative effects through its auto-oxidation and inhibition of glutathione peroxidase (GPx) (49) and was found to have thrombogenic effects on arterial endothelial cells (50), suggesting that hyperhomocysteinemia induces vascular events. In IBD patients, the incidence of arterial and venous thromboembolic events was reported to be 1-8% (51, 52), and our results suggest that a relationship exists between homocysteine and the risk of vascular events in UC patients. In addition, significant alterations in the levels of citrulline and urea, which are components of urea cycle-related metabolites, were also found in the UC patients in the present study (Fig 1A, 1B, Supplemental Table 2), and in a previous study the UC patients demonstrated significantly decreased plasma levels of ornithine and citrulline compared with the HV (31). In the present study, the serum level of arginine, which is another component of the urea cycle, was positively correlated with the disease activity of UC, and arginine was found to exert anti-inflammatory effects in DSS-treated mice (53, 54). Therefore, our results suggest that UC patients suffer from a disorder that affects the metabolic pathways of the urea cycle.

In conclusion, using multiple logistic regression analysis we established novel and non-invasive serum metabolomics-based diagnostic and assessment models for UC. Our models demonstrated higher accuracy and/or stronger correlations with disease activity than previously reported biomarker candidates for UC. In addition, several metabolites displayed characteristic alterations in UC that varied according to disease activity. Our GC/MS based-metabolomic research will aid the elucidation of the pathogenesis of UC although IBD is one of the multifactorial disorders, and might lead to the development of a novel method for selecting appropriate treatments according to UC status.

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Each authors made the following substantial contributions. Michitaka Kohashi carried out drafting the manuscript, care of patients, acquisition of data, statistical analysis and interpretation of data. Shin Nishiumi carried out drafting the manuscript, metabolomics analysis, interpretation of data and obtaining funding. Makoto Ooi, Tomoo Yoshie, Koji kamikozuru, Yoko Yokoyama, Ken Fukunaga and Shiro Nakamura carried out care of patients and acquisition of data. Atsuki Matsubara, Makoto Suzuki and Namiko Hoshi carried out technical support. Takeshi Azuma carried out obtaining funding and administrative support. Masaru Yoshida carried out obtaining funding and study supervision. All authors read and approved the final manuscript.

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Figure legends

Figure 1. Metabolites that displayed altered serum levels according to the presence/absence of UC and disease activity

Twenty-four metabolites showed significantly decreased **(A)** or increased **(B)** serum levels between the UCa and UCr, and between the UCa/UCr and HV, in both the training and validation sets. Data are shown as the mean ±standard deviation. P-values were calculated using the Wilcoxon rank sum test; *P < 0.05, **P < 0.01 ***P< 0.001.

Figure 2. ROC curve for diagnostic model A

ROC curve for the diagnostic model constructed based on the training set. Its AUC and cut-off values were 0.9881{95% confidence interval (95% CI): 0.9644-0.9961} and 0.6586, respectively.

Figure 3. ROC curve for diagnostic model B

ROC curve for diagnostic model B constructed based on the training set. Its AUC and cut-off values were 0.9650{95% confidence interval (95% CI): 0.9214-0.9848} and 0.6526, respectively.

Figure 4. ROC curve for assessment model A

ROC curve for assessment model A constructed based on the training set. Its AUC and cut-off values were 0.9667{95% confidence interval (95% CI): 0.8327-0.9777} and 0.4214, respectively.

Figure 5. Correlation between the values assigned by assessment model A and the CAI in the validation set

A scatterplot with a regression line showing the correlation between the values assigned by assessment model A and the CAI in the validation set. {Spearman's rank correlation coefficient (r_s) = 0.7371 (P < 0.001), value assigned by assessment model A = 0.067 + 0.081 × CAI score}. The values assigned by assessment model A exhibited a positive correlation with the CAI.

Figure 6. Prospective monitoring of UC with assessment model A

In our study, 7 UC patients were prospectively monitored. The seven upper graphs show the alterations in the values assigned to the 7 UC patients by assessment model A, respectively. Among them, 4 patients (A-D) achieved remission after treatment **(A)**, and 3 patients (E-G) suffered flare-up **(B)**.

Table 1. Characteristics of the patients in the training and validation sets

	Training set		Validation set		Training & validation set
	UC	**HV**	**UC**	**HV**	**CD**
N (male/female)	60(21/39)	60(23/37)	60(22/38)	60(23/37)	39(26/13)
Age [a)]	42.8±14.1/16-72	45.4±11.1/26-71	41.9±15.0/13-77	45.4±10.8/27-70	35.4±12.0/13-58
Years with disease [a)]	8.7±7.3/0.2-23	-	7.9±6.0/0.3-21	-	9.8±8.9/0.3-32
Disease location					
UC: P/LS/PC	7/23/30	-	7/24/29	-	-
CD: S/C/Both	-	-	-	-	10/4/25
Disease activity [a)]					
CAI	3.7±3.6/0-12	-	3.6±3.8/0-15	-	-
CDAI	-	-	-	-	1202.1±98.6/0-287
Remission/active	34/26	-	34/26	-	29/10
Daily medication					
Salicylates	56	-	50	-	29
Prednisolone	14	-	20	-	2
6-Mercaptopurine	1	-	2	-	2
Azathioprine	13	-	12	-	17
Tacrolimus	3	-	3	-	0
Anti-TNF-α agents	4	-	5	-	25
Enteral nutrition	-	-		-	21

[a)]: Age, years with disease and disease activity are shown as the mean ± standard deviation together with the range. P: proctitis, LC: left-sided colitis, PC: pan-colitis, S: small bowel, C: colon, Both: small bowel & colon, CAI: Rachmilewitz index (a clinical activity index), CDAI: Crohn's disease activity index.

Table 2. Variables selected by the stepwise method

Metabolite	Coefficient	Standard error	P-value	Lower 95% CI	Upper 95% CI
(Intercept)	0.84	2.03	0.6802	-3.29	4.94
Taurine	-116.34	37.26	0.0018	-204.86	-55.76
S-Benzyl-L-cysteine	-335.31	116.46	0.004	-607.59	-142.98
Maleic acid	10801.09	5924.86	0.0683	162.81	24165.11
N-Acetyl-L-glutamine	2220.85	1371.78	0.1055	241.26	5001.77

P-values were calculated using the Wald test.

Table 3. Diagnostic performance of our predictive model (Diagnostic model A)

	Training set	Validation set	Additional validation set
	UC+HV	UC+HV	CD
Sensitivity (%)	93.33%	95.00%	-
Specificity (%)	95.00%	98.33%	0.26%
Accuracy (%)	94.17%	96.67%	-
PPV	94.91%	98.28%	-
NPV	93.44%	95.16%	-
LR+	18.67	57.00	-
LR-	0.07	0.05	-

PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio. The accuracy of our diagnostic model was calculated using the cut-off value obtained by ROC analysis. The cut-off value of the model was 0.6586. In an additional validation set study, the diagnostic model was used to assign values to 39 CD patients.

Table 4. Variables selected by the stepwise method

Metabolite	Coefficient	Standard error	P-value	Lower 95% CI	Upper 95% CI
(Intercept)	-1.36	1.15	0.2393	-3.86	0.82
Oxalate	2281.82	773.27	0.0032	1148.43	4446.28
3-Hydroxybutyrate	35.00	18.89	0.0640	8.40	84.47
Ribulose	-570.81	250.43	0.0227	-1142.15	-151.30
1,6-Anhydroglucose	-279.26	260.87	0.2844	-1051.20	-1.92

P-values were calculated using the Wald test.

Table 5. Diagnostic performance of our predictive model (Diagnostic model B)

	Training set	**Additional validation set**
	UC+CD	**UC**
Sensitivity (%)	85.00%	83.33%
Specificity (%)	97.44	-
Accuracy (%)	86.87%	-
PPV	98.08%	-
NPV	80.85%	-
LR+	33.15	-
LR-	0.15	-

PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio. The accuracy of our diagnostic model was calculated using the cut-off value obtained from ROC analysis of the training set. The cut-off value was 0.6586. In an additional validation set study, diagnostic model B was used to assign values to the UC patients in the validation set.

Table 6. Variables selected by the stepwise method

Metabolite	**Coefficient**	**Standard error**	**P-value**	**Lower 95% CI**	**Upper 95% CI**
(Intercept)	1.64	1.12	0.1439	-0.55	4.17
Histidine	-380.17	105.29	0.0003	-638.95	-208.44
p-Hydroxybenzoic acid	2559.38	1377.28	0.0631	865.61	6269.25

P-values were calculated using the Wald test.

Table 7. Diagnostic performance of our predictive model (Assessment model A)

	Training set	**Validation set**
	UCr+UCa	**UCr+UCa**
Sensitivity (%)	84.62%	84.62%
Specificity (%)	88.23%	91.18%
Accuracy (%)	86.67%	88.33%
PPV	84.62%	88.57%
NPV	88.23%	95.16%
LR+	7.19	9.58
LR-	0.17	0.16

PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio. The accuracy of our diagnostic model was calculated using the cut-off value obtained from ROC analysis of the training set.

Figure 1-A.

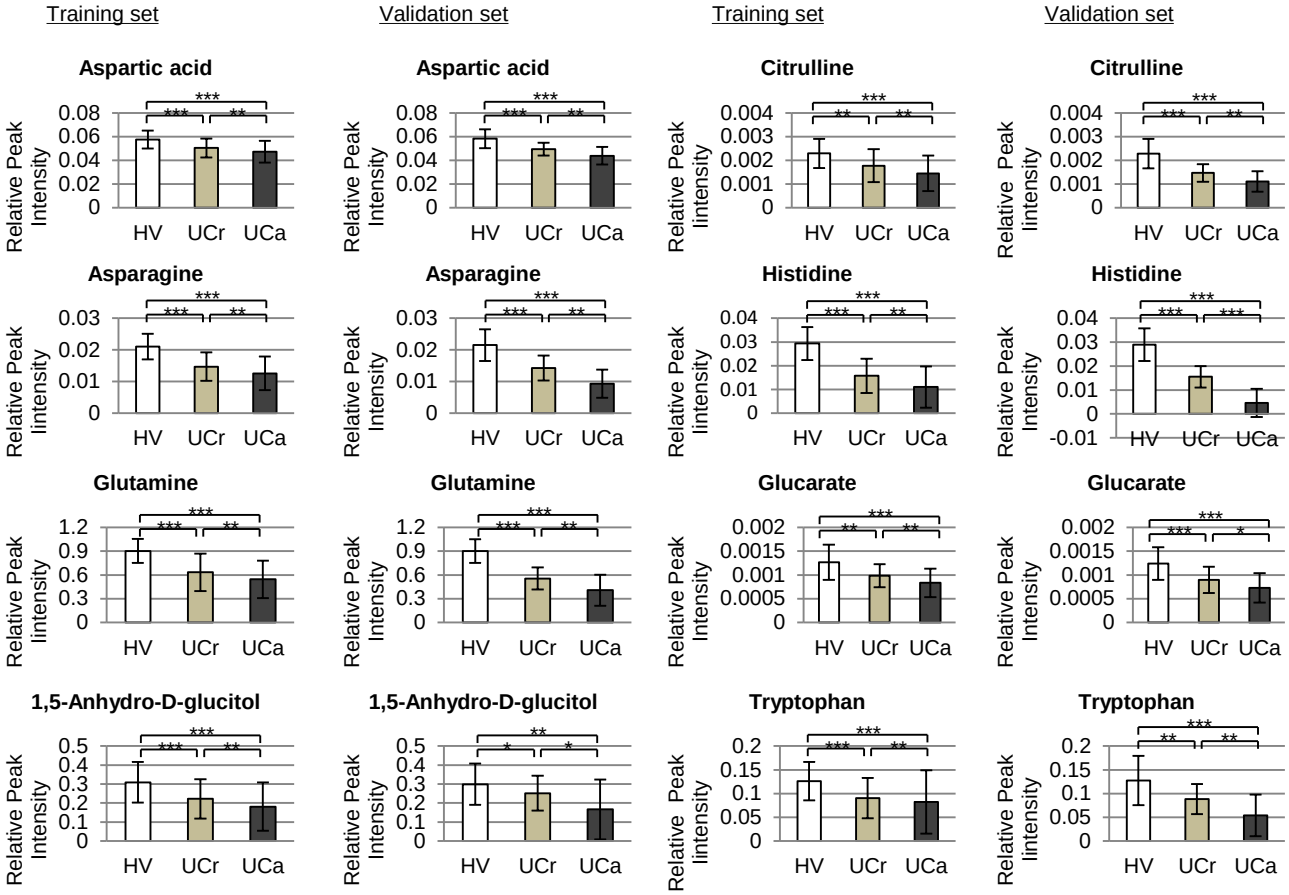
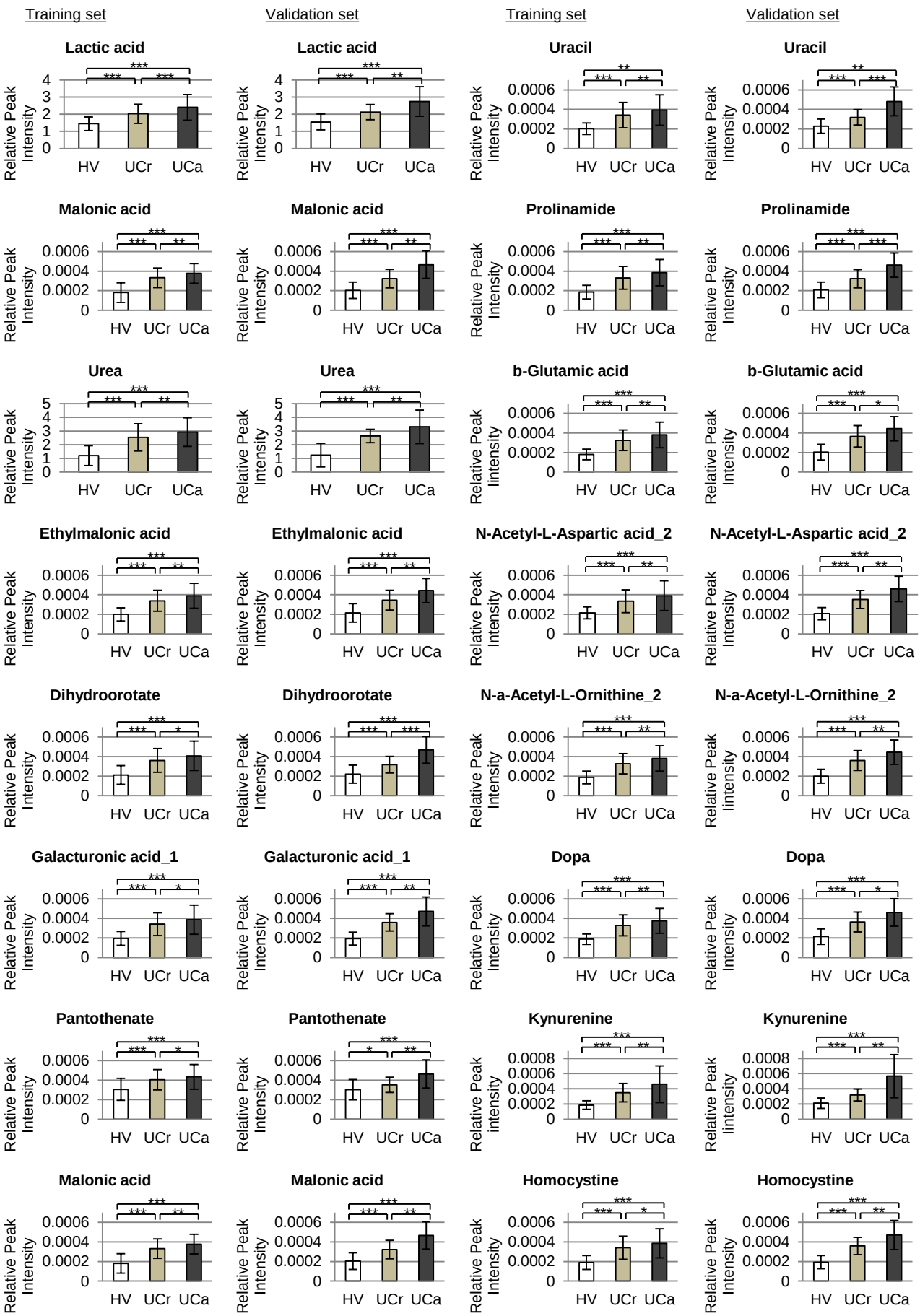

Training set
Validation set
Training set
Validation set
Aspartic acid
Aspartic acid
Citrulline
Citrulline
Asparagine
Asparagine
Histidine
Histidine
Glutamine
Glutamine
Glucarate
Glucarate
1,5-Anhydro-D-glucitol
1,5-Anhydro-D-glucitol
Tryptophan
Tryptophan
Relative Peak Intensity
HV
UCr
UCa

Figure 1-B.


Training set
Validation set
Training set
Validation set
Lactic acid
Lactic acid
Uracil
Uracil
Malonic acid
Malonic acid
Prolinamide
Prolinamide
Urea
Urea
b-Glutamic acid
b-Glutamic acid
Ethylmalonic acid
Ethylmalonic acid
N-Acetyl-L-Aspartic acid_2
N-Acetyl-L-Aspartic acid_2
Dihydroorotate
Dihydroorotate
N-a-Acetyl-L-Ornithine_2
N-a-Acetyl-L-Ornithine_2
Galacturonic acid_1
Galacturonic acid_1
Dopa
Dopa
Pantothenate
Pantothenate
Kynurenine
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Malonic acid
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Homocystine
Homocystine
Relative Peak Intensity
HV
UCr
UCa

Figure 2.

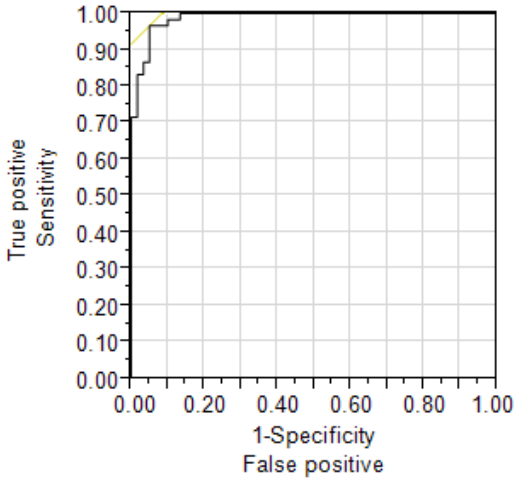


Figure 3.

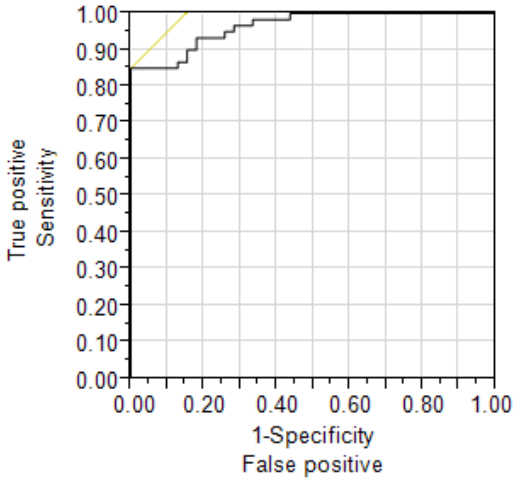


Figure 4.

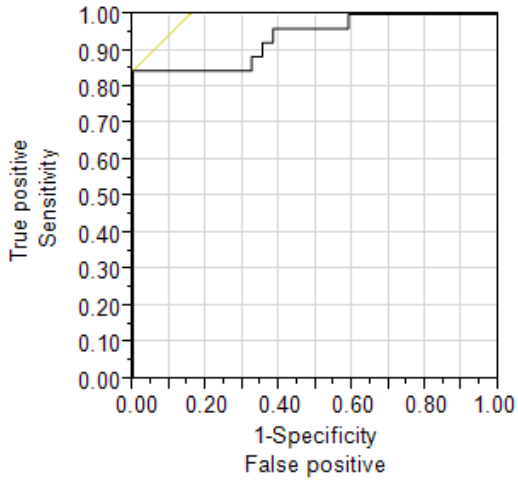


Figure 5.

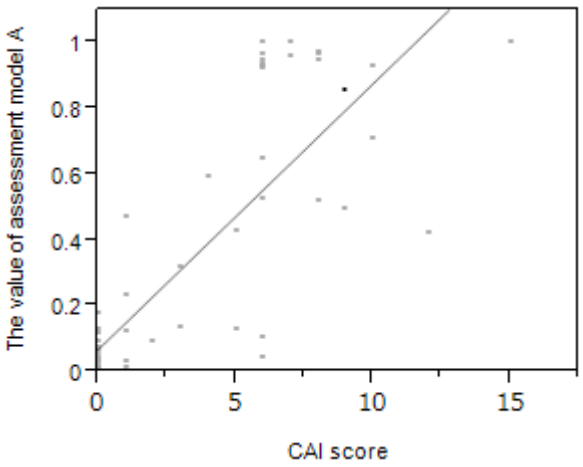


Figure 6-A.

Prospective monitoring of 4 patients (A-D) achieved remission after treatment

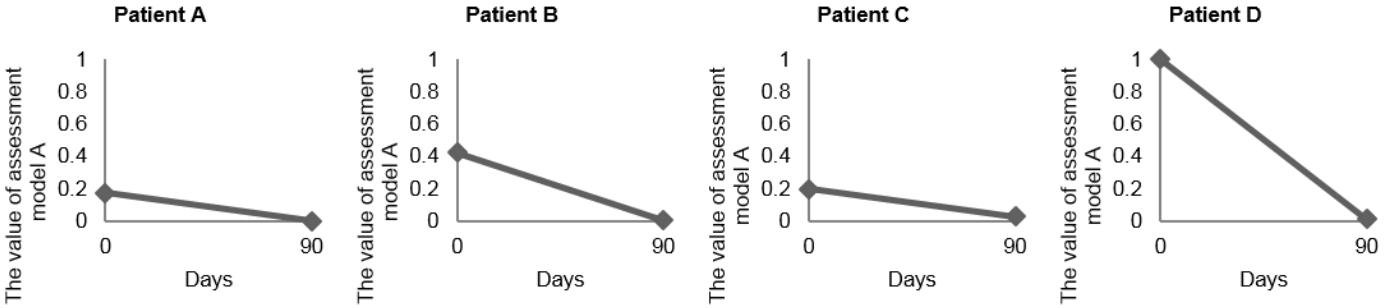
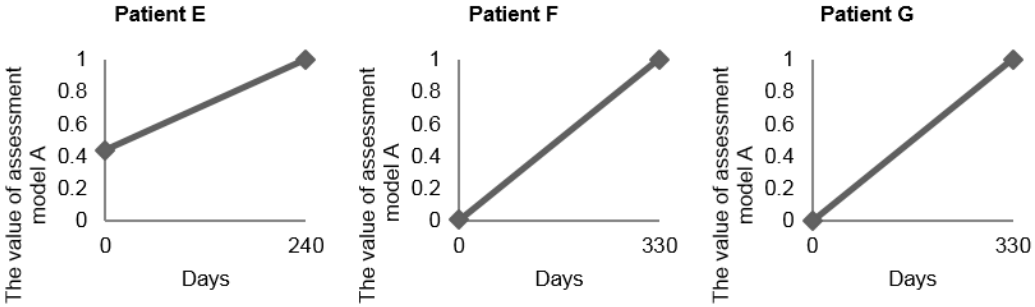


Figure 6-B.

Prospective monitoring of 3 patients (E-G) suffered flare-up



Supplemental Table 1. Comparison of the serum levels of all metabolites detected by GC/MS between the UC patients and HV

	Training set		Validation set	
	F.I. UC/HV	P-value UC vs. HV	F.I. UC/HV	P-value UC vs. HV
Pyruvate+oxalacetic acid	1.32	0.2670	0.99	0.0783
Lactic acid	1.66	<.0001	1.55	<.0001
Glycolic acid	0.94	0.0220	0.92	0.1043
Alanine (2TMS)	1.00	0.2536	0.92	0.0135
Hydroxybutyrate	1.50	0.0238	1.01	0.6236
Oxalate	0.72	<.0001	0.80	0.0012
Sarcosine	0.75	0.0003	0.63	<.0001
2-Aminoisobutyrate	0.93	0.1619	0.85	0.0029
3-Hydroxybutyrate	4.18	0.3199	1.75	0.2164
2-Aminobutyric acid	0.87	0.0043	0.73	<.0001
Ketoisoleucine_1	1.15	0.5514	0.93	0.0288
Malonic acid	2.09	<.0001	1.89	<.0001
3-Hydroxyisovaleric acid	1.42	0.0033	1.08	0.2223
Valine (2TMS)	0.74	<.0001	0.71	<.0001
Urea	2.44	<.0001	2.37	<.0001
Oxamic acid	0.84	0.6162	2.96	0.6015
2-Aminoethanol	0.75	<.0001	0.72	<.0001
n-Caprylic acid	0.89	<.0001	0.74	<.0001
Phosphate	0.75	<.0001	0.69	<.0001
Glycerol	2.21	0.7910	2.14	0.0621
Ethylmalonic acid	1.95	<.0001	1.81	<.0001
Isoleucine	0.82	0.0004	0.71	<.0001
Threonine (2TMS)	0.87	0.0079	0.75	<.0001
Proline	0.81	0.0022	0.79	0.001
Maleic acid	2.02	<.0001	2.07	<.0001
Glycine (3TMS)	0.98	0.1957	0.94	0.357
Succinic acid (or aldehyde)	0.77	<.0001	0.71	<.0001
Catechol	1.90	<.0001	1.72	<.0001
Glyceric acid	0.86	0.0066	0.85	0.0027
Uracil	1.94	<.0001	1.70	<.0001
Fumaric acid	0.65	<.0001	0.60	<.0001
Serine (3TMS)	0.61	<.0001	0.62	<.0001
Nonanoic acid (C9)	0.47	<.0001	0.48	<.0001
4-Methylbenzoic acid	1.56	<.0001	1.22	<.0001
Glutaric acid	0.86	0.0783	0.93	0.4204
Maleamic acid	1.18	0.0003	0.99	0.0360
Prolinamide	2.07	<.0001	1.84	<.0001
Homoserine	0.86	0.0024	0.85	0.0017
Malic acid	0.77	<.0001	0.68	<.0001

Threitol	0.91	0.2366	1.00	0.7708
meso-Erythritol	1.01	0.3199	3.20	0.0537
Adipic acid	1.10	0.0284	1.33	0.0004
Acetylsalicylic acid	6.43	<.0001	0.63	<.0001
Aspartic acid	0.82	<.0001	0.81	<.0001
Methionine	0.82	<.0001	0.73	<.0001
trans-4-Hydroxy-L-proline	0.67	<.0001	0.59	<.0001
4-Aminobutyric acid	2.10	<.0001	1.45	0.0013
Pyrogallol	0.49	0.6273	0.68	0.2647
Creatinine	0.80	0.0015	0.78	0.0016
Acetoacetic acid	0.25	<.0001	0.26	<.0001
β-Glutamic acid	2.11	<.0001	1.96	<.0001
Glutamic acid	0.79	<.0001	0.70	<.0001
Phenylalanine	0.90	0.0002	0.79	<.0001
5-Aminovaleric acid	0.88	0.1699	0.72	0.0023
p-Hydroxybenzoic acid	2.07	<.0001	1.84	<.0001
Xylose_2	2.12	0.0035	2.46	0.1651
threo-β-Hydroxyaspartic acid	1.25	<.0001	1.40	<.0001
Lyxose_2	1.01	0.4055	0.99	0.0017
Arabinose	1.00	0.5067	1.04	0.5237
Lauric acid	0.91	0.0161	0.77	<.0001
N-Acetyl-L-aspartic acid_2	1.81	<.0001	1.92	<.0001
Homocysteine_1	1.92	<.0001	1.70	<.0001
Ribose	1.21	0.7548	1.44	0.0925
Ribulose	0.87	0.1100	1.00	0.2471
Asparagine	0.60	<.0001	0.56	<.0001
Taurine	0.18	<.0001	0.25	<.0001
Cysteine sulfonic acid	0.96	0.6919	0.95	0.3879
Xylitol	0.97	0.2031	0.94	0.0245
1,6-Anhydroglucose	1.45	0.0010	1.93	<.0001
Arabitol	0.76	0.0666	0.93	0.0765
Ribitol	1.25	0.3123	0.96	0.4388
Aconitate	1.10	0.9812	0.92	0.0013
N-Acetyl-L-glutamate_1	3.32	<.0001	3.34	0.0003
Glutamine	0.60	<.0001	0.54	<.0001
4-Hydroxymandelate	0.55	<.0001	0.43	<.0001
Dihydroorotate	1.92	<.0001	1.73	<.0001
O-Phosphoethanolamine	0.35	<.0001	0.34	<.0001
Theanine_2	0.58	0.0020	0.63	0.0017
Glycyl-glycine_1	0.62	<.0001	0.71	<.0001
Citric acid + isocitric acid	0.70	<.0001	0.70	<.0001
Ornithine	0.87	0.0065	0.84	0.0002
Hypoxanthine	0.84	0.0027	0.93	0.081

Citrulline	0.63	<.0001	0.58	<.0001
1,5-Anhydro-D-glucitol	0.59	<.0001	0.72	0.001
Tagatose_2 (or psicose_2)	0.74	<.0001	0.59	<.0001
Fructose_1	1.07	0.9561	1.13	0.1124
2-Dehydro-D-gluconate_1	1.20	<.0001	1.28	<.0001
Glucose_1	1.89	<.0001	1.82	<.0001
Sebacic acid	1.46	0.0292	1.81	0.2580
Galactose_2	2.05	<.0001	1.00	0.0055
Mannitol	1.08	0.6498	2.89	0.0103
Lysine (4TMS)	0.96	0.0053	0.85	0.0008
Galactosamine_1	1.03	0.0369	0.93	0.0394
Histidine	0.37	<.0001	0.37	<.0001
Glucuronate_1	0.77	<.0001	0.69	<.0001
5-Keto-D-gluconate_2	0.86	0.7036	0.89	0.4673
Galacturonic acid_1	1.98	<.0001	2.10	<.0001
Tyrosine	0.82	<.0001	0.68	<.0001
Pantothenate	1.42	<.0001	1.32	<.0001
N-α-Acetyl-L-ornithine_2	2.05	<.0001	1.99	<.0001
Glucarate	0.66	<.0001	0.66	<.0001
S-Benzyl-L-cysteine_1	0.20	<.0001	0.23	<.0001
Xanthine	0.82	0.0031	0.80	0.0001
Palmitoleate	0.75	0.0003	0.44	<.0001
Inositol	0.81	0.0001	0.78	<.0001
Dopa	2.00	<.0001	1.90	<.0001
Uric acid	0.63	<.0001	0.59	<.0001
N-α-Acetyl-L-lysine_2	5.89	0.0493	5.89	0.8194
Heptadecanoate	0.58	0.0004	0.63	0.0525
Kynurenine	2.46	<.0001	2.01	<.0001
Cystathionine	0.71	0.5305	0.72	0.7829
Cystamine	2.12	<.0001	3.41	<.0001
Tryptophan	0.65	<.0001	0.58	<.0001
Elaidic acid	0.81	0.9352	0.55	0.0355
Homocysteine	2.03	<.0001	2.11	<.0001

Values are shown as fold induction (F.I.) values, which were obtained by comparing the peak intensity values of the UC patients with those of the HV. The peak intensity values for each quantified ion have been normalized to that of 2-isopropylmalic acid as an internal standard. P-values were calculated using the Wilcoxon rank sum test.

Supplemental Table 2. Comparison of the serum levels of all metabolites detected by GC/MS among UCa patients, UCr patients, and HV

	Training set						Validation set					
	F.I. UCr/HV	P-value UCr vs. HV	F.I. UCa/HV	P-value UCa vs. HV	F.I. UCa/UCr	P-value UCa vs. UCr	F.I. UCr/HV	P-value UCr vs. HV	F.I. UCa/HV	P-value UCa vs. HV	F.I. UCa/UCr	P-value UCa vs. UCr
Pyruvate+oxalacetic acid	0.97	0.4064	1.76	0.0028	1.81	0.0043	0.78	0.0004	1.28	0.2859	1.64	0.0136
Lactic acid	1.40	<.0001	2.00	<.0001	1.43	<.0001	1.37	<.0001	1.77	<.0001	1.29	0.0002
Glycolic acid	0.97	0.5011	0.90	0.001	0.93	0.0633	1.02	0.8103	0.78	0.0088	0.76	0.0212
Alanine (2TMS)	1.01	0.7086	0.99	0.111	0.98	0.4973	0.92	0.0201	0.93	0.1008	1.01	0.994
Hydroxybutyrate	1.04	0.3311	2.11	<.0001	2.03	<.0001	0.82	0.0242	1.26	0.0703	1.53	0.0028
Oxalate	0.93	0.0544	0.44	<.0001	0.48	<.0001	0.97	0.2332	0.57	<.0001	0.58	0.0002
Sarcosine	0.90	0.2211	0.55	<.0001	0.61	0.0011	0.70	0.0016	0.52	<.0001	0.74	0.0432
2-Aminoisobutyrate	0.85	0.0021	1.02	0.2417	1.19	0.0071	0.77	<.0001	0.96	0.7456	1.25	0.0039
3-Hydroxybutyrate	2.33	0.1578	6.61	0.0005	2.84	0.0008	0.81	0.0036	2.97	0.206	3.67	0.0092
2-Aminobutyric acid	0.77	<.0001	1.01	0.7173	1.32	0.0119	0.69	<.0001	0.78	0.0042	1.13	0.1723
Ketoisoleucine_1	0.90	0.0227	1.47	0.0001	1.63	0.0002	0.75	<.0001	1.16	0.1361	1.55	<.0001
Malonic acid	1.84	<.0001	2.42	<.0001	1.32	0.0013	1.58	<.0001	2.29	<.0001	1.45	0.0002
3-Hydroxyisovaleric acid	0.91	0.0885	2.10	0.0012	2.32	0.0059	0.74	0.6623	1.53	0.097	2.06	0.13
Valine (2TMS)	0.76	<.0001	0.71	<.0001	0.94	0.6599	0.74	<.0001	0.66	<.0001	0.90	0.1055
Urea	2.11	<.0001	2.87	<.0001	1.36	0.0013	2.13	<.0001	2.68	<.0001	1.26	0.0003
Oxamic acid	0.95	0.7262	0.71	0.6349	0.75	0.2729	0.57	0.3311	6.09	0.8251	10.72	0.5261
2-Aminoethanol	0.65	<.0001	0.89	0.0499	1.38	0.0002	0.63	<.0001	0.83	0.0075	1.32	0.0021
n-Caprylic acid	1.01	0.0139	0.75	<.0001	0.74	0.0204	0.70	<.0001	0.80	0.0004	1.14	0.1586
Phosphate	0.75	<.0001	0.76	<.0001	1.01	0.6278	0.69	<.0001	0.69	<.0001	1.00	0.7711
Glycerol	0.76	0.0002	4.12	<.0001	5.43	<.0001	0.64	<.0001	4.10	0.0249	6.42	0.0004
Ethylmalonic acid	1.70	<.0001	2.29	<.0001	1.35	0.0005	1.61	<.0001	2.07	<.0001	1.29	0.002
Isoleucine	0.84	0.0022	0.80	0.0065	0.95	0.4248	0.73	<.0001	0.67	<.0001	0.92	0.4423
Threonine (2TMS)	0.83	0.0015	0.93	0.3351	1.12	0.0348	0.74	<.0001	0.76	0.0004	1.02	0.6173

Proline	0.86	0.065	0.75	0.001	0.88	0.1226	0.85	0.0524	0.72	0.0004	0.85	0.022
Maleic acid	1.78	<.0001	2.32	<.0001	1.31	0.0018	1.87	<.0001	2.33	<.0001	1.25	0.0289
Glycine (3TMS)	0.90	0.002	1.09	0.1713	1.21	0.0092	0.94	0.1928	0.94	0.9288	1.00	0.5861
Succinic acid (or aldehyde)	0.82	0.0003	0.70	<.0001	0.86	0.0323	0.72	<.0001	0.70	<.0001	0.97	0.0933
Catechol	1.84	<.0001	1.98	<.0001	1.08	0.15	1.68	<.0001	1.76	<.0001	1.05	0.0388
Glyceric acid	0.94	0.2181	0.74	0.0007	0.79	0.0229	0.94	0.2363	0.72	<.0001	0.76	0.0047
Uracil	1.69	<.0001	2.28	<.0001	1.35	0.0035	1.39	<.0001	2.10	<.0001	1.51	<.0001
Fumaric acid	0.78	<.0001	0.49	<.0001	0.63	<.0001	0.63	<.0001	0.56	<.0001	0.89	0.1918
Serine (3TMS)	0.60	<.0001	0.62	<.0001	1.04	0.9584	0.67	<.0001	0.56	<.0001	0.84	0.0822
Nonanoic acid (C9)	0.51	<.0001	0.42	<.0001	0.82	0.0323	0.50	<.0001	0.45	<.0001	0.89	0.0962
4-Methylbenzoic acid	1.55	<.0001	1.58	<.0001	1.02	0.2075	1.27	0.0043	1.16	0.0001	0.92	0.2795
Glutaric acid	0.80	0.0252	0.93	0.6349	1.15	0.1458	0.78	0.0616	1.13	0.4324	1.44	0.0007
Maleamic acid	1.07	0.0247	1.33	0.0002	1.24	0.0047	0.95	0.151	1.03	0.0418	1.09	0.1377
Prolinamide	1.79	<.0001	2.44	<.0001	1.37	0.0007	1.55	<.0001	2.23	<.0001	1.43	<.0001
Homoserine	0.81	0.0006	0.94	0.1803	1.16	0.1121	0.82	0.0016	0.87	0.0633	1.06	0.1631
Malic acid	0.86	0.001	0.65	<.0001	0.76	0.0654	0.67	<.0001	0.69	<.0001	1.03	0.7371
Threitol	1.05	0.5845	0.72	0.0055	0.69	0.0065	1.03	1	0.95	0.6018	0.93	0.5261
meso-Erythritol	1.18	0.9969	0.78	0.0732	0.66	0.1631	2.23	0.2301	4.48	0.0437	2.01	0.4973
Adipic acid	0.99	0.4109	1.23	0.0033	1.25	0.013	0.99	0.0487	1.78	<.0001	1.79	0.0012
Acetylsalicylic acid	9.97	<.0001	1.80	<.0001	0.18	0.1543	0.49	0.0031	0.81	<.0001	1.66	0.0002
Aspartic acid	0.88	<.0001	0.75	<.0001	0.86	0.0011	0.85	<.0001	0.75	<.0001	0.89	0.0025
Methionine	0.74	<.0001	0.93	0.011	1.25	0.0796	0.67	<.0001	0.80	0.0019	1.19	0.1226
trans-4-Hydroxy-L-proline	0.67	<.0001	0.67	<.0001	1.00	0.1377	0.70	0.0001	0.45	<.0001	0.64	0.0009
4-Aminobutyric acid	1.77	0.0192	2.52	<.0001	1.43	0.0001	1.05	0.429	1.98	<.0001	1.88	<.0001
Pyrogallol	0.55	0.8164	0.40	0.5567	0.72	1	0.68	0.376	0.69	0.3495	1.00	0.8287
Creatinine	0.87	0.1444	0.70	<.0001	0.81	0.0534	0.83	0.0544	0.72	0.0008	0.88	0.314
Acetoacetic acid	0.24	<.0001	0.27	<.0001	1.11	0.119	0.25	<.0001	0.27	<.0001	1.09	0.0962
β-Glutamic acid	1.81	<.0001	2.51	<.0001	1.39	0.0002	1.79	<.0001	2.18	<.0001	1.22	0.0173

Glutamic acid	0.62	<.0001	1.01	0.1598	1.61	0.0188	0.65	<.0001	0.76	0.0155	1.18	0.2241
Phenylalanine	0.83	<.0001	1.00	0.0933	1.22	0.6278	0.79	<.0001	0.80	<.0001	1.02	0.9109
5-Aminovaleric acid	0.73	0.0117	1.07	0.5823	1.47	0.0026	0.59	0.0002	0.89	0.3212	1.52	0.0004
p-Hydroxybenzoic acid	0.92	0.0013	3.58	<.0001	3.90	0.0002	1.22	<.0001	2.64	<.0001	2.17	0.0022
Xylose_2	1.91	0.2793	2.40	<.0001	1.25	0.0085	1.58	0.7381	3.62	0.0373	2.29	0.2075
threo-β-Hydroxyaspartic acid	1.18	0.0133	1.34	<.0001	1.14	0.0962	1.10	0.076	1.79	<.0001	1.63	0.0016
Lyxose_2	0.95	0.1128	1.09	0.6894	1.15	0.2416	0.83	<.0001	1.19	0.8105	1.43	0.0034
Arabinose	0.87	0.0058	1.17	0.0356	1.35	0.0059	0.91	0.0247	1.21	0.1242	1.33	0.0101
Lauric acid	0.95	0.2066	0.85	0.0052	0.90	0.2075	0.75	<.0001	0.79	0.0001	1.06	0.7597
N-Acetyl-L-aspartic acid_2	1.56	<.0001	2.15	<.0001	1.38	0.0016	1.70	<.0001	2.22	<.0001	1.31	0.0008
Homocysteine_1	1.70	<.0001	2.20	<.0001	1.29	0.0031	1.44	<.0001	2.04	<.0001	1.41	0.0005
Ribose	0.95	0.6284	1.55	0.2533	1.62	0.4603	1.12	0.3593	1.85	0.0557	1.65	0.336
Ribulose	0.82	0.0584	0.95	0.5504	1.16	0.5457	0.83	0.0339	1.22	0.6484	1.46	0.2356
Asparagine	0.70	<.0001	0.46	<.0001	0.67	0.0002	0.66	<.0001	0.43	<.0001	0.65	<.0001
Taurine	0.19	<.0001	0.17	<.0001	0.94	0.2021	0.25	<.0001	0.26	<.0001	1.01	0.0676
Cysteine sulfonic acid	0.96	0.7086	0.97	0.796	1.02	0.6707	0.90	0.1849	1.03	0.9737	1.15	0.1024
Xylitol	1.06	0.376	0.84	0.0008	0.79	0.0016	0.95	0.1146	0.92	0.0324	0.97	0.9584
1,6-Anhydroglucose	1.65	0.0043	1.19	0.0122	0.72	0.8873	1.68	0.0003	2.25	0.0001	1.34	0.2729
Arabitol	1.03	0.249	0.40	<.0001	0.39	<.0001	1.00	0.9655	0.83	0.0018	0.83	0.0141
Ribitol	1.49	0.0389	0.94	0.5134	0.63	0.1458	1.07	0.9718	0.82	0.1803	0.77	0.1969
Aconitate	1.23	0.0976	0.92	0.0427	0.75	0.0037	0.98	0.0945	0.84	0.0002	0.86	0.0403
N-Acetyl-L-glutamate_1	1.93	0.0162	5.14	<.0001	2.66	0.0001	1.39	0.343	5.89	<.0001	4.25	<.0001
Glutamine	0.70	<.0001	0.48	<.0001	0.68	0.0014	0.62	<.0001	0.45	<.0001	0.73	0.0004
4-Hydroxymandelate	0.60	0.0013	0.47	0.0015	0.78	0.6491	0.42	<.0001	0.45	<.0001	1.07	0.0181
Dihydroorotate	1.70	<.0001	2.21	<.0001	1.30	0.0125	1.44	<.0001	2.12	<.0001	1.48	<.0001
O-Phosphoethanolamine	0.33	<.0001	0.37	<.0001	1.09	0.9109	0.32	<.0001	0.36	<.0001	1.12	0.3748
Theanine_2	0.54	0.0037	0.64	0.0381	1.19	0.0417	0.54	0.0012	0.75	0.0795	1.39	0.0025
Glycyl-glycine_1	0.69	<.0001	0.53	<.0001	0.77	0.0022	0.87	0.124	0.50	<.0001	0.57	<.0001

Citric acid + isocitric acid	0.77	0.0001	0.60	<.0001	0.78	0.0229	0.72	<.0001	0.68	0.0001	0.95	0.1676
Ornithine	0.69	<.0001	1.10	0.5567	1.60	<.0001	0.67	<.0001	1.07	0.9064	1.59	0.0017
Hypoxanthine	0.78	0.0008	0.91	0.1655	1.17	0.314	0.91	0.0813	0.97	0.2988	1.07	0.9109
Citrulline	0.78	0.0002	0.45	<.0001	0.58	<.0001	0.65	<.0001	0.49	<.0001	0.76	0.0007
1,5-Anhydro-D-glucitol	0.72	0.0002	0.41	<.0001	0.58	0.0092	0.84	0.0444	0.56	0.0005	0.66	0.0229
Tagatose_2 (or psicose_2)	0.76	<.0001	0.71	0.0002	0.93	0.1155	0.61	<.0001	0.56	<.0001	0.91	0.0311
Fructose_1	1.38	0.0232	0.68	0.0091	0.49	0.001	1.54	<.0001	0.58	0.0027	0.38	<.0001
2-Dehydro-D-gluconate_1	0.72	<.0001	1.84	<.0001	2.57	0.0023	0.66	<.0001	2.09	<.0001	3.19	0.0041
Glucose_1	1.80	<.0001	2.01	<.0001	1.11	0.1121	1.70	<.0001	1.99	<.0001	1.17	0.0009
Sebacic acid	0.62	0.0087	2.56	0.4435	4.12	0.2664	0.62	0.0205	3.36	0.4604	5.45	0.0011
Galactose_2	1.45	<.0001	2.83	0.0007	1.95	0.2129	0.96	0.0496	1.05	0.0088	1.09	0.8991
Mannitol	1.02	0.402	1.16	0.8545	1.15	0.4694	3.07	0.1673	2.66	0.0033	0.87	0.3285
Lysine (4TMS)	0.74	<.0001	1.23	0.3032	1.66	0.0002	0.68	<.0001	1.08	0.2306	1.59	0.0014
Galactosamine_1	1.01	0.5162	1.06	0.0031	1.05	0.0167	0.96	0.402	0.90	0.0073	0.94	0.0612
Histidine	0.54	<.0001	0.16	<.0001	0.31	<.0001	0.54	<.0001	0.16	<.0001	0.30	<.0001
Glucuronate_1	0.90	0.0001	0.61	<.0001	0.68	0.3435	0.68	<.0001	0.70	0.0003	1.03	0.8638
5-Keto-D-gluconate_2	0.79	0.1578	0.96	0.3166	1.21	0.0267	0.70	0.0058	1.13	0.0467	1.62	<.0001
Galacturonic acid_1	1.75	<.0001	2.29	<.0001	1.31	0.013	1.85	<.0001	2.43	<.0001	1.31	0.0023
Tyrosine	0.88	0.0024	0.73	<.0001	0.83	0.1088	0.69	<.0001	0.67	<.0001	0.97	0.3993
Pantothenate	1.32	<.0001	1.55	<.0001	1.17	0.0361	1.16	0.0346	1.53	<.0001	1.32	0.0011
N-α-Acetyl-L-ornithine_2	1.75	<.0001	2.43	<.0001	1.38	0.0002	1.81	<.0001	2.24	<.0001	1.24	0.0101
Glucarate	0.78	0.0002	0.50	<.0001	0.65	<.0001	0.72	<.0001	0.59	<.0001	0.81	0.0257
S-Benzyl-L-cysteine_1	0.10	<.0001	0.33	<.0001	3.15	0.5759	0.09	<.0001	0.41	0.0002	4.33	0.0654
Xanthine	0.84	0.0169	0.79	0.0147	0.94	0.6173	0.84	0.0042	0.76	0.0005	0.91	0.3512
Palmitoleate	0.65	0.0005	0.87	0.02	1.33	0.1458	0.43	<.0001	0.44	<.0001	1.02	0.5068
Inositol	0.79	<.0001	0.82	0.0488	1.03	0.5657	0.70	<.0001	0.88	0.0125	1.25	0.0962
Dopa	1.75	<.0001	2.32	<.0001	1.33	0.002	1.70	<.0001	2.16	<.0001	1.27	0.0119
Uric acid	0.72	<.0001	0.52	<.0001	0.73	0.0311	0.64	<.0001	0.53	<.0001	0.83	0.1586

N-α-Acetyl-L-lysine_2	8.40	0.1849	2.60	0.0533	0.31	0.9465	8.27	0.6738	2.78	0.3592	0.34	0.3512
Heptadecanoate	0.57	0.0033	0.59	0.0052	1.02	0.336	0.60	0.0505	0.67	0.2572	1.13	0.011
Kynurenine	1.87	<.0001	3.24	<.0001	1.74	<.0001	1.51	<.0001	2.68	<.0001	1.78	<.0001
Cystathionine	0.60	0.978	0.86	0.2775	1.44	0.0278	0.56	0.1279	0.93	0.0205	1.66	<.0001
Cystamine	1.26	0.0012	3.24	<.0001	2.58	0.0167	1.16	0.0102	6.36	<.0001	5.47	0.0001
Tryptophan	0.71	<.0001	0.57	<.0001	0.80	0.0047	0.69	0.0003	0.42	<.0001	0.61	0.0003
Elaidic acid	0.80	0.9092	0.82	0.9962	1.03	0.0612	0.49	0.0308	0.64	0.238	1.31	0.0035
Homocysteine	1.79	<.0001	2.34	<.0001	1.31	0.013	1.86	<.0001	2.43	<.0001	1.31	0.0023

Disease activity was assessed with the Rachmilewitz index; i.e., a clinical activity index (CAI). Active disease was defined as a CAI of ≥ 6 for UC (UC in the active phase: UCa). Remission was defined as a CAI of < 6 for UC (UC in remission: UCr).Values are represented as fold induction (F.I.) values of peak intensity values in the comparisons among the UCr, UCa, and HV. The peak intensity values for each quantified ion were normalized to that of 2-isopropylmalic acid as an internal standard. P-values were calculated using the Wilcoxon rank sum test.

Supplemental Table 3. Spearman's rank correlation coefficients (r_s) for the relationships between the CAI and the 24 metabolites that exhibited significant alterations according to disease activity or the presence/absence of disease

	Training set		**Validation set**	
	r_s	**P-value**	**r_s**	**P-value**
Aspartic acid	-0.2993	0.0202	-0.3817	0.0026
Asparagine	-0.3969	0.0017	-0.641	<.0001
Glutamine	-0.3329	0.0094	-0.5559	<.0001
Citrulline	-0.5551	<.0001	-0.4361	0.0005
1,5-Anhydro-D-glucitol	-0.3523	0.0058	-0.3474	0.0065
Histidine	-0.5249	<.0001	-0.7436	<.0001
Glucarate	-0.4733	0.0001	-0.266	0.04
Tryptophan	-0.4472	0.0003	-0.5568	<.0001
Lactic acid	0.5978	<.0001	0.5281	<.0001
Malonic acid	0.3538	0.0056	0.5087	<.0001
Urea	0.3201	0.0127	0.5125	<.0001
Ethylmalonic acid	0.3618	0.0045	0.5348	<.0001
Maleic acid	0.3469	0.0066	0.278	0.0315
Uracil	0.3214	0.0123	0.6098	<.0001
Prolinamide	0.3831	0.0025	0.6258	<.0001
β-Glutamic acid	0.4199	0.0008	0.4164	0.0009
N-Acetyl-L-aspartic acid_2	0.3143	0.0145	0.5373	<.0001
Dihydroorotate	0.304	0.0182	0.6999	<.0001
Galacturonic acid_1	0.2793	0.0307	0.3949	0.0018
Pantothenate	0.2344	NS	0.4724	0.0001
N-α-Acetyl-L-ornithine_2	0.422	0.0008	0.4213	0.0008
Dopa	0.3705	0.0036	0.3323	0.0095
Kynurenine	0.4229	0.0008	0.583	<.0001
Homocysteine	0.2793	0.0307	0.3949	0.0018

NS: not significant. Spearman's rank correlation coefficients (r_s) were calculated for the relationships between the CAI and the 24 metabolites that exhibited significant alterations according to disease activity or the presence/absence of disease.

Supplemental Table 4. Twenty-two metabolites selected from a volcano plot of UC versus HV

	log2(F.I.) (X axis)	**-log10(P-value) (Y axis)**
Malonic acid	1.062041005	>12
Urea	1.284873133	>12
Maleic acid	1.010995484	>12
Nonanoic acid (C9)	-1.082985088	>12
Prolinamide	1.050293668	>12
Acetylsalicylic acid	2.684350319	>12
4-Aminobutyric acid	1.067392629	5.287906147
Acetoacetic acid	-1.997662002	7.070581074
β-Glutamic acid	1.079983692	>12
p-Hydroxybenzoic acid	1.052647743	6.382789905
Xylose_2	1.086430825	2.457102093
Taurine	-2.466213592	>12
N-Acetyl-L-glutamate_1	1.732777541	6.240105626
O-Phosphoethanolamine	-1.524368073	8.585026652
Galactose_2	1.034690833	5.462999913
Histidine	-1.415784243	>12
N-α-Acetyl-L-ornithine_2	1.033225873	>12
S-Benzyl-L-cysteine_1	-2.307528402	7.913640169
N-α-Acetyl-L-lysine_2	2.557824764	1.306773807
Kynurenine	1.300074675	>12
Cystamine	1.082590369	6.275642196
Homocysteine	1.021748676	>12

The X and Y axes of the Volcano plot showed Log2 fold induction (F.I.) values and –log10 p-values, respectively. The above 22 metabolites met the following criteria: fold induction > 2.0; i.e., |log2 of F.I.| > 1, and p-value > 0.05; i.e., –log10 of p value > 1.301029996. P-values were calculated using Wilcoxon rank sum test.

Supplemental Table 5. VIF of the variables in diagnostic model A

Variable	VIF
Taurine	1.77
S-Benzyl-L-cysteine	1.12
N-Acetyl-L-glutamine	1.32
Maleic acid	1.97

The 4 metabolites; i.e., taurine, S-benzyl-L-cysteine, maleic acid, and N-acetyl-L-glutamine that made up diagnostic model A, which was able to discriminate the UC patients from HV as follows:

p=1/[1+e-{0.83-116.3(Taurine)-335.3(s-Benzyl-L-cysteine)+10801.1(Maleic acid)+2220.8(n-Acetyl-L-glutamine)}].

Supplemental Table 6. Nine metabolites selected from a volcano plot of UC versus CD

	log2(fold change)	log10(p-value)
Oxalate	-1.659772104	9.000000000
3-Hydroxybutyrate	-3.014294754	3.317757241
Acetylsalicylic acid	-1.448047145	3.248290357
p-Hydroxybenzoic acid	1.294445194	7.183758700
Ribulose	1.338250558	7.552841969
1,6-Anhydroglucose	2.617149363	3.900771841
N-Acetyl-L-glutamate_1	1.229194931	8.431798276
2-Dehydro-D-gluconate_1	1.018103046	6.181906131
Histidine	-1.910157875	4.937997461

The X and Y axes of the Volcano plot showed Log2 fold induction (F.I.) values and –log10 p-values, respectively. The above 9 metabolites met the following criteria: fold induction > 2.0; i.e., |log2 of F.I.| > 1, and p-value > 0.05; i.e., –log10 of p value > 1.301029996. P-values were calculated using the Wilcoxon rank sum test.

Supplemental Table 7. VIF of the variables in diagnostic model B

Variable	VIF
Oxalate	1.21
3-Hydroxybutyrate	1.06
Ribulose	1.37
1,6-Anhydroglucose	1.15

The 4 metabolites; i.e., oxalate, 3-hydroxy-butyrate, ribulose and 1,6-anhydroglucose, that made up diagnostic model B, which was able to discriminate between the UC patients and CD patients as follows:

p=1/[1+e-{1.36-2281.82(Oxalate)-35.00(3-Hydroxybutyrate)-570.81(Ribulose)+279.26(1,6-Anhydroglucose)}].

Supplemental Table 8. Twelve metabolites selected from a volcano plot of UCr versus UCa

	log2(F.I.)	**log10(P-value)**
Hydroxybutyrate	1.019579642	5.694326254
Oxalate	-1.058554613	6.019451261
3-Hydroxybutyrate	1.507059903	3.114396706
3-Hydroxyisovaleric acid	1.211874346	2.227839107
Glycerol	2.440845122	6.873219423
p-Hydroxybenzoic acid	1.963311328	3.781411984
Arabitol	-1.35185713	4.952047928
N-Acetyl-L-glutamate_1	1.409605033	3.991857356
Fructose_1	-1.02785869	2.998161403
2-Dehydro-D-gluconate_1	1.359687026	2.641410567
Histidine	-1.701649185	5.378532901
Cystamine	1.367684057	1.778504581

The X and Y axes of the Volcano plot showed Log2 fold induction (F.I.) values and –log10 p-values, respectively. The above 12 metabolites met the following criteria: fold induction > 2.0; i.e., |log2 of F.I.| > 1, and p-value > 0.05; i.e., –log10 of p value > 1.301029996. P-values were calculated using the Wilcoxon rank sum test.

Supplemental Table 9. VIF of the variables in assessment model A

Variable	**VIF**
Histidine	1.01
p-Hydroxybenzoic acid	1.01

The 2 metabolites; i.e., histidine and p-hydroxybenzoic acid, that made up assessment model A, which was found to be useful for prospectively monitoring UC as follows: p=1/[1+e-{-1.64+380.17(histidine)-2559.38(p-hydroxybenzoic acid

Supplemental Table 10. Additional information of the patients in the training and validation sets

	Training set		Validation set		Training & validation set
	UC	**HV**	**UC**	**HV**	**CD**
Body weight (kg) [a)]	54.1±9.1/36.8-76.0	58.2±11.0/40.6-84.9 [b)]	56.8±11.0/32.9-90.0	57.4±10.7/41.9-86.5 [b)]	57.2±9.8/36.0-73.0
BMI [a)]	20.6±2.8/14.9-27.6	21.5±2.6/17.3-28.3	21.3±3.3/14.7-34.7	21.9±2.7/17.3-27.8	20.7±3.0/14.1-26.7
Daily medication					
Salicylates (N)	56	0	50	0	29
Pentasa (N)	30	-	30	-	29
dose / dose range (mg/day) [a)]	1583±1709/0-4000	-	1544±1677/0-4000	-	2130±1311/0-3000
Asacol (N)	22	-	16	-	0
dose / dose range (mg/day) [a)]	1260±1688/0-3600	-	980±1585/0-3600	-	-
SASP (N)	4	-	4	-	0
dose / dose range (mg/day) [a)]	183±747/0-4000	-	233±958/0-4500	-	-
Prednisolone (N)	14	0	20	0	2
Total dose for 1 year (mg) [a)]	749±1197/0-5200	-	568±1004/0-5087	-	62.7±279.4
Total dose for 6 months (mg) [a)]	436±828/0-3520	-	345±654/0-3057	-	27.6±136.6
Total dose for 3 months (mg) [a)]	234±612/0-3520	-	183±391/0-1960	-	12.9±68.2
Total dose for 1 month (mg) [a)]	135±431/0-2960	-	72.1±195/0-1150	-	4.3±22.7
Current dose (mg/day) [a)]	5.8±15.0/0-80	-	3.3±11.7/0-80	-	0.2±0.8
6-mercaptopurine (N)	1	0	2	0	2
dose / dose range (mg/day) [a)]	0.5±1.5/0-30	-	1.3±7.5	-	1.7±7.5/0-40
dosing period (month) [a)]	1.7±12.8	-	0.5±3.4	-	2.4±15.3
Azathioprine (N)	13	0	12	0	17
dose / dose range (mg/day) [a)]	10±21.7/0-100	-	10.8±20.8	-	18.6±25.4
dosing period (mg) [a)]	4.3±10.2	-	6.4±12.4	-	14.5±20.6
Tacrolimus (N)	3	0	3	0	0

dose / dose range (mg/day) [a)]	0.4±1.5	-	0.5±2.3	-	0
dosing period (month) [a)]	0.8±3.5	-	0.5±3.2	-	0
Anti-TNF-α agents (N)	4	0	5	0	25
Infliximab (N)	4	-	5	-	19
dose / dose range (mg/body) [a)]	18.7±72.8/0-380	-	22.4±75.6	-	165.5±188.2/0-400
dosing period (month) [a)]	0.3±1.3	-	0.8±3.8	-	20.8±24.3
adalimumab (N)	0	0	0	0	6
dose / dose range (mg/body) [a)]	-	-	-	-	6.15±14.6/0-40
dosing period (month) [a)]	-	-	-	-	1.1±3.6
Enteral nutrition (N)	0	0	0	0	21
dose / dose range (kcal/day) [a)]	-	-	-	-	415±535/0-2100
dosing period (month) [a)]	-	-	-	-	16.9±37.3
Food restriction therapy (N)	7	0	4	0	1
period (day) [a)]	1.7±5.7	-	0.7±3.1	-	1.1±6.6
Blood chemical findings					
Total protein (g/dl) [a)]	6.9±0.6/5.3-7.9	7.2±0.4/6.5-8.3 [c)]	6.9±0.5/4.7-8.0	7.2±0.4/6.4-8.4 [d)]	7.3±1.2/8.6-5.4
Albumin (g/dl) [a)]	4.0±0.7/1.9-5.0	4.6±0.2/4.2-5.0 [c)]	4.1±0.6/1.7-4.8	4.6±0.2/4.1-5.1 [d)]	3.9±0.7/1.9-4.7
Total cholesterol (mg/dl) [a)]	188±38.9/109-274	200±34.5/123-262	183±37.2/74-269	200±25.5/140-259	152.8±38.2/55-255

BMI: body mass index, SASP: sulfasalazine, P: proctitis, LC: left-sided colitis, PC: pan-colitis, S: small bowel, C: colon, Both: small bowel & colon, CAI: Rachmilewitz index (a clinical activity index), CDAI: Crohn's disease activity index. [a)]: Body weight, BMI, dose and dosing period of each medication including enteral nutrition, period of food restriction test and data of biochemical findings are shown as the mean ± standard deviation together with the range. We could not obtain all data on the clinical record and reports of medical check-ups from all the subjects, and deficient data existed. Therefore, this table is composed of data obtained in this study. **[b)]**: The bodyweight data of HV in training and validation set was shown using 57 HV samples except 3 samples. **[c)]**: Serum total protein and albumin levels of HV were shown using 44 samples except 16 samples. **[d)]**: Serum total protein and albumin levels of HV were shown using 42 samples except 18 samples. As far prednisolone, previous medication status of several samples was unclear because of their long disease durations and complicated treatment histories in several hospitals. Therefore, only reliable information we could check in our medical records was shown in this table; current dose and total dose within 1, 3, 6 months and 1 year. In our study, salicylates were medicated over entire disease duration in almost all cases except a few allergic cases, so their durations are not shown.

Supplemental Table 11. Spearman's rank correlation coefficients (r_s) for the relationships between the various factor; body weight, body mass index and blood chemical findings, and all metabolites detected by GC-MS in the sera of HV.

	Bw		**BMI**		**TP**		**Alb**		**T-chol**	
	r_s	**P-value**	**r_s**	**P-value**	**r_s**	**P-value**	**r_s**	**P-value**	**r_s**	**P-value**
Pyruvate+oxalacetic acid	0.1529	0.1044	0.2098	0.0215	0.1835	0.0888	0.2944	0.0056	0.0682	0.4712
Lactic acid	0.0813	0.3901	0.1171	0.2027	0.0426	0.6950	0.0009	0.9932	-0.0399	0.6735
Glycolic acid	0.3574	<.0001	0.3425	0.0001	-0.2253	0.0359	-0.1460	0.1771	-0.0064	0.9464
Alanine (2TMS)	0.2883	0.0019	0.2744	0.0024	0.1894	0.0789	0.2226	0.0382	0.1733	0.0652
Hydroxybutyrate	0.1306	0.1661	0.2617	0.0039	0.3677	0.0005	0.2562	0.0166	0.0099	0.9166
Oxalate	-0.0094	0.9209	-0.0294	0.7496	0.0644	0.5532	0.0726	0.5041	-0.0541	0.5673
Sarcosine	0.0594	0.5300	0.0467	0.6126	0.1496	0.1667	0.1392	0.1984	0.2125	0.0232
2-Aminoisobutyrate	0.2116	0.0238	0.1411	0.1242	0.0115	0.9161	0.0858	0.4296	0.0161	0.8654
3-Hydroxybutyrate	-0.2487	0.0076	-0.1123	0.2222	0.2165	0.0440	-0.0216	0.8429	-0.1164	0.2176
2-Aminobutyric acid	0.1430	0.1290	0.1209	0.1883	0.0389	0.7203	0.1186	0.2741	0.1696	0.0713
Ketoisoleucine_1	0.4835	<.0001	0.4505	<.0001	0.2403	0.0250	0.4167	<.0001	0.0620	0.5120
Malonic acid	0.1312	0.1640	0.0830	0.3675	0.0429	0.6932	0.0690	0.5254	-0.0667	0.4808
3-Hydroxyisovaleric acid	0.2408	0.0099	0.1738	0.0576	0.1625	0.1326	0.2394	0.0256	-0.0083	0.9299
Valine (2TMS)	0.5273	<.0001	0.4224	<.0001	0.0644	0.5532	0.2941	0.0057	0.0898	0.3423
Urea	0.0398	0.6745	0.0732	0.4266	-0.0053	0.9615	-0.0019	0.9863	-0.1038	0.2719
Oxamic acid	-0.2477	0.0079	-0.2552	0.0049	0.0294	0.7872	-0.1198	0.2692	0.0523	0.5808
2-Aminoethanol	0.3385	0.0002	0.3466	0.0001	0.0547	0.6150	0.0543	0.6176	0.2571	0.0058
n-Caprylic acid	-0.0643	0.4965	0.0026	0.9772	0.0416	0.7019	0.1063	0.3270	0.1520	0.1064
Phosphate	-0.2218	0.0177	-0.0589	0.5230	0.0499	0.6463	0.0411	0.7054	0.0058	0.9510
Glycerol	-0.3103	0.0008	-0.1218	0.1850	0.1871	0.0826	-0.0016	0.9879	-0.0847	0.3702
Ethylmalonic acid	0.1022	0.2792	0.1444	0.1157	0.0505	0.6423	-0.0090	0.9342	-0.1721	0.0671
Isoleucine	0.5053	<.0001	0.3963	<.0001	0.0647	0.5515	0.2640	0.0135	0.0718	0.4480

Threonine (2TMS)	0.4989	<.0001	0.4032	<.0001	0.0494	0.6497	0.2538	0.0177	0.0826	0.3821
Proline	0.2993	0.0012	0.2704	0.0028	-0.0751	0.4891	0.1306	0.2280	0.0263	0.7812
Maleic acid	0.1099	0.2445	0.1510	0.0997	-0.0346	0.7505	-0.0680	0.5316	-0.1403	0.1366
Glycine (3TMS)	0.0209	0.8255	0.0377	0.6824	-0.1703	0.1148	-0.0864	0.4259	0.1001	0.2893
Succinic acid (or aldehyde)	-0.1412	0.1339	0.0137	0.8820	0.1193	0.2712	0.1203	0.2670	0.1605	0.0880
Catechol	0.0340	0.7199	0.0364	0.6932	0.0446	0.6820	0.1665	0.1233	-0.0114	0.9043
Glyceric acid	0.1863	0.0472	0.1504	0.1010	-0.2198	0.0408	-0.0238	0.8269	0.1102	0.2432
Uracil	0.1824	0.0521	0.1864	0.0415	-0.0449	0.6795	-0.0076	0.9442	-0.2215	0.0179
Fumaric acid	-0.0795	0.4005	0.0326	0.7235	0.2806	0.0085	0.2425	0.0236	0.1549	0.0999
Serine (3TMS)	-0.0958	0.3106	-0.1472	0.1085	0.0125	0.9083	0.2184	0.0421	0.1538	0.1023
Nonanoic acid (C9)	0.2934	0.0015	0.2216	0.0150	0.0925	0.3943	0.1814	0.0926	0.2413	0.0097
4-Methylbenzoic acid	0.1274	0.1769	0.1571	0.0867	0.0096	0.9296	0.0693	0.5238	0.0152	0.8722
Glutaric acid	0.2369	0.0111	0.2469	0.0066	0.1215	0.2622	0.0659	0.5443	0.2058	0.0281
Maleamic acid	0.1365	0.1474	0.1272	0.1662	-0.1737	0.1076	-0.0581	0.5930	0.0926	0.3273
Prolinamide	0.0074	0.9380	0.0107	0.9073	-0.1017	0.3487	-0.1705	0.1143	-0.1242	0.1882
Homoserine	0.0907	0.3372	0.0564	0.5405	0.0510	0.6392	0.1726	0.1100	0.0980	0.2994
Malic acid	0.0235	0.8039	0.0794	0.3888	0.2597	0.0151	0.2766	0.0095	0.0732	0.4390
Threitol	0.1617	0.0856	0.1373	0.1348	0.0098	0.9286	0.1307	0.2275	0.1300	0.1679
meso-Erythritol	0.2151	0.0215	0.1789	0.0506	-0.0213	0.8445	0.1444	0.1821	0.1758	0.0613
Adipic acid	-0.1673	0.0751	-0.1182	0.1985	0.0039	0.9716	0.1046	0.3351	-0.1062	0.2605
Acetylsalicylic acid	0.0752	0.4266	0.1385	0.1313	0.0642	0.5545	0.1467	0.1753	-0.0620	0.5125
Aspartic acid	0.0294	0.7565	0.0553	0.5483	-0.0130	0.9051	0.1017	0.3484	0.1070	0.2571
Methionine	0.3898	<.0001	0.2680	0.0031	-0.0414	0.7036	0.1330	0.2193	0.0845	0.3713
trans-4-Hydroxy-L-proline	0.2091	0.0256	0.1601	0.0807	-0.1403	0.1949	-0.0040	0.9705	-0.0567	0.5488
4-Aminobutyric acid	0.1687	0.0728	0.1832	0.0452	-0.0221	0.8389	0.1945	0.0710	0.2380	0.0108
Pyrogallol	-0.0399	0.6732	-0.0234	0.8001	-0.0478	0.6601	-0.0775	0.4754	0.0689	0.4661

Creatinine	0.3056	0.0009	0.2833	0.0017	0.0882	0.4165	0.1374	0.2045	0.1472	0.1180
Acetoacetic acid	0.3566	<.0001	0.2642	0.0036	0.0348	0.7491	0.2543	0.0175	0.1535	0.1030
β-Glutamic acid	0.0925	0.3276	0.1335	0.1461	-0.0365	0.7369	-0.1098	0.3113	-0.2108	0.0244
Glutamic acid	0.3135	0.0007	0.3339	0.0002	0.2406	0.0248	0.3015	0.0045	0.0928	0.3260
Phenylalanine	0.3131	0.0007	0.3664	<.0001	0.1505	0.1642	0.1947	0.0707	0.1900	0.0428
5-Aminovaleric acid	0.1227	0.1933	0.1174	0.2014	-0.1217	0.2616	0.0138	0.8992	0.1453	0.1230
p-Hydroxybenzoic acid	-0.2116	0.0238	-0.1121	0.2229	0.0047	0.9658	-0.0871	0.4227	-0.1144	0.2256
Xylose_2	0.1074	0.2553	0.1086	0.2376	0.0041	0.9698	-0.0673	0.5355	-0.1308	0.1655
threo-β-Hydroxyaspartic acid	0.2234	0.0169	0.1717	0.0607	-0.0486	0.6545	0.0953	0.3799	0.2048	0.0289
Lyxose_2	0.0936	0.3220	0.1476	0.1076	0.0137	0.8998	0.0484	0.6565	0.2608	0.0051
Arabinose	-0.0231	0.8071	-0.0341	0.7117	0.1095	0.3127	0.1539	0.1547	0.0464	0.6237
Lauric acid	-0.4411	<.0001	-0.3193	0.0004	0.0944	0.3846	0.0105	0.9228	-0.0921	0.3296
N-Acetyl-L-aspartic acid_2	-0.0688	0.4670	-0.0353	0.7022	-0.0132	0.9033	-0.1467	0.1750	-0.1359	0.1492
Homocysteine_1	0.2085	0.0260	0.1615	0.0781	-0.0611	0.5738	0.1149	0.2894	-0.0237	0.8022
Ribose	0.2370	0.0111	0.2132	0.0194	0.1739	0.1073	0.1477	0.1723	0.0981	0.2990
Ribulose	0.1508	0.1092	0.1617	0.0778	0.1354	0.2110	0.1005	0.3544	0.1495	0.1124
Asparagine	0.2222	0.0175	0.1211	0.1877	-0.0623	0.5664	0.1511	0.1625	0.0690	0.4655
Taurine	-0.0562	0.5524	0.0400	0.6643	0.2287	0.0331	0.1871	0.0827	0.1687	0.0727
Cysteine sulfonic acid	0.1252	0.1845	0.1240	0.1773	-0.0236	0.8284	0.1683	0.1192	0.0285	0.7632
Xylitol	0.3760	<.0001	0.4194	<.0001	0.1171	0.2802	0.2069	0.0545	0.0049	0.9584
1,6-Anhydroglucose	-0.0665	0.4819	-0.0524	0.5695	-0.1354	0.2110	0.0182	0.8669	0.1007	0.2862
Arabitol	0.2959	0.0014	0.1874	0.0404	0.0483	0.6566	0.1616	0.1349	0.1057	0.2631
Ribitol	0.3542	0.0001	0.2598	0.0042	0.1178	0.2770	0.2312	0.0312	-0.0879	0.3522
Aconitate	0.0182	0.8473	0.0805	0.3823	0.1241	0.2521	0.0738	0.4972	0.2615	0.0050
N-Acetyl-L-glutamate_1	0.2158	0.0211	0.2061	0.0239	-0.0613	0.5726	0.1973	0.0670	-0.0375	0.6922
Glutamine	0.0944	0.3180	0.0044	0.9623	-0.0447	0.6809	0.1476	0.1726	0.2252	0.0160

4-Hydroxymandelate	-0.0888	0.3475	-0.1180	0.1992	-0.1983	0.0656	-0.0930	0.3917	0.0545	0.5645
Dihydroorotate	0.0966	0.3065	0.0769	0.4041	-0.1245	0.2507	-0.1173	0.2791	-0.2348	0.0119
O-Phosphoethanolamine	-0.1684	0.0733	-0.0932	0.3111	0.0630	0.5620	0.1138	0.2939	-0.0530	0.5754
Theanine_2	0.3119	0.0007	0.2807	0.0019	0.0985	0.3641	0.1257	0.2461	0.1676	0.0747
Glycyl-glycine_1	0.0856	0.3650	0.1422	0.1212	0.0935	0.3889	0.0203	0.8517	0.0960	0.3094
Citric acid + isocitric acid	-0.2774	0.0028	-0.2149	0.0184	0.1832	0.0894	0.0308	0.7772	0.1282	0.1741
Ornithine	0.1904	0.0425	0.1693	0.0645	0.0499	0.6461	0.2330	0.0299	0.1411	0.1343
Hypoxanthine	0.1888	0.0442	0.2331	0.0104	0.1432	0.1858	0.1487	0.1693	0.0809	0.3923
Citrulline	0.1905	0.0423	0.1041	0.2576	0.0864	0.4260	0.1303	0.2290	0.1950	0.0376
1,5-Anhydro-D-glucitol	0.3449	0.0002	0.3031	0.0008	0.0525	0.6291	0.0729	0.5024	0.0636	0.5014
Tagatose_2 (or psicose_2)	-0.1292	0.1707	-0.0651	0.4802	0.1295	0.2318	0.0670	0.5376	0.0385	0.6845
Fructose_1	0.1712	0.0685	0.0821	0.3726	-0.2776	0.0092	0.0517	0.6344	0.0118	0.9009
2-Dehydro-D-gluconate_1	0.2035	0.0299	0.2168	0.0174	-0.0195	0.8576	0.0719	0.5078	0.1494	0.1126
Glucose_1	0.0842	0.3733	0.1141	0.2145	0.0406	0.7090	-0.0041	0.9698	-0.0390	0.6804
Sebacic acid	0.2484	0.0077	0.2449	0.0070	-0.0800	0.4616	0.0733	0.5001	-0.0177	0.8515
Galactose_2	0.0891	0.3456	0.0544	0.5553	-0.0262	0.8095	0.0074	0.9460	-0.1549	0.0998
Mannitol	0.1302	0.1675	0.0636	0.4904	-0.0847	0.4354	0.0802	0.4604	0.0801	0.3970
Lysine (4TMS)	0.4870	<.0001	0.3765	<.0001	0.0424	0.6965	0.2893	0.0066	0.0903	0.3392
Galactosamine_1	0.1606	0.0878	0.1384	0.1316	0.0206	0.8500	0.2237	0.0372	0.0040	0.9660
Histidine	0.2970	0.0013	0.2140	0.0189	-0.0458	0.6736	0.1519	0.1603	0.1321	0.1612
Glucuronate_1	0.3183	0.0006	0.2963	0.0010	0.2065	0.0550	0.3325	0.0017	0.3121	0.0007
5-Keto-D-gluconate_2	-0.3920	<.0001	-0.3805	<.0001	-0.0181	0.8681	-0.1941	0.0716	-0.0780	0.4095
Galacturonic acid_1	-0.0127	0.8932	-0.0102	0.9118	-0.0007	0.9951	0.0871	0.4225	0.1245	0.1869
Tyrosine	0.4218	<.0001	0.4288	<.0001	0.0228	0.8339	0.0916	0.3990	0.1038	0.2716
Pantothenate	0.1377	0.1439	0.1460	0.1117	-0.0143	0.8956	0.0885	0.4152	0.1309	0.1649
N-α-Acetyl-L-ornithine_2	0.0903	0.3393	0.0661	0.4733	0.0469	0.6665	-0.0323	0.7667	-0.0308	0.7451

Glucarate	0.1039	0.2715	0.0840	0.3618	-0.0697	0.5211	0.1086	0.3168	0.0828	0.3813
S-Benzyl-L-cysteine_1	0.3117	0.0007	0.2527	0.0054	0.1224	0.2586	0.1754	0.1041	0.1902	0.0427
Xanthine	0.2643	0.0045	0.3420	0.0001	0.2556	0.0169	0.2038	0.0583	0.1389	0.1405
Palmitoleate	-0.2339	0.0123	-0.1222	0.1835	0.2326	0.0302	0.0951	0.3808	0.0098	0.9178
Inositol	0.0995	0.2921	0.1203	0.1907	0.1008	0.3530	0.1440	0.1832	0.1904	0.0424
Dopa	0.1540	0.1019	0.1928	0.0349	0.0200	0.8540	0.1110	0.3062	0.0270	0.7756
Uric acid	0.5227	<.0001	0.4896	<.0001	0.0448	0.6802	0.3149	0.0030	0.1218	0.1967
N-α-Acetyl-L-lysine_2	0.2358	0.0116	0.2757	0.0023	0.1934	0.0727	0.2399	0.0252	0.1172	0.2144
Heptadecanoate	0.0509	0.5905	0.0567	0.5384	-0.0437	0.6876	0.1166	0.2821	0.0840	0.3740
Kynurenine	0.2216	0.0178	0.2131	0.0194	-0.0153	0.8881	0.1443	0.1825	0.0115	0.9034
Cystathionine	0.2413	0.0097	0.2521	0.0055	0.0384	0.7237	0.0834	0.4427	0.1236	0.1900
Cystamine	0.3516	0.0001	0.4018	<.0001	-0.0187	0.8636	0.0519	0.6330	0.0865	0.3602
Tryptophan	0.2922	0.0016	0.2001	0.0284	-0.0285	0.7931	0.2119	0.0488	0.2501	0.0073
Elaidic acid	-0.2076	0.0266	-0.0702	0.4464	0.2392	0.0256	0.0699	0.5201	0.0393	0.6780
Homocysteine	0.0587	0.5347	0.0333	0.7180	0.0228	0.8338	0.1049	0.3338	0.0816	0.3880

Spearman's rank correlation coefficients (r_s) were calculated for the relationships between the various factors; i.e., body weight (Bw, kg) and body mass index (BMI), or blood chemical findings; i.e., the serum levels of total protein (TP, g/dl), albumin (Alb, g/dl) and total cholesterol (T-chol, mg/dl), and the all metabolites detected by GC-MS in HV. The data of Bw, TP and Alb could not be obtained from reports of medical check-ups of all the subjects; Bw was not measured in 3 of 120 samples. The serum levels of TP and Alb were not measured in 34 of 120 samples. Therefore, this table is composed of only data obtained in this study.

Supplemental Table 12. Spearman's rank correlation coefficients (r_s) for the relationships between medications; i.e., salicylates and azathioprine, and all metabolites detected by GC-MS in the sera of UCr.

	salicylates						azathioprine	
	Pentasa		Asacol		SASP			
	r_s	P-value	r_s	P-value	r_s	P-value	r_s	P-value
Pyruvate+oxalacetic acid	-0.0383	0.8526	0.4364	0.2797	0.2554	0.5415	0.0976	0.5335
Lactic acid	0.1822	0.3731	0.5455	0.1619	0.7407	0.0356	0.2874	0.0617
Glycolic acid	0.3629	0.0684	-0.4364	0.2797	-0.3193	0.4408	0.1972	0.2050
Alanine (2TMS)	-0.0515	0.8025	0.1091	0.7970	-0.1277	0.7631	0.0371	0.8131
Hydroxybutyrate	0.0115	0.9556	-0.3273	0.4287	-0.0383	0.9282	-0.1451	0.3531
Oxalate	-0.0522	0.7999	-0.2182	0.6036	-0.0639	0.8806	-0.1239	0.4284
Sarcosine	-0.0341	0.8685	0.1091	0.7970	-0.1660	0.6944	-0.1223	0.4346
2-Aminoisobutyrate	-0.3107	0.1224	-0.6547	0.0781	-0.1149	0.7864	-0.2028	0.1920
3-Hydroxybutyrate	0.0164	0.9367	-0.2182	0.6036	0.3576	0.3845	-0.2074	0.1821
2-Aminobutyric acid	0.0338	0.8698	-0.3273	0.4287	-0.3448	0.4029	-0.1576	0.3129
Ketoisoleucine_1	0.1226	0.5507	0.3273	0.4287	0.2554	0.5415	0.2199	0.1565
Malonic acid	0.2205	0.2791	-0.6547	0.0781	0.0639	0.8806	0.2830	0.0659
3-Hydroxyisovaleric acid	0.1641	0.4232	-0.4364	0.2797	0.1405	0.7401	0.2224	0.1517
Valine (2TMS)	-0.0432	0.8341	0.0000	1.0000	-0.3959	0.3316	-0.0876	0.5766
Urea	0.2703	0.1817	-0.1091	0.7970	0.4980	0.2091	0.2328	0.1330
Oxamic acid	0.0798	0.6985	-0.6547	0.0781	0.3448	0.4029	0.2811	0.0678
2-Aminoethanol	-0.0522	0.7999	-0.2182	0.6036	-0.4214	0.2984	-0.0888	0.5711
n-Caprylic acid	0.1331	0.5170	0.3273	0.4287	-0.1405	0.7401	-0.0719	0.6469
Phosphate	0.0031	0.9879	0.1091	0.7970	0.6257	0.0970	0.0606	0.6995
Glycerol	0.2010	0.3249	0.0000	1.0000	0.4853	0.2229	-0.1147	0.4641
Ethylmalonic acid	0.1811	0.3759	0.0000	1.0000	0.8428	0.0086	0.2680	0.0823
Isoleucine	-0.0599	0.7713	0.1091	0.7970	-0.5108	0.1958	-0.0708	0.6521

Threonine (2TMS)	-0.0822	0.6897	0.1091	0.7970	-0.3703	0.3665	-0.0790	0.6144
Proline	-0.2170	0.2870	0.0000	1.0000	-0.3320	0.4217	-0.1287	0.4108
Maleic acid	0.1703	0.4055	0.0000	1.0000	0.7024	0.0521	0.2124	0.1715
Glycine (3TMS)	-0.1292	0.5292	-0.4364	0.2797	-0.7790	0.0227	0.0393	0.8026
Succinic acid (or aldehyde)	-0.1898	0.3530	-0.1091	0.7970	-0.0766	0.8569	-0.1123	0.4735
Catechol	0.6681	0.0002	-0.2182	0.6036	0.5236	0.1829	-0.0591	0.7066
Glyceric acid	0.0456	0.8248	0.1091	0.7970	-0.1022	0.8098	0.2400	0.1211
Uracil	0.0188	0.9273	-0.3273	0.4287	0.0511	0.9044	0.2345	0.1302
Fumaric acid	-0.2734	0.1765	-0.1091	0.7970	-0.1916	0.6495	0.0617	0.6942
Serine (3TMS)	-0.1634	0.4252	-0.4364	0.2797	-0.3065	0.4603	-0.1847	0.2358
Nonanoic acid (C9)	0.2790	0.1675	0.3273	0.4287	0.4853	0.2229	0.1371	0.3806
4-Methylbenzoic acid	0.2577	0.2036	0.4364	0.2797	0.8556	0.0067	-0.0497	0.7517
Glutaric acid	0.0815	0.6922	0.0000	1.0000	0.2043	0.6274	0.0056	0.9713
Maleamic acid	-0.1846	0.3666	-0.7638	0.0274	0.0000	1.0000	-0.0639	0.6842
Prolinamide	0.2480	0.2219	-0.3273	0.4287	0.0639	0.8806	0.2722	0.0774
Homoserine	0.0791	0.7010	-0.2182	0.6036	-0.0894	0.8333	0.0891	0.5701
Malic acid	-0.2076	0.3089	-0.1091	0.7970	-0.4470	0.2669	0.1490	0.3402
Threitol	-0.1616	0.4303	0.3273	0.4287	-0.8939	0.0028	0.1471	0.3464
meso-Erythritol	0.1376	0.5027	-0.2182	0.6036	-0.3320	0.4217	-0.1410	0.3671
Adipic acid	0.2661	0.1888	-0.1091	0.7970	0.6385	0.0884	-0.0016	0.9917
Acetylsalicylic acid	0.3281	0.1018	0.6547	0.0781	0.1405	0.7401	0.0247	0.8750
Aspartic acid	0.0070	0.9731	-0.1091	0.7970	-0.2299	0.5839	0.0980	0.5319
Methionine	-0.0832	0.6860	-0.1091	0.7970	-0.3320	0.4217	0.0223	0.8870
trans-4-Hydroxy-L-proline	-0.0226	0.9126	-0.2182	0.6036	-0.7534	0.0309	-0.1591	0.3083
4-Aminobutyric acid	-0.3535	0.0764	0.0000	1.0000	-0.7918	0.0192	0.0073	0.9631
Pyrogallol	-0.0766	0.7098	0.0000	1.0000	-0.4725	0.2371	0.0312	0.8424

Creatinine	0.0077	0.9704	-0.1091	0.7970	-0.4725	0.2371	-0.0092	0.9535
Acetoacetic acid	0.3225	0.1081	0.1091	0.7970	0.3959	0.3316	-0.0394	0.8020
β-Glutamic acid	0.3253	0.1049	-0.1091	0.7970	0.7024	0.0521	0.2520	0.1030
Glutamic acid	0.0596	0.7726	0.2182	0.6036	-0.6385	0.0884	-0.1094	0.4850
Phenylalanine	-0.1324	0.5192	0.1091	0.7970	-0.3703	0.3665	-0.0730	0.6417
5-Aminovaleric acid	0.1700	0.4065	-0.2182	0.6036	-0.2937	0.4801	-0.1810	0.2454
p-Hydroxybenzoic acid	0.3577	0.0728	0.2182	0.6036	0.7790	0.0227	0.0751	0.6320
Xylose_2	-0.0418	0.8393	-0.2182	0.6036	-0.7024	0.0521	0.1722	0.2694
threo-β-Hydroxyaspartic acid	0.0056	0.9784	-0.1091	0.7970	-0.0383	0.9282	0.3363	0.0274
Lyxose_2	0.0132	0.9488	-0.8729	0.0047	0.3065	0.4603	0.0681	0.6643
Arabinose	-0.0888	0.6661	-0.3273	0.4287	-0.7790	0.0227	-0.1853	0.2342
Lauric acid	-0.0871	0.6723	-0.1091	0.7970	0.0894	0.8333	-0.2056	0.1859
N-Acetyl-L-aspartic acid_2	0.2118	0.2990	0.2182	0.6036	0.6385	0.0884	0.0745	0.6349
Homocysteine_1	0.2679	0.1859	-0.2182	0.6036	0.6385	0.0884	0.2782	0.0708
Ribose	-0.0571	0.7816	-0.1091	0.7970	-0.6002	0.1157	-0.1673	0.2834
Ribulose	-0.2494	0.2192	0.0000	1.0000	-0.2682	0.5208	-0.1217	0.4370
Asparagine	-0.2929	0.1464	-0.6547	0.0781	-0.6385	0.0884	0.0222	0.8876
Taurine	-0.0038	0.9852	0.1091	0.7970	-0.0383	0.9282	-0.2830	0.0659
Cysteine sulfonic acid	-0.3020	0.1338	-0.3273	0.4287	0.1660	0.6944	0.3052	0.0466
Xylitol	0.1376	0.5027	0.1091	0.7970	0.5236	0.1829	0.0133	0.9326
1,6-Anhydroglucose	0.0307	0.8818	-0.1091	0.7970	-0.2937	0.4801	0.0942	0.5479
Arabitol	0.1059	0.6067	0.1091	0.7970	0.2682	0.5208	-0.1152	0.4621
Ribitol	-0.0132	0.9488	0.3273	0.4287	0.3065	0.4603	0.0785	0.6167
Aconitate	0.0829	0.6872	0.5455	0.1619	-0.4853	0.2229	0.0147	0.9256
N-Acetyl-L-glutamate_1	0.2995	0.1371	-0.1091	0.7970	-0.0128	0.9761	0.0327	0.8349
Glutamine	-0.0947	0.6453	-0.1091	0.7970	-0.3448	0.4029	-0.0237	0.8800

4-Hydroxymandelate	-0.2790	0.1675	-0.5455	0.1619	-0.8301	0.0108	-0.0447	0.7761
Dihydroorotate	0.1794	0.3806	-0.5455	0.1619	-0.2043	0.6274	0.1420	0.3637
O-Phosphoethanolamine	-0.0098	0.9623	0.1091	0.7970	0.4214	0.2984	0.0763	0.6269
Theanine_2	0.1978	0.3326	0.5455	0.1619	0.6385	0.0884	0.1099	0.4830
Glycyl-glycine_1	0.0324	0.8752	-0.1091	0.7970	-0.3703	0.3665	-0.1334	0.3939
Citric acid + isocitric acid	-0.1209	0.5564	0.2182	0.6036	-0.2682	0.5208	0.0573	0.7150
Ornithine	-0.0195	0.9247	-0.2182	0.6036	-0.5491	0.1586	-0.0680	0.6649
Hypoxanthine	0.1843	0.3676	0.3273	0.4287	-0.1916	0.6495	-0.0613	0.6960
Citrulline	-0.1459	0.4768	-0.3273	0.4287	-0.7790	0.0227	-0.0957	0.5415
1,5-Anhydro-D-glucitol	0.0906	0.6600	-0.2182	0.6036	-0.6641	0.0725	-0.0982	0.5309
Tagatose_2 (or psicose_2)	-0.0247	0.9046	0.0000	1.0000	0.2937	0.4801	-0.1557	0.3188
Fructose_1	-0.0195	0.9247	0.4364	0.2797	-0.3448	0.4029	-0.0898	0.5668
2-Dehydro-D-gluconate_1	0.0254	0.9019	0.1091	0.7970	0.0255	0.9521	-0.2311	0.1360
Glucose_1	0.2807	0.1648	-0.1091	0.7970	0.4980	0.2091	0.2274	0.1424
Sebacic acid	0.2139	0.2942	0.1091	0.7970	0.2937	0.4801	-0.0754	0.6309
Galactose_2	0.2166	0.2878	0.8729	0.0047	0.7407	0.0356	0.1423	0.3628
Mannitol	0.1250	0.5428	0.0000	1.0000	-0.4086	0.3148	-0.1781	0.2531
Lysine (4TMS)	-0.2083	0.3072	-0.5455	0.1619	-0.8301	0.0108	-0.0161	0.9186
Galactosamine_1	0.2198	0.2807	-0.2182	0.6036	-0.1277	0.7631	-0.0236	0.8807
Histidine	-0.2264	0.2661	-0.1091	0.7970	-0.4980	0.2091	-0.1465	0.3485
Glucuronate_1	-0.2274	0.2638	-0.1091	0.7970	0.2809	0.5003	-0.3410	0.0253
5-Keto-D-gluconate_2	0.0920	0.6550	-0.7638	0.0274	-0.0511	0.9044	0.2351	0.1291
Galacturonic acid_1	0.1449	0.4800	0.2182	0.6036	0.6385	0.0884	0.0583	0.7102
Tyrosine	-0.0331	0.8725	-0.2182	0.6036	-0.5619	0.1472	0.0749	0.6331
Pantothenate	0.0902	0.6612	-0.2182	0.6036	0.4086	0.3148	0.0632	0.6871
N-α-Acetyl-L-ornithine_2	0.3243	0.1061	-0.1091	0.7970	0.7024	0.0521	0.2520	0.1030

Glucarate	0.0784	0.7035	0.1091	0.7970	-0.2299	0.5839	-0.0957	0.5415
S-Benzyl-L-cysteine_1	0.2302	0.2578	0.3273	0.4287	0.4086	0.3148	0.2128	0.1708
Xanthine	0.2853	0.1578	0.1091	0.7970	-0.0511	0.9044	-0.0856	0.5854
Palmitoleate	0.0568	0.7829	-0.4364	0.2797	0.5491	0.1586	-0.0015	0.9924
Inositol	-0.2104	0.3023	-0.4364	0.2797	-0.3703	0.3665	-0.1017	0.5162
Dopa	0.2870	0.1551	0.2182	0.6036	0.7024	0.0521	0.2406	0.1201
Uric acid	0.0313	0.8792	0.2182	0.6036	-0.1916	0.6495	0.0083	0.9580
N-α-Acetyl-L-lysine_2	0.1069	0.6031	-0.7638	0.0274	-0.2682	0.5208	0.3465	0.0228
Heptadecanoate	-0.1794	0.3806	-0.1091	0.7970	0.2554	0.5415	-0.1508	0.3345
Kynurenine	0.1947	0.3405	-0.2182	0.6036	-0.0894	0.8333	0.1277	0.4144
Cystathionine	0.0850	0.6798	-0.6547	0.0781	0.1532	0.7171	0.2180	0.1602
Cystamine	0.3379	0.0914	-0.2182	0.6036	0.3576	0.3845	0.1357	0.3855
Tryptophan	-0.0167	0.9354	-0.1091	0.7970	-0.1277	0.7631	0.0144	0.9268
Elaidic acid	0.2783	0.1686	0.0000	1.0000	0.3703	0.3665	0.0623	0.6912
Homocysteine	0.1449	0.4800	0.2182	0.6036	0.6385	0.0884	0.0583	0.7102

Spearman's rank correlation coefficients (r_s) were calculated for the relationships between current dose including 0 mg of medication; i.e., each salicylates; Pentasa (N=26), Asacol (N=8) and SASP (N=8), or azathioprine (N=39) without steroid, other immunomodulatory agents and biological agents, and all metabolites detected by GC-MS in the sera of UCr. As far steroid only, previous medication status of several samples is unclear because of their long disease durations and complicated treatment histories in several hospitals. However, we could check those of steroid within a year in our medical records reliably. Therefore, the sample not medicated with steroid within the range of a year was treated as a non-administrated sample. As far salicylates, correlation coefficients (r_s) of dose of each salicylates without other medication including immunomoduratoy agents were calculated. All samples of the patients medicated with azathioprine were compared with those with salicylates, so correlation coefficients (r_s) of dose of azathioprine with salicylates were calculated.

Supplemental Table 13. Spearman's rank correlation coefficients (r_s) for the relationships between steroid and all metabolites detected by GC-MS in the sera of UCr.

	Current dose		Total dose							
			for 1 month		for 3 months		for 6 months		for 1 year	
	r_s	P-value	r_s	P-value	r_s	P-value	r_s	P-value	r_s	P-value
Pyruvate+oxalacetic acid	0.3033	0.0453	0.3015	0.0467	0.3303	0.0305	0.2645	0.0865	0.2825	0.0664
Lactic acid	0.4176	0.0048	0.4161	0.0050	0.4288	0.0041	0.4443	0.0028	0.4662	0.0016
Glycolic acid	-0.1205	0.4358	-0.1131	0.4650	-0.1015	0.5172	-0.0090	0.9541	0.0254	0.8718
Alanine (2TMS)	0.0859	0.5793	0.0685	0.6585	0.0887	0.5715	0.0864	0.5815	0.0578	0.7126
Hydroxybutyrate	0.3393	0.0243	0.3466	0.0212	0.3446	0.0236	0.2558	0.0978	0.2180	0.1602
Oxalate	-0.1399	0.3650	-0.1296	0.4018	-0.1083	0.4895	-0.0386	0.8057	0.0449	0.7748
Sarcosine	0.1413	0.3602	0.1466	0.3423	0.1702	0.2753	0.0898	0.5667	0.0169	0.9143
2-Aminoisobutyrate	-0.1705	0.2685	-0.1765	0.2518	-0.1576	0.3127	-0.1349	0.3883	-0.0863	0.5821
3-Hydroxybutyrate	0.3090	0.0413	0.3139	0.0380	0.3100	0.0430	0.2812	0.0678	0.2618	0.0899
2-Aminobutyric acid	0.0899	0.5616	0.0821	0.5962	0.0845	0.5902	-0.0231	0.8829	-0.0830	0.5968
Ketoisoleucine_1	0.3024	0.0460	0.3065	0.0430	0.3261	0.0329	0.2603	0.0919	0.2531	0.1015
Malonic acid	0.1637	0.2885	0.1707	0.2679	0.1697	0.2767	0.1975	0.2042	0.2716	0.0781
3-Hydroxyisovaleric acid	0.0838	0.5887	0.0869	0.5747	0.0669	0.6698	0.0729	0.6421	0.1150	0.4627
Valine (2TMS)	0.1404	0.3634	0.1329	0.3898	0.1574	0.3134	0.0544	0.7291	-0.0178	0.9098
Urea	0.1323	0.3918	0.1298	0.4009	0.1378	0.3780	0.1971	0.2053	0.2663	0.0844
Oxamic acid	0.1647	0.2853	0.1668	0.2792	0.1872	0.2293	0.1488	0.3409	0.1146	0.4645
2-Aminoethanol	-0.0344	0.8246	-0.0468	0.7630	-0.0243	0.8770	-0.0742	0.6362	-0.0994	0.5258
n-Caprylic acid	-0.0999	0.5190	-0.0853	0.5820	-0.0607	0.6992	-0.1133	0.4693	-0.0692	0.6594
Phosphate	0.1693	0.2718	0.1760	0.2531	0.1767	0.2570	0.2498	0.1062	0.2218	0.1529
Glycerol	0.2326	0.1287	0.2392	0.1178	0.2589	0.0937	0.2031	0.1916	0.1766	0.2572
Ethylmalonic acid	0.1008	0.5150	0.1071	0.4888	0.1053	0.5017	0.1223	0.4348	0.1537	0.3251
Isoleucine	0.1791	0.2446	0.1805	0.2410	0.2000	0.1985	0.0896	0.5677	0.0316	0.8406

Threonine (2TMS)	0.1477	0.3387	0.1498	0.3318	0.1689	0.2789	0.0598	0.7034	0.0000	1.0000
Proline	-0.0912	0.5559	-0.0986	0.5241	-0.0842	0.5913	-0.1359	0.3849	-0.1715	0.2715
Maleic acid	0.1669	0.2790	0.1579	0.3059	0.1571	0.3142	0.1526	0.3287	0.1675	0.2830
Glycine (3TMS)	-0.1301	0.3999	-0.1418	0.3587	-0.1261	0.4205	-0.0352	0.8225	-0.0892	0.5695
Succinic acid (or aldehyde)	-0.0450	0.7717	-0.0560	0.7181	-0.0612	0.6969	-0.0413	0.7924	-0.0113	0.9424
Catechol	0.0538	0.7289	0.0591	0.7033	0.0669	0.6698	0.1231	0.4317	0.1223	0.4345
Glyceric acid	0.1058	0.4945	0.1146	0.4589	0.0822	0.6002	0.1658	0.2879	0.2296	0.1387
Uracil	0.1449	0.3481	0.1427	0.3555	0.1158	0.4597	0.1919	0.2176	0.2587	0.0939
Fumaric acid	0.0960	0.5355	0.0988	0.5236	0.1228	0.4327	0.0860	0.5836	0.0087	0.9560
Serine (3TMS)	0.0118	0.9393	0.0019	0.9903	-0.0208	0.8947	-0.0873	0.5779	-0.1608	0.3029
Nonanoic acid (C9)	0.1787	0.2459	0.1843	0.2311	0.2143	0.1676	0.1786	0.2517	0.1159	0.4593
4-Methylbenzoic acid	0.0357	0.8181	0.0243	0.8754	0.0190	0.9035	0.0114	0.9422	-0.0222	0.8874
Glutaric acid	0.0827	0.5935	0.0901	0.5607	0.1060	0.4986	0.1788	0.2514	0.1824	0.2417
Maleamic acid	0.4625	0.0016	0.4627	0.0016	0.4559	0.0021	0.4914	0.0008	0.4687	0.0015
Prolinamide	0.1472	0.3402	0.1546	0.3162	0.1539	0.3245	0.1780	0.2533	0.2525	0.1024
Homoserine	0.1022	0.5091	0.1001	0.5181	0.0855	0.5858	0.1198	0.4442	0.1731	0.2671
Malic acid	0.3982	0.0074	0.4024	0.0068	0.4025	0.0075	0.3257	0.0331	0.2442	0.1145
Threitol	-0.2440	0.1104	-0.2411	0.1149	-0.2263	0.1445	-0.1340	0.3916	-0.1375	0.3794
meso-Erythritol	0.0876	0.5720	0.0960	0.5352	0.1211	0.4394	0.0594	0.7050	0.0803	0.6087
Adipic acid	-0.0314	0.8395	-0.0396	0.7987	-0.0479	0.7605	0.0294	0.8517	0.0972	0.5352
Acetylsalicylic acid	-0.0144	0.9260	-0.0126	0.9351	0.0155	0.9212	0.0190	0.9036	0.0474	0.7629
Aspartic acid	0.1670	0.2787	0.1496	0.3326	0.1519	0.3309	0.1270	0.4172	0.0672	0.6686
Methionine	0.1166	0.4509	0.0978	0.5276	0.0877	0.5759	0.0240	0.8788	-0.0387	0.8054
trans-4-Hydroxy-L-proline	-0.1002	0.5175	-0.1179	0.4460	-0.1098	0.4834	-0.1534	0.3261	-0.1639	0.2935
4-Aminobutyric acid	0.0273	0.8604	0.0315	0.8389	0.0376	0.8108	0.0913	0.5606	0.1564	0.3166
Pyrogallol	-0.1605	0.2981	-0.1682	0.2750	-0.1526	0.3285	-0.0902	0.5651	-0.1230	0.4320

Creatinine	-0.0486	0.7542	-0.0652	0.6741	-0.0779	0.6193	-0.1521	0.3302	-0.2011	0.1960
Acetoacetic acid	-0.0772	0.6186	-0.0835	0.5899	-0.1208	0.4403	-0.1459	0.3506	-0.1090	0.4866
β-Glutamic acid	0.2440	0.1104	0.2443	0.1100	0.2514	0.1039	0.2445	0.1140	0.3156	0.0392
Glutamic acid	0.2855	0.0603	0.2730	0.0730	0.2466	0.1109	0.2331	0.1325	0.2545	0.0996
Phenylalanine	0.2244	0.1431	0.2102	0.1709	0.2043	0.1889	0.1373	0.3800	0.0934	0.5513
5-Aminovaleric acid	0.0711	0.6463	0.0640	0.6797	0.0659	0.6745	-0.0261	0.8682	-0.0845	0.5899
p-Hydroxybenzoic acid	0.0736	0.6349	0.0729	0.6382	0.0915	0.5596	0.1581	0.3113	0.1659	0.2876
Xylose_2	0.2278	0.1369	0.2373	0.1209	0.2251	0.1468	0.1732	0.2666	0.1686	0.2798
threo-β-Hydroxyaspartic acid	-0.0236	0.8790	-0.0201	0.8971	-0.0188	0.9048	-0.0648	0.6796	-0.0888	0.5714
Lyxose_2	0.0609	0.6948	0.0509	0.7427	0.0298	0.8494	0.0194	0.9018	0.0198	0.8997
Arabinose	0.0077	0.9605	-0.0083	0.9575	-0.0055	0.9720	-0.0773	0.6223	-0.0214	0.8919
Lauric acid	-0.1798	0.2427	-0.1685	0.2744	-0.1664	0.2862	-0.1877	0.2281	-0.0959	0.5408
N-Acetyl-L-aspartic acid_2	-0.0086	0.9557	-0.0154	0.9212	-0.0135	0.9314	0.0734	0.6399	0.0956	0.5418
Homocysteine_1	0.1359	0.3791	0.1429	0.3546	0.1429	0.3608	0.1627	0.2973	0.2013	0.1955
Ribose	-0.0865	0.5766	-0.0900	0.5612	-0.0669	0.6698	-0.1497	0.3379	-0.1584	0.3104
Ribulose	-0.2269	0.1386	-0.2452	0.1086	-0.2348	0.1295	-0.3082	0.0444	-0.3572	0.0187
Asparagine	-0.0099	0.9490	-0.0245	0.8748	-0.0281	0.8582	-0.1177	0.4523	-0.1969	0.2058
Taurine	0.0265	0.8646	0.0250	0.8718	-0.0020	0.9898	-0.0653	0.6774	-0.1303	0.4048
Cysteine sulfonic acid	0.1282	0.4069	0.1360	0.3788	0.1353	0.3869	0.1647	0.2914	0.2124	0.1714
Xylitol	0.2298	0.1334	0.2347	0.1251	0.2193	0.1577	0.1186	0.4487	0.1061	0.4983
1,6-Anhydroglucose	0.0052	0.9733	-0.0086	0.9557	0.0115	0.9415	0.0590	0.7073	0.0823	0.5998
Arabitol	0.1026	0.5076	0.1118	0.4702	0.1531	0.3269	0.1293	0.4086	0.0734	0.6399
Ribitol	0.3211	0.0336	0.3252	0.0312	0.3323	0.0295	0.3042	0.0473	0.2382	0.1239
Aconitate	0.1381	0.3712	0.1232	0.4256	0.1629	0.2966	0.0994	0.5261	0.0970	0.5361
N-Acetyl-L-glutamate_1	0.2276	0.1373	0.2339	0.1265	0.2561	0.0973	0.1514	0.3325	0.1335	0.3935
Glutamine	-0.1703	0.2691	-0.1840	0.2317	-0.1800	0.2482	-0.2224	0.1517	-0.2809	0.0680

4-Hydroxymandelate	-0.1595	0.3010	-0.1708	0.2676	-0.1431	0.3599	-0.1663	0.2865	-0.1597	0.3063
Dihydroorotate	0.0013	0.9933	0.0084	0.9569	0.0195	0.9010	0.1021	0.5149	0.1806	0.2464
O-Phosphoethanolamine	-0.0661	0.6701	-0.0512	0.7416	-0.0328	0.8344	-0.0162	0.9178	0.0391	0.8032
Theanine_2	0.1793	0.2443	0.1746	0.2570	0.1835	0.2389	0.2322	0.1341	0.3003	0.0504
Glycyl-glycine_1	-0.0130	0.9333	-0.0038	0.9806	0.0000	1.0000	0.0870	0.5790	0.1221	0.4353
Citric acid + isocitric acid	-0.2204	0.1506	-0.2306	0.1321	-0.2068	0.1834	-0.2193	0.1577	-0.2124	0.1714
Ornithine	-0.0254	0.8700	-0.0445	0.7741	-0.0266	0.8657	-0.0001	0.9994	-0.0087	0.9560
Hypoxanthine	0.0002	0.9988	0.0119	0.9387	0.0005	0.9975	-0.0015	0.9922	0.0385	0.8065
Citrulline	-0.1614	0.2952	-0.1763	0.2524	-0.1734	0.2660	-0.1866	0.2308	-0.1862	0.2319
1,5-Anhydro-D-glucitol	-0.2308	0.1318	-0.2335	0.1271	-0.2368	0.1262	-0.1838	0.2381	-0.1428	0.3610
Tagatose_2 (or psicose_2)	0.0422	0.7857	0.0549	0.7232	0.0459	0.7702	0.0548	0.7268	0.0674	0.6676
Fructose_1	0.0086	0.9557	0.0274	0.8598	0.0218	0.8896	-0.0721	0.6459	-0.1050	0.5028
2-Dehydro-D-gluconate_1	0.3707	0.0132	0.3706	0.0133	0.3546	0.0196	0.2476	0.1095	0.1811	0.2452
Glucose_1	0.1319	0.3935	0.1294	0.4027	0.1386	0.3754	0.1934	0.2139	0.2583	0.0945
Sebacic acid	-0.0069	0.9648	-0.0139	0.9284	-0.0010	0.9949	-0.0029	0.9851	0.0151	0.9233
Galactose_2	0.1776	0.2488	0.1793	0.2441	0.1792	0.2502	0.0944	0.5470	0.0865	0.5811
Mannitol	-0.0718	0.6430	-0.0782	0.6139	-0.0629	0.6886	-0.0534	0.7336	-0.1061	0.4983
Lysine (4TMS)	0.0228	0.8832	0.0047	0.9757	-0.0098	0.9504	-0.0898	0.5667	-0.1495	0.3387
Galactosamine_1	0.2303	0.1326	0.2302	0.1327	0.2323	0.1338	0.1139	0.4670	0.0787	0.6157
Histidine	-0.3309	0.0283	-0.3407	0.0236	-0.3429	0.0244	-0.4163	0.0055	-0.4685	0.0015
Glucuronate_1	0.1095	0.4791	0.1028	0.5068	0.1291	0.4094	0.0209	0.8941	0.0194	0.9020
5-Keto-D-gluconate_2	0.0783	0.6132	0.0861	0.5783	0.0965	0.5382	0.0998	0.5242	0.1315	0.4007
Galacturonic acid_1	0.0591	0.7032	0.0516	0.7393	0.0561	0.7207	0.1409	0.3673	0.1526	0.3286
Tyrosine	-0.0087	0.9551	-0.0169	0.9133	-0.0095	0.9517	-0.1038	0.5076	-0.1103	0.4812
Pantothenate	0.0567	0.7146	0.0790	0.6101	0.0805	0.6081	0.1655	0.2889	0.1722	0.2696
N-α-Acetyl-L-ornithine_2	0.2440	0.1104	0.2443	0.1100	0.2514	0.1039	0.2445	0.1140	0.3156	0.0392

Glucarate	-0.1755	0.2546	-0.1687	0.2737	-0.1729	0.2674	-0.2281	0.1413	-0.2118	0.1728
S-Benzyl-L-cysteine_1	-0.1470	0.3410	-0.1537	0.3192	-0.1574	0.3134	-0.1773	0.2552	-0.1998	0.1991
Xanthine	0.2783	0.0674	0.2761	0.0697	0.2546	0.0994	0.1656	0.2886	0.2060	0.1851
Palmitoleate	0.0796	0.6073	0.0849	0.5836	0.0637	0.6851	0.1299	0.4064	0.1003	0.5221
Inositol	-0.1541	0.3180	-0.1675	0.2771	-0.1757	0.2598	-0.2557	0.0980	-0.2689	0.0812
Dopa	0.2440	0.1104	0.2443	0.1100	0.2514	0.1039	0.2397	0.1216	0.3107	0.0425
Uric acid	0.0657	0.6718	0.0582	0.7073	0.0321	0.8382	-0.0408	0.7953	0.0033	0.9831
N-α-Acetyl-L-lysine_2	0.2555	0.0942	0.2614	0.0865	0.2368	0.1262	0.1556	0.3190	0.2302	0.1375
Heptadecanoate	0.0703	0.6502	0.0671	0.6652	0.0361	0.8183	0.0637	0.6851	0.1112	0.4777
Kynurenine	-0.0766	0.6213	-0.0787	0.6117	-0.0762	0.6272	0.0268	0.8647	0.0621	0.6926
Cystathionine	0.0973	0.5300	0.0940	0.5438	0.1130	0.4705	0.1665	0.2858	0.1481	0.3431
Cystamine	0.2797	0.0659	0.2764	0.0693	0.2732	0.0763	0.3572	0.0187	0.4166	0.0055
Tryptophan	0.0606	0.6959	0.0478	0.7578	0.0226	0.8858	-0.0229	0.8841	-0.0463	0.7683
Elaidic acid	0.1914	0.2132	0.2077	0.1762	0.2010	0.1962	0.2644	0.0867	0.2952	0.0547
Homocysteine	0.0591	0.7032	0.0516	0.7393	0.0561	0.7207	0.1409	0.3673	0.1526	0.3286

Spearman's rank correlation coefficients (r_s) were calculated for the relationships between each dose of prednisolone (N=44); current dose or total dose including 0 mg within 1, 3, 6 months or 1 year, and all metabolites detected by GC-MS in the sera of UCr. All samples were evaluated about only salicylates but not immunomoduratory agents and biological agents.

Supplemental Table 14. Characteristics of the UC patients in food restriction therapy group and non-food restriction therapy group.

	food restriction therapy	**non-food restriction therapy**	**P value**
N (male/female)	4(1/3)	4(1/3)	-
Age [a)]	34.2±18.3/13-51	29.2±8.1/22-41	0.1824
BMI [a)]	17.8±2.4/15.0-20.4	22.9±8.2/18.4-34.7	0.3173
Disease location			
UC: P/LS/PC	0/0/4	1/2/1	-
Disease activity			
CAI [a)]	8.3±1.3/7-10	8.5±1.3/7-10	0.2260
remission/active	0/4	0/4	
food restriction therapy			
duration (days) [a)]	9.5±8.5/3-21	-	-
Daily medication			
5-Aminosalicylates			
Pentasa [a)]	3750±500/3000-4000	4000±0/4000	0.4795
Asacol [a)]	0±0	3600±0/4000	-
SASP [a)]	0±0	0±0	-
Predonisolone [a)]	0±0	0±0	-
6-Mercaptopurine [a)]	0±0	0±0	-
Azathioprine [a)]	0±0	0±0	-
Tacrolimus [a)]	0±0	0±0	-
Anti-TNF-α agents [a)]	0±0	0±0	-

[a)]: Age, BMI, disease activity, days with food restriction therapy and current dose of medication are shown as the mean ± standard deviation together with the range. P: proctitis, LC: left-sided colitis, PC: pan-colitis, CAI: Rachmilewitz index (a clinical activity index). P-values were calculated using the Wilcoxon rank sum test.

Supplemental Table 15. Comparison of the serum levels of all metabolites detected by GC-MS between food restriction therapy group and non-food restriction therapy group

	F.I. food restriction therapy /non-food restriction therapy	P-value food restriction therapy vs. non-food restriction therapy
Pyruvate+oxalacetic acid	1.4	0.0668
Lactic acid	1.0	0.4047
Glycolic acid	0.8	1.0000
Alanine (2TMS)	1.5	0.0668
Hydroxybutyrate	1.9	0.4047
Oxalate	1.5	0.4047
Sarcosine	0.9	0.1336
2-Aminoisobutyrate	1.0	0.4047
3-Hydroxybutyrate	6.0	0.4047
2-Aminobutyric acid	1.9	0.4047
Ketoisoleucine_1	1.0	0.2433
Malonic acid	0.9	0.8676
3-Hydroxyisovaleric acid	10.7	0.4047
Valine (2TMS)	1.0	0.4047
Urea	0.8	0.6171
Oxamic acid	1.2	0.1336
2-Aminoethanol	1.7	0.1336
n-Caprylic acid	1.0	1.0000
Phosphate	1.4	0.2433
Glycerol	0.5	0.2433
Ethylmalonic acid	0.7	0.6171
Isoleucine	1.1	0.8676
Threonine (2TMS)	1.1	0.6171
Proline	1.0	0.8676
Maleic acid	1.0	0.8676
Glycine (3TMS)	1.5	0.2433
Succinic acid (or aldehyde)	1.5	0.1336
Catechol	1.0	0.8676
Glyceric acid	1.5	0.6171
Uracil	1.1	0.8676
Fumaric acid	1.4	0.8676
Serine (3TMS)	1.7	1.0000
Nonanoic acid (C9)	1.3	0.1336
4-Methylbenzoic acid	0.7	0.2433
Glutaric acid	1.1	1.0000
Maleamic acid	0.8	0.6171
Prolinamide	0.7	0.6171

Homoserine	0.9	1.0000
Malic acid	2.2	0.2433
Threitol	0.6	0.1336
meso-Erythritol	1.2	0.8676
Adipic acid	0.8	0.8676
Acetylsalicylic acid	0.9	0.8676
Aspartic acid	1.2	0.6171
Methionine	1.6	0.1336
trans-4-Hydroxy-L-proline	1.1	1.0000
4-Aminobutyric acid	2.3	0.0668
Pyrogallol	1.1	0.8676
Creatinine	1.6	1.0000
Acetoacetic acid	0.9	1.0000
β-Glutamic acid	0.7	0.6171
Glutamic acid	2.3	0.6171
Phenylalanine	1.6	0.2433
5-Aminovaleric acid	1.3	0.6171
p-Hydroxybenzoic acid	4.8	0.4047
Xylose_2	0.3	0.8676
threo-β-Hydroxyaspartic acid	0.9	0.4047
Lyxose_2	1.0	0.1336
Arabinose	1.2	0.8676
Lauric acid	1.4	0.0668
N-Acetyl-L-aspartic acid_2	0.8	0.8676
Homocysteine_1	0.9	0.8676
Ribose	2.0	0.0668
Ribulose	1.3	0.1336
Asparagine	1.3	0.4047
Taurine	3.0	1.0000
Cysteine sulfonic acid	0.9	0.6171
Xylitol	1.2	0.6171
1,6-Anhydroglucose	0.4	0.6171
Arabitol	1.0	0.1336
Ribitol	1.4	0.4047
Aconitate	1.0	1.0000
N-Acetyl-L-glutamate_1	1.1	0.2433
Glutamine	1.2	0.6171
4-Hydroxymandelate	1.1	0.8676
Dihydroorotate	0.8	0.8676
O-Phosphoethanolamine	1.0	0.8676
Theanine_2	0.9	0.6171
Glycyl-glycine_1	0.8	0.4047

Citric acid + isocitric acid	1.7	0.2433
Ornithine	1.2	1.0000
Hypoxanthine	2.1	0.1336
Citrulline	0.9	0.2433
1,5-Anhydro-D-glucitol	0.9	0.4047
Tagatose_2 (or psicose_2)	0.7	0.8676
Fructose_1	1.5	0.8676
2-Dehydro-D-gluconate_1	2.1	0.2433
Glucose_1	0.9	0.8676
Sebacic acid	1.8	0.0668
Galactose_2	2.1	0.2433
Mannitol	1.2	0.6171
Lysine (4TMS)	1.0	0.2433
Galactosamine_1	1.2	0.8676
Histidine	1.0	0.0668
Glucuronate_1	1.2	0.2433
5-Keto-D-gluconate_2	0.7	0.8676
Galacturonic acid_1	1.1	0.6171
Tyrosine	0.9	0.4047
Pantothenate	0.9	0.8676
N-α-Acetyl-L-ornithine_2	0.7	0.6171
Glucarate	0.9	0.0668
S-Benzyl-L-cysteine_1	1.2	0.1336
Xanthine	1.9	0.0668
Palmitoleate	3.2	0.1336
Inositol	1.8	0.4047
Dopa	1.0	0.8676
Uric acid	1.4	1.0000
N-α-Acetyl-L-lysine_2	2.0	0.1336
Heptadecanoate	1.0	0.8676
Kynurenine	0.7	0.8676
Cystathionine	1.1	0.8676
Cystamine	0.9	0.4047
Tryptophan	0.4	0.8676
Elaidic acid	0.7	0.2433
Homocysteine	1.1	0.6171

We picked up UC patients' samples randomly (N=8) and divided them into two groups; food restriction therapy group and non-food restriction therapy group which various points such as age, gender ratio, BMI, medicated condition and clinical activity were matched. The detail data of two groups is shown in Supplemental table 15. Values are shown as fold induction (F.I.) values, which were obtained by comparing the peak intensity values of food restriction therapy group patients with those of non-food restriction therapy group. The peak intensity values for each quantified ion have been normalized to that of 2-isopropylmalic acid as an internal standard. P-values were calculated using the Wilcoxon rank sum test.

Supplemental Table 16. Characteristics of the CD patients in infliximab administration group and non-administration group.

	administration group	non-administration group	P value
N (male/female)	7(5/2)	7(5/2)	-
Age [a)]	46.5±8.8/30-58	37.2±11.3/28-53	0.2003
BMI [a)]	20.9±2.3/19.6-23.5	21.6±3.0/15.5-26.3	0.3379
Disease location			
CD: S/C/Both	3/1/2	3/0/4	-
Disease activity			
CDAI* [a)]	67.7±48.8/9.5-136.3	66.1±44.6/29.4-147.4	0.848
remission/active	7/0	7/0	
Daily medication			
Pentasa [a)]	1500±1341/0-3000	1500±1379/0-3000	0.8915
Predonisolone [a)]	0±0	0±0	-
6-Mercaptopurine [a)]	0±0	0±0	-
Azathioprine [a)]	14.2±37.9/0-100	17.8±18.8/0-50	0.2029
Tacrolimus [a)]			-
infliximab [a)]	290.3±49.1	0±0	-
Enteral nutrition [a)]	471±515/0-900	557±801/0-2100	0.8358

[a)]: Age, BMI, disease activity, and current dose of medication including infliximab are shown as the mean ± standard deviation together with the range. S: small bowel, C: colon, Both: small bowel & colon, CDAI: Crohn's disease activity index. P-values were calculated using the Wilcoxon rank sum test.

Supplemental Table 17. Comparison of the serum levels of all metabolites detected by GC-MS between infliximab administration group and non-administration group

	F.I. adnimistration / non-adnimistration	P-value adnimistration vs. non-administration
Pyruvate+Oxalacetic acid	1.3	0.2774
Lactic acid	1.2	0.0845
Glycolic acid	1.1	0.6547
Alanine(2TMS)	1.0	0.8480
HydroxyButyrate	1.2	0.9491
Oxalate	0.9	0.6547
Sarcosine	1.0	0.4822
2-Aminoisobutyrate	1.1	0.1102
3-Hydroxy-Butyrate	0.6	0.1102
2-Aminobutyric acid	1.0	0.8480
Ketoisoleucine_1	1.1	0.5653
Malonic acid	1.2	0.0639
3-Hydroxyisovaleric acid	1.1	0.8480
Valine(2TMS)	0.9	0.8480
Urea	1.1	0.0476
Oxamic acid	1.9	0.2248
2-Aminoethanol	0.9	0.8480
n-Caprylic acid	1.2	0.5653
Phosphate	0.7	0.1797
Glycerol	0.5	0.0476
Ethylmalonic acid	1.0	0.9491
Isoleucine	1.0	0.6547
Threonine(2TMS)	1.0	0.7494
Proline	1.0	0.6547
Maleic acid	1.2	0.0639
Glycine(3TMS)	1.1	0.4062
Succinic acid(or aldehyde)	1.0	0.8480
Catechol	1.3	0.2248
Glyceric acid	0.9	0.2774
Uracil	1.2	0.0639
Fumaric acid	1.2	0.1797
Serine(3TMS)	1.1	0.8480
Nonanoic acid(C9)	1.1	0.5653
4-Methyl Benzoic acid	1.2	0.0181
Glutaric acid	1.2	0.1797
Maleamic acid	1.2	0.1417
Prolinamide	1.0	0.9491

homoserine	1.1	0.5653
Malic acid	0.9	0.7494
Threitol	1.2	0.3379
meso-erythritol	0.2	0.6547
Adipic acid	1.2	0.2248
Acetylsalicylic acid	0.8	0.4822
Aspartic acid	1.0	0.5653
Methionine	1.0	0.5653
trans-4-Hydroxy-L-proline	1.0	0.9491
4-Aminobutyric acid	0.9	0.5653
Pyrogallol	0.9	0.0476
Creatinine	1.3	0.0845
Acetoacetic acid	1.1	0.2248
β-Glutamic acid	1.0	0.9491
Glutamic acid	0.8	0.2248
Phenylalanine	1.0	0.7494
5-Aminovaleric acid	1.0	0.4062
p-Hydroxybenzoic acid	0.7	0.0845
Xylose_2	0.8	0.3379
threo-β-Hydroxyaspartic acid	1.2	0.3379
Lyxose_2	1.0	0.8480
Arabinose	1.1	0.9491
Lauric acid	0.5	0.0845
N-Acetyl-L-Aspartic acid_2	1.0	0.9491
Homocysteine_1	1.2	0.0639
Ribose	0.5	0.1417
Ribulose	1.0	0.9491
Asparagine	1.2	0.1797
Taurine	1.2	0.5653
Cysteine Sulfonic acid	1.0	0.9491
Xylitol	1.2	0.4822
1,6-Anhydroglucose	0.9	0.5653
Arabitol	2.0	0.5653
Ribitol	1.0	0.9491
Aconitate	1.1	0.1797
N-Acetyl-L-Glutamate_1	1.1	0.6547
Glutamine	1.0	0.8480
4-HydroxyMandelate	1.1	0.2248
Dihydroorotate	1.0	0.9491
O-Phosphoethanolamine	1.1	0.5653
Theanine_2	1.6	0.1417
Glycyl-Glycine_1	1.1	0.2248

Citric acid + Isocitric acid	1.1	0.9491
Ornithine	1.3	0.0350
Hypoxanthine	0.6	0.1797
Citrulline	0.9	0.5653
1,5-Anhydro-D-glucitol	1.0	0.9491
Tagatose_2(or Psicose_2)	1.3	0.3379
Fructose_1	1.1	0.6547
2-Dehydro-D-gluconate_1	291.4	0.2774
Glucose_1	1.1	0.1102
Sebacic acid	13.5	0.4822
Galactose_2	0.8	0.2774
Mannitol	3.4	0.1102
Lysine(4TMS)	0.9	0.9491
Galactosamine_1	1.1	0.1797
Histidine	3.2	0.0845
Glucuronate_1	0.7	0.2774
5-Keto-D-Gluconate_2	1.2	0.2774
Galacturonic acid_1	1.2	0.0639
Tyrosine	0.9	0.7494
Pantothenate	1.0	0.9491
N-α-Acetyl-L-ornithine_2	1.0	0.9491
Glucarate	1.1	0.3379
S-Benzyl-L-Cysteine_1	10.9	0.7494
Xanthine	0.8	0.4822
Palmitoleate	0.3	0.0253
Inositol	1.0	0.6547
Dopa	1.2	0.0639
Uric acid	1.1	0.5653
N-α-Acetyl-L-lysine_2	0.3	0.1797
Heptadecanoate	1.2	0.2248
Kynurenine	0.9	0.6547
Cystathionine	0.9	0.6547
Cystamine	1.0	0.9491
Tryptophan	0.6	0.1102
Elaidic acid	1.2	0.1797
Homocystine	1.2	0.0639

As far infliximab, the number of CD patient's samples was sufficient for statistical analysis, so we used CD patients' data. We picked up CD patients' samples (N=14) randomly and divided them into two groups; food restriction therapy group and non-food restriction therapy group which various points such as age, gender ratio, BMI, medicated condition and clinical activity were matched. The detail data of two groups was shown in Supplemental table 17. Values are shown as fold induction (F.I.) values, which were obtained by comparing the peak intensity values of administration group patients with those of non-administration therapy group. The peak intensity values for each quantified ion have been normalized to that of 2-isopropylmalic acid as an internal standard. P-values were calculated using the Wilcoxon rank sum test.

Supplemental Table 18. Spearman's rank correlation coefficients (r_s) for the relationships between daily total calorie of enteral nutrition therapy and all metabolites detected by GC-MS in the sera of CD in remission.

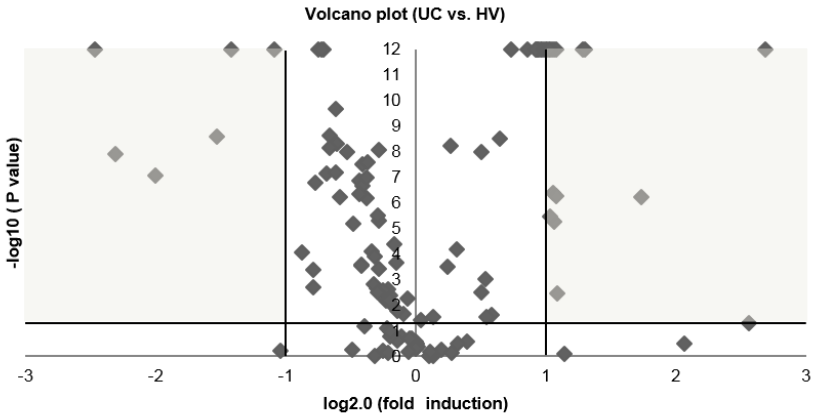
	r_s	P-value
Pyruvate+oxalacetic acid	0.2648	0.6121
Lactic acid	-0.2648	0.6121
Glycolic acid	-0.1765	0.7379
Alanine (2TMS)	0.7945	0.0590
Hydroxybutyrate	0.6179	0.1911
Oxalate	-0.4414	0.3809
Sarcosine	-0.0883	0.8679
2-Aminoisobutyrate	-0.8827	0.0198
3-Hydroxybutyrate	0.5296	0.2798
2-Aminobutyric acid	0.8827	0.0198
Ketoisoleucine_1	-0.0883	0.8679
Malonic acid	-0.0883	0.8679
3-Hydroxyisovaleric acid	-0.1765	0.7379
Valine (2TMS)	0.6179	0.1911
Urea	-0.7062	0.1168
Oxamic acid	-0.3531	0.4924
2-Aminoethanol	0.1765	0.7379
n-Caprylic acid	-0.1765	0.7379
Phosphate	0.2648	0.6121
Glycerol	0.5296	0.2798
Ethylmalonic acid	-0.7945	0.0590
Isoleucine	0.3531	0.4924
Threonine (2TMS)	0.3531	0.4924
Proline	0.2648	0.6121
Maleic acid	-0.0883	0.8679
Glycine (3TMS)	0.2648	0.6121
Succinic acid (or aldehyde)	0.5296	0.2798
Catechol	-0.2648	0.6121
Glyceric acid	0.8827	0.0198
Uracil	-0.0883	0.8679
Fumaric acid	0.0000	1.0000
Serine (3TMS)	0.3531	0.4924
Nonanoic acid (C9)	0.0883	0.8679
4-Methylbenzoic acid	-0.4414	0.3809
Glutaric acid	-0.9710	0.0012
Maleamic acid	-0.2648	0.6121
Prolinamide	-0.7945	0.0590
Homoserine	-0.5296	0.2798
Malic acid	0.0000	1.0000

Threitol	-0.7062	0.1168
meso-Erythritol	-0.5296	0.2798
Adipic acid	-0.0883	0.8679
Acetylsalicylic acid	-0.3531	0.4924
Aspartic acid	0.0883	0.8679
Methionine	0.7945	0.0590
trans-4-Hydroxy-L-proline	0.2648	0.6121
4-Aminobutyric acid	0.6179	0.1911
Pyrogallol	0.5296	0.2798
Creatinine	-0.6179	0.1911
Acetoacetic acid	-0.4414	0.3809
β-Glutamic acid	-0.7945	0.0590
Glutamic acid	0.6179	0.1911
Phenylalanine	0.7062	0.1168
5-Aminovaleric acid	-0.2648	0.6121
p-Hydroxybenzoic acid	0.6179	0.1911
Xylose_2	0.4414	0.3809
threo-β-Hydroxyaspartic acid	-0.4414	0.3809
Lyxose_2	0.1765	0.7379
Arabinose	-0.7062	0.1168
Lauric acid	-0.3531	0.4924
N-Acetyl-L-aspartic acid_2	-0.7945	0.0590
Homocysteine_1	-0.0883	0.8679
Ribose	0.6179	0.1911
Ribulose	-0.0883	0.8679
Asparagine	0.3531	0.4924
Taurine	-0.0883	0.8679
Cysteine sulfonic acid	-0.7945	0.0590
Xylitol	-0.0883	0.8679
1,6-Anhydroglucose	0.7945	0.0590
Arabitol	-0.7945	0.0590
Ribitol	-0.2648	0.6121
Aconitate	-0.4414	0.3809
N-Acetyl-L-glutamate_1	-0.5296	0.2798
Glutamine	0.4414	0.3809
4-Hydroxymandelate	0.0000	1.0000
Dihydroorotate	-0.7945	0.0590
O-Phosphoethanolamine	-0.5296	0.2798
Theanine_2	0.2648	0.6121
Glycyl-glycine_1	-0.5296	0.2798
Citric acid + isocitric acid	0.7062	0.1168
Ornithine	0.0000	1.0000

Hypoxanthine	0.0000	1.0000
Citrulline	0.7945	0.0590
1,5-Anhydro-D-glucitol	0.6179	0.1911
Tagatose_2 (or psicose_2)	0.2648	0.6121
Fructose_1	0.3531	0.4924
2-Dehydro-D-gluconate_1	-0.3531	0.4924
Glucose_1	0.2648	0.6121
Sebacic acid	-0.6179	0.1911
Galactose_2	-0.1765	0.7379
Mannitol	-0.1765	0.7379
Lysine (4TMS)	0.9710	0.0012
Galactosamine_1	0.0883	0.8679
Histidine	0.7062	0.1168
Glucuronate_1	0.6179	0.1911
5-Keto-D-gluconate_2	-0.5296	0.2798
Galacturonic acid_1	-0.0883	0.8679
Tyrosine	0.7945	0.0590
Pantothenate	-0.7945	0.0590
N-α-Acetyl-L-ornithine_2	-0.7945	0.0590
Glucarate	0.1765	0.7379
S-Benzyl-L-cysteine_1	-0.5296	0.2798
Xanthine	0.5296	0.2798
Palmitoleate	0.2648	0.6121
Inositol	0.8827	0.0198
Dopa	-0.0883	0.8679
Uric acid	0.6179	0.1911
N-α-Acetyl-L-lysine_2	-0.5296	0.2798
Heptadecanoate	-0.1765	0.7379
Kynurenine	0.0000	1.0000
Cystathionine	0.8827	0.0198
Cystamine	-0.5296	0.2798
Tryptophan	0.8827	0.0198
Elaidic acid	-0.3531	0.4924
Homocysteine	-0.0883	0.8679

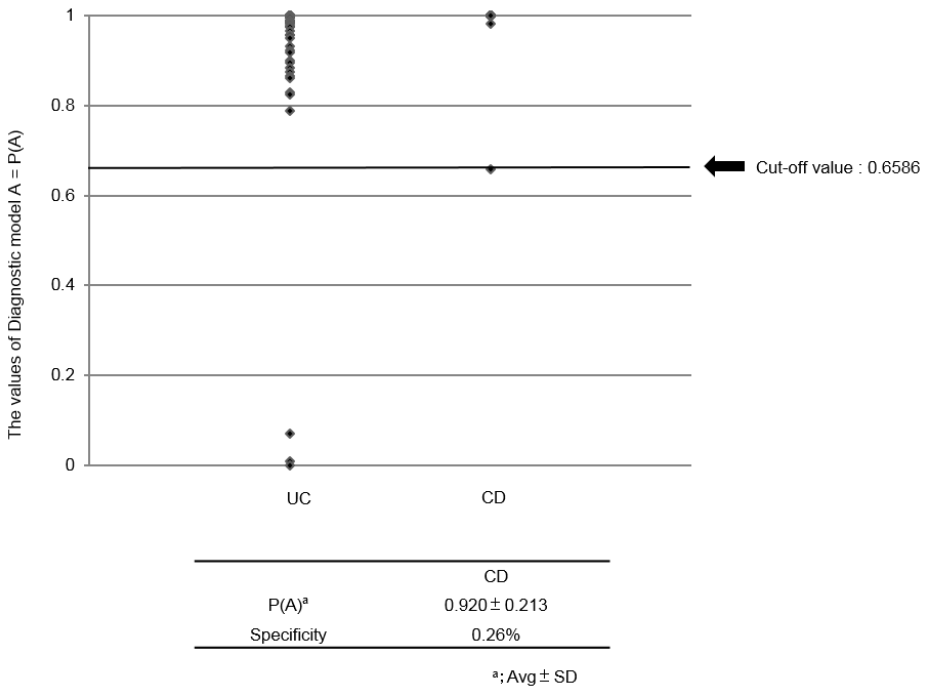
Spearman's rank correlation coefficients (r_s) were calculated for the relationships between daily total calorie including 0 kcal of enteral nutrition; Elental, amino acid formulation (N=6), and all metabolites detected by GC-MS in the sera of CDr. All samples were evaluated about only salicylates but not steroid, immunomoduratory agents and biological agents. As far steroid only, previous medication status of several samples is unclear because of their long disease durations and complicated treatment histories in several hospitals. However, we could check those of steroid within a year in our medical records reliably. Therefore, the sample not medicated with steroid within the range of a year was treated as a non-administrated sample.

Supplemental Figure 1.



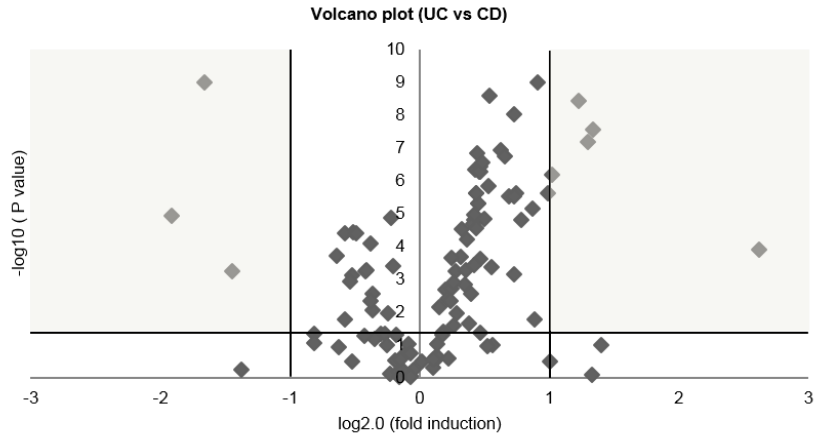
Supplemental Figure 1. Volcano plot of UC versus HV
Volcano plot showing the alterations in the levels of all metabolites between the UC patients and HV according to GC/MS. P-values obtained with the Wilcoxon rank sum test and fold change values are plotted on the y- and x-axes, respectively. Metabolites that met the pre-defined criteria; i.e., a p-value of less than 0.05 and a fold induction value of greater than 2.0 are included in the gray area. We selected 22 metabolites using these criteria.

Supplemental Figure 2.



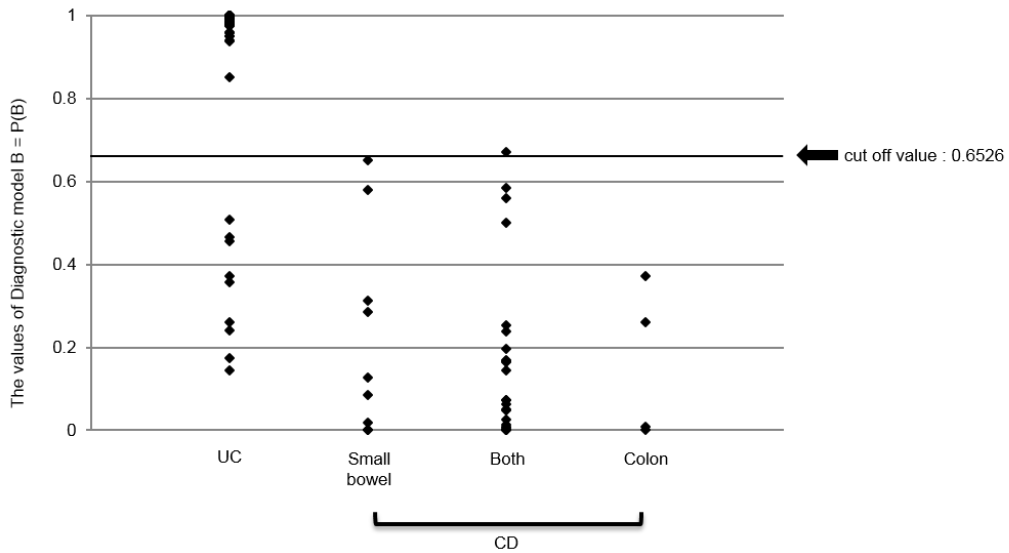
Supplemental Figure 2. Scatterplots of the values assigned by diagnostic model A to the UC patients in the validation set and the CD patients
Scatterplots showing the values assigned by diagnostic model A to UC patients from the validation set and 39 CD patients according to their serum metabolite profiles. The figure shows that almost all of the UC patients were assigned values above the cut-off threshold. However, almost all of the CD patients were also assigned values above the cut-off threshold; i.e., were false-positives. Among the CD patients, the specificity of this model was only 0.26%, so it can not be used to distinguish between UC and CD.

Supplemental Figure 3.



Supplemental Figure 3. Volcano plot of UC versus CD
Volcano plot showing the alterations in the levels of all metabolites between the UC patients and CD patients according to GC/MS. P-values obtained with the Wilcoxon rank sum test and fold change values are plotted on the y- and x-axes, respectively. Metabolites that met the pre-defined criteria; i.e., a p-value of less than 0.05 and a fold induction value of greater than 2.0 are included in the gray area. We selected 9 metabolites using these criteria.

Supplemental Figure 4.

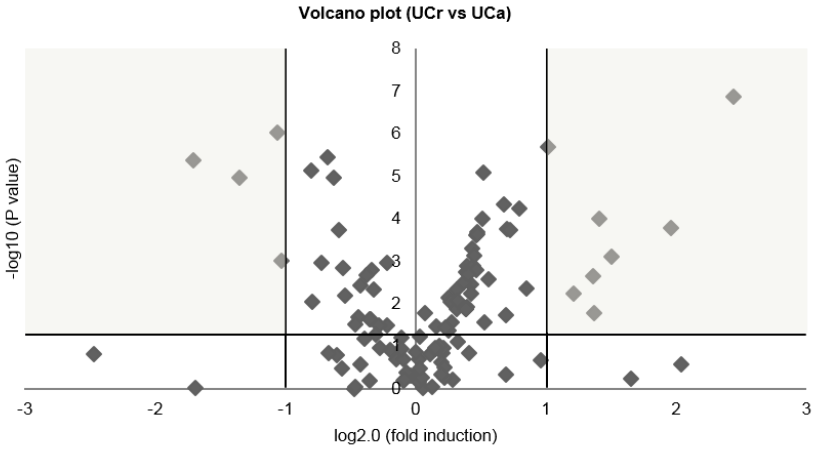


disease location	Small bowel (N=10)	Both (N=25)	Colon (N=4)
P(B)[a]	0.206 ± 0.245	0.161 ± 0.204	0.161 ± 0.186
Specificity	100%	96%	100%

Both; small bowel and colon [a]; Avg ± SD

Supplemental Figure 4. Scatterplots of the values assigned to UC patients from the training set and CD patients by diagnostic model B
The scatterplots show the values assigned to the UC patients in the training set and 39 CD patients by diagnostic model B based on their serum metabolite profiles. The figure shows that almost all of the CD patients were assigned values that fell below the cut-off threshold. The specificity of the model at various CD disease locations; i.e., the small bowel, colon, and both were 100%, 100%, and 96%, respectively. This shows that diagnostic model B was able to discriminate UC from CD, regardless of disease location.

Supplemental Figure 5.



Supplemental Figure 5. Volcano plot of UCr versus UCa
Volcano plot showing the alterations in the levels of all metabolites between the UC patients and CD patients according to GC/MS. P-values obtained with the Wilcoxon rank sum test and fold change values are plotted on the y- and x-axes, respectively. Metabolites that met the pre-defined criteria; i.e., a p-value of less than 0.05 and a fold induction value of greater than 2.0 are included in the gray area. We selected 12 metabolites using these criteria.