

Effects of Empagliflozin on Gut Microbiota in Heart Failure with a Preserved Ejection Fraction: The Design of a Pragmatic Randomized, Open-Label Controlled Trial (EMPAGUM)

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Abstract: Although empagliflozin has been recommended for individuals with heart failure, its effects on heart failure with preserved ejection fraction (HFpEF) remain uncertain from a physiopathological standpoint. The metabolites produced by gut microbiota have been shown to have a crucial role in the development of heart failure. Sodium-glucose cotransporter-2 inhibitors (SGLT2) have been shown to change the make-up of the gut microbiota in rodent studies. There is mixed evidence from similar studies investigating whether or not SGLT2 can affect the microbiota in the human gut. This trial is a pragmatic, randomized, open-label controlled study with empagliflozin as an intervention. We will enroll 100 patients with HFpEF and randomly assign them to one of two groups to receive either empagliflozin or a placebo. Patients in the Empagliflozin group will be given 10 mg of the drug daily, while those in the Control group will not be given empagliflozin or any other SGLT2. The purpose of the trial is to validate the changes that occur in gut microbiota in patients with HFpEF who take empagliflozin and to investigate the function of gut microbiota and their metabolites in the process.

Keywords: derived metabolite, gut microbiota, heart failure, HF, myocardial fibrosis, SGLT2, short-chain fatty acids, SCFAs, sodium-glucose cotransporter-2 inhibitor

Introduction

Heart failure (HF) is a complex clinical disease characterized by symptoms and indications caused by any anatomical or functional impairment of ventricular filling or blood ejection.¹ As one of the major cardiac disorders, HF has a significant morbidity and mortality rate around the world. According to data from a multicenter observational study, the combined endpoint of mortality or HF hospitalization after one year was 36% for acute heart failure (AHF) and 14.5% for chronic heart failure (CHF).² There were 1.2 million heart failure hospitalizations in the United States in 2017, involving 924,000 patients. This represents a 26% increase over the last decade, and the figure is expected to rise more in the future.¹ Heart failure can be categorized into several phenotypes based on the left ventricular ejection fraction (LVEF), with HFpEF accounting for more than half of all patients.³ HFpEF is a complex biological process that includes systemic inflammation, epicardial adipose tissue buildup, cardiac fibrosis, and vascular dysfunction, among other metabolic pathways.⁴⁻⁶ However, the mechanisms and therapeutic strategies for it require additional experimental data.⁷

Intestinal flora is a complex collection of microbial communities that are intimately linked to the human body and participate in a variety of biological processes.⁸ Patients with heart failure frequently have decreased cardiac output as well as peripheral circulation congestion. In this circumstance, intestinal ischemia and edema cause a disruption in gut flora, resulting in an overproduction of inflammatory agents and toxic metabolites, which accelerates the HF process.^{9–11} However, new evidence suggests that gut metabolites play a role in various HF pathways. Some of them (such as SCFAs) have potential protective effects, whereas others (uremic toxins) have the inverse effect.¹²

Empagliflozin, a novel type of hypoglycemic medication, is an SGLT2 that blocks glucose reabsorption by the kidneys. As a result, more glucose is excreted in the urine, decreasing blood glucose levels. However, multiple research have revealed other biological effects of SGLT2. SGLT2 has been shown in animal models to reduce sodium consumption, inhibit NO production, enhance cardiac energy metabolism, and regulate inflammation.^{13,14} The EMPEROR-preserved trial is a large-scale Phase III clinical trial examining the effect of empagliflozin on the prognosis of patients with HFpEF. According to current research, empagliflozin may be effective in this type, with a 21% reduction in the key composite endpoint of time to HF hospitalization or cardiovascular mortality.¹⁵ However, the mechanisms by which SGLT2 works on HF are not completely understood. Recent studies have found that SGLT2 impacts the intestinal microbiota in animal models.^{16,17}

Given the growing importance of intestinal microbiota in HF and the effects of SGLT2 on intestinal microbiota and HF, we are conducting a clinical trial to assess the effects of empagliflozin on gut microbiota in patients with HFpEF under real-world conditions, and this paper describes the study's design (NCT05584319).

Aim of the Study

The purpose of this study is to investigate the effects of empagliflozin on intestinal microbiota and associated metabolites in individuals with HFpEF.

Study Oversight

The trial is a pragmatic, randomized, open-label controlled study with empagliflozin as an intervention. We will enroll 100 patients with HFpEF and divide them into two groups. Patients in the Empagliflozin group will receive 10 mg of empagliflozin daily, whereas patients in the Control group will not receive empagliflozin or any other SGLT2.

Study Participants and Inclusion/Exclusion Criteria

The trial will enroll 100 participants with HFpEF between November 2022 and October 2024. After signing the informed consent form, patients who meet the inclusion criteria will be randomly assigned to either the Empagliflozin Group or the Control Group (Table 1).

Study Process and Follow-Up

The study is a prospective open-label randomized controlled trial conducted at Shengjing Hospital in Shenyang, China. After a week of screening, participants who meet the inclusion criteria and sign the informed consent form will be randomly assigned to either the Empagliflozin or the Control groups. Randomization will be conducted using RANDOM.ORG (an online randomization website). Participants having a randomized figure between 1–50 will be assigned to the Empagliflozin group, while the others will be assigned to the Control group.

The observation course will continue for 6 months, during which time efficacy and safety assessments will be carried out. Laboratory investigations, imaging examinations, physical examinations, and questionnaires will be used to assess the patients' prognosis at both the beginning and end of the trial. The following are the specifics:

Laboratory Examinations

Various biomarkers, including but not limited to C-reactive protein (CRP) and soluble suppression of tumorigenicity 2 (ST2); end-organ functional assessments (liver function, renal function tests), and serum level of N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) will be evaluated. NT-proBNP will be evaluated using the Hiscl-5000 high-sensitivity chemiluminescence immunoanalyzer (Japan) by Hissen Micron, and sST2 will be assessed using the Shanghai Meilian Co., Ltd. enzyme-linked immunosorbent assay (ELISA). Liver function, renal function tests, and other

Table 1 Inclusion/Exclusion Criteria for the Trial

Inclusion Criteria	<ol style="list-style-type: none"> 1. Age \geq 18 years old, BMI 18.5–27.9kg/m. 2. Patients with heart failure and EF > 40%, New York Heart Association classification (NYHA) class II–IV and evidence of structural heart disease (ie left ventricular hypertrophy or left atrial enlargement by the recent echocardiogram within the last 12 months before enrollment) 3. NT-proBNP \geq 300 pg/mL for patients without AF, or \geq 600 pg/mL for patients with atrial fibrillation (AF) 4. Signed and dated written informed consent form (ICF).
Exclusion Criteria	<ol style="list-style-type: none"> 1. Current use or prior use of a SGLT2-i in 3 months. 2. Patients with type I diabetes mellitus. 3. History of ketoacidosis. 4. Impaired renal function with estimated glomerular filtration rate (eGFR)$<$20mL/min/1.73m². 5. On a diet or with a recent diet plan adjustment. 6. Have gastrointestinal diseases in active stages (eg malabsorptive conditions such as irritable bowel syndrome, coeliac). 7. Combination of severe infectious diseases (eg Severe myocarditis, severe pneumonia, and severe urinary tract infection) 8. Use of systemic antibiotics within past 30 days. 9. Acute decompensated heart failure. 10. Severe diseases in other systems (eg severe liver insufficiency, moderate-severe anemia, malignant tumors, hematological diseases). 11. Known or suspected allergic to the active or inactive ingredients of the drug under study. 12. Admitted percutaneous coronary intervention (PCI), or cardiac surgery, cardiac resynchronization (CRT), or other surgery within past 90 days. 13. Arranging to receive cardiovascular revascularization (percutaneous intervention or surgery) or major heart surgery (coronary artery bypass transplantation, valve replacement, ventricular aids, heart transplantation, CRT or any other surgery requiring thoracotomy or transcatheter aortic valve replacement) in 30 days. 14. Symptomatic hypotension with systolic pressure \leq 90mmHg. 15. Systolic pressure \geq 180mmHg which cannot be controlled with drugs. 16. Currently enrolled in another investigational device or drug trial. 17. Women who are pregnant, nursing, or who plan to become pregnant while in the trial.

biochemical tests will be measured and rechecked in the Shengjing Hospital core laboratory using an automated analyzer (AU5800; Beckman Coulter, Inc., Carlsbad, CA, United States). At baseline and every six months, participants will provide feces samples in the hospital, which will subsequently be frozen at -80°C until further use. In the relevant laboratory, gut microbiomes will be tested using 16S rRNA sequencing.

Imaging Examinations

ECG and echocardiography will be utilized to assess the patients' cardiac function in this study (Philips Diagnostic Ultrasound System and Transducers, USA; Iricon Aricon Digital ECG Machine for ECG-12C, China).

Physical Examinations

The New York Heart Association functional class, in conjunction with other physical examinations, will be used to evaluate patients' heart function.

Questionnaires

The Kansas City Cardiomyopathy Questionnaire (KCCQ) will be used to assess patients' quality of life in relation to heart failure, and the Food Frequency Questionnaire-25 (FFQ25) will be used to assess patients' nutrition status.

Investigators will keep track of the relevant syndrome for the entire six months, and all patients will be followed up by phone on a monthly basis. MACE events such as hospital readmission, all causes of mortality, other concomitant diseases, and drug conditions will be noted (Figure 1).

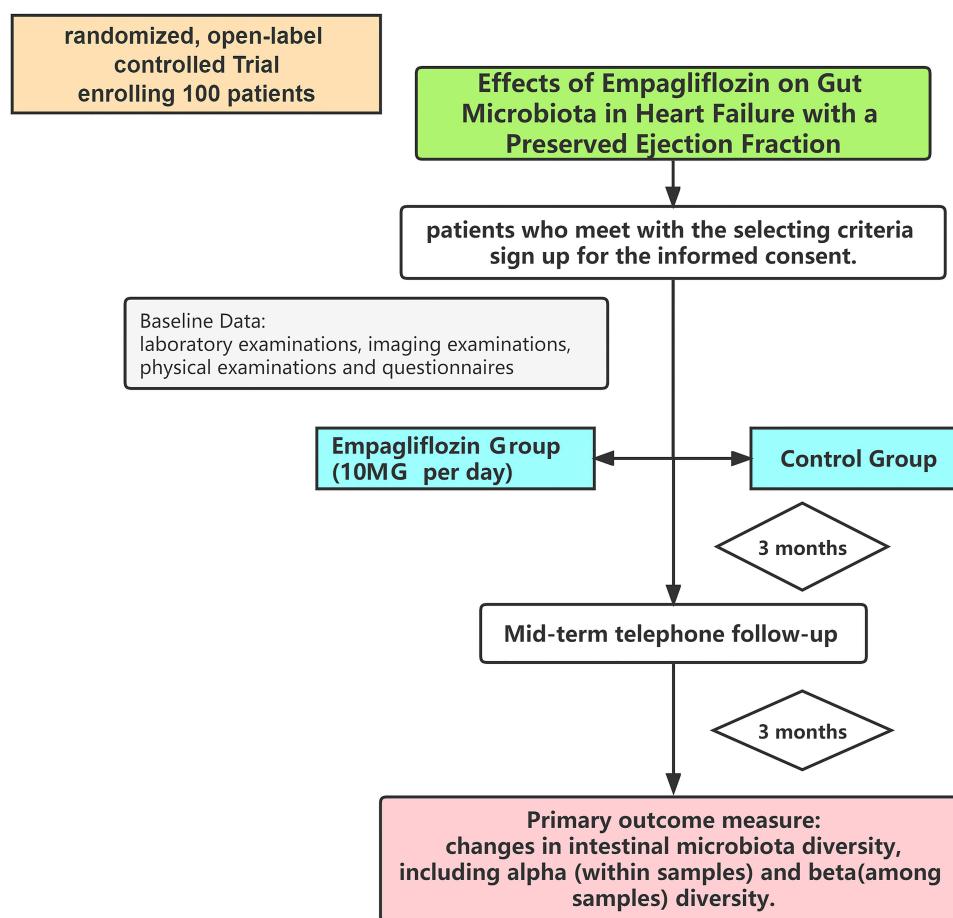


Figure 1 Trial procedure.

Outcome Measures

Primary Outcome Measure

The primary outcome will be changes in gut microbiota diversity, including alpha (within samples) and beta (across samples) diversity. Furthermore, using general linear models, the effects of empagliflozin as an intervention on changes in gut flora abundance will be studied, with changes in OUT abundance as the outcome and empagliflozin intervention as the exposure. The specifics are as follows:

The CTAB/SDS method will be used to extract total genomic DNA from the samples. The 16s rRNA genes will next be amplified and purified using the AxyPrepDNA Gel Extraction Kit (AXYGEN). The quality of sequencing libraries will be evaluated using the Qubit@ 2.0 Fluorometer (Thermo- Scientific) and the Agilent Bioanalyzer 2100 system. Finally, the library will be sequenced using an Illumina NovaSeq6000 platform to generate 250bp paired-end reads.

Sequence analysis will be performed using the UPARSE software package and the UPARSE-OUT and UPARSE-OTUref algorithms to recognize and compare the biological characteristics of the samples. Alpha (within samples) and beta (across samples) diversity will be analyzed using in-house Perl scripts. Sequences with $\geq 97\%$ similarity will be allocated to the same OTUs. The STAMP software will be used to confirm differences in individual taxonomic abundances between the two groups.

Aside from changes in OTU abundance in patients, other indicators such as inflammation factors will be investigated to detect correlations between numerous biomarkers and gut flora altered by empagliflozin, with potential confounders such as age, gender, and BMI being addressed.

Secondary Outcome Measures

- 1) Major Adverse Cardiovascular Events (MACE): Mace is defined as cardiovascular death and cardiovascular related readmission.
- 2) Serum NT-proBNP level.
- 3) Serum sST2 level.
- 4) Serum SCFAs level.

Sample Size Calculation

Since no clinical trials have been undertaken on how SGLT2 impacts the gut microbiome in patients with HF, determining the probable effect sample size is difficult. Experiments on mice and rats administered sGLT2 revealed alterations in the gut microbiome composition, supporting the hypothesis that SGLT2 can influence gut flora.^{17,18} Furthermore, with a sample size of 69 and a 6-week intervention duration, Vijay et al discovered that fiber or omega-3 intervention relates to changes in the gut microbiota and relative metabolites. In contrast, no significant changes in gut microbiome alpha diversity or composition were observed in a 12-week treatment study with dapagliflozin for patients with type 2 diabetes.¹⁹ As a result, the inconsistent experimental results make estimating the sample size more challenging.

As empagliflozin has been licensed for clinical use by the Food and Drug Administration (FDA), this trial is designed as a pragmatic randomized controlled trial (pRCT) to assess drug efficacy in real-world settings. This trial also includes adaptive designs, and the efficiency of the outcomes will be assessed during the process to ensure the sample size is effective.

Discussion

Chronic systemic inflammatory conditions in patients with HF trigger the release of inflammatory factors and signaling molecules such as IL-6 and neprilysin, which results in water-sodium retention.^{20,21} As a result, the blood supply to the digestive tract is interrupted, leading to increased permeability of the gut mucosa and flora displacement.⁹ Previous clinical studies suggest that patients with HFpEF may have altered gut flora and an elevated inflammatory status.^{11,20} Huang et al discovered a rise in the number of microbiota linked with inflammation, such as Enterococcus and Lactobacillus, whereas an anti-inflammatory effect (*Lachnospira butyricococcus* and others) was related with

a reduction.²² Gut flora fractions such as LPS and related metabolites enter the circulation through the compromised intestinal mucosal barrier in HF, triggering additional inflammatory stimuli and worsening HF.²³

Furthermore, accumulating evidence suggests that metabolites generated from gut microbiota, including short chain fatty acids (SCFAs), trimethylamine N-oxide (TMAO), secondary bile acids (BAs), and uremic toxins, contribute in various pathways relevant to the HF development process.^{24–30} SCFAs, among other metabolites, have been shown to protect against HF. SCFAs are produced by gut microbiota (*Bacteroides*, *Bifidobacterium*, and *Faecalibacterium*) as byproducts of dietary fiber fermentation to supply energy sources.³¹ SCFAs are required for the maintenance of the intestinal mucosal barrier and are linked to the promotion of intestinal immune activity. A growing number of studies indicate that SCFAs are involved in a variety of processes. SCFAs were reported to improve post-infarction cardiac repair by triggering CX3CR1+ monocyte infiltration in the peri-infarct zone.³² According to Pluznick et al, SCFAs (acetic acid/propionic acid) reduce myocardial fibrosis and restrict ventricular remodeling.³³

Empagliflozin is a sodium-glucose transporter 2 inhibitor that inhibits glucose reabsorption in the kidney, hence excreting excess glucose from the urine and lowering blood glucose levels, and has been used as a novel form of hypoglycemic medication. However, as the research continues, the impacts of SGLT2 will be extended. Empagliflozin had good efficacy in HFpEF among 5988 patients in the EMPEROR-preserved trial, lowering composite endpoint events by 21%.³⁴ In laboratory and clinical trials, SGLT2 was reported to be capable of alleviating inflammation induced by adipose tissue, reducing EAT volume, and attenuating cardiac fibrosis in both atrial and ventricular tissues.^{35,36} However, the specific mechanisms for this have yet to be discovered. Recent studies in mice and rats suggest that SGLT2 may influence the composition of the gut microbiome.^{18,37}

Prior studies on how gut flora and metabolites affect HF, as well as the unknown mechanism of SGLT2, led to the following questions: What's the connection between SGLT2 and gut flora? Is there a link between SGLT2, gut flora, and heart failure? The ongoing study aims to evaluate changes in gut microbiota in patients using empagliflozin who do not have a lower ejection fraction and to investigate the role of gut microbiota and their metabolites in the process. The study will look at the differences in gut microbiota abundance and the diversity of gut flora in individuals with HFpEF who have been taking empagliflozin for 6 months. Significantly, sST2, a fibrosis index, will be evaluated in the trial along with serum SCFA levels and gut microbiota producing SCFAs (such as *Bacteroides*, *Bifidobacterium*, and *Faecalibacterium*) to see if SGLT2 could alleviate cardiac fibrosis via gut microbiota.

The discovery of a prior clinical trial involving 69 healthy participants who were given omega-3 or inulin fibers in SCFAs generating gut microbiomes strengthens the chances of reaching the intended goal. Changes in gut microbiota and SCFAs were shown to be linked with cardiovascular risk factors and cytokines in this particular trial.³⁸ There were no significant changes in the gut microbiome alpha diversity or composition in a double-blind randomized trial of 44 patients with type 2 diabetes who were given dapagliflozin for 12 weeks.¹⁹ Due to the obvious discrepancy between clinical trials and laboratory studies,^{17,18,37} it is critical to determine if SGLT2 can alter the gut microbiome.

Furthermore, this study will look at how empagliflozin affects the gut flora of patients with HFpEF, as well as fibrosis-related factor (sST2), serum SCFAs, and SCFA-producing microbiota. Based on the information presented above, a putative SGLT2-gut-HF axis could be established. As a result, future study into the SGLT2 and gut microbiota processes may yield some concise new approaches for the clinical treatment of HFpEF. Furthermore, this trial aims to have a longer observation time of 6 months by combining the pRCT approach with other methods such as adaptive design to improve the efficiency of the outcomes.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki (as was revised in 2013). This controlled trial was approved by the Ethics Committee of the Shengjing Hospital of China Medical University (No. 2022PS999K), and registered in the Clinical Trial Register website (<https://clinicaltrials.gov/ct2/home>, NCT05584319, 10-8-2022). Informed consent will be obtained from all subjects involved in the study.

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Disclosure

The authors declare that they have no competing interests.

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