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Anthocyanins from purple corn affect gut microbiota and metabolome in inflammatory bowel disease patients under infliximab infusion: the SiCURA pilot study

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ABSTRACT

Nowadays, inflammatory bowel disease (IBD)-patient therapies are mainly based on corticosteroid, thiopurine, and immunomodulator treatments. Patients with active disease, that do not respond to corticosteroid and/or thiopurine treatment, can switch to the usage of the chimeric monoclonal antibody infliximab (IFX). However, to date, no treatment appeared to be conclusive in lowering the incidence of IBD relapses. With the aim to increase the effectiveness of IFX treatment, we combined it with an adjuvant purple corn supplementation enriched in anthocyanins. IBD-patients were enrolled before they underwent to the IFX-infusion, and they were allocated in 2 different study arms. Patients in the intervention-arm followed a dietary supplementation with purple corn water-soluble extract, whereas control patients had a daily consumption of red fruit tea. 16S rDNA gene-sequencing and high-resolution mass-spectrometry metabo-lipidomics analyses were conducted on stool and sera samples, respectively. As a result, the experimental intervention mainly affected the serum metabolome of IBD-patients by decreasing the concentration of specific lipids. Focusing on IBD patient annotated taxa, a significant decrease in *Lactobacillus* and *Bifidobacterium* relative abundances was found. As far as it concerns the ulcerative colitis patient subset, the experimental intervention led to a decrease in *Alistipes* and Erysipelotrichaceae UCG-003 genus abundances and a concomitant *Parabacteroides* increase. On the contrary, after treatment, Crohn's disease patients did not exhibit metataxonomics differences at the genus level. At the end of the treatment that led to a reshaped microbiota community, the gathered data paves the way for the usage of a specifically designed probiotic supplementation as a valuable strategy for IBD-patients under IFX infusion.

1. Introduction

Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD), is a severe, chronic, debilitating and relapsing inflammatory disorder involving the

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gastrointestinal tract. IBD is characterized by a multifactorial etiology, influenced both by genetic and environmental factors, coupled with the constant presence of an immunological and inflammatory component. The pathology onset is featured by a multifactorial background that reveals an inappropriate host's immune response against environmental, luminal, and microbial antigens[1]. In fact, several studies exerted how the strict relationship occurring between the pathology onset/progression and the gut microbes may lead to both an unbalanced microbiota composition and to an altered microbiota relative activity[2]. However, whether the dysbiosis acts as the triggering cause or if it is the downstream result of the chronic intestinal inflammation remains a questionable issue.

The IBD medical treatment is usually thought to target inflammatory mediators directly. As a matter of fact, corticosteroids, thiopurines, and immunomodulators represent the most applied therapies in IBD[3]. Furthermore, based upon the Food and Drug Administration approval in 1998, the delivery of chimeric monoclonal antibodies binding the tumor necrosis factor (TNF), commonly known as infliximab (IFX) infusion, has became a strategy that can be applied as the initial or the advanced therapy. However, due to the huge heterogenicity of IBD-based symptoms, IFX infusions may lead to disease remission and mucosal healing associated with decreased circulating biomarkers of disease activity. These account for fecal calprotectin, fecal lactoferrin, serum C-reactive protein, and fecal S100A12. However, a significant percentage of IFX-treated patients are primary no-responders or, lose over time their response to treatment[4]. Frequently, patients experience disease-free time frames followed by disease relapses[5], which constitute a dramatic event for those of them who achieve disease remission. Literature evidence suggest how the disease relapse may potentially be triggered by persistent dysbiosis, potentially sustained by discrete levels of pro-inflammatory residuals[5].

Epidemiological and experimental data support the existence of a consolidated axis between dysbiosis and IBD. While germ-free mice are protected from IBD development, recent evidence support how germ-free mice exposed to the dysbiotic microbiota of their IBD-mothers, developed a non-completely mature intestinal immune system[6-7]. Even the increased prevalence of IBD in Eastern societies suggests that changes in lifestyle and dietary habits might contribute to disease onset and, for this reason, adjuvant therapies can be useful in avoiding the disease progression *via* microbiota and microbiome modulation[8]. In common practice, nutritional therapy has been adopted in different pathologies and previous studies evaluated its impact in IBD too[9]. Although likely ineffective in the case of patients with active IBD pathology, the nutritional therapy approach would seem the most appropriate adjuvant therapy in supporting dysbiosis resolution and, resulted also helpful in preventing disease relapse. To the best of our knowledge, no previous study concerning IBD evaluated the effect of IFX therapy coupled with a nutritional intervention on gut microbiota. The chosen dietary supplementation was tested in our patient cohort and aimed at supporting the role of oxidative stress as an IBD-associated component[10]. Dietary antioxidants, belonging to the class of polyphenols, act as iron-sequestering agents that reduce the host metal bioavailability or impact the host health status through modulating its gut microbiome metabolism[11]. As a beneficial effect linked to the host, dietary antioxidants promote the reduction of cytoplasmic iron concentration, support the expression of anti-inflammatory/tissue repair genes like secretory leukocyte protease inhibitor (Slpi) and heme oxygenase-1 (HO-1)[12] and, contribute to suppress the inflammatory cascade implied in free radical formation[13].

As far as it concerns the effect on the microbiota, iron is a full-fledged growth-inhibitor factor still poorly investigated[11].

Being aware of these considerations, we designed this pilot interventional study with the aim to address the contribute of an antioxidant-enriched complementary nutrition, that was helpful in improving the clinical response of IBD patients to IFX infusion. In our analyses, we evaluated the effect of the administered treatment in terms of both fecal metataxonomics and serum metabolomics.

2. Materials and methods

2.1 Study design

This work relied on the Soluzioni Innovative per la gestione del paziente e il follow up terapeutico della Colite UlceRosA (SiCURA) study, for which the complete design was previously detailed[14]. The study was approved by the Institutional Review Board (Number 333, 31 July 2019) of the National Institute of Gastroenterology "S. de Bellis" (Castellana Grotte BA, Italy) who verified the accordance with the Declaration of Helsinki guidelines. All patients provided their signed informed consent.

IBD patients were enrolled before the beginning of the IFX infusion and randomized in 2 groups. Patients underwent a total of 5 infusions administered in a time frame of 22 weeks. For the trial, a specific dietary regimen was created by medical personnel using the WinFood software (Medimatica Srl Unipersonale, Colonnella, Italy) and provided to patients allocated in both arms. Detailing differences between arms, the intervention group (NI) had a daily intake of a water-soluble extract produced from *B1 Pl1* purple corn cobs titrated at 4% of anthocyanins, which was packaged in single-dose bags by Sveba Srl (Appiano Gentile, CO, Italy). In details, the purple corn extract was previously characterized[15]. Although the absolute concentrations of single molecules were not quantified, the anthocyanidin identified peaks were relative to cyanidin-glucoside, pelargonidin-glucoside, peonidin-glucoside, cyanidin-malonylglucoside, pelargonidin-malonylglucoside, peonidin-malonylglucoside, cyanidin-dimalonylglucoside[15].

Following the same dietary regimen, the control group (SD) differed for a daily intake of a commercial red fruit tea instead of the purple corn extract. Even the red fruit tea was characterized, and it provided 2 mg gallic acid equivalents of total polyphenols per gram of dry weight (d.w.), of which the esteemed anthocyanin content was 0.5 mg cyanidin 3-glucoside equivalents per gram d.w.

Hence, 34 patients delivered fecal and serum samples at the end of the trial for metataxonomics and metabolomics profiling, respectively.

2.2 Fecal sampling and DNA extraction and sequencing

Feces were collected in sterile stool-containers and stored by patients at 4 °C in the case of fecal sampling carried out on the same day of delivery, otherwise stored at −20 °C by those patients who

collected the feces the day before delivery. After delivery to the laboratory and before exploratory experiments, all samples were stored at –20 °C. Aliquots (1 g) of each stool samples were shipped on dry ice to Genomix4Life Srl (C/O Laboratory of Molecular and Genomic Medicine-Campus of Medicine and Surgery, Baronissi, Salerno, Italy, spin-off of the University of Salerno, Fisciano, Italy) to carry out the 16S rDNA gene amplicon sequencing. The total DNA was extracted from stool with the Invimag Stool kit (Stratec Molecular GmbH, Berlin, Germany) following the manufacturer's instructions, while distilled water was used as extraction negative control. To guarantee the patient privacy, exception made for the assigned ID-number, no clinical or personal information was provided. The 16S rRNA gene amplification was performed using the forward 5′-CCT ACG GGNGGC WGC AG-3′ and reverse 5′-GAC TAC HVGGG TAT CTA ATC C-3′ primers, which targeted the hypervariable V3−V4 regions of the 16S rRNA gene. The sequencing was performed on a MiSeq platform (Illumina, San Diego, CA) in a 2 × 300 paired-end format and procedures followed to obtain libraries and to check the sequence quality were previously detailed[16].

2.3 Bioinformatics analysis

In silico bioinformatics analyses, including read quality check and taxa assignment, relied on the QIIME2[17] microbiome platform (version 2020.8). QIIME plugin q2-deblur was used for the 16S denoising step. The SILVA 138 SSU database (www.arb-silva.de/documentation/release-138/; accessed online: 2023, January) was used to infer the taxonomy starting from the ASV-table.

2.4 Sample collection and high-resolution mass spectrometry (HRMS) analysis of lipids and metabolites

Patients underwent to fasting venous sampling. The samples were collected into 5 mL Vacutainers Gel Tubes (Becton Dickinson, Franklin Lakes, NJ, USA). After blood clot formation and centrifugation, each serum sample was stored at –80 °C in the institute biobank, until the HMRS analysis. Polar metabolites and lipids were extracted as previously described[18]. The HRMS analyses were performed in flow injection analysis (FIA) by using an Ultimate 3000 UHPLC (Thermo, Bremen, Germany) coupled to a SolariX XR 7T (Bruker Daltonics, Bremen, Germany). Flow rate was set to 10 μL/min, and increased in the washing step to 300 μL/min. The instrument was tuned with a standard solution of sodium trifluoroacetate (NaTFA). Mass spectra were recorded in broad-band mode in the range *m/z* 150–1 500 for lipids, whereas *m/z* 90–800 was used for polar metabolites, with a 10 ms accumulation of ions, 64 scans were acquired using 4 million data points (4 M), with an approximate resolution of 400.000 at *m/z* 400. Drying gas (nitrogen) was set at 2 mL/min, with a drying temperature of 150 °C. Funnel amplitude was set to 90 V (polar metabolites) or 100 V (lipids), transfer was set at 0.6 MHz, and TOF 0.7 s. Both positive and negative ESI ionization were employed in separate runs. Five replicates of each injection were carried out. The instrument was controlled by Bruker FTMS Control (Bruker). FIA-FT-ICR data extraction, alignment, filtering, and annotation was performed with Metaboscape 2021 (Bruker) as previously detailed[19].

2.5 Statistical analysis

Range scaled normalized matrices by means (*Z*-scores) were used for principal component analysis (PCA) analysis and for the clustering based on complete algorithm of Euclidean distances. These were computed in R-environment using the FactoMineR-package (Multivariate Exploratory Data Analysis and Data Mining) version 2.4 available in the CRAN repository (https://cran.r-project.org/web/packages/FactoMineR/index.html). Significant features based on false discovery rate (FDR) correction, fold change (FC), and *t*-tests statistical analyses, have been graphically rendered as a volcano plot by using EnhancedVolcano R-package in MetaboAnalyst R-software (version 2.0.0). For features reaching the significancy (FC > 3 and *P*-value < 0.05) the relative boxplots were also graphically elaborated. Partial least square differential analysis (PLS-DA) was run in R-environment by using PLSR. Anal in MetaboAnalystR-software (version 2.0.0). The multivariable associations between treatment and 16S rRNA gene data abundances at genus levels and metabolomics variables, were computed using the general linear model implemented in MaAsLin2 R-package (https://huttenhower.sph.harvard.edu/maaslin). The β-diversity was inspected by running a PERMANOVA on Bray-Curtis and Jaccard indices and was obtained by using the specific QIIME2 conda plugins. Correlations were computed by using the R "cor.test" package (https://www.rdocumentation.org/packages/stats/versions/3.6.2/topics/cor.test) by setting the Spearman's rank as the correlation test to be used.

3. Results

3.1 Baseline characteristics

Although the entire cohort description was previously detailed[14], being here used a subset of the SiCURA cohort, we specifically accounted for patients with a broad heterogeneity in terms of age and gender. According to the IBD-diagnosis, the present study accounted for 16 CD-diagnosed and 18 UC-diagnosed patients. Following the "Montreal Classification", the CD phenotype was inflammatory in 46.67% of patients, structuring in 40.00%, and penetrating in 13.33%. Moreover, the ileal disease was diagnosed in 13.33% of CD patients, the colic phenotype was found in 6.67%, while the 80.00% of CD patients had an ileocolic phenotype. Among UC patients, 13.33% had a distal UC while the 66.67% had a pancolitis disease phenotype. At the enrolment, ongoing steroid therapy was assessed in the 50.00% of patients.

3.2 Data-fusion and sample inspection

After merging metataxonomics and metabolomics data into a unique dataset, the cohort was inspected based on PCA to evaluate the occurrence of possible confounding factors. The first 3 principal components (PC) were plotted (PC1: 22.1%, PC2: 10.2%, and PC3: 8.2%; Fig. 1A) and this allowed for assessing how, due to significantly different profiles based on high negative values of PC1 or PC3, 3 IBD-patients allocated in the SD-arm were in fact outliers. A dendrogram accounting for Euclidean distances and Ward's clustering were used to graphically support the exclusion of the 3 samples (i.e., 03IBD.SD, 48IBD.SD, and 51IBD.SD; Fig. 1B).

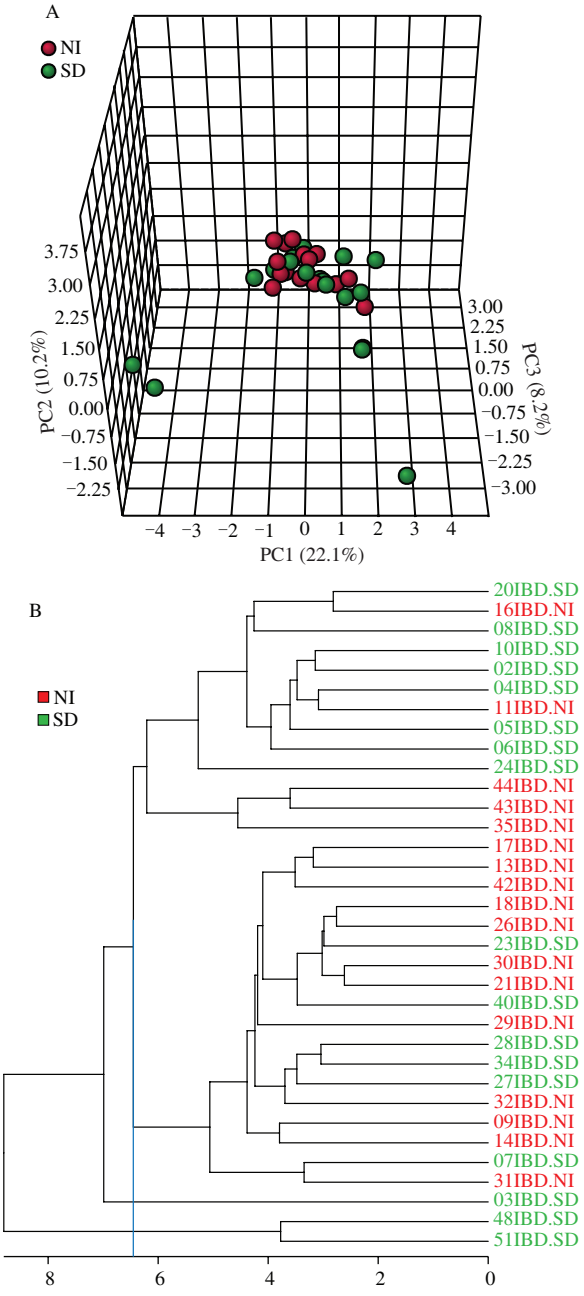

Fig. 1 (A) PCA from merged metataxonomics and metabolomics results. Taxa and metabolite data were obtained from biological samples (stool and serum, respectively) delivered by IBD volunteers after following the standard diet (SD) or the NI with antioxidants. (B) IBD patient clustering based on Euclidean distances. Metataxonomics abundances and metabolomics concentrations from IBD volunteers were both used to obtain the clustering based on the complete algorithm of Euclidean distances.

3.3 *Effects of antioxidant supplementation in IBD patients*

The α- and β-diversity metrics did not revealed differences between IBD patient group who underwent SD or NI treatments. Specifically, α-diversity median values for the Faith's PD index in SD and NI groups were 9.18 (IQR: 7.56–10.33) and 9.89 (IQR: 8.25–11.84), respectively. The β-diversity, inspected by running a PERMANOVA on Bray-Curtis and Jaccard indices, showed for all taxonomic levels the absence of statistically significant differences between SD and NI IBD-groups (Table S1).

The effect of the present nutritional intervention (NI) was evaluated by profiling the gut microbiota and sera metabolite compositions in a cohort of IBD patients. Specifically, metataxonomics genera with a relative abundance higher than 0.1% were combined with serum metabolite concentrations. Compared against IBD-patients under SD, serum lysophosphatidyl choline (LPC) 16:1 concentration increased in NI (Fig. 2; Fig. S1). Oppositely, 7 metabolites, i.e., 2-ethylidracrylic acid, triacylglycerols (TG) 50:4, TG 52:4, TG 54:5, γ-glutamyl glutamine, deoxycholic acid glycine conjugate, and acylcarnitine (CAR) 8:0, were significantly decreased in NI. Based on genus relative abundance, the NI treatment determined a significant decrease in *Lactobacillus* and *Bifidobacterium* relative abundances. Five additional features reached the significance ($P < 0.05$; Fig. 2) but were excluded because of the fold change occurring between the SD and NI arms (lower than the set threshold).

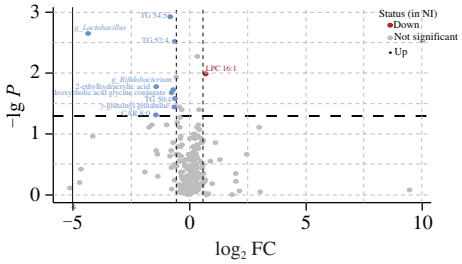

Fig. 2 Volcano plot showing significantly different features ($P < 0.05$ and fold change > 3) obtained from fecal metataxonomics and serum metabolomics in IBD volunteers under SD or after NI with antioxidants. Taking into account the NI treatment, increased and decreased features have been graphically rendered as red or blue dots, respectively. Not significant features (ns) have been rendered as grey dots.

3.4 *Effect of antioxidant supplementation on the CD patients*

To test the multivariable association between taxa abundances and metabolomics variables the MaAsLin2 regression model was applied. The model finds out the differences existing between SD-CD and CD-NI groups. 17 features were significantly affected by the NI administration and mainly concerned metabolomics features (Table 1). Specifically, 4 different metabolites were positively associated to NI, while 12 metabolites and 1 taxon (*Bifidobacterium*) resulted to be negatively associated.

To define which set of features allowed for a better discrimination of CD patients under SD, with respect to those who completed the NI treatment, a PLS-DA was carried out. Although 2 CD-NI samples had positive values relative to the first component, the PLS-DA score-plot weighted up on the 95% of the confidence interval (95% CI) (Fig. 3A) supported the separation of CD-SD and CD-NI clouds. Exception made for 1 taxon (*Bifidobacterium*), the top 15 variable importance in projection (VIP) items exclusively account for metabolites with relative scores ranging between 1.6 and 3.0 (Fig. 3B). Importantly, 9 out of these 15 high scored VIPs mainly supported the clustering of CD-NI samples, while the remaining five metabolites and *Bifidobacterium* supported the CD-SD cloud clustering. Overall, the PLS-DA results supported a general decrease of glycerophospholipids (phosphatidylcholines (PCs), diacyl-glycerophosphate (PA), LPC and lys-phosphatidylserine (LPS)) in CD-SD patients. Oppositely, the same metabolite pattern was

increased in CD-NI patients. Noteworthy, some members of the glycerophospholipid class, such as PCs, LPC (16:0), LPS (21:0), and PA (42:3) showed the highest VIP scores (Fig. 3B).

Table 1
Effect of NI with antioxidants in CD patients.

Feature	Trend	N.not.0	*P*-value
PA 42:3	↑	14	0.003
PC 38:5	↑	14	0.002
PC O-32:0	↑	14	0.021
ST 29:2;O_2	↑	14	0.003
6-(6-Aminohexanamido) hexanoic acid	↓	14	0.034
CE 16:0	↓	14	0.039
CE 19:0	↓	14	0.038
Cer 42:1;O_2	↓	14	0.038
FA 18:1	↓	14	0.032
FA 18:2	↓	14	0.022
FA 19:1;O_2	↓	14	0.033
L-Glutamic acid	↓	14	0.015
ST 24:2;O_4	↓	14	0.020
TG 42:0	↓	14	0.041
TG 54:5	↓	14	0.042
TG 63:5	↓	14	0.013
Bifidobacterium	↓	11	0.014

Note: Only significant associations (*P*-value < 0.05) between NI and variables from fecal metataxonomics and serum metabolomics (both listed in the "feature" column) have been shown. Features significantly modulated by NI (compared with CD-SD) were flagged with upwards and downwards arrow symbols "↑" or "↓", and indicated an increasing or decreasing trend, respectively. N.not.0 stands for the number of CD patients having the variable different from 0.

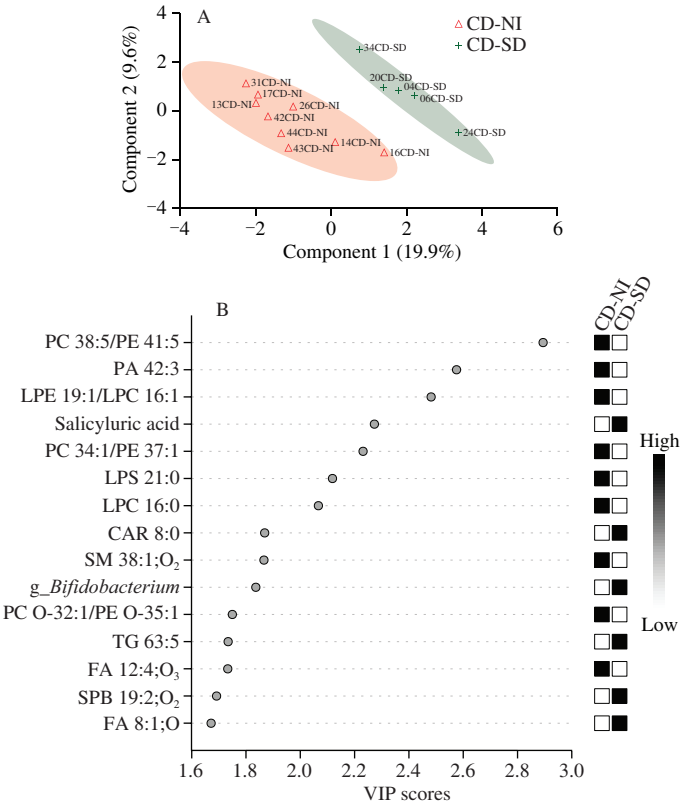

Fig. 3 PLS-DA in CD subset. (A) Score plot of CD volunteers who delivered samples (feces and blood) after following the SD (green cloud) and the NI (red cloud) with antioxidants. Clouds were weighted up on the 95% CI. (B) VIP scores, ranging from black (higher score) to white (lower score). Variables included both the concentration of serum metabolites and the relative abundance of the gut taxa.

3.5 Effects of antioxidant supplementation on the UC patients

The MaAsLin2 regression model was run to evaluate differences existing between UC patients under SD and those who concluded the NI treatment. 13 features resulted to be significantly affected by the NI treatment and, most of them (12 out of 13) emerged from the metabolomics profiling (Table 2). Specifically, 1 metabolite (CAR 8:0) and 1 taxon (*Parabacteroides*) exhibited an increasing trend after the NI supplementation, whereas eleven metabolites were significantly decreased.

Table 2
Effect of NI with antioxidants in UC patients.

Feature	Trend	N.not.0	*P*-value
Parabacteroides	↑	11	0.021
CAR 5:0	↑	17	0.038
CE 20:5	↓	17	0.024
FA 20:4	↓	17	0.049
LPC 18:1	↓	17	0.005
MG 16:0	↓	17	0.009
PC 34:1	↓	17	< 0.001
PC 34:2	↓	17	0.009
PC 36:4	↓	17	0.010
PC 38:5	↓	17	0.001
SM 34:2	↓	17	< 0.001
ST 24:2;O_4	↓	17	0.044
TG 50:1	↓	17	< 0.001

Note: Only significant associations (*P*-value < 0.05) between NI and variables from fecal metataxonomics and serum metabolomics analyses (both listed in the "feature" column) have been shown. Features significantly modulated by NI (compared with UC-SD) were flagged with upwards and downwards arrow symbols "↑" or "↓", and indicated an increasing or decreasing trend, respectively. N.not.0 stands for the number of CD patients having the variable different from 0.

A further PLS-DA was carried out to define which features had an impact on UC patients that followed the NI treatment. The PLS-DA score-plot (Fig. 4A) based on the 95% CI of UC-SD and UC-NI samples revealed 2 separate clouds without any overlap. This time, the top 15 VIPs accounted for features with a score higher than 1.8 (Fig. 4B) and, principally relied on metabolomics variables. In fact, only 3 microbial genera were included in the top 15 VIPs (i.e., *Parabacteroides*, *Alistipes*, and Erysipelotrichaceae UCG-003). Based on VIP score ranking, the first 6 features were metabolites useful in differentiating the UC patients who followed SD. The following 6 ranked features (7^{th}–12^{th}) accounted for 3 metabolites and 3 bacterial genera matching UC-NI patient profiles. Evenly, the last 3 features (13^{th}–15^{th}) are all statistically linked to the UC phenotype under SD treatment instead. Among the significant metabo-lipidomics features, the PLS-DA highlighted an important modulation of TG, whose levels resulted to be negatively modulated by the NI treatment.

3.6 Gut microbiota and serum metabo-lipidome statistical correlation

In order to evaluate the simultaneous effect of NI on gut microbiota and serum metabo-lipome we first made use of a Spearman rank correlation test run on the complete matrix including both taxa and metabolites. In a second step, we searched statically significant correlations (correlation factor |0.5|, and $P < 0.05$) dealing

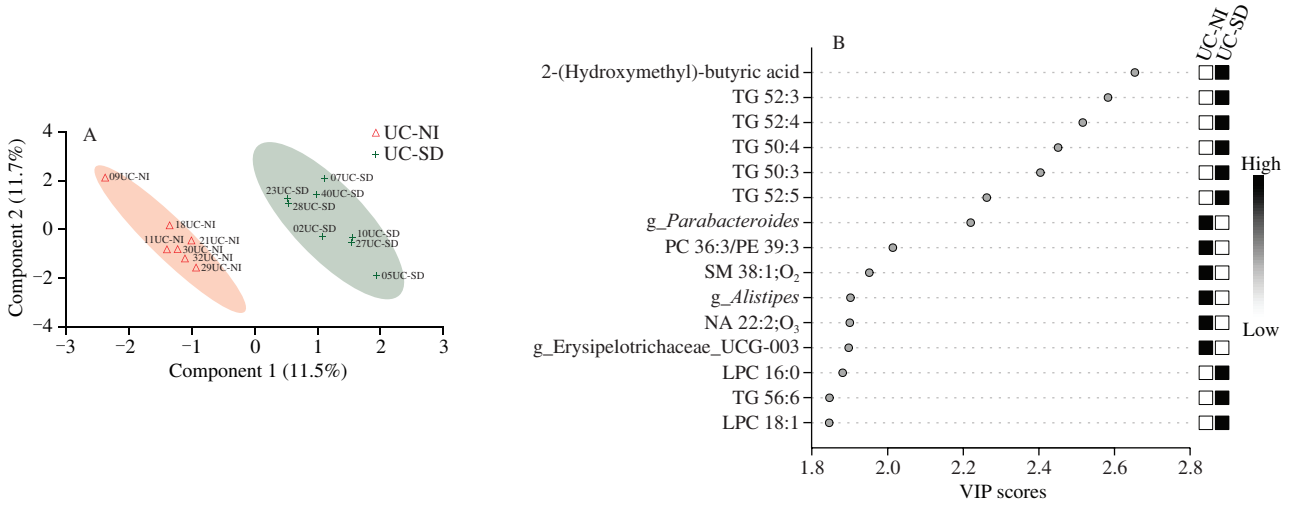

Fig. 4 PLS-DA in the UC subset. (A) Score plot of UC volunteers who delivered samples (feces and blood) after SD (green cloud) and NI (red cloud) with antioxidants. Clouds were weighted on the 95% CI. (B) VIP scores, ranging from black (high score) to white (low score). Variables included both the concentration of serum metabolites and the relative abundance of the gut taxa.

with taxa emerged as biomarkers in the previous reported analyses (Table S2).

Among statistically significant positive correlations, *Lactobacillus* taxa correlated with salicylglycine, *Bifidobacterium* with SM 42:3;O2, salicylglycine, and glycyldeoxycholate, while *Alisptipes* with *p*-cresol sulfate and cholesteryl arachidonate (CE 20:4).

Among negative correlations only Erysipelotrichaceae UCG-003 correlated with 9-hydroxy-5*Z*-nonenoic acid. Noteworthy, *Parabacteroides* did not show any statistically significant correlation.

4. Discussion

The burden of IBD is rapidly growing worldwide and its impact on the healthcare systems[20] is pushing towards the need to timely identify efficient treatments able to reverse this tendency. Nowadays, although biological strategies are mainly based on the infusion of anti-TNF, the 50%–80% of UC and about the 67% of CD patients suffer from clinical relapses[21-22]. Therefore, the development of new and more efficient drugs is one of the most intriguing research areas, while marginal attention has been paid to adjuvant strategies accounting for nutritional-derived interventions. In this field, we recently published a single-center study that focused the attention on the efficacy of purple corn extract useful in reducing circulating inflammation markers in IBD patients whose diet was supplemented by adding an adjuvant during the IFX infusion[14]. In fact, C-reactive protein (CRP), ceruloplasmin, and TNF levels were significantly lower in the group receiving this experimental extract. Importantly, to complete our overview, we here evaluated the impact of this innovative intervention on the composition and activity of the gut microbiota.

From a microbiologic point of view, gut dysbiosis is a common hallmark of IBD. This is sustained by the evidence coming from human and mouse model studies that both reveal changes in the relative abundance of several bacterial taxa colonizing the intestine[23]. However, no previous studies explored gut microbiota modifications in the of IBD patients under anti-TNF-based therapies combined with antioxidant-enriched nutritional regimens. To explore this key issue, the present single-center study accounted for a purple corn extract supplementation that was simultaneously administered to patients starting the IFX infusion[14]. This allowed us to address how the NI supplementation determined beneficial clinical outcomes and led the groundwork for the purple corn nutritional supplementation usage as a suitable adjuvant therapy to be delivered during the induction of disease remission. To assess microbiota and metabo-lipidome profiles, stools and sera from our cohort patient subsets were gathered and analysed. Although these data refer to a limited number of samples, and although they are based on a single center of enrolment, the analysed IBD patients under the NI administration showed a significant decrease of *Lactobacillus* and *Bifidobacterium* abundances. The still open debate existing on the involvement of both these taxa in IBD requires further specific in-depth analysis to be solved. In fact, *Lactobacillus* and *Bifidobacterium* are commonly used as probiotics[24-25] and their effectiveness in ameliorating IBD symptoms is supported by clinical trials[26]. On the other hand, cross-sectional studies revealed an increased abundance of *Lactobacillus*[27-28], *Bifidobacterium*[29], or both the genera[30] in the active phase of IBD. Thus, in line with literature evidence supporting the great interspecies heterogeneity and the niche-specialized adaptation of probiotics to different pathognomonic conditions[31], the gut microbiota of IBD patients cannot be associated with a general fingerprint of probiotic species. When untreated UC models were used to evaluate the relationship between colitis and dietary antioxidants, these genera showed controversial behavior[28]. *Bifidobacterium* was harbored by an antioxidant enriched chow, whereas *Lactobacillus* decreased after administering the same chow during active colitis. This data rely upon UC models that have been already deeply explored, whereas studies involving CD models (SAMP1/YitFc mouse strain) are less advanced and not yet conclusive[32]. Reasonably, more studies involving health-promoting bacteria specifically targeting the IBD-treatment are still needed. In the correlation analysis between these two health promoting bacteria and the detected metabolites, both the taxa were positively correlated with salicylglycine, a conjugated linoleic acid (CLA). Both *Lactobacillus* and *Bifidobacterium* have been found to be involved in the bioconversion of linoleic acid, whose derivatives, i.e. CLA, exhibited anti-inflammatory, anti-hypertensive, and anticarcinogenic properties[33]. A recent publication highlighted a positive interaction between *Lacticaseibacillus rhamnosus*

and salicylic acid able to exert a cytotoxic effect on human colon cancer cells[34].

In our experimental set, gut microbiota fingerprint mainly accounted for differences in relative abundance of specific genera. While the trio made by *Alistipes*, Erysipelotrichaceae UCG-003, and *Parabacteroides* contributed to identify NI-treated patients within the UC subgroup in PLS-DA, only the last-mentioned taxon was confirmed to be strict associated with NI treatment when the MaAsLin regression model was run. This result reflects previous findings on antioxidant supplementation with resveratrol[35], anthocyanins[36] or *Vitis davidii* Foex seed extract[37], that are all in line in describing an increase of *Parabacteroides*. Noteworthy, the synthesis of short chain fatty acids (SCFA) via arabinoxylans metabolism was previously determined in *Parabacteroides* genome[38]. Therefore, considering the decreased abundance of *Lactobacillus* and *Bifidobacterium* in our patients, it is tempting to speculate about a possible positive cause-effect connection between the NI supplementation and SCFA synthesis pathways. Moreover, in a previous pediatric study, *Parabacteroides* was detected in the UC-patient remission group that underwent fecal microbiota transplantation (FMT) compared to ongoing UC-child group, and its presence in feces was detected to be shared with donors[39]. On the other hand, as far as it concerns *Alistipes* a recent large study[40] explored the IBD microbiota in terms of metagenomics and metatranscriptomics data both useful in assessing a negative correlation between this taxa and the disease severity. *Alistipes* contributed, almost alone, to the methylerythritol phosphate (MEP) pathway transcription that pivotally supported the immune response through human γδ T cells activation, allowing for a better response against inflammation in IBD patients[41]. The abundance of *Alistipes* is negatively affected by high fat diet consumption in mice[42]. Oppositely other evidence, in line with our results, found a positive correlation between *Alistipes* and arachidonic acid[43]. However, the same study focused on extracellular antibiotic resistance genes, defined *Alistipes* among the pattern of opportunistic pathogenic bacteria. Because of this still open debate, the role of this genus in IBD needs to be further explored.

Looking at the NI treatment results, the metabo-lipidomics profiling revealed the modulation of numerous metabolite classes, in particular lipids. Considering that CD patients reported alterations in lipid profiles as a hallmark of the IBD[44], Tefas et al.[45] enlightened an important change in glycerophospholipid levels and considered this decrease as one of the most contributing factors. Moreover, decreased glycerophospholipids were also involved in chronic fatigue, as well as in low remission rates[46]. Our results, instead, highlighted how the NI increased the concentration of various glycerophospholipids subclasses (PC, LPC, LPS, PA). Similar outcomes also emerged when exclusive enteral nutrition was administered and these data revealed, besides the improvement in clinical response, the increase of glycerophospholipids levels in plasma of CD patients[47]. On the other hand, UC-SD patients were here characterized by high levels of numerous TG. This molecules were previously reported to be increased or unchanged in other IBD cohorts despite the treatment[48], while the NI-treatment here used in combination with IFX-infusion allowed for decreasing their concentrations. The absence of concordant results between CD and UC equally treated with the NI highlights the difficulty in determining a unique NI-based fingerprint. However, considering the complexity of factors affecting metabolome, we might speculate on glycerophospholipids as potential markers useful in discriminating IBD patients during active inflammation and remission phases.

5. Conclusion

Polyphenol adjuvants combined with IFX infusion may support disease remission in IBD patients even though the setting of a larger cohort study is of major importance. The present study highlighted the need for a further exploration of the nutritional adjuvants-microbiome data as a key point for the definition of innovative therapies. Based on our outcomes, the effectiveness of nutritional management in IBD remission maintenance and relapse prevention should be firstly considered. Moreover, looking at the decrease in *Bifidobacterium* and *Lactobacillus* abundance, the hypothesis of an *ad hoc* defined probiotics supplementation (during or after treatment) needs to be deeply investigated.

Conflict of interest

The authors declare no conflict of interest.

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Ethical Considerations

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (Number 333, 31 July 2019) of the National Institute of Gastroenterology IRCCS "S. de Bellis", Institute of Research, Castellana Grotte (BA), Italy. Informed consent was obtained from all subjects involved in the study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2023.9250036.

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