Synbiotics and Conditioned Fecal Microbiota Transplantation to Treat Non-Alcoholic Steatohepatitis.

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Primary objective To investigate the therapeutic potential of A. soehngenii and pasteurized A. muciniphila combined with B. animalis subsp. lactis and FOS with and without conditioned vegan LFMT capsules to reduce NASH in patients with fibrotic NASH…

Ethical review	Approved WMO
Status	Recruiting
Health condition type	Hepatic and hepatobiliary disorders
Study type	Interventional

Summary

ID

NL-OMON51470

Source
ToetsingOnline

Brief title
SYNCH-trial

Condition

- Hepatic and hepatobiliary disorders

Synonym
non-alcoholic fatty liver disease, non-alcoholic steatohepatitis

Research involving
Human

Sponsors and support

Primary sponsor: Amsterdam UMC
Source(s) of monetary or material Support: A-mansia Biotech,Caelus Health,Het onderzoek wordt hoofdzakelijk gesponsord door een TKI-LSH PPP beurs van Health Holland

(TKI: Top Consortia for Knowledge and Innovation LSH: Life Sciences & Health; PPP: public-private partnerships). Daarnaast dragen de bedrijven Caelus Health (producent van A. soehngenii en leverancier van Bifidobacterium animalis) en A-mansia Biotech (leverancier van A. muciniphila) ook financieel bij aan het Consortium.

Intervention

Keyword: Fecal microbiota transplantation, Non-alcoholic steatohepatitis, Probiotics, Synbiotics

Outcome measures

Primary outcome

Improvement of liver histology in subjects with NASH and fibrosis stage 0-3,

with improvement defined as reduction of steatohepatitis by >=1 SAF-A point and

no worsening of liver fibrosis, or improvement in >= 1 stage liver fibrosis and

no worsening of steatohepatitis.

Secondary outcome

- non-invasive outcomes of NAFLD, i.e. multiparametric MRI of liver and

surrounding subcutaneous adipose tissue (MRI-PDFF, MR elastography, corrected

T1), FibroScan Elastography and Controlled Attenuation Parameters, and plasma

panel Enhanced Liver Fibrosis (ELF) panel, and plasma Pro-C3 concentrations.

- liver gene expression profile: lipogenic, inflammatory and fibrogenic pathways

- Liver pathology, histopathological features, immunofluorescence and

assessment of pathophysiological proteins

- blood markers of NAFLD and metabolic syndrome, namely: liver enzymes (i.e.

alanine amino transferase (ALT), aspartate amino transferase (AST), gamma

glutamyl transferase (GGT), alkaline phosphatase (ALP)), inflammatory blood

markers (i.e. leukocytes, monocytes, CRP, Il-1(β), Il-6, Il-11, Il-17, Il-32,

TNF-α, IFN-γ, other cytokines), SCFA (i.e. propionate, butyrate, acetate),

lactate, lipids (i.e. LDL, HDL, triglycerides, total cholesterol), FGF21, adiponectin, leptin, lipopolysaccharides and zonulin, and metabolomic and lipodomic panels.

- fecal microbiota composition, microbiome read outs (composition, engraftment, strain tracking) en metabolites, fecal albumin.
- glycemic control, insulin resistance, HOMA-index (HOMA-IR), body weight/BMI, waist circumference and percentage body fat
- MetSy criteria / %
- quality of life (general (SF36) and NAFLD/NASH-specific (CDLQ-NAFLD)).

Other study parameters

- BMI
- Waist circumference
- Percentage body fat
- Comorbidities (e.g. diabetes)
- Smoking, yes/no
- Alcohol intake
- Polypharmacy (defined as chronic use of >= 5 different medications)
- Daily caloric intake
- Daily fat consumption

Study description

Background summary

The prevalence and severity of NAFLD - excessive hepatic fat accumulation without excessive alcohol consumption - are increasing at an alarming rate, because more and more patients are living longer with the two main factors that drive NAFLD: obesity and T2DM. 20-30% of the global population including The Netherlands7 has some stage of NAFLD8, and in T2DM, this is staggering 60-80%. NAFLD is regarded as the hepatic component of the metabolic syndrome (MetSy) and T2DM. It can silently progress from simple steatosis to NASH (prevalence 2-3%) and NASH-related fibrosis, and ultimately to cirrhosis and hepatocellular carcinoma (fig.1)

Progression into the fibrotic stages of NAFLD is strongly associated with liver-related and overall mortality and increased risk of atherosclerotic cardiovascular disease, the latter most likely via induction of dyslipidemia. Although NAFLD-NASH has a complex pathogenesis with a multiplicity of pathways, it is thought that insulin resistance is a crucial trigger since it causes lipolysis in peripheral adipose tissue, resulting in increased hepatopetal free fatty acid (FFA) flux. This drives hepatic lipotoxicity, triggering NASH, necroinflammation and activation of hepatic stellate cells, initiating fibrogenesis.

To date, no treatment is available for progressive NAFLD stages.However, based on insights from observational studies and animal experiments, the gut microbiome and its metabolites are emerging as an innovative approach to treat this challenging metabolic liver disease, Potentially detrimental alterations in gut microbial composition have frequently been observed in NAFLD: abberant microbiota composition, often depleted in butyrate producers, reduced diversity, small intestinal bacterial overgrowth and signs of increased gut permeability. This project aims to study the effects of the combination of bacterial strains on NAFLD.

We recently published the first vegan fecal microbiota transplantation study in patients with NASH and aberrant microbial composition. This pilot study found anti-inflammatory effects of vegan FMT into NASH patients, with a trend towards reduced hepatic necro-inflammation. Upon vegan FMT engraftment, the gut microbiome of the NASH patients was enriched in Anaerobutyricum soehngenii (also/formerly known as Eubacterium hallii) and other butyrate-producing Lachnospiracae. Furthermore, in obese mice, A. soehngenii treatment improved insulin sensitivity and reduced intrahepatic triglyceride content. Of note, A. soehngenii is well known to not only convert sugars but also lactate and acetate into butyrate, a short chain fatty acid that may protect against NASH. Butyrate may reduce the inflammatory component of NAFLD by promoting differentiation of regulatory T cells. It also has an anti-migratory effects on neutrophils31, and in the gut, it protects epithelial barrier integrity, both potentially protective effects on NASH. Moreover, since lactate is found to be

increased in T2DM; the efficient conversion of lactate into butyrate by A. soehngenii is expected to contribute to its beneficial actions.

Another species thought to be benifical is B.animalis subsp. lactis of which the well-studied strain BLC1 is marketed as a probiotic and well characterized. In vitro studies have shown that this strain can form a trophic chain with A. soehngenii to accelerate butyrate production from FOS. In addition, the BLC1 strain can in conjuction with A. soehngenii improve butyrate production in a model of the small intestine. Moreover, B.animalis subsp lactis with FOS has been shown to improve intestinal discomfort and promote anti-inflammatory properties.

Pasteurized A. muciniphila is involved in host immunological homeostasis at the gut mucosa, improvement of gut barrier function and contributes to metabolic health. Indeed, in a study assessing the effects of pasteurized A. muciniphila on diet-induced metabolic disorders in mice, it was not only found that pasteurization did not diminish the beneficial effects, but it also unexpectedly enhanced the beneficial impact of A. muciniphila on insulin resistance and dyslipidemia. In addition, a randomized, double-blind, placebo-controlled proof-of-concept study in overweight/obese insulin-resistant human volunteers showed that the daily supplementation with pasteurized A. muciniphila at a daily dose of 10^10 cells (based upon CFU count) for 12 weeks improved several metabolic parameters such as insulin sensitivity, insulinemia, plasma total cholesterol, as well as relevant blood markers for liver dysfunction and inflammation while serum lipopolysaccharide (LPS) levels were decreased, indicative of improved barrier function. A recent study in a high fat induced fatty liver mouse model showed that administration of A. muciniphila cells prevented fatty liver disease by regulation of the expression of genes that regulate fat synthesis and inflammation in the liver. Since these studies all found that pasteurized A. muciniphila cells were similar or more efficient than the same amount of live A. muciniphila cells, we aim to use pasteurized A. muciniphila ATCC BAA-835T cells in the present study. Of importance, these cells have recently received EFSA approval based on the extensive toxicological safety assessment.

Recently studies found an interesting mechanism by which A. soehngenii may reduce NASH. In patients with MetSy, of which NAFLD is considered the hepatic equivalent, duodenal administration of A. soehngenii strongly increased glucagon-like peptide 1 (GLP1) secretion from the enteric endocrine L-cells, potentially relating to the observed reduced plasma glucose fluctuations. Of note, A. muciniphila administration induced GLP1 secretion in a mouse model. Interestingly, clinical evidence is mounting that enhancing GLP1 may be a plausible approach to reduce NASH. In a subset of patients from the phase 2A LEAN trial with a GLP1-receptor agonist, it was shown that in addition to weight loss and improved glycemic control, insulin-resistance driven hepatopetal FFA-flux - the main contributor to lipid overload in NAFLD - was reduced. In a recent Ph2B trial with a next generation GLP1 receptor agonist,

NASH histology improved and signs of fibrosis regression were noted.

Candidate therapeutic bacteria may be potentiated when combined with prebiotics, i.e. non-digestible food substances, which can be fermented by bacteria, promoting their growth. Such combinations are termed synbiotics. Early evidence from murine studies and pilot studies with synbiotic cocktails in NASH patients underscore the feasibility of this approach. NASH-fibrosis was reduced by a cocktail of 4 prebiotics and 4 probiotics in a high fat-choline-deficient diet mouse model. In addition, ALT and TNF-a were reduced in patients with NAFLD who received a synbiotic cocktail of 7 bacterial strains with the prebiotic fructo-oligosaccharides (FOS; oligomer of <10 fructose molecules). In another pilot study in patients with NASH, a 12-week treatment with a synbiotic cocktail of 5 probiotic bacteria and fructo-oligosaccharides reduced ALT and liver stiffness on elastography. Together these reports are supportive, yet the murine study did not decipher any mechanisms underlying the protective effects and the human studies e.g. did not provide liver histology, the gold standard in therapeutic development for NASH.

Furthermore, FOS is degraded to lactate and acetate by Bifidobacteria spp. such as Bifidobacterium longum or B. animalis subsp. lactis, a well-studied strain applied as a probiotic. The generated lactate and acetate has shown to be effectively converted by A. soehngenii into butyrate in in vitro experiments, including simulated ileum and colon models at ProDigest. Such a cross-feeding mechanism can play a role in the colon ecosystem and contribute to the combined bifidogenic/butyrogenic effect observed after addition of FOS to the diet. In this context a recent human intervention study should be noted where administration of B.animalis subsp. lactis with FOS was found to improve intestinal discomfort and promote anti-inflammatory properties.

In our study, we will apply the complementary mode of actions of the individual components in the synbiotic treatment, the established effects of their combinations, and their expected impact on both upper intestinal tract and colon. We hypothesize that for NASH, synbiotic combination treatment will change the tipping point in the gut microbiome in order to reduce steatohepatitis and liver fibrosis.

Study objective

Primary objective
To investigate the therapeutic potential of A. soehngenii and pasteurized A. muciniphila combined with B. animalis subsp. lactis and FOS with and without conditioned vegan LFMT capsules to reduce NASH in patients with fibrotic NASH.

Secondary objective
To investigate the mechanisms of A. soehngenii and pasteurized A. muciniphila combined with B. animalis subsp. lactis and FOS with and without conditioned

vegan LFMT capsules in reducing NASH in patients with fibrotic NASH.

Study design

Double-blind randomized placebo-controlled intervention study.

Intervention

Participants will either receive 3x 21 LFMT capsules on 1 day and daily 2 LFMT-capsules, or the same amount of placebo capsules.

All participants will daily ingest 10^9 A. soehngenii CH-106 cells, 10^10 B. animalis subsp. lactis BLC1 (commercially available Sacco SRL) and 3x10^10 pasteurized A.muciniphila cells and 5 grams of FOS.

Study burden and risks

Benefits

NAFLD and NASH have an enormous and increasing prevalence: 20-30% of the gobal population has NAFLD to some extent. NAFLD-related livercirrosis is a growing indication for liver transplantation and the incidence of NASH-related hepatocellular carcinoma is increasing. The disease progresses slowly and NASH-liver fibrosis is strongly associated with atherosclerotic cardiovascular disease and liver-related and overall mortality. Nevertheless, there is no registered or proven treatment for progressed stages of NAFLD-NASH.
As previously stated, extensive prior research associates the gut microbiome with NAFLD-NASH. Regardless, these studies did not entail an treatment targeting the microbiome to reduce NAFLD-NASH, which would be a big step in the field. Based on available literature on treatment with synbiotics in NAFLD/NASH-patients and our pilot study on FMT in NAFLD/NASH-patients, this trial with adequate sample size endeavours to study if modification of the gut microbiome can attenuate NASH and liver fibrosis.
The benefits the study entails will be discussed in three sections. First, we expect a short term benefit for participants. Our underpowered study in which we administered FMT in NAFLD, showed a trend to reduction of necro-inflammation (i.e. inflammation and ballooning) in histopathological examination. Inflammation and ballooning are the most important predictors of progression of NAFLD-NASH, and subsequent secondary complications.9 Half of the individuals will receive FMT, and we expect that this already will attenuate NASH. All participants will, in addition, be treated with a complementary synbiotic combination. Multiple small clinical trials already showed a beneficial effect of synbiotics on NAFLD-NASH, without entailing serious side effects. Therefore, we expect an beneficial effect of the synbiotics in both study arms.
Second, participants will be able to benefit from the development of a treatment for NASH. As stated, NASH is a slowly progressing, chronic disease,

without an effective treatment. If this study leads to a treatment of NASH, it is likely that participants in the future will receive the investigated treatment. Moreover, participants will assist in further understanding of the pathophysiology of NASH and its relation to the gut microbiome, possibly rendering further leads for the development of a therapy for NASH. Furthermore, the current study and its subsequent insights could not only lead to a treatment, but could also be the basis for preventive measures such as effective and relative easily applicable lifestyle and dietary interventions targeting the gut microbiome.
Last, the current study might benefit health on a population scale. As mentioned, the prevalence of NAFLD and NASH is high and increasing subsequent to the increase of its two main risk factors: obesity and T2DM. Fibrotic-NASH is strongly associated with atherosclerotic cardiovascular events and liver-specific and overall mortality. Considering the enormous prevalence, this study and its possibilty to develop an effective treatment has the potential to benefit the global population on an tremendous scale. Furthermore, in comparison to medicinal alternatives currently investigated in the phase 2 and 3 trials for the treatment of NASH, FMT and synbiotics give a significant reduction of adverse effects.

Risks and burdens

The study protocol is quite intense for participants. In summary, the burden consists of the ingestion of LFMT capsules (3x on study visits 21 capsules, daily 2 capsules) and daily ingestion of pre- en probiotica for 168 days, 2x a liver biopsy, 2x a MRI and fibroscan, 4x 7 days of continuous glucose measurements and 5x blood withdrawal. The risk for participants will mainly be due to liver biopsies, with a complication rate of approximately 1 in 1000 per procedure. Below, the risks and burdens are discussed more extensively.

FMT and synbiotics
In the literature and our center, FMT procedures have not been associated with (serious) adverse events. Literature concerning the treatment with LFMT capsules reports a lower number of adverse events in comparison to the infusion of FMT via duodenal tube. The most prevalent side effects are mild, self-resolving abdominal pain, nausea, diarrhea, and flatulence.
In regard to possible infectious transmission, fecesdonors are extensively screened to mitigate the risk of potential infections by the FMT. Moreover, fecesdonors are screened again after 2 months (during the storage of the capsules) prior to administration of the LFMT capsules to the participants. Participants with an immune deficiency are excluded from the studie. In our center, FMT procedures have not been associated with adverse events, and donors are extensively screened to mitigate the risk of potential infections by the FMT procedure.

The provided synbiotics, moreover, are well-researched and deemed safe in both toxicological studies and trials in humans, with no serious side effects

reported.

The predominant burden of the LFMT capsules and synbiotics will consist of taking the capsules. On the days of the study visits (3x), participants will ingests 21 capsules of FMT and 3 probiotic capsules. Daily, participants will take 2 LFMT capsules, 3 probiotic capsules, and 5 grams of the fructo-oligosaccharide powder. The ingestion of the bulk of 21 LFMT capsules on the days of the study visits will be spread over the day to mitigate the theoretical risk of clotting of the capsules in the stomach (which might prevent normal passage to the duodenum). All capsules are designed to open in the duodenum, therefore the content of the capsules will not reach the stomach or a higher part of the digestive tract.

Liver biopsies
To date, liver biopsy is still the golden standard in the assessment of progression or reduction of NASH, and there is no alternative. In therapy development for NASH, the American FDA, for example, demands liver biopsy in clinical trials. This study will, however, contribute to the further development and validation of multiparametric MRI as a substitute for biopsy by comparing the two.
An experienced interventional radiologist will perform ultrasound-guided liver biopsy. Ultrasound-guided percutaneous liver biopsy is a safe method with very low the risk of complications (< 1 per 1000 persons) comprising mostly bleeding from the biopsy site. Moreover, local hemostasis after the procedure can be observed, and patients will be screened for bleeding disorders. Liver biopsy will be performed under local anesthesia, which can cause short discomfort.

Continuous glucose monitoring
Participants will have a continuous glucose monitoring-device (Freestyle libre) installed for 4 times 7 days, in order to investigate the relation with GLP1. This is usually well tolerated (as we see in diabetic patients), however the prick of the installment can be unpleasant.

Blood withdrawal
Blood withdrawal, which happens five times, can be unpleasant, and can cause self-limiting (sub)cutaneous hemorrhage/bruising.

Diaries and questionnaires
Last, participants have to keep a daily diary of food-intake, and have to fill out two questionnaires on quality of life at baseline and week 24.

Contacts

Public

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

- age 18-75 years
- biopsy-proven NASH obtained up to 32 weeks before screening: SAF Steatosis score >=1, Activity >=2, Fibrosis <4; 50% of participants should at least have NASH fibrosis stage 1, 2 or 3 according to the NASH CRN fibrosis staging system based on tandem reading of two expert liver pathologists
- fluency in Dutch or English
- subjects should be able to understand the information and give informed consent

Exclusion criteria

- Current or history of significant alcohol consumption for a period of more than 3 consecutive months within 1 year before screening (significant alcohol consumption is defined as more than 2 units/day for females and more than 3 units/day for males, on average)

- liver cirrhosis or hepatocellular carcinoma
- hepatitis B and/or C
- auto-immune hepatitis
- Wilson*s disease
- primary sclerosing cholangitis
- primary biliary cholangitis
- alpha-1-antitripsine deficiency and hemochromatosis
- history of liver transplant, current placement on a liver transplant list
- use of pre-, pro- or synbiotics
- use of systemic antibiotics 3 month prior to randomization
- prior or planned bariatric surgery
- active GLP-1 receptor agonist treated diabetes mellitus
- bleeding disorder
- International normalized ratio (INR) of prothrombin time >1.4 or platelet count <100 109/L at screening
- anti-platelet/coagulant therapy use which cannot be temporarily discontinued
- any major cardiovascular event within 6 months prior to screening (e.g. myocardial infarction, cerebrovascular accident)
- prolonged compromised immunity (e.g. recent cytotoxic chemotherapy, HIV-infection with a CD4 count < 240)
- active or prior history of invasive malignancy (except for curatively treated in situ carcinomas [e.g., cervix] or non-melanoma skin cancer) unless a complete remission was achieved
- surgery scheduled for the trial duration period, except for minor surgical procedures, in the opinion of the investigator
- pregnant or nursing women
- any condition which, in the investigator*s opinion, might jeopardize subject*s safety or compliance with the protocol,
- participation in another concomitant clinical trial.

Study design

Design

Study phase:	2
Study type:	Interventional
Intervention model:	Parallel
Allocation:	Randomized controlled trial
Masking:	Double blinded (masking used)
Control:	Placebo
Primary purpose:	Treatment

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	17-11-2022
Enrollment:	52
Type:	Actual

Ethics review

Approved WMO Date:	15-08-2022
Application type:	First submission
Review commission:	METC Amsterdam UMC
Approved WMO Date:	11-11-2022
Application type:	Amendment
Review commission:	METC Amsterdam UMC
Approved WMO Date:	28-04-2023
Application type:	Amendment
Review commission:	METC Amsterdam UMC

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL81001.018.22