Study Protocol and Statistical Analysis Plan (SAP)

Official Title: The Crohn's Disease Exclusion Diet With Early Dairy Introduction Plus Partial Enteral Nutrition (CD-EDEN) in Adult Patients With Crohn's Disease

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Study Protocol

Background

Crohn's disease (CD) is a chronic relapsing inflammatory bowel disease (IBD), that may affect any part of the gastrointestinal tract, but most commonly the terminal ileum and colon. Its clinical manifestations usually include diarrhea, abdominal pain, fatigue, and unintentional weight loss. CD has a significant impact on patients' quality of life (1).

The incidence of IBD has markedly increased in the Western world during the 20th century, in parallel with the urbanization and industrialization of societies. At the beginning of the 21st century, the incidence started to increase in newly industrialized countries too, suggesting the role of environmental factors in IBD emergence (2,3).

Current epidemiological trends indicate that prevalence continues to grow due to decades of high incidence and low mortality, thanks to advances in medical science. In many westernized countries, IBD prevalence exceeds 0.3%, which translates to a high burden of IBD. More specifically, recent data, published in 2021, shows that the incidence of CD in Europe ranged between 0.4 to 22.8 per 100,000 person-years (4), while in Greece the incidence of CD is about 2.0 to 3.6 per 100,000 per year (5). The predicted value of IBD prevalence in the Western world is 1% by 2030, based on forecasting models. Prevalence of IBD in advanced developing countriesis currently low but is expected to rise in the following decades. The evolving global burden of IBD is expected to challenge the health-care systems in managing these complex and costly diseases (2-4).

The disease etiology is complex and not fully understood. Accumulated data indicate that environmental factors, including diet, might play a key role in the pathogenesis and inflammation, through mechanisms involving gut dysbiosis, in genetically susceptible individuals (6).

Epidemiological data suggest that following a Mediterranean diet, which represents the "pre-industrial" dietary pattern, is associated with decreased risk for CD, whereas a Western diet, rich in animal fat, processed foods and food additives, seems to be linked with an increased disease risk (2).

Current evidence-based nutritional guidelines for CD, arise mainly from epidemiological data and generally suggest a “healthy diet”, characterized by an increased consumption of nutrient-dense foods along with a restricted intake of saturated and trans-fats, emulsifiers, thickeners, processed foods, unpasteurized dairy. According to the Dietary Guidance from the International Organization for the Study of Inflammatory Bowel Diseases (IOIBD), consensus was not reached for pasteurized dairy consumption among patients with CD. Based to evidence from epidemiology and animal models, the authors clarify that it is prudent for Crohn’s disease patients to avoid lactose, given that lactase deficiency and lactose intolerance is common among patients with IBD. It was also noted that it could be prudent to reduce dairy fat and processed dairy rich in maltodextrins and emulsifiers (7). To date, no clear and strong recommendation can be given to follow a specific diet for inducing remission in patients with active CD, whereas the importance of a personalized nutritional approach is highlighted (8). Exclusive Enteral Nutrition (EEN) is the only well-established dietary therapy in CD and is commonly used for the induction of remission in mild to moderate pediatric CD (8-9, 12-13). Evidence according to EEN efficacy in adults is not consistent, which could more likely be explained by the compliance difficulty rather that by mechanistic differences (10-12). It is of interest, that fecal inflammation increases rapidly after food re-introduction following EEN in children (14). In this context, partial enteral nutrition (PEN) has sparked scientific interest as a possible maintenance dietary strategy. PEN has been shown to be beneficial in maintaining remission, but poor compliance due to low formula palatability and patients’ fatigue remains an important barrier in the clinical practice. Moreover, PEN is inferior to EEN in inducing remission (13,15). Sigall Boneh and her colleagues evaluated a novel dietary intervention that combined PEN with an elimination diet (19). The Crohn’s disease exclusion diet (CDED) is a whole-food, high protein, low saturated fat, low taurine, low heme, low food additives diet with exposure to fiber mainly from fruits, vegetables and complex carbohydrates. More specifically, CDED eliminates specific dietary components hypothesized to induce dysbiosis and appears to be effective in inducing and maintaining remission both in pediatric and adult population (16-19).

Currently, most of the available strategies to meet goals of medical therapy for CD require immune suppression. In the era of newer biological agents, safety and efficacy in managing CD has increased whereas, eventually, most patients require dose escalation and combination therapy to tackle with loss of response, which comes at a higher cost and with increased health risks (22, 23). It has already been shown that CDED plus PEN could be an effective strategy to obtain remission in patients failing biological therapy (17), while a recent pilot study indicated efficacy in using CDED as a dietary monotherapy for induction and maintenance of remission in patients with mild-to-moderate active CD (18). Despite the promising findings, CDED should be further explored in powered randomized controlled trials, including patients with wide disease phenotypes, clinical activity and concurrent drug therapies. Moreover, the exposure to certain of the eliminated foods should be tested in order fortailored nutrition advice to be feasible.

In light of the growing evidence linking diet with CD outcomes and given the patients' strong interest in ways to manage their disease through diet (12,20), the establishment of a palatable and as flexible as possible dietary pattern, not merely for inducing remission, but also as a feasible maintenance strategy is one of the main priorities in CD research at present.

Objectives

The initial hypothesis is that Crohn's disease may occur via an order of events involving dysbiosis and genetically determined or environmentally acquired defects in innate immunity. It has also been hypothesized that the mechanism of EEN for induction of remission acts by the elimination of exposure to dietary components that may cause a defect in bacterial clearance or dysbiosis. Based on that premise, Crohn's Disease Exclusion Diet (CDED), has been developed, as

The aim of this study is to explore the role of the CDED combined with PEN in both inducing and maintaining remission of CD in adults, taking into consideration important objective biomarkers such as faecal calprotectin (FC) (21) along with patient reported outcomes. More precisely, our primary goal is to investigate whether CDED with PEN could lead to a significant decrease in feacal calprotectin concentration. In

view of the existing dietary guidelines for adult Crohn's disease patients (7,8) and considering that dairy constitute the main dietary source of calcium, researchers wish to challenge patients in remission with a gradually increasing exposure to dairy products, to assess tolerability, prospecting for the development of a personalized remission maintenance diet based on the CDED principles. Our aim is to examine the proportion of patients with significant feacal inflammation elevation following the dairy introduction.

We intent to investigate the effectiveness and feasibility of this dietary strategy in terms of changes in clinical and biochemical indices, patient's quality of life and nutritional status as well as patients' adherence and acceptance of the diet. Finally, we plan to re-evaluate patients' dietary habits and clinical outcomes, 6 months after the beginning of the study to address the established dietary adaptations, as well as the sustainability and durability of potential benefits of the dietary intervention.

Design and Methods

The proposed project is a randomized controlled clinical trial which is held in Evangelismos General Hospital of Athensin collaboration with the Department of Food Science & Human Nutrition of Agricultural University of Athens. Briefly, eligible patients are adults with established Crohn's disease with ileal and/or colonic involvement who receive their IBD care at the hospital's Gastroenterology Unit (see eligibility criteria below). Patients with active mild-to-moderate disease, defined by aHarvey–Bradshaw Index (HBI) score of 5–14 points, or inactive disease (HBI<5) are eligible for enrolment. Detailed information about the purpose and the study procedures will be given to all patients and consent form will be signed. The study protocol has been approved by the Ethics committee of the hospital.

Patients will be randomly assigned 1:1 to either CDED plus PEN plus dairy products or PEN plus usual nutritional care. In detail, half of the patients in remission will follow the first stage diet (CDED+PEN) for 4 weeks and will continue with CDED + PEN + dairy products for another 4 weeks. The other half will be given PEN plus the usual advice for a healthy dietary pattern. PEN will cover the 50% of total energy requirements for the first 4 weeks and the 25% during the second 4 weeks. Half of the patients with active disease will follow the first stage diet (CDED + PEN 50%) for 6 weeks

and then, the responders will continue with adding the dairy product while PEN will cover 25% of the total energy requirements, for 4 more weeks. The other half will be given PEN plus the usual advice for a low residue diet. Low lactose, low fat and low additive dairy foods will be given, namely Greek yogurt 2% fat and light yellow cheese with 10-12% fat, in calculated portions to meet tailored energy, protein and calcium requirements. After the end of the intervention period, patients will be advised to re-introduce foods that were eliminated and to follow a maintenance diet. All participants will attend study visits at the beginning, in the middle (week 8 or week 10, for patients entering the study in remission or with active symptoms, respectively) and at the end of the study, at which points questionnaires will be completed, anthropometric measurements will be conducted and biological samples will be collected. The last follow-up will take place in week 24. In the meanwhile, phone calls by a researcher dietitian will be held to assess adherence and support the patients.24-hour diet recallsand the modified Medication Adherence Report Scale (MARS) questionnaire (16) will be used to address compliance along with the physician's assessment.

At length, our methodological approach includes:

i) **Anthropometry**

(1) Weight (kg) measurement, [Time Frame: Baseline, week 4, week 8 (Baseline, week 6, week 10 for patients entering the study with active disease), week 24]

(2) Height (m) measurement, [Time Frame: Baseline],

(3) Body mass index (BMI) (kg/m^2) calculation

(4) Waist, neck and midarm circumferences (cm), triceps skinfold (mm) measurement [Time Frame: Baseline, week 8/w10]

(5) Body composition analysis (Fat mass, fat %, fat free mass, phase angle) by Bioelectrical Impedance Analysis (BIA) [Time Frame: Baseline, week 8/10]

(6) Handgrip strength (kg) assessment by dynamometer [Time Frame: Baseline, week 8/w10]

ii) **Dietary habits assessment**, using a semi-quantitative food frequency questionnaire (FFQ), to assess dietary habits andadherence to the Mediterranean diet (MD-score) [Time Frame: Baseline, week 24] and 24-

recalls [Time Frame: Baseline, week 2/w3, week 4/w6, week 6/w8, week 8/w10]

iii) **Physical activity assessment**, using HPAQ questionnaire [Time Frame: Baseline, week 8/w10]

iv) **Health-related quality of lifeassessment**, defined by the self-administered 32-item Inflammatory Bowel Disease questionnaire (IBDQ) score. Higher IBDQ scores indicate better outcomes. Clinical response defined as increase ≥16 points [Time Frame: Baseline, week 8/w10]

v) **Medical history** (e.g.,prior GI surgery, comorbidities) data collection and **disease activity assessment** by the HBI. Clinical response is defined as baseline HBI score decrease of ≥ 3[Time Frame: Baseline, week 4/w6, week 8/w10]

vi) **Blood and Feacal samples collection** by specializes staff [Time Frame: Baseline, week 4/w6, week 8/w10]

(1) **Blood samples analysis** by the hospital's lab to estimate serum **CRP** and **albumin**

(2) **Feacal samples analysis** by certified labs to estimate **Feacal calprotectin** concentration (μg/g)

vii) **Nutrition education** according to the intervention principals and **prescription of nutritional supplements** (liquid formulas-PEN) [Time Frame: Baseline]. Support line for patients' support will be available.

viii) **Handle–analysis** of the questionnaires and **data entry** in electronic databases [Microsoft® Excel, IBM SPSS Statistics®, Nutritionist Pro®]

ix) **Statistical analysis/interpretation of results**.

<u>Eligibility Criteria:</u>

Inclusion Criteria:

- Participants must have an established diagnosis of CD
- Individuals able to give informed consent and willingness to participate

Exclusion Criteria:

- Age < 18 years old
- Previous extensive bowel resection
- Reported pregnancy or lactation
- Current stoma or abscess
- Clinically significant stricture
- Introduction of or change in dose of drug therapy within the past 8 weeks

- Comorbidities including diabetes or coeliac disease, or other concomitant serious comorbidity e.g. significant psychiatric, hepatic, renal, endocrine, respiratory, neurological, cardiovascular, neoplastic or other autoimmune disease
- Food allergies or intolerances, which do not permit participation in the study
- Any proven current infection such as positive stool cultures or positive tests for parasites or C. difficile.

The proposed project has a duration of thirty-six (36) months. The 1st phase that has already been completed included preparation and printing of questionnaires, creation of recipes based on the principles of the dietary intervention. The recruitment process is anticipated to start in September 2022 and to be completed by March 2024.

Statistical Analysis Plan (SAP)

Based on pilot data (18) we performed a sample size calculation using the GPower 3.0.10 software (http://www.softpedia.com/get/Science-CAD/G-Power.shtml). A sample of 140 individuals is sufficient to achieve statistical power > 80 %, at a significance level of a = 0,05 for two-sided statistical tests. All continuous variables will be assessed for normality with the Kolmogorov–Smirnov test (cutoff $p < 0.01$), to use parametric or non-parametric tests, as appropriate. Chi-squared or Fischer's test will be used to analyze categorical variables. Continuous variables will be analyzed by using Mann Whitney U test and t-test for paired-samples or the Wilcoxon signed rank). For paired measures (e.g. FC concentration) before and after the intervention, we will calculate the percentage change for each patient relative to baseline. Logistic regression models will be used to explore how age, sex, phenotypic factors (disease location and activity), MD-scoreat baseline, adherence and intervention grouping would contribute to significant HBI or FC concentration drop.

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