TITLE:
The effect of probiotics in non-alcoholic fatty liver disease and steatohepatitis measured by transient elastography

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Study Protocol and Statistical Analysis Plan

Title: Effect of probiotics in non-alcoholic fatty liver disease and steatohepatitis measured by transient elastography (PRONE Study)

FDA IND Exempt

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BACKGROUND AND SIGNIFICANCE

Non-alcoholic fatty liver disease (NAFLD) and Non-alcoholic steatohepatitis (NASH) are usually conditions without symptoms where inflammation in the liver can progress to end-stage liver disease (Cirrhosis). Current standard of care for these conditions include control of metabolic syndrome which includes but is not limited to a patient's high blood pressure (hypertension), high cholesterol (hyperlipidemia), high blood sugar (hyperglycemia) and excess fat around the waist (central obesity and waist circumference) with lifestyle modifications including diet, exercise and medications.

Previous trials have identified equivocal results with Vitamin E and Pioglitazone (an oral diabetic drug). Some patients do not see results and we still see increased liver enzymes known as transaminases. Other studies suggest that there is a role for the Mediterranean diet in managing NAFLD, but further research is indicated. While the mechanism for inflammation on the liver in NAFLD and NASH is not completely understood, the American Association for the Study of Liver Diseases currently suspects it may be connected with the metabolism in the bowel and subsequent hepatic (liver) circulation.

The bacteria of the intestines (microbiome or gut flora) may play a role in the inflammatory cascade through the bloodstream that affects the liver [1]. Related studies on hepatic function following administration of probiotics by Wong et al. (2013) and Aller (2011) over 6 and 3 months duration demonstrated statistically significant changes.

OBJECTIVES

Our hypothesis is that introducing a benign probiotic (beneficial intestinal bacteria) using Align probiotics will cause improvement in hepatocellular (liver tissue) injury and fibrosis. Align is a once daily over-the-counter defined by the Food and Drug Administration as a new dietary ingredient. Each Align capsule contains one billion beneficial live bacteria/CFU, known as Bifidobacterium 35624. Our goal is to provide data that probiotics may be a viable treatment option in the future for clinicians for NAFLD and NASH and to establish a link that the intestinal microbiome plays a role in liver inflammation.

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Primary Endpoint:

To determine if probiotic use improves fibrosis stage on transient elastography

Secondary Endpoints:

To determine if probiotic use improves:

1) Liver Enzymes (AST and ALT)
2) Hemoglobin A1C (Diabetic Marker)
3) BMI (Body Mass Index)
4) Lipids (LDL)

METHODS

NAFLD and NASH patients seen in office consultation at South Oakland Gastroenterology Associates in Farmington Hills, Michigan from 2019-2021 who have confirmed diagnosis of NAFLD or NASH and fit the eligibility requirements will be approached to participate in the double-blind, placebo-controlled study.

After preparation and randomization by the Beaumont research pharmacy, the probiotic and placebo capsules will be delivered to the office in groups of 10 bottles at a time. Capsule bottles will be numbered consecutively and pharmacy will keep the randomization log; patients and researchers will be blinded. The bottles will be stored in a locked cabinet in the clinic. Only pharmacy will hold the randomization key. A log will be used to track the receipt and distribution of the capsules.

After informed consent is obtained, subjects will be counseled on diet and exercise and a baseline transient elastography exam will be ordered and baseline liver enzymes (AST and ALT) will be drawn, including hemoglobin A1C, lipids in the form of LDL and assessment of BMI (all standard of care tests). The patients will receive information on a standardized Mediterranean diet based nutrition program as well as direction for recommended 30 minutes of aerobic exercise 3 times weekly. Our recommended nutrition program limits excessive alcohol to avoid a confounding variable.

Patients will be randomized by pharmacy into 2 groups, a control group and a treatment group. The treatment group will be provided with a 6 month supply of probiotic supplementation; the control group will be given a placebo for 6 months along with instructions on how to take the supplement. Subjects will receive a 1 month (+/- 1 week) phone call follow up to ensure adherence to study instructions and daily oral intake of supplement and again at 3 months (+/- 1 week).

Study participation will end at 6 months (+/- 2 weeks) after the repeat of liver enzymes, hemoglobin A1C, lipid panel, assessment of BMI and transient elastography as completed at scheduled clinical appointment.

TABLE OF EVENTS

	Pretreatment	1 month +/-1 week	3 month +/-1 week	6 month +/-2 weeks
Consent	X			
Capsules dispensed	X			
Diet & exercise plan explained	X	X	X	
Labs drawn	X			X
Liver scan done	X			X
Phone call		X	X	
Remaining capsules returned				X

RISKS AND BENEFITS

The FDA has identified the study as minimal risk and exempt of the need for an IND number.

There are currently no alternative treatments or procedures indicated in this disease process, hence, the purpose of the study. However, potential benefits to subjects from exercise (walking) intervention and nutritional coaching may improve the elasticity and function of the liver.

Potential risks include GI symptoms related to probiotic use such as gas and bloating. The recommended exercise is walking.

VI. ELIGIBILITY CRITERIA

Inclusion Criteria:

- Diagnosis of NAFLD and/or NASH
- Subject aged 18 and older
- Non-pregnant - Self-reported
- Subject with decision making capacity to understand and consent to study procedures
- Ability to follow study related activities regarding medications, diet and exercise

Exclusion Criteria:

- Without diagnosis of NAFLD or NASH

- History of liver disease from other causes, including but not limited to hepatitis, autoimmune, alcohol use, fatty liver of pregnancy, Wilson's disease, primary or secondary hemochromatosis
- Patients aged less than 18 years
- Self-reported pregnant patients
- Inability to understand, follow and consent to study procedures
- Received a liver scan greater than 2 months prior to enrollment
- Patient lost more than 5 pounds of weight in the last 2 months
- Hepatic decompensation defined as gastrointestinal bleeding, ascites, hepatic encephalopathy
- Inability to engage in exercise
- Currently immunocompromised or taking immunosuppressive drugs
- Milk protein allergy
- Recent or active chemotherapy for malignancy
- Gastrointestinal malignancy
- Gastrointestinal disease such as Ulcerative Colitis, Crohn's Disease as these alter the microbiome
- Recent antibiotic therapy (within 6 months)
- Known allergy to probiotics
- History of major gastrointestinal surgery such as resection of the colon
- No concomitant use of probiotic from any source (i.e., kefir, certain yogurts, live culture sauerkraut)
- Any implanted battery operated device (i.e. AICD, pacemaker, loop recorder, cochlear implant)

DATA COLLECTION

Any changes to the liver will be documented using a non-invasive scan called transient elastography. Elastography is a technique similar to ultrasound that measures the stiffness and density of the liver and is a safer alternative to a liver biopsy. This is a standard of care procedure will be done at 0 months and 6 months. Along with the scans, the results of liver enzymes, standard of care laboratory tests used to follow the disease progression for patients with NAFLD and NASH, will be reviewed. These labs include AST and ALT, Hemoglobin A1C, BMI (Body Mass Index) and Lipids (LDL), also standard of care.

DATA ANALYSIS

A. Sample Size Consideration

Using paired measurements, a two-tail alpha of 0.05, a power level of 0.8, and assuming a large effect size (contingency coefficient of 0.447), it is estimated that 40 subjects (20 per group) would detect statistically significant between-group changes in liver function. Related studies on hepatic function (Wong, et al., 2013 and Aller, et al. 2011) following administration of probiotics over 6 and 3 months

duration demonstrated statistically significant changes with estimated large effect sizes using 20 and 28 subjects, respectively. A sample size of approximately 40 is expected to hold statistical significance, however, to account for dropouts or potential removal of subjects from study, a goal of 80 subjects are expected to be enrolled to ensure adequate sample size.

B. Statistical Methodology

Categorical data will be summarized as counts and percentages, and continuous data as means with corresponding standard deviations. Pre- post- results will use the chi-square test for association for nonparametric data or analysis of covariance for parametric data. Because multiple measures will be obtained for achieving the secondary objectives, multivariate analyses will be used for exploring overall treatment effects. Throughout this study, a p-value<0.05 (two-tail) will be considered statistically significant.

DATA AND SAFETY MONITORING PLAN

The Principal Investigator and the co-investigators will do safety monitoring. Ongoing assessment of subject safety will be monitored during subject phone calls as detailed in methodology. Any reported problems will be discussed with Medical Monitor/ Principle Investigator. Subjects with any signs or symptoms of hepatic decompensation or elevated liver enzymes beyond three times the upper limit of normal will be removed from the study, the study will be aborted for that subject and immediate diagnostic and/or therapeutic modalities will be delivered. It should be noted that our study is defined as minimal risk as also identified by the FDA with the exemption of need for an IND number.

Any subject found to be noncompliant with the protocol will be removed from the study and asked to return their medications to the office. The medications will then be returned to the research pharmacist.

All data will be kept electronically in a secure SharePoint site. Hard copies will be kept in a locked file located in a locked office until the study ends. Hard copies will then be scanned into the SharePoint site and shredded per Beaumont policy.

REFERENCES

[1] The Liver Meeting, Post-Graduate Seminar, New Research, American Association for the Study of Liver Diseases, Washington, D.C., 2017

Velasco, Nicolas; Contreras, Alvaro; Grassi, Bruno. The Mediterranean diet, hepatic steatosis and nonalcoholic fatty liver disease. Current Opinion in Clinical Nutrition & Metabolic Care. 17(5):453-457, September 2014.

Anania C, Perla FM, Olivero F, Pacifico L, Chiesa C. Mediterranean diet and nonalcoholic fatty liver disease. World J Gastroenterol. 2018;24(19):2083–2094. doi:10.3748/wjg.v24.i19.2083

Attachments

A. PRONE Study Informed Consent Form and Authorization for Disclosure of Protected Health Information

B. PRONE Study FDA 2018-1186 Signed Final Response

C. PRONE Study Nutrition Program

D. PRONE Study Exercise suggestions

E. PRONE Study Pharmacy Budget Estimate