Modulation of gut microbiota through nutritional interventions in Behçet's syndrome patients (the MAMBA study): study protocol for a randomized controlled trial

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Study protocol

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Abstract

Background

Behçet's syndrome (BS) is a systemic inflammatory disorder of unknown etiology, characterized by a wide range of potential clinical manifestations. Recent evidences suggest that the gut microbiota (GM) in BS shows low biodiversity with a significant depletion in butyrate producers. The aim of the present project is to investigate whether a dietary intervention could ameliorate the clinical manifestations and modulate the GM of patients with BS.

Methods

This is a randomized, open, cross-over study involving 90 BS patients who will be randomized to follow a 3-months dietary profile with either: lacto-ovo-vegetarian diet (VD), Mediterranean diet (MD) or Mediterranean diet supplemented with butyrate (MD-Bt). The VD will contain inulin and resistant starch-rich foods, eggs and dairy, in addition to plant-based food, but will not contain meat, poultry or fish. The MD will contain all food categories and will provide 2 portions per week of fish and 3 portions per week of fresh and processed meat. The MD-Bt will be similar to the MD but supplemented with 1.8 g/day of oral butyrate. The three different dietary patterns will be isocaloric and related to subject’s nutritional requirements. Anthropometric measurements, body composition, blood and fecal samples will be obtained from each participant at the beginning and at the end of each intervention phase. The primary outcomes will be represented by the change from baseline of the BS gastrointestinal and systemic symptoms. Changes from baseline of GM composition, SCFA production, inflammatory and antioxidant profile will be considered as secondary outcomes.

Discussion

BS is a rare disease, and, actually, not all the available treatments are target therapies. A supportive treatment based on dietary and lifestyle issues, able to restore immune system homeostasis, could have a high impact on costs sustainability for the treatment of such a chronic and disabling inflammatory condition.

Introduction

Behçet's syndrome (BS) is a systemic inflammatory disorder characterized by a wide range of potential clinical manifestations with no gold-standard therapy. BS is usually not a life-threatening condition; however, a higher mortality can be associated with vascular-thrombotic and neurological manifestations [1, 2]. Although BS pathogenesis is currently unknown, it has been recently classified among systemic vasculites [3]. Gut microbiota (GM) is recognized to deeply influence our metabolic and immunological health, and specific dysbiotic (i.e. perturbed) GM configurations have been indicating a fascinating link between intestinal microbes and health status. A recent study from our group showed, for the first time, that a peculiar dysbiosis of the GM ecosystem is present also in patients with BS, corresponding to

specific changes in the profiles of short-chain fatty acid (SCFA) production [4]. In particular, the GM layout in BS showed low biodiversity, in line with several other chronic disorders [5–7]. Moreover, a significant depletion of well-known butyrate producers, i.e. Roseburia and Subdoligranulum, and a consistent decrease in butyrate production in BS patients was demonstrated. Butyrate - which is the preferred fuel for colonocytes - is able to induce T regulatory cell differentiation via several mechanisms, so the butyrate impairment in BS patients could favor a reduced T regulatory cell-mediated control, thus promoting a powerful immuno-pathological T cell response [8] as suggested by the higher Th1/Th17 cells percentage at gut mucosal level observed in BS patients [9].

In this context, over the last years, growing evidence suggested that high-fiber dietary patterns are able to promote a more favorable GM profile, and are key mediators of microbial diversity [10, 11]. Lacto-ovo-vegetarian diet is characterized by abstention from consuming meat and meat products, poultry, seafood and flesh from any other animal and by a large amount of plant-derived foods. This dietary pattern has been largely demonstrated to be beneficial for both patients with an established disease and for subjects with traditional risk factors for chronic diseases [12]. Indeed, dietary patterns rich in plant-based food have been found to promote a healthier GM profile, due to the high amount of dietary fiber. These fermentable substrates are sources of metabolic fuel for GM fermentation that, in turn, results in end-products - mainly SCFAs - that are key microbial metabolites with a multifactorial role on the host health [13]. In particular, it has recently been demonstrated that high adherence to a lacto-ovo-vegetarian diet - including high intake of non-refined cereals, fruit, vegetables and legumes - is associated with a beneficial GM profile, with enrichment in fiber-degrading bacteria and increase in fecal SCFAs [14]. In a similar way, other dietary patterns rich in plant-based food have been shown to modulate GM dysbiosis, by supporting the recovery of a balanced microbial community of health-promoting SCFA-producing members with the decrease in pro-inflammatory pathobiont groups [15, 16]. Furthermore, current evidence indicates that the consumption of certain fibers - such as inulin and resistant starch - leads to specific GM rearrangements with consequent butyrate hyperproduction in humans [17]. All these findings let us hypothesize that the adherence to a controlled dietary profile enriched in substrates with potential for butyrate production, may select for butyrate-producing bacteria – such as Roseburia spp. and Faecalibacterium prausnitzii – so reversing the pro-inflammatory dysbiosis observed in BS.

Thus, the aim of the present project is to conduct an open, randomized, cross-over dietary intervention trial in order to investigate whether a lacto-ovo-vegetarian diet enriched in substrates with potential for butyrate production or a Mediterranean diet supplemented with butyrate could be beneficial for the GM and for the amelioration of the clinical manifestations and disease severity of BS patients.

Methods: Participants, Interventions And Outcomes

Study design

The randomized, open, cross-over clinical trial will be conducted at the Careggi University Hospital, Florence, Italy. A cross-over design will be implemented to allow comparison of a lacto-ovo-vegetarian

diet (VD), Mediterranean diet supplemented with butyrate (MD-Bt) and Mediterranean diet without any supplement (MD), as control, within the same individual. Participants will act as their own controls in cross-over studies, so individual differences will be controlled for, making the error variance smaller and subsequently reducing the sample size required to find a significant effect due to increased statistical power. The study design follows the SPIRIT guidelines (see Figure 1 and supplementary file #1).

Eligibility criteria

Inclusion criteria include diagnosis of BS, age 18-65 years, willing to give informed consent, and willing to participate in a study where one of the proposed dietary profile is a vegetarian pattern.

Exclusion criteria include pregnancy or lactation, concomitant presence of serious illness or unstable condition (other immune-mediated or autoimmune diseases, including inflammatory bowel diseases); chronic viral infections; malignancies, recent myocardial infarction, chronic liver disease, current or recent (past 6 months) participation in weight loss treatment program or use of weight loss medication, and adoption of a vegetarian diet for the past 3 months. Antibiotic, prebiotic or probiotic use in the past 3 months.

Interventions and participant timeline

This clinical randomized study will use a cross-over design with three intervention periods and two wash-out. After a 2-week run-in period, the eligible participants (N=90) will be randomly assigned to follow a 3-months dietary profile with either: VD, MD or MD-Bt. The VD will contain inulin and resistant starch-rich foods, eggs and dairy, in addition to plant-based food, but will not contain meat, poultry or fish. The MD will contain all the food categories and will provide 2 portions per week of fish and 3 portions per week of fresh and processed meat (1 of which fresh or processed red meat). The MD-Bt will be similar to the MD but supplemented with 1.8 g/day of oral butyrate. The three different dietary patterns will be isocaloric and related to the subject's nutritional requirements and will derive about 50-55% of energy from carbohydrates, <30% from fats, and 15-20% from proteins. Participants will prepare their meals or eat at restaurants. Alcoholic beverages will be limited to two per day for men and one per day for women.

Study design is depicted in Figure 2. There will be six clinical evaluations of the study population: at baseline, before starting the nutritional interventions (T0), three months after the onset of the first nutritional intervention (T1), at the end of the first wash-out period, lasting 1.5 month, when patients will be allowed to come back to their normal eating habits and at the onset of the second intervention (T2), at 7.5 months after the onset of the study and at the end of the second nutritional intervention (T3), at the beginning of the third nutritional intervention and at the end of the wash-out period, lasting again 1.5 month (T4), and at the end of the third nutritional intervention (T5).

During the baseline visit, participants will be instructed on the objectives and methods of the clinical trial and will be asked not to alter their physical exercise habits during the study. Anthropometric measurements, body composition, blood, urine and stool samples will be obtained from each participant

at the beginning and at the end of each intervention phase. BS clinical manifestations and disease severity, especially for symptoms involving gastrointestinal system, will be assessed using a modified version of two questionnaires originally computed for Inflammatory Bowel Disease patients: The Global Assessment of Improvement Scale (GAI) and The Symptom Severity Scale (SSS). Disease activity will be assessed by the validated Behçet Disease Current Activity Form (BDCAF), which consists of objective and subjective items, and considers the symptoms present over the 4 weeks prior to assessment. Each participant will have to complete a 3-day dietary record (two weekdays and a weekend day) before starting and a dietician will analyze all 3-day dietary records using a country-specific food-nutrient database.

Outcome measures

Primary outcomes

Primary outcomes will be assessed through validated questionnaires, in order to analyze BS progression. Used metric will be changes in means from the beginning to the end of each dietary intervention. They will include:

- *Severity of gastrointestinal symptoms*, assessed by the SSS modified form. The SSS is a multidimensional rating scale assessing overall symptoms' severity on a Visual Analogue Scale (VAS). An overall score will be calculated from six items: pain severity, pain frequency, abdominal bloating, bowel habit dissatisfaction, abdominal heaviness, and life interference. The modified SSS ranges from 0 to 600, with higher scores meaning more severe symptoms.
- *Improvement of gastrointestinal-related BS symptoms*, assessed by the GAI modified form. The GAI will assess BS improvement of symptoms using a 7-point scale, with higher scores meaning an improvement of the symptoms. The severity of abdominal pain, severity of abdominal distention, satisfaction with bowel habits, severity of headache, severity of exhaustion, severity of nausea, attention disorder, muscle/joint pain, and quality of life will be investigated in response to the following question: “Compared to the way you felt before you entered the study, have your symptoms over the past 7 days been: 1) “Substantially Worse”, 2) ”Moderately Worse, 3) ”Slightly Worse”, 4) ”No Change”, 5) ”Slightly Improved”, 6) ”Moderately Improved” or 7) “Substantially Improved”.
- *Disease severity of BS*, assessed by the BDCAF. The BDCAF will assess the presence of oral and genital ulceration, skin, joint and gastrointestinal involvement, presence of fatigue and headache with a 5-point scale according to the duration of symptoms, with 0 meaning no symptoms and 4 meaning symptoms for 4 weeks. The presence of eye, large vessel or central nervous system (CNS) involvement will be documented with "yes/no" answers. In addition, patients will be asked to rate on a 7-point scale how active they felt. Similarly, clinicians will complete a 7-rating scale to assess their opinion of overall disease activity, with lower scores representing better outcomes.

Secondary outcomes:

Secondary outcomes will be measured in blood and stool samples. Used metric will be changes in means from the beginning to the end of each dietary intervention. Regarding stool samples, we will evaluate:

- *Changes of GM composition from baseline*, assessed by 16S rRNA gene-based next-generation sequencing on Illumina MiSeq platform. Total microbial DNA will be extracted from feces using the repeated bead-beating plus column method, as previously described [18]. The V3 and V4 hypervariable regions of the 16S rRNA gene will be sequenced following the Illumina protocol for 16S Metagenomic Sequencing Library Preparation.
- *Fecal SCFA change from baseline*, assessed by Gas Chromatography - Mass Spectrometry system (GC-MS). The metabolomic analysis of fecal waters will be performed after sample preparation involving solid phase microextraction (SPME), followed by GC-MS analysis to detect the volatile metabolites [4].
- *Inflammatory profile change in feces from baseline*, assessed by cytofluorimetric approach. In particular, superficial (CD3, CD4, CD8) and intracitoplasmic (transforming growth factor (TGF)-ß, interferon (IFN)-γ, interleukin (IL)-4, IL-9, IL-10, IL-17, IL-10, IL-22, FoxP3) cell marker analysis will be measured. The cells will be analysed by a BD FACScan Cytofluorimeter using the Diva software (BD Biosciences, San Jose, USA). The concentration of cytokines, chemokines and growth factors, including interleukins, IFN-γ, tumor necrosis factor (TNF)-α, growth-regulated oncogene-α (Gro-α), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1ß (MIP-1ß), granulocyte colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) will be determined in the fecal solutions according to the methodology of Munoz-González et al. [19].

In blood, we will evaluate:

- *Inflammatory profile change from baseline*, assessed by cytofluorimetric approach. It will be assessed with the Bio-Plex cytokine assay (Bio-Rad Laboratories Inc., Hercules, CA, USA), according to the manufacturer's instructions. In particular, IL-1ra, IL-4, IL-6, IL-8, IL-10, IL-12, IL-17, MCP-1, MIP-1ß, vascular endothelial growth factor (VEGF), TNF-α, IFN-γ, IFN-γ-induced protein (IP)-10, will be measured.
- *Lipid peroxidation markers' change from baseline*, assessed by It will be estimated using the Thiobarbituric Acid Reactive Substances (TBARS) assay kit. [20]
- *Plasma total antioxidant capacity's (TAC) change from baseline*, assessed by fluorometry, using the oxygen radical absorbance capacity. [21]
- *Reactive oxygen species (ROS) change from baseline*, assessed by flow cytometry. In particular, leukocyte subpopulations (lymphocyte, monocyte, and granulocyte) ROS will be measured. [22]

Sample size calculation

Due to the lack of dietary intervention trials on BS and to the fact that gastrointestinal symptoms are quite similar in BS and in the inflammatory bowel syndrome, on the basis of a previously published trial

[23], a sample size of 80 BS patients was required to achieve power of 80% (beta) with alpha = 0.05, to detect a 50-points difference in the mean of SSS (the primary outcome), between VD and MD interventions. We will recruit 10 extra volunteers (for a total of N=90) as we assume that not all our patients would be compliant with the treatments and in case of loss to follow-up. Losses will be included in the intention-to-treat but not in the per-protocol analyses.

Recruitment and randomization

Participants will be recruited from the Behçet Center and Lupus Clinic, Careggi University Hospital, Florence, Italy, or using advertisements on local media, newspapers, social media and websites. After approval and completion of the initial assessment, the subjects will be formally included in the study and randomized with a 1:1:1 randomization to the three intervention arms through a web-based online randomization procedure. No adaptive randomization procedures will be performed. The random allocation sequence will be produced and managed by an independent staff member who is outside of the project, to code the treatments, and maintain the key to this code until data collection is completed.

Blinding

Although full blinding of both participants and dieticians to treatments in this study is not possible because of obvious differences between the intervention diets, several strategies will be employed to reduce the risk of bias. First of all, the treatment allocation for each patient will not be revealed until the patient has irrevocably been entered into the trial, to avoid selection bias. In addition, outcome measures in the present study cannot be easily influenced by the observer. Furthermore, trial personnel who will enrol participants, data collectors, outcome assessors and data analysts will be blinded to treatment allocation, and an employee outside of the research team will insert data into the computer in separate datasheets. On the other hand, making the trial open rather than blinded may improve recruitment. Unblinding will be permissible only when knowledge of the treatment will be absolutely essential for further management of the patient.

Data collection

Follow-up assessments and data collection will be undertaken at the Unit of Clinical Nutrition of the Careggi University Hospital, Florence, Italy, by trial personnel. All subjects will be examined between 7.30 and 11.30 a.m. after a 12 hours-fasting period.

Compliance

Compliance with the interventions will be achieved using behavior change strategies including self-monitoring, and regular phone calls for dietary counseling. In particular, participants will receive at least one unannounced phone call during each intervention, in which participants will recall his or her 24-h diet period. Furthermore, participants will be provided with a detailed one-week menu plan for each dietary period with all foods expressed in weight and/or volume measures, and a hand-out containing details on their assigned diet, including food groups that can be included and ones that should be avoided. The

vegetarian menu plan will include also recipes for preparing meals. Participants may discontinue the intervention or withdraw from the study for the following reasons: (1) at the request of the participant; (2) if the investigator considers that a participant's health will be compromised due to adverse events or concomitant illness that develop after entering the study. Participants prematurely discontinued from the study before the 3-month evaluation will have the baseline clinical and laboratory evaluations performed.

Anthropometric measurements and body composition

Weight and height will be measured using a stadiometer. BMI will be calculated as weight (kg) / height $(m)^2$. Patients will be classified as overweight if their BMI is more than 25 kg/m^2 but less than 30 kg/m^2, and obese if their BMI is 30 kg/m^2 or more. Body composition will be determined with a bioelectrical impedance analyzer device (TANITA, model BC 420 MA) at the beginning and at the end of each intervention phase.

Blood samples

Blood samples will be collected at each patient clinical evaluation. Blood samples will be centrifuged at 3,000 rpm for 15 minutes to yield serum, aliquoted, and then stored at -80°C until analyses.

Stool samples

Fecal samples (four or five scoops totaling 4 g) will be collected in sterile containers before and after each intervention phase - for a total of six fecal samples for each participant - and immediately frozen at −20 °C, before being transferred to −80 °C until analysis. Stool sample collection kits, including containers and instructions, will be provided for the participants.

Storage of biological specimens

The storage of biological specimens will be performed under appropriate conditions, according to standard methods. Blood samples will be aliquoted and stored at −80°C for 4 years before being used or destroyed. Stored samples will be used exclusively for research purposes upon consent of the donor. Sample destruction will be appropriately documented.

Data management

Data will be collected on an electronic database. Identifiable data or other documents will not be recorded in the database and participants will be identified by a unique trial ID only. Hard copies of data sheets linking the participant identification number to the person's contact details will be kept securely in a locked filing cabinet in a locked office, accessible only to key research team members. Participant files and other source data (including copies of protocols, questionnaires, original reports of test results, correspondence, records of informed consent, and other documents pertaining to the conduct of the study) will be kept for the maximum period of time permitted by the institution. All data of the project will be stored in the DASH-IN infrastructure, which is developed by ENPADASI. Thereby we will adhere to the

FAIR principles (Findable, Accessible, Interoperable and Reusable). The data will be made open access upon publication. Within Europe the ELIXIR infrastructure is coordinating data stewardship and management activities in the life sciences.

Multiple strategies will be employed to improve data quality during data collection, including an accurate recruitment, a structured and time-limited protocol, the inclusion of a run-in period, the limitation of the burden and inconvenience of data collection on the participants, the development of a trusting and collaborative relationship between research units and participants and double data entry.

Statistical analysis

Outcomes will be analyzed within each group using paired comparison t-tests to test whether the changes will be statistically significant. The absolute change will be estimated with independent t sample tests. One-way analysis of variance will be used for testing differences between changes in VD, MD-Bt and MD intervention groups. A linear regression analysis, adjusting for confounders will be performed in order to compare the effect of the three different treatments. Data for the general linear model for repeated measurements will be reported as geometric means with their standard errors. A P-value < 0.05 will be considered statistically significant. Statistical analyses will be performed using SPSS software for Macintosh (SPSS Inc., Chicago, IL, US).

For the GM analysis, raw sequences will be processed using a pipeline that combines PANDAseq [24] and QIIME [25]. The UCLUST software [26] will be used to bin high-quality reads into operational taxonomic units (OTUs) at 0.97 similarity threshold through an open-reference strategy. Taxonomy will be assigned through the RDP classifier, using the Greengenes database as a reference. Alpha-diversity and rarefaction curves will be computed using different metrics, including the Faith's phylogenetic diversity, the number of observed OTUs and the Shannon index. Beta-diversity will be estimated by weighted and unweighted UniFrac distances, which will be used as input for Principal Coordinates Analysis (PCoA). All statistical analysis will be performed using R and the packages vegan, stats and made4. The significance of data separation in the PCoA will be tested using a permutation test with pseudo-F ratios (function Adonis of vegan). Wilcoxon test for paired data will be used to assess significant differences in alpha diversity and taxon relative abundance between groups, while Kruskal–Wallis test will be used for multiple comparisons. P-values will be corrected for false discovery rate (FDR, Benjamini-Hochberg). A p value < 0.05 will be considered statistically significant.

Monitoring

Given the limited objectives and its short-term nature, this trial will be monitored on a regular basis by the protocol team and the local Institutional Review Board, without the use of a formal data monitoring committee. Each month, the protocol team will provide the local Institutional Review Board with a monitoring report, including a review of activities, progress, difficulties and issues of concern. No ad interim analysis will be performed. Data access will be restricted to trained staff with unique password-protected accounts. Adverse events such as unfavourable and unintended signs, abnormal laboratory

findings, symptoms, or diseases temporally associated with the intervention diet will be collected from the time of randomisation until the final 12-month follow-up visit for each participant, whether or not considered related to the intervention study. All adverse events will be followed up until they are resolved.

Discussion

BS is a systemic inflammatory disorder characterized by a wide range of potential clinical manifestations affecting different organs and tissues [1], with the higher risk of mortality due to vascular-thrombotic and neurological affections [2]. The etiology still remains unknown, and although various mechanisms have been proposed, it is not yet clear whether the microbiome has a role in this process [27]. To date, no studies are available that evaluate the effects of a nutritional intervention on BS. Thus, the aim of the project will be to understand the effect of a VD, MD-Bt or MD on BS manifestations and, possibly, the role of the intestinal microbiota as a mediator of diet effects on BS patients. BS is a rare disease, and all available treatments are not specific tailored therapies. Beyond the real progress made in the past decade for the treatment of this disease, management of BS remains a challenge for physicians. Increasing evidences are demonstrating that it is a peculiar disorder with an extremely wide spectrum of clinical features that can respond to certain specific treatments, which however, can be ineffective on other manifestations or even worsen some features. A treatment based on dietary and lifestyle issues, able to restore immune system homeostasis, could have a high impact on costs sustainability for the treatment of such a chronic and disabling inflammatory condition.

Trial Status

The trial has received all necessary regulatory approvals. The current approved protocol version is 2.0 (version date April 16, 2018). We anticipate a September 16, 2019 start date for recruitment and a June 15, 2020 recruitment completion date. At the time of manuscript submission, the enrolment of BS subjects is ongoing.

Abbreviations

BS (Behçet's syndrome); GM (gut microbiota); SCFAs (short-chain fatty acids); VD (lacto-ovo-vegetarian diet); MD-Bt (Mediterranean diet supplemented with butyrate); MD (Mediterranean diet); BMI (Body Mass Index); GAI (Global assessment of improvement scale); SSS (Symptom severity scale); BDCA (Behçet disease current activity form); VAS (visual analogue scale); CNS (central nervous system); GC-MS (gas chromatography - mass spectrometry); SPME (solid phase microextraction); TIC (total ion current scan); MS/MS (tandem mass); SIM (single ion monitoring); SRM (selected ion monitoring); TGF-ß (transforming growth factor beta); IFN-γ (interferon-gamma); IL-1ra (interleukin-1ra); IL-4 (interleukin-4); IL-6 (interleukin-6); IL-8 (interleukin-8); IL-9 (interleukin-9); IL-10 (interleukin-10); IL-12 (interleukin-12); IL-17 (interleukin-17); IL-22 (interleukin-22); FoxP3 (forkhead box P3); TNF-α (tumor necrosis factor alpha); Gro-α (growth-regulated oncogene-α); MCP-1 (monocyte chemoattractant protein-1); MIP-1ß (macrophage inflammatory protein-1ß); G-CSF (granulocyte colony stimulating factor); GM-CSF (granulocyte-macrophage colony

stimulating factor); VEGF (vascular endothelial growth factor); IP-10 (IFN-γ-inducible protein 10); TBARS (thiobarbituric acid reactive substances); TAC (total antioxidant capacity); ROS (reactive oxygen species); IBS (inflammatory bowel syndrome)

Declarations

Authors' contributions

GP participated in the design of the study, wrote the study protocol and prepared the final version of the manuscript. She is on the protocol team. She is currently screening and instructing subjects regarding the protocol and is involved in the enrollment of participants and the assignment of the interventions. MD participated in the design of the study and in the writing of the study protocol. She is on the protocol team. She is currently screening and instructing subjects regarding the protocol and is involved in the enrollment of participants and the assignment of the interventions. CF participated in the design of the study and critical revision of the manuscript for important intellectual content. She is responsible for the oxidative stress analyses. She is on the protocol team. MB participated in the design of the study and in writing the study protocol and is responsible for the oxidative stress analyses. He is on the protocol team. ST participated in the design of the study and in writing the study protocol and participated in the critical revision of the manuscript. She is responsible for the analyses of microbiota and SCFA profiles. She is on the protocol team. GE conceived the study, participated in the design of the study and critical revision of the manuscript. He is responsible for protocol team, recruitment and clinical evaluation of the patients. FS conceived the study, participated in the design of the study and wrote the study protocol. He is responsible for protocol team, recruitment, clinical evaluation of the patients and blood analyses. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The results from this clinical trial have the potential for immediate public health applicability. The target audience will be reached through publications, oral presentations, and seminars. Data analysis and manuscript preparation will occur during the last 6 months of this proposed trial. All plans for dissemination of study results will be discussed with the investigators before implementation. Any amendments to the protocol and information provided to participants will be submitted to the Ethics Committee for approval prior to implementation. Substantial amendments may only be implemented after written approval has been obtained whereas non-substantial amendments can be implemented

without written approval from the Ethics Committee. The Chief Investigator will have to ensure that the participant's privacy is maintained. Data and source document will be stored in such a way that they can be accessed at a later date for the purposes of monitoring or inspection by the Ethics Committee. At the end of the study, participants will be able to request a copy of the results of the study from the Chief Investigator. The results from the trial will be submitted for publication in a peer-reviewed journal irrespective of the outcome. The final report will follow the CONSORT 2010 guidelines. Authorship of presentations and reports related to the study will be in the name of the collaborative group.

Ethics approval and consent to participate

The Tuscany Regional Ethics Committee of the University Hospital of Careggi, Florence has reviewed and approved the version 2.0 of the study protocol of April 16, 2018 (12773_SPE). The study will be conducted in accordance with the Declaration of Helsinki and the Data Protection Act. Trial personnel will obtain informed consent from each participant prior to inclusion. All patients will be free to withdraw from the study at any time. Ethics Committee's approval included the trial protocol, information sheet and consent form, questionnaires, interviews, any other written information that will be provided to the participants and any advertisements that will be used during the study. The trial is registered at the clinical trial registration (clinicaltrials.gov: NCT03962335, registered on May 21, 2019) in accordance with the International Committee of Medical Journal editors (ICMJE) requirements. Given the nature of the study, post-trial provisions are not required.

Consent for publication

Results from this study will be presented in publications and meetings but will not contain any identifying information. Consent for publication as well as sample storage for future research will be obtained from participants in the study.

Competing interests

The Authors declare that they have no competing interests and the research is not being supported by any commercial organization.

References

1. Skef W, Hamilton MJ, Arayssi T. Gastrointestinal Behçet¿s disease: A review. World J Gastroenterol 2015; 21: 3801-12
2. Emmi G, Bettiol A, Silvestri E, Di Scala G, Becatti M, Fiorillo C, Prisco D. Vascular Behçet's syndrome: an update. Intern Emerg Med 2019; 14(5): 645-652
3. Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum. 2013; 65(1): 1-11
4. Consolandi C, Turroni S, Emmi G, Severgnini M, Fiori J, Peano C et al. Behçet's syndrome patients exhibit specific microbiome signature. Autoimmun Rev 2015; 14: 269- 276

5. Neish AS. Microbes in gastrointestinal health and disease. Gastroenterology 2009; 136: 65-80.
6. Candela M, Biagi E, Turroni S, Maccaferri S, Figini P, Brigidi P. Dynamic efficiency of the human intestinal microbiota. Crit Rev Microbiol 2013: 1-7
7. Lynch SV, Pedersen O. The Human Intestinal Microbiome in Health and Disease. N Engl J Med. 2016; 375(24): 2369-2379
8. Kosiewicz M, Dryden GW, Chhabra A, Alard P. Relationship between gut microbiota and development of T cell associated disease. FEBS Lett 2014; 5793: 224-5
9. Emmi G, Silvestri E, Della Bella C, Grassi A, Benagiano M, Cianchi F et al. Cytotoxic Th1 and Th17 cells infiltrate the intestinal mucosa of Behcet patients and exhibit high levels of TNF-α in early phases of the disease. Medicine (Baltimore). 2016 Dec; 95(49): e5516
10. Zmora N, Suez J, Elinav E. You are what you eat: diet, health and the gut microbiota. Nat Rev Gastroenterol Hepatol. 2019;16(1): 35-56
11. Kolodziejczyk AA, Zheng D, Elinav E. Diet-microbiota interactions and personalized nutrition. Nat Rev Microbiol. 2019; 17(12): 742-753.
12. Dinu M, Abbate R, Gensini GF, Casini A, Sofi F. Vegetarian, vegan diets and multiple health outcomes: a systematic review with meta-analysis of observational studies. Crit Rev Food Sci Nutr 2017; 57:3640–3649
13. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes. 2017; 8(2): 172-184
14. Kabeerdoss J, Devi RS, Mary RR, Ramakrishna BS. Faecal microbiota composition in vegetarians: comparison with omnivores in a cohort of young women in southern India. Br J Nutr 2012; 108: 953,957
15. Haro C, Montes-Borrego M, Rangel-Zúñiga OA, Alcalá-Díaz JF, Gómez-Delgado F, Pérez-Martínez P et al. Two Healthy Diets Modulate Gut Microbial Community Improving Insulin Sensitivity in a Human Obese Population. J Clin Endocrinol Metab 2016; 101: 233-42
16. Candela M, Biagi E, Soverini M, Consolandi C, Quercia S, Severgnini M, et al. Modulation of gut microbiota dysbioses in type 2 diabetic patients by macrobiotic Ma-Pi 2 diet. Br J Nutr 2016; 116: 80-93
17. Candela M, Maccaferri S, Turroni S, Carnevali P, Brigidi P. Functional intestinal microbiome, new frontiers in prebiotic design. Int J Food Microbiol 2010; 140: 93- 101
18. Biagi E, Franceschi C, Rampelli S, Severgnini M, Ostan R, Turroni S, et al. Gut Microbiota and Extreme Longevity. Curr Biol. 2016; 26(11): 1480-5
19. Munoz-Gonzalez I, Jiménez-Girón A, Martín-Álvarez PJ, Bartolomé B, Moreno-Arribas MV. Profiling of microbial-derived phenolic metabolites in human faces after moderate red wine intake. J Agr Food Chem 2013; 61: 9470-9
20. Whittaker A, Sofi F, Luisi ML, Rafanelli E, Fiorillo C, Becatti M, et al. An organic khorasan wheat-based replacement diet improves risk profile of patients with acute coronary syndrome: a randomized

crossover trial. Nutrients. 2015 May 11;7(5):3401-15. doi: 10.3390/nu7053401

21. Whittaker A, Dinu M, Cesari F, Gori AM, Fiorillo C, Becatti M, et al. A khorasan wheat-based replacement diet improves risk profile of patients with type 2 diabetes mellitus (T2DM): a randomized crossover trial. Eur J Nutr. 2017 Apr;56(3):1191-1200.
22. Sofi F, Dinu M, Pagliai G, Cesari F, Gori AM, Sereni A, et al. Low-Calorie Vegetarian Versus Mediterranean Diets for Reducing Body Weight and Improving Cardiovascular Risk Profile: CARDIVEG Study (Cardiovascular Prevention With Vegetarian Diet). Circulation. 2018 Mar 13;137(11):1103-1113.
23. Pedersen N, Ankersen DV, Felding M, Wachmann H, Végh Z, Molzen L, et al. Low-FODMAP diet reduces irritable bowel symptoms in patients with inflammatory bowel disease. World J Gastroenterol. 2017; 23(18): 3356–3366
24. Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD. PANDAseq: paired-end assembler for illumina sequences. BMC Bioinformatics. 2012;13:31
25. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335–6.
26. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26(19):2460–1
27. Mumcu G, Direskeneli H. Triggering agents and microbiome as environmental factors on Behçet's syndrome. Intern Emerg Med. 2019; 14(5): 653-660

Figures

	STUDY PERIOD						
	Enrolment	Allocation	Post-allocation				Close-out
PROTOCOL ACTIVITY	*day-14 to 1*	day 0	*mo 3*	*mo 4.5*	*mo 7.5*	*mo 9*	*mo 12*
TIMEPOINT	$-t_1$	t_0	t_1	t_2	t_3	t_4	t_5
ENROLMENT:							
Eligibility screen	X						
Informed consent	X						
Demographic details	X						
3-day dietary records	X						
Allocation		X					
INTERVENTIONS:							
Vegetarian diet		← – – – →		← ····· →		← ——— →	
Mediterranean diet + butyrate		← ····· →		← ——— →		← – – – →	
Mediterranean diet		← ——— →		← – – – →		← ····· →	
ASSESSMENTS:							
Anthropometric measurements		X	X	X	X	X	X
Body composition		X	X	X	X	X	X
Blood samples		X	X	X	X	X	X
Stool samples		X	X	X	X	X	X
SSS Questionnaire		X	X	X	X	X	X
GAI Questionnaire		X	X	X	X	X	X
BDCA Questionnaire		X	X	X	X	X	X
24-hour dietary recall		← ——— →		← ——— →		← ——— →	

Figure 1

Schedule of enrolment, interventions, and assessments for participants

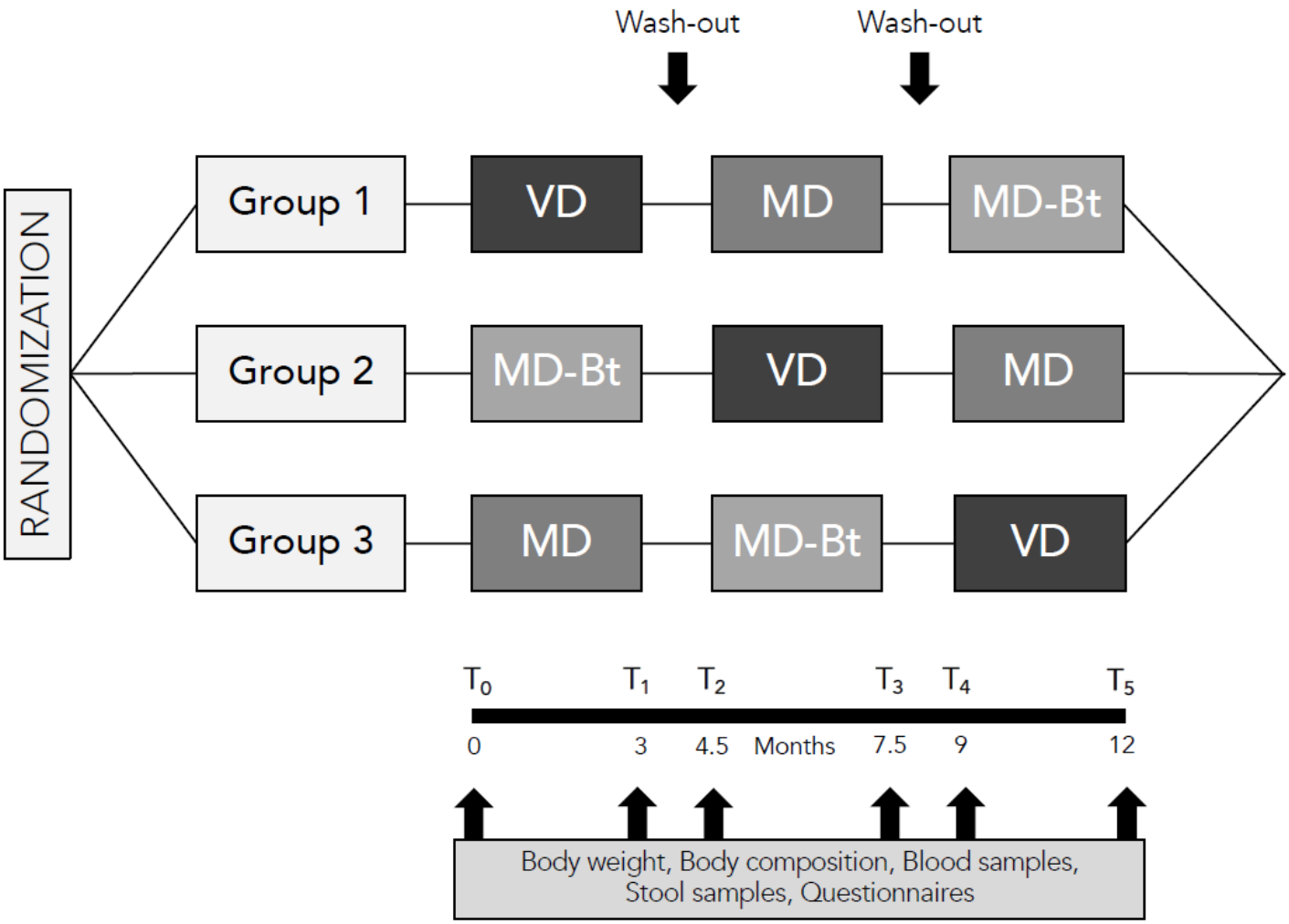

Figure 2

Study design

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Suppl.2.docx
- Suppl.1SPIRITChecklist.doc