PROTOCOL TITLE

Open-Label Prospective Randomized Control Trial to Investigate the Efficacy of Omega-3 Fatty Acid Therapy in Preventing Gastrointestinal Bleeding in Patients with Continuous-Flow Left Ventricular Assist Device

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Open-Label Prospective Randomized Control Trial to Investigate the Efficacy of Omega-3 Fatty Acid Therapy in Preventing Gastrointestinal Bleeding in Patients with Continuous-Flow Left Ventricular Assist Device

PRINCIPAL INVESTIGATOR

Gene Kim, MD
Department of Medicine, Section of Cardiology
(773) 702-9396

OBJECTIVES

Aim 1: We will evaluate whether high-dose omega-3 fatty acid (O3FA) supplementation alters markers of angiogenesis and inflammation in patients with continuous-flow left ventricular assist devices (CF-LVAD).

- **Aim 1a** will assess the effect of high-dose O3FA in patients who have not experienced an episode of gastrointestinal (GI) bleeding. Patients will be enrolled up to 3 months following CF-LVAD implantation and randomized to high-dose O3FA or none. Markers of angiogenesis and inflammation will be assessed prior to and following initiation of high-dose O3FA therapy.
- **Aim 1b** will assess the effect of high-dose O3FA in CF-LVAD patients who have already experienced a GI bleeding event. Patients who have experienced a prior bleeding event will be enrolled and randomized to high-dose O3FA or none. Markers of angiogenesis and inflammation will be assessed prior to and following initiation of high-dose O3FA therapy.

Aim 2: We will assess the changes that occur to the microbiome of CF-LVAD patients following O3FA supplementation.

Aim 3: We will assess the effect of high-dose O3FA supplementation on GI bleeding events in both the population without prior GI bleeding events, and the population with prior GI bleeding events.

BACKGROUND

Continuous-flow left ventricular assist devices (CF-LVAD) have become the mainstay of therapy for advanced heart failure, both as bridge to transplantation and as destination therapy, allowing significant improvement in survival. However, this therapy is limited by a high adverse event profile, specifically those related to hemocompatibility.

One of the leading causes for readmission after CF-LVAD implantation is gastrointestinal (GI) bleeding, representing nearly 30% of both early and late readmissions following device implantation[1]. Multiple reports found that 25-40% of the patients will experience GI bleeding, with 15.9% in the first 6 months in the MOMENTUM 3 trial, and 35.1% in the ENDURANCE trial [2, 3]. Originally, pathophysiology of GI bleeding was attributed to Von Willebrand factor deficiency [4, 5]. More recently, focus has shifted to the development of arteriovenous malformations (AVMs) in the GI tract as a major contributor to the development of GI bleeding in patients supported with CF-LVADs [6].

The process of angiogenesis is controlled by angiopoietins 1 and 2 (Ang-1 and Ang-2), and vascular endothelial growth factor (VEG-F). Ang-1 leads to normal angiogenesis in conjunction with VEG-F, while Ang-2 promotes dysfunctional vessel growth with an expression that is highly regulated by the thrombin receptor PAR-1. Our group recently demonstrated that an abnormal ratio of Ang-1 and Ang-2 has a key role in abnormal angiogenesis, due to elevated Ang-2 and reduced Ang-1[7]. CF-LVAD patients with Ang-2 levels above the mean had more nonsurgical bleeding events compared to those with levels below the mean [7]. Furthermore, we found that tumor necrosis factor-α (TNF-α) was significantly higher in serum of patients with CF-LVAD compared to patients with heart failure or heart transplantation, and contributes to abnormal angiogenesis by killing pericytes and reducing Ang-1 levels [8]. We have confirmed these findings in a larger cohort from the PREVENT biobank. 114 paired samples obtained before and 3 months after CF-LVAD implantation were analyzed. 69% of patients with increased levels of Ang-2 and TNF-α experienced significant GI bleeding 6 months after implantation while only 38% of patients with Ang-2 and TNF-α levels below the mean had GI bleeding (p=0.02).

To further implicate the role of dysregulated angiogenesis in GI bleeding in CF-LVAD patients, Houston et al. evaluated the use of angiotensin-converting enzyme inhibitor (ACEI) and angiotensin receptor blocker (ARB) therapy in reducing the risk of GI bleeding. Ang-2 expression has previously been shown to be induced through angiotensin II signaling [9]. Houston et al showed that of the patients who did not receive angiotensin II antagonism, 48% experienced GI bleeding. In contrast, only 24% of the patients who received ACEI or ARB experienced GI bleeding [10].

In the most recent J-MACS (Japanese registry for Mechanically Assisted Circulatory Support registry) report, GI bleeding occurred in 4 of 259 patients followed during a 5-year period [11]. This is in stark contrast to the latest INTERMACS update that reports a rate of 16.24 events of GI bleeding per 100 patient months in the first 3 months after implantation, and 4.08 events per 100 patient months in the 3-12 months after implantation [12]. What accounts for this difference? One possibility may lie in the Japanese diet, which is rich in omega 3 fatty acids (O3FA) compared to the diet of patients in the United States. We performed a retrospective study in a cohort of 166 patients with CF-LVAD to assess this theory. Patients who received high-dose O3FA as part of a recovery protocol were compared to those who did not. The frequency of GI bleeding was significantly lower in the O3FA group compared with the control group (0.077 ± 0.419 events/year vs. 0.305 ± 0.847 events/year, p = 0.033), leading to a higher 1-year event-free rate in the O3FA group compared to the control group (97% vs. 79%, p = 0.046).

In the past decade, there has been an emergence of interest in the human gut microbiota. Using next-generation sequencing (NSG), studies have been able to characterize with great detail the gut microbiome in humans. Further studies have shown that the microbiota is affected by patient characteristics (age, sex, body mass index, lifestyle), diet, and pathologic diseases [13]. In particular, O3FA and its effect on the gut microbiota has been studied, showing that an increase in dietary or supplemental O3FA changes the fingerprint of the microbiome [14-16]. O3FA and the microbiome also have a presence in the cancer literature. It is postulated that O3FA play a role in the prevention and treatment of cancer by reduction in cell proliferation, cell survival, angiogenesis, and inflammation [17].

The data presented thus far shows a possible link between O3FA, GI bleeding, and abnormal angiogenesis, and may be related to the microbiome in CF-LVAD patients. This prospective randomized study aims to evaluate the effect of supplemental O3FA on the frequency of GI bleeding in patients implanted with CF-LVAD both as primary and secondary prevention.

Additionally, we will investigate the effect of O3FA on angiogenesis parameters (Ang-1, Ang-2, VEGF, TNF-α) and the microbiome.

STUDY ENDPOINTS
The primary endpoint will be a change in angiogenesis markers resulting in dysregulated angiogenesis, as described above. Secondary endpoints include rate of GIB and improvement in inflammatory markers.

STUDY INTERVENTION
The study intervention being evaluated is high-dose O3FA (4,000 mg), which is an over-the-counter supplement. Heart failure patients are routinely instructed to take an O3FA supplement for heart health. The research team will fund the supplement, which will be stored in the heart failure office. Once a patient is enrolled, a total of 1 year of supplement will be given to the patient for consumption at home.

PROCEDURES INVOLVED
We will perform an open-label, prospective, randomized control study in which 120 CF-LVAD patients are enrolled. We plan to have 2 separate arms: primary prevention and secondary prevention (60 in each arm). In the primary prevention arm, 60 patients who have received CF-LVAD implantation and have not experienced any episodes of GI bleeding will be assigned to an O3FA group or a control group, with 30 patients in each group. In the secondary prevention arm, 60 LVAD patients who have experienced at least 1 episode of GI bleeding after CF-LVAD implantation will be assigned to an O3FA group or a control group, with 30 patients in each group. Randomization will be performed using computer software, and initiation of therapy will start within 24 hours of randomization. O3FA will be given at a dose of 4,000 mg orally per day, and all patients will receive guideline-directed medical therapy during the study period.

The following study procedures will be performed:

Baseline
Subject to sign consent
Verify inclusion/exclusion criteria
Record medical history and medications
Collect stool sample
Collect blood sample
Randomization
For 03FA subjects:
-Provide O3FA supplement
-Subjects will be instructed to take 4 pills a day

1.5 months post-randomization
Record medical history and medications
Record adverse events
For 03FA subjects:
-Provide O3FA supplement
-Subjects will be instructed to take 4 pills a day
-Study drug compliance check

3 months post-randomization
Record medical history and medications
Collect stool sample
Collect blood sample
Record adverse events
For 03FA subjects:
-Provide O3FA supplement
-Subjects will be instructed to take 4 pills a day
-Study drug compliance check

4.5 months post-randomization
Record medical history and medications
Record adverse events
For 03FA subjects:
-Provide O3FA supplement
-Subjects will be instructed to take 4 pills a day
-Study drug compliance check

6 months post-randomization
Record medical history and medications
Collect stool sample
Collect blood sample
Record adverse events
For 03FA subjects:
-Provide O3FA supplement
-Subjects will be instructed to take 4 pills a day
-Study drug compliance check

7.5 months post-randomization
Record medical history and medications
Record adverse events
For 03FA subjects:
-Provide O3FA supplement
-Subjects will be instructed to take 4 pills a day
-Study drug compliance check

9 months post-randomization
Record medical history and medications
Record adverse events
For 03FA subjects:
-Provide O3FA supplement
-Subjects will be instructed to take 4 pills a day
-Study drug compliance check

10.5 months post-randomization
Record medical history and medications
Record adverse events
For 03FA subjects:
-Provide O3FA supplement
-Subjects will be instructed to take 4 pills a day
-Study drug compliance check

1 year post-randomization
Record medical history and medications
Collect stool sample
Collect blood sample
Record adverse events
For 03FA subjects:
-No more O3FA supplements will be provided by the study
-Study drug compliance check

Stool Collection for Microbiome Sequencing Analysis
Stool will be collected in the hospital prior to CF-LVAD implantation, as most patients have prolonged hospitalizations prior to implantation. Additional stool samples will be collected at home after CF-LVAD implantation at 3 months, 6 months, and 1 year. Stool samples will be collected using home stool sample kits. The stool will be kept cool in anaerobic conditions and will be shipped directly to Dr. Jack Gilbert's laboratory at the University of Chicago for microbiome analysis. For whole stool samples, we will employ the BioCollector at home stool sampling kit (BioCollective, Denver CO, USA). Smaller stool samples will be collected using a non-invasive double-tipped sterile polyester culture swabs (Becton-Dickinson catalog # BD 220135). All samples will be shipped straight to Dr. Gilbert's lab for aliquot and storage at -80C until DNA extraction of microbiome samples. Microbiome DNA will be isolated with the MoBio Powersoil kit with the addition of mechanical lysis. DNA samples will be subsequently quantified by Quant-iT PicoGreen dsDNA Assay (Life Technologies) and normalized to a concentration of 50 pg/mL. Whole-genome shotgun sequencing libraries will be prepared according to the manufacturer's instructions using the Nextera XT DNA Library Preparation kit (Illumina) with 100-250 pg input DNA. Libraries will be pooled by transferring equal volumes of each library using a Labcyte Echo 550 liquid handler. The concentrations and insert size ranges for each pooled library will be checked using an Agilent Bioanalyzer DNA 1000 kit (Agilent Technologies). Libraries will be subsequently sequenced on the Illumina HiSeq 2500 platform in paired-end mode (2x151bp) targeting ~10Gb of sequences per sample.

Blood Collection
Complete metabolic panel including liver function tests, complete blood count, lipid profile, and inflammatory markers (hs-CRP and ESR) will be collected as standard of care. Approximately 20mL of peripheral blood will be collected and tested for markers of angiogenesis using a multiplex immunoassay system (Biorad). The angiogenesis factors to be measured are angiopoetin-2, epidermal growth factor (EGF), endoglin, sCD40L, vascular endothelial growth factor A, C and D, (VEGF-A, -C, -D), placental growth factor (PlGF), heparin-binding EGF-like growth factor (HB-EGF), plasminogen activator inhibitor-1 (PAI-1), insulin-like growth factor binding protein (*IGFBP*)-*1*, transforming growth factor alpha (TGF-α), and urokinase-type plasminogen activator (uPA). The inflammatory markers to be measured are Interleukin-6 (IL-6), Interleukin-8, Interleukin-18 (IL-18), sCD40L, soluble Fas ligand (sFASL), and TNFα.

Data will be collected from the subjects' medical records for up to 2 years post-randomization to collect outcomes and adverse events.

GIB episodes will be defined by INTERMACS:

An episode of <u>SUSPECTED INTERNAL OR EXTERNAL BLEEDING</u> that results in 1 or more of the following:

1. Death
2. Re-operation
3. Hospitalization
4. Transfusion of red blood cells as follows:
    - During first 7 days post implant (≥4 units packed red blood cells within any 24-hour period in patients ≥50kg or ≥20cc/kg packed red blood cells within any 24-hour period in patients <50kg)
    - After 7 days post implant (transfusion of packed red blood cells (PRBC) after 7 days following implant with the investigator recording the number of units given.)

During the 1-year study period, clinical events including GIB and other major adverse events will be recorded and evaluated on a regular basis.

DATA AND SPECIMEN BANKING

Peripheral blood collected from consented participants will be stored in refrigerators at -80C for the duration of the study. All members of the research team will have access to the specimens.

Stool samples collected from consented participants will be shipped straight to Dr. Gilbert's laboratory for microbiome analysis and storage. All samples will receive a designated study code. This code will be linked to the patient's data.

All samples, blood and stool, will be stored indefinitely for future research related to heart failure, GI bleeding, Omega-3 supplements, and microbiome.

STUDY TIMELINES

Each subject will actively participate in the study for 1 year after randomization. We will collect data from the subjects' medical records for an additional year. It will take approximately 6 months to enroll all study participants. Once the study is complete, it will take approximately 3 months to complete the primary analysis.

INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria

1. Subject has signed consent
2. Age ≥ 18 years
3. Subjects with a CF-LVAD or are scheduled to receive a CF-LVAD implant

Exclusion Criteria:

1. Psychiatric disorder or disease, irreversible cognitive dysfunction or psychosocial issues that might impair compliance with the study.
2. Patients already taking O3FA.

RECRUITMENT METHODS

Patients with advanced heart failure will be seen in clinic, in the cardiac catheterization laboratory, or inpatient wards or intensive care units. If the patient goes on to have an evaluation for LVAD during their clinical course, a member of the research team will approach the patient. We currently implant approximately 60 CF-LVAD per year at the University of

Chicago. Recruitment from the recently implanted cohort and newly implanted patients should provide us with sufficient patients for this study.

COMPENSATION FOR PARTICIPATION IN RESEARCH ACTIVITIES
There will be no compensation to participants in this study.

WITHDRAWAL OF PARTICIPANTS
Participants can be withdrawn without consent if they are not compliant with taking O3FA daily. They will be notified of withdrawal and all their data will be permanently erased. If participants decide to withdraw from the study, all of the O3FA will be returned to the research team, and the all of their data will be permanently erased.

RISKS TO PARTICIPANTS
There is a slight risk associated with vein punctures. We will make every effort to obtain the samples during a standard of care blood draw. If this is not possible, the risks associated with a blood draw include pain, dizziness, fainting, infection (rare), and redness. There is also a risk of loss of confidentiality, which will be minimized by efforts to secure the data.

POTENTIAL BENEFITS TO PARTICIPANTS
There will be no direct benefit to patient. We hope that the existence of this data will facilitate fundamental and significant research in advanced heart failure, ultimately benefitting patients in the future.

STATISTICAL ANALYSIS, DATA MANAGEMENT, AND CONFIDENTIALITY
Statistical analysis will be performed using SPSS Statistics 22 (SPSS Inc, Chicago, IL, USA). Variables with 2-tailed p value <0.05 will be considered significant. Clinical parameters and outcomes including rates of GI bleeding, angiogenesis-related biomarkers, inflammatory markers, and microbiome data will be compared between the O3FA and control groups, both among the primary and secondary prevention arms using unpaired t-test or Mann-Whitney U test as appropriate. A necessary patient number for each group is calculated as 26 (total 104) by power analysis with 0.05 of α, 0.8 of effect size, 0.8 of $1-\beta$.

A master list maintaining patient names and code numbers will be stored in locked files. A password-protected computer located in the heart failure main office will contain a database for data collected in this study. Case report forms (CRF's) will be generated and filled out for each subject enrolled in this study.

There is a risk of loss of confidentiality. If a patient agrees to participate, and signs an informed consent, a signed copy of the consent will be given to them, a copy placed in their record and then the data will be abstracted into an electronic database. The database will be within the Department of Medicine, Section of Cardiology. The data will be password protected and covered by all of the firewalls and security features of the Department of Medicine.

The investigators seek to minimize these risks by labeling the study related data and specimens with a study code. This study code will be linked with the patient's data. Only the investigators

and their designated staff will have access to the databases and the link between study code numbers and patient identifying information.

Data and samples may be utilized by faculties who have the proper IRB approval to do so, and whose study objective meets with the overall objective of this research.

We may share coded data and/or samples outside of the institution in collaboration efforts. An IRB amendment will be submitted to the IRB for review if this happens. Additionally, if NIH funding is obtained for any proposed study, it is possible that the PI will be required to provide a copy of coded data to the NIH for public use. In either case, direct patient identifiers will never be shared.

ECONOMIC BURDEN TO PARTICIPANTS

There will be no economic burden to the participants.

CONSENT PROCESS

The consent process will take place in either clinic, cardiac catheterization laboratory, and inpatient wards or intensive care units. Patients will be given an informed consent and allowed as much time as needed to read all components and be provided time to formulate questions. If questions arise, the PI or a member of the research team will provide answers to the patient regarding participation in the research study.

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