

Title: Efficacy of the Autoimmune Protocol Diet for Inflammatory Bowel Disease

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Study Protocol and Statistical Analysis Plan

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Sponsor: Scripps Health

Responsible Party: Principal Investigator, Gauree G. Konijeti, MD, MPH

Affiliation: Scripps Health

Study Start: September 1, 2016 (actual)

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1. **SPECIFIC AIM:** The aim of this study is to determine the potential efficacy of dietary therapy, the Autoimmune Protocol Diet (AIP Diet), in adult patients with active Crohn's disease (CD) or ulcerative colitis (UC).
2. **RESEARCH DESIGN/METHODS:**
 - A. Primary Outcome Measure:** Change in clinical disease activity for CD and UC from baseline to 11 weeks (study completion)
 - For CD, clinical activity will be defined by the Harvey Bradshaw Index (HBI). Clinical remission is defined as a HBI < 5, Clinical response is defined as a reduction in the HBI of at least 3 points.
 - For UC, the Mayo Clinic Score (MCS) will be used to assess disease activity. Clinical remission is defined as a MCS of ≤ 2 and no subscore > 1. Clinical response is defined as a reduction in the MCS of at least 3 points and a decrease of 30% from the baseline score, with a decrease of at least 1 point on the rectal bleeding subscale or an absolute rectal bleeding score of 0 or 1.
 - B. Secondary Outcome Measures:**
 - For CD, changes in endoscopic activity will be defined by the Simple Endoscopic Score in Crohn's Disease (SES-CD) from most recent endoscopic evaluation prior to starting treatment with the AIP diet to 11 weeks (study completion). We will assess (a) Endoscopic remission, defined as an SES-CD of 0-2, and (b) Endoscopic response, defined as a decrease of 50% from the baseline SES-CD
 - For patients with small intestinal CD not amenable to endoscopic evaluation, changes in inflammatory activity will be assessed by radiographic changes as measured by CT or MR enterography or video capsule endoscopy at baseline and 11 weeks (study completion).
 - For patients with UC, changes in endoscopic activity will be defined by the Mayo Clinic endoscopic subscore that comprises the MCS: We will assess (a) Endoscopic remission, defined as a Mayo endoscopic subscore of 0 or 1, and (b) Endoscopic response, defined as improvement in the Mayo endoscopic subscore by 1 point.
 - Change in common laboratory measures, histologic activity, micronutrient levels (vitamin D, iron studies), use of steroids, and any adverse events resulting in flare or hospitalization from baseline to 11 weeks
 - Where possible, assess changes in RNA expression from intestinal mucosal biopsy specimens from baseline to 11 weeks
 - Assess changes in fecal microbial populations from baseline to 11 weeks, and compare with fecal microbial populations from IBD controls in clinical and endoscopic remission
 - C. Study Design:** Twenty participants with CD or UC with mild or moderate disease activity, as defined by HBI ≥ 5 for CD or Partial Mayo Clinic Score ≥ 3 for UC, ages 18 and over will enroll into this study for treatment with the AIP diet. Each patient will receive an initial evaluation including a physical exam, medication review, nutritional guidance and post treatment evaluations. We will also identify 10 patients with CD or UC in clinical and endoscopic remission and obtain a single stool sample for fecal calprotectin (inflammatory marker in stool) and microbiome analysis as our positive controls.
- **Inclusion Criteria (Participants):**
 1. At least 18 years of age

2. Able to provide written informed consent prior to screening and to comply with the requirements of the study protocol.
3. Established diagnosis of small bowel CD or colonic CD or ulcerative colitis
4. Confirmation of active CD or UC with recent (within 3 months) objective evidence of endoscopically active disease on colonoscopy or CT/MR enterography or video capsule endoscopy
5. Any medications being currently used for IBD must remain stable during the study period with the exception of tapering of corticosteroids.
6. Current disease activity defined as a Harvey Bradshaw index ≥ 5 at baseline for CD or Partial Mayo Score ≥ 3 for UC
7. Established Facebook account
8. Comfortable with internet-based surveys and email

- Exclusion Criteria (Participants):

1. If female, is pregnant or is breast feeding
2. Known celiac disease or subjects with a positive screen for celiac disease (elevated tissue transglutaminase antibodies)
3. Inability to provide informed consent or unwilling to participate
4. Evidence of untreated infection (e.g. Clostridium difficile)
5. Presence of stoma or J pouch
6. Bowel surgery within 12 weeks prior to enrollment and/or has surgery planned or deemed likely for IBD during the study period
7. Use of oral or intravenous antibiotics within 4 weeks prior to study initiation
8. Use of tube or enteral feeding, elemental diet, or parenteral alimentation within 4 weeks prior to study initiation

- Study Process: (Note: SOC indicates standard of care)

- Enrollment: Enrollment will take place over 2 months prior to study initiation. Participants will be screened by SCMG gastroenterologists, and informed consent will be obtained by the study coordinator (SC). At enrollment, the SC will document relevant history and physical examination, current prescription and nonprescription medications, and any imaging, endoscopic, and histologic evaluation completed for IBD. Participants will undergo SOC stool testing for common infections (unless completed within 3 months) and one 5mL blood draw into PAX tube for future genotyping.
- 1 week prior to study initiation: Participants will undergo SOC testing for CBC, HFP, vitamin D, iron studies, CRP, and fecal calprotectin. They will also establish online membership in the "SAD (Standard American Diet)-to-AIP (Autoimmune Paleo) Diet Transition Program" run by Angela Alt, and receive two books on AIP diet and recipes ("The Paleo Approach: Reverse Autoimmune Disease and Heal Your Body" by Ballantyne and "The Autoimmune Paleo Cookbook: An Allergen-Free Approach to Managing Chronic Illness" by Trescott).
- Study initiation/Treatment with AIP diet: Intervention will be based on a 6-week transition to the AIP diet and 6-week maintenance phase on the AIP diet (no food group reintroduction allowed), using the "SAD-to-AIP Diet Transition Program." Patients will receive certified health coaching and one-on-one and group guidance by a registered dietitian, along with group-based coaching through a private and secret Facebook group. During the study, participants will record daily dietary intake, which

- will be reviewed by program team. They will also complete surveys regarding feasibility, tolerability, and adherence to AIP diet every 3 weeks during the study.
- Use of medications/supplements during study: Any medications, vitamins or supplements that participants are taking for at least 1 month prior to study initiation may be continued during the study period. During the 12 week study, however, participants will not be allowed to add, remove, or change doses any medications, vitamins or supplements, unless there are concerns related to IBD activity or flare, side effects or potential toxicity. The only exception to medication change is that steroid tapering will be allowed during the 12 week period. Any unanticipated changes to a patient's medication regimen will be recorded.
 - Physician follow-up during study: Each participant will have clinical follow-up with their SCMG gastroenterologist during the week prior to enrollment, at 6 weeks, and at 11 weeks. Routine history and physical examination, as well as medication assessment, will be performed at these visits. In addition patients will undergo SOC blood work for CBC, HFP, vitamin D, iron studies, and CRP at 12 weeks. Fecal calprotectin will be performed at weeks 6 and 11.
 - Intestinal biopsy and RNA sequencing: RNA-sequencing is a widely used next-generation sequencing technology which is used to study the expression patterns of genes in intestinal samples. A typical RNA-seq analysis pipeline involves demultiplexing of samples and quality control checks followed by alignment to a reference genome and finally counting the sequences aligned to various genes. The counts are then used to quantify expression levels of genes or compare samples using differential expression analysis. Where possible, patients undergoing endoscopic assessment at baseline will have 2 intestinal biopsy specimens collected in RNAlater for RNA sequencing. All patients will undergo standard of care endoscopic or radiographic reassessment of IBD activity at 11 weeks (study completion), with 2 intestinal biopsy specimens collected in RNAlater.
 - Microbiome Analysis: 16S ribosomal RNA sequencing is a commonly used method to identify and quantify microbial species in a microbiome sample. Sequencing can be performed using an Illumina MiSeq machine which outputs anywhere from 10-20 Million paired-end 300 bp reads. Analysis will be performed using "mothur," an open-source software, with subsequent pipeline steps obtained to estimate species diversity and subtypes. Stool assessment for microbiome analysis from participants will be performed during the week prior to study initiation, and weeks 2, 4, 6, and 11. Stool assessment from positive controls will be performed during enrollment or the study period.

D. Statistical Analysis:

Analyses for the primary outcome will be conducted for participants with CD or UC and for the entire cohort. Paired t test will be used to examine differences between baseline and week 6 or 11 outcomes among those with follow-up data at those time points. Fisher's exact test will be used to examine whether IBD type is associated with the primary outcome. Survey data will be analyzed using descriptive statistics, including frequencies, percentages, means, and modes, as well as ANOVA. Relationships between bivariate categorical variables, including diet adherence and IBD type, will be tested using Chi-Square or Fisher's Exact Test, as appropriate.

RNA analyses: To identify genes which are differentially expressed due to the dietary intervention, we will first control for patient level differences using a Bayesian hierarchical mixture model, which estimates the log fold change for each gene and calculates a probability of the gene being differentially expressed ($P(DE)$). Significant genes will be selected considering a 5% Type 1 error rate i.e. $P(DE) > 0.95$. Gene Ontology enrichment will be done for significantly up-regulated and down-regulated gene using DAVID. DAVID is a web-based application that ranks biologically relevant gene sets enriched in the gene list being interrogated using a modified Fisher Exact test.

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We will also identify at least 10 patients with CD or UC in clinical, endoscopic, and histologic remission and obtain a single stool sample using mail-in FTA cards for microbiome analysis as our positive controls. Stool assessment from positive controls will be performed during study enrollment or the study period to avoid any temporal bias. In this way we will be able to characterize whether, within 11 weeks of the AIP diet, the microbiome profile changes over time due to dietary intervention are on a trajectory towards normalization based on changes in the degree of similarity with profiles from IBD patients already in remission. Diversity within individual samples and similarity between individuals and time-points will be measured using diversity metrics commonly used in ecological studies. These summary metrics will be utilized to determine whether clinical response can be correlated to microbiome normalization or other diversity changes.