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Rationale and Design of the EMPYREAN Study: Investigation on the Effect of Empagliflozin on Cardiac Sympathetic and Parasympathetic Nerve Activity in Japanese Patients with Type 2 Diabetes

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Study protocol

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Abstract

Background: A sodium glucose cotransporter 2 (SGLT2) inhibitor was recently found to reduce heart failure hospitalization in the EMPA-REG OUTCOMES trial. We have hypothesized that autonomic nerve activity may be modulated by SGLT2 inhibition. The current study aims to investigate the impact of empagliflozin on sympathetic and parasympathetic nerve activity in patients with type 2 diabetes mellitus.

Methods: This ongoing study is a prospective, randomized, open-label, multicenter investigation of 134 patients with type 2 diabetes mellitus. The patients are randomly allocated to receive either empagliflozin or sitagliptin with the treatment goal of the Japan Diabetes Society guidelines. Ambulatory electrocardiographic monitoring is performed at the baseline and at 12 and 24 weeks of treatment. Analyses of heart rate variability are performed using the MemCalc method, which is a combination of the maximum entropy method for spectral analysis and the non-linear least squares method for square analysis. The primary endpoint is the change in the low frequency (LF; 0.04-0.15 Hz) / high frequency (HF; 0.15-0.4 Hz) ratio from baseline to 24 weeks.

Discussion: This investigation on the effect of EMPagliflozin on cardiac sYmpathetic and parasympathetic neRve activity in JapanEse pAtieNts with type 2 diabetes (EMPYREAN study) offers an important opportunity to understand the impact of SGLT2 inhibition on autonomic nerve activity in patients with type 2 diabetes.

Trial Registration: UMIN Clinical Trials Registry identifier UMIN000029194. Registered 19 September 2017, https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000033375

Background

A resurgence of interest on the association between heart failure and type 2 diabetes mellitus (T2DM) (1) has emerged with the increasing prevalence of patients concomitantly afflicted with both diseases. T2DM frequently coexists with cardiovascular disease to raise the risk of heart failure (2) and heart failure-related complications, including death (3). The leading cause of death in T2DM is cardiovascular mortality, which is reportedly associated with diabetic cardiac autonomic neuropathy (4–5).

Heart rate variability (HRV), which is measured by the variation between two consecutive beats, is the golden standard to evaluate cardiac autonomic function for various conditions, including myocardial infarction (6–7), cardiac transplantation (8), heart failure (9), and diabetic neuropathy (10–13). Impaired HRV is a marker of cardiovascular risk (14) and is often used for early detection of cardiac autonomic neuropathy in T2DM patients (15). Low-frequency (LF: 0.04–0.15 Hz) and high-frequency (HF: 0.15–0.4 Hz) are components of the frequency domain parameters of HRV. High-frequency is mediated by parasympathetic activity, and LF values are derived from both sympathetic and parasympathetic activities on the heart, but with sympathetic predominance. LF/HF ratio represents sympathovagal balance, in which higher value indicates sympathetic predominance (16). Although the prognostic

significance of impaired HRV in T2DM has been assessed in several studies (10–13), the impact of anti-diabetic drugs on cardiac autonomic function has not been well established.

The recent EMPA-REG OUTCOME trial on T2DM patients clarified the prognostic impact of the antihyperglycemic agent empagliflozin, a sodium glucose cotransporter 2 (SGLT2) inhibitor, as reducing mortality and heart failure hospitalization risk (17). Although SGLT2 inhibitors may have potential applications beyond T2DM, such as for heart failure, the mechanisms underlying the cardioprotective effects of SGLT2 inhibitors remain incompletely understood. A significant reduction in arterial blood pressure and a significant increase in hematocrit suggestive of plasma volume reduction were both observed in the absence of increased heart rate in patients treated with empagliflozin in the EMPA-REG trial (17). Moreover, incidence of sudden death was less in pooled empagliflozin group than placebo (1.1% vs. 1.6%) (17). Thus, the present investigation on the effect of EMPagligflozin on cardiac sYmpathetic and parasympathetic nerve activity in JapanEse pAtieNts with type 2 diabetes (EMPYREAN study; UMIN Clinical Trials Registry identifier UMIN000029194) has been designed to investigate our hypothesis that empagliflozin modulates cardiac autonomic function of patients with T2DM.

Methods

The EMPYREAN study is an ongoing multicenter, randomized, open-label (empagliflozin vs. sitagliptin), assessor-blinded, active controlled using sitagliptin, parallel-group clinical trial planned to test the hypothesis that empagliflozin improves autonomic disturbance in patients with T2DM during 24 weeks of treatment. Before patient enrollment, the study protocol was approved by the certified review board of Shinshu University School of Medicine. This study is being conducted in accordance with the Declaration of Helsinki. Written informed consent is obtained from each patient before enrollment. Personal information about potential and enrolled participants is kept confidential, and subject data are de-identified using participant numbers.

Inclusion and exclusion criteria

We aim to recruit a total of 134 participants across approximately 20 sites in Japan. Recruitment for the trial began in December 2017 and will end in April 2020. Eligible participants for the trial are patients with T2DM aged 20 to 74 years who meet the enrollment criteria detailed in Table 1. Briefly, eligible patients include those with a diagnosis of T2DM, HbA1c ranging from 6.5% to 10.0% (7.0% to 10.0% for patients treated with sulfonylurea or glinide), no anti-diabetic therapy with SGLT2 inhibitors or dipeptidyl peptidase 4 inhibitor (DPP4-I) agents for ≥12 weeks prior to randomization, and body mass index ≥18.5 kg/m^2 and ≤40 kg/m^2 at screening.

Trial design and follow-up

Eligible patients undergo a 4-week screening period (Figure 1), during which time background glucose-lowering therapy is continued unchanged. The purpose of the screening period is to evaluate participants' willingness and ability to adhere to the treatment and follow-up planned in the trial and evaluate baseline

clinical characteristics. Blood/urine sampling (on-site and central measurement), electrocardiogram (ECG) recording, the Schellong test, and 24-hour Holter ECG are also performed. Details of the blood/urine sampling for on-site monitoring and central measurement are listed in Table 2. HbA1c is evaluated by on-site management of diabetes and by central measurement as an exploratory outcome. Following the screening period, patients still meeting the inclusion/exclusion criteria are randomized (1:1) to receive either empagliflozin 10 mg or sitagliptin 50 mg once daily in addition to their background therapy. Treatment allocation is centrally performed with a minimization algorithm implementing a random component by the data management group of the data center. Baseline heart rate (<75 count/min or ≥75 count/min), age (<50 years or ≥50 years), HbA1c (<8% or ≥8%), and treatment center are considered as balancing factors. Background glucose-lowering therapy is to remain unchanged for the 24 weeks after randomization if possible, although rescue therapy can be initiated. During this period, empagliflozin (10-25 mg/day) or sitagliptin (50-100 mg/day) can be adjusted to achieve desired glycemic control at the investigator's discretion for the best standard of care according to local guidelines (18). Twenty-four hour Holter ECG and blood sampling (on-site/central measurement) are performed at 12 and 24 weeks of treatment (Table 3). HRV is analyzed in the time-frequency domain as shown in Table 4.

Endpoints

The primary outcome of this study is the change in the LF/HF ratio from the baseline to the end of the study (24 weeks). The key secondary outcomes include the changes in LF, HF, and LF/HF ratio from baseline to 12 weeks and changes in LF and HF from baseline to 24 weeks. The following variables are evaluated for changes from baseline to 12 and 24 weeks: average of all N-N intervals, standard deviation of N-N intervals (SDNN), standard deviation of the averages of N-N intervals for all 5-minute segments of a 24-hour recording, mean of the standard deviations of N-N intervals for all 5-minute segments of a 24-hour recording, root mean square of successive differences between adjacent N-N intervals (rMSSD), percentage of differences between adjacent N-N intervals that are greater than 50 ms, incidence of premature heartbeat and arrhythmia events, body weight and body mass index, and HbA1c. The changes from baseline to 12 and 24 weeks in waist circumference, serum catecholamines, thyroid-stimulating hormone, fT4, fT3, brain natriuretic peptide (BNP), N-terminal-pro-BNP, pro-BNP, and atrial natriuretic peptide (ANP) are investigated as exploratory outcomes. Details of the outcomes are described in Table 5.

Holter ECG

All data from 24-hour Holter ECG are sent to the Core Laboratory for analysis by an unrelated physician in a blinded manner (MemCalc/CHIRAM, Suwa Trust GMS, Tokyo, Japan) to obtain the LF (0.04–0.15 Hz) and HF (0.15–0.4 Hz) components of HRV (measured in absolute units; i.e., ms^2). The total power of HRV is also calculated for regression analysis as a global marker of cardiac autonomic function. From the electrocardiographic recordings, the statistical and geometric time domain indices are calculated from the N-N intervals noted above (Table 5). Frequency domain variables including the total, LF, and HF powers as well as the LF/HF ratio are derived from spectral analysis of successive N-N intervals.

Safety

Throughout the study, safety information is collected by recording such serious adverse events as all-cause mortality, fatal events, and adverse conditions requiring admission or prolongation of admission regardless of the causal relationship with the trial drugs or protocol, in addition to pre-defined adverse events of special interest including hepatic injury, decreased renal function, metabolic acidosis, ketoacidosis, diabetic ketoacidosis, and events involving lower limb amputation. When investigators identify any adverse event, its severity or grade, procedures conducted, outcomes, and relationship to the study drug are recorded and assessed. Investigators promptly report the occurrence of adverse events to the secretariat who then immediately informs the principal investigator for relay to Nippon Boehringer Ingelheim and the Data and Safety Monitoring Board (DSMB). The DSMB consists of an authorized endocrinologist and clinical epidemiologists with relevant expertise. Blinded to treatment allocation, the DSMB independently evaluates safety during the trial, assesses the necessity for any revisions to the trial design, and validates any decisions to continue the trial. If needed, the DSMB makes recommendations on safety issues to the principal investigator.

Study monitoring

Risk-based monitoring of the study sites is implemented to ensure that this study is properly conducted. A monitoring protocol has been separately created for the detailed monitoring-related plan. Auditing by an independent third party is also conducted to ensure the reliability of study results. Auditing is performed according to a separately specified, documented procedure. Records and medical information identifying the patients are kept confidential during monitoring and auditing. When new safety information-related issues arise, the Protocol Steering Committee or the DSMB discuss the issue, including study discontinuation or continuation, and the ethical review committee at each study site confirms each patient’s intention to continue participation in the study.

Statistical considerations

Sample size estimation

To date, no data are available on the impact of SGLT-2 inhibitors on cardiac autonomic nerve activity. Thus, we referred to a previous study which evaluated the LF/HF ratio of patients treated with amlodipine or verapamil. The mean difference and standard deviation of the LF/HF ratio was 0.15±0.25; thus, the effect size was 0.6 (19) in the study. Accordingly, we considered a conservative effect size of 0.5, alpha error of 5%, and power of 80%. To allow for approximately 10% drop-out, 67 patients are required per group, i.e., 134 participants in total.

Statistical analysis plan

The primary efficacy population has been defined as a full analysis set (patients who were randomly assigned to a group and received Holter ECG at least once). The change in the LF/HF ratio at 24 weeks is examined via mixed model repeated measures analysis for comparisons between the efficacy of the

treatments (empagliflozin and sitagliptin). Inter-group comparisons of the least squares means at 24 weeks are performed as well. Missing data are not imputed. The covariates included in the model are: treatment group, time point, interaction between treatment group and time point, baseline heart rate, age, and HbA1c. Additionally, unstructured correlation is assigned via the restricted maximum likelihood concerning the correlation structure, and the Kenward-Roger method is used to calculate the degree of freedom (20). When difficulties (e.g., convergence is not attained) arise, first-order autoregressive covariance is used followed by compound symmetry. Similar analytical methods are applied to the secondary endpoints. The safety population has been defined as a safety analysis set (patients who were randomly assigned to a group and received at least one dose of the study treatment). Descriptive analysis is performed for the frequency of adverse events. A two-sided P-value of <0.05 is considered statistically significant. All statistical analyses are performed using SAS Version 9.4 software (SAS Institute, Cary, NC).

Discussion

The EMPYREAN, identically designed, phase IV studies will shed light on the impact of empagliflogin on cardiac autonomic function in patients with T2DM in addition to conventional therapy. Although SGLT-2 inhibitors have been recommended for the treatment of T2DM and provide favorable cardiovascular outcomes, their mechanism of improving morbidity and mortality are not well investigated. Furthermore, while empagliflogin decreased systolic blood pressure and increased hematocrit after administration, substantial declines in HR were also observed in the EMPA-REG outcome study (17). This raised the possibility that empagliflogin might modulate cardiac autonomic function in patients with T2DM.

In the present ongoing study, a DPP4-I is being administered for treatment of the control group. An increasing number of patients with T2DM are receiving DPP4-I agents, which are a new therapeutic class of oral anti-hyperglycemic drugs for T2DM. These agents have been shown to reduce HbA1c levels without increasing the risk of hypoglycemia or weight gain (21). The use of DPP4-I drugs for T2DM has now been firmly established in clinical practice. However, in comparisons with a placebo, the DPP4-I sitagliptin could not reduce cardiovascular mortality in the TECOS trial (22). Thus, comparing SGLT-2 and DPP4-I drugs enables us to evaluate the additional impact of SGLT-2 inhibition on the cardiovascular system with a sufficient decrease in HbA1c that is equivalent to that with a DPP4-I and with a low hypoglycemic risk. In the EMPA-REG OUTCOME trial, an Asian cohort that underwent empagliflogin treatment displayed better cardiovascular outcomes than did a non-Asian cohort (17). Thus, our Japanese population appears preferable for investigation of the impact of empagliflogin on the cardiovascular system.

In terms of the observation period of the EMPYREAN study, 24 weeks have been considered necessary. In the TAKEDA INSIGHT trial, patients showed body weight loss until 24 weeks (23) under SGLT-2 inhibition by dapagliflogin. In those patients, weight loss with diuresis was obvious in the first 4 weeks, followed next by body weight loss with visceral fat reduction. Arterial blood pressure and HbA1c decreased from 12 weeks after the treatment, which was maintained until 24 weeks.

Conclusions

The results of the current EMPYREAN study are expected to provide key pathophysiological insights regarding the cardio-protective effect of empagliflozin. Findings from the EMPYREAN study will greatly facilitate clinical decision-making for patients with T2DM.

Abbreviations

ANP: atrial natriuretic peptide; BNP: brain natriuretic peptide; DPP4: dipeptidyl peptidase 4; DSMB: data and safety monitoring board; EMPYREAN: the effect of empagliflozin on cardiac sympathetic and parasympathetic nerve activity in Japanese patients with type 2 diabetes; ECG: electrocardiogram; HRV: heart rate variability; HF: high frequency; LF: low frequency; rMSSD: root mean square of successive differences between adjacent N-N intervals; SDNN: standard deviation of N-N intervals; SGLT2: sodium glucose cotransporter 2; T2DM: type 2 diabetes mellitus

Declarations

Ethics approval and consent to participate

This study was approved by the certified review board of the Shinshu University School of Medicine. This study has met all ethical standards as required for the study of human subjects.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

Dr. Hiramitsu has received lecture fee from MSD and Boehringer Ingelheim. Dr. Onishi has received lecture fee from Boehringer Ingelheim. Dr. Kuwahara has received lecture fee from Boehringer Ingelheim. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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Authors' contributions

HM conceived and designed the project and wrote the manuscript. IM conceived and designed the project. SY conceived and designed the project and substantively revised the manuscript. K Oba provided significant study design and statistical support and substantively revised the manuscript. WS acquired and managed the data and substantively revised the manuscript. KU conceived and designed the project and substantively revised the manuscript. SU, YS, MW, M Komatsu, KU, YN, CS, SH, S Yonemitsu, M Konda, and K Onishi made substantial conrtibutions to design. KK conceived the project and substantively revised the manuscript. All authors read and approved the final manuscript.

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Not applicable

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Tables

Table 1. Inclusion and Exclusion Criteria

Inclusion criteria
All of the following criteria must be met:

- Subjects with T2DM on a diet and exercise regimen who have HbA1c ≥6.5% and ≤10.0% at screening and during run-in phase
- SGLT2i/DPP4-naïve (no anti-diabetic therapy with SGLT2 inhibitors or DPP4 inhibitors for ≥12 weeks prior to randomization)
- Age ≥20 years and <75 years
- Body mass index ≥18.5 kg/m^2 and ≤40 kg/m^2 at screening
- Signed and dated written informed consent prior to screening

Exclusion criteria
- Treatment with insulin or glucagon-like peptide-1 receptor agonist
- Neuropathy evidenced by orthostatic hypotension, diabetic neuropathy, or autonomic disturbance
- Proliferative retinopathy
- Estimated glomerular filtration rate <45 mL/min/1.73m^2 (a) or creatinine clearance <50 mL/min (b) at screening or during run-in phase
- Treatment with prohibited drugs
- Chronic obstructive lung disease under treatment
- Sleep apnea syndrome
- Indication of liver disease defined by serum levels of alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase above 3 times the upper limit of normal during screening or run-in phase
- Acute coronary syndrome or stroke within 12 weeks prior to informed consent
- Planned cardiac surgery or angioplasty within 12 weeks prior to informed consent
- Surgical intervention for obesity within 2 years prior to informed consent
- Any uncontrolled endocrine disorder apart from T2DM
- Alcohol or drug abuse within 12 weeks of informed consent that would interfere with trial participation or any ongoing condition leading to decreased compliance with study procedures or study drug intake
- Treatment with anti-obesity drugs
- Atrial fibrillation or atrial flutter
- Implanted permanent pacemaker
- Frequent premature atrial/ventricular contraction (according to the Minnesota Code Classification System for electrocardiographic findings)
- Bundle branch block
- Sick sinus syndrome or atrio-ventricular block more than the 2nd degree
- Pre-menopausal women who were nursing, pregnant, or requesting maternity
- Medical history of cancer and/or treatment for cancer within the last 5 years
- Contraindications to background therapy according to the local label
- Treatment with systemic steroids, hyperthyroidism, or hypothyroidism under treatment
- Intake of an investigational drug in another trial within 30 days prior to informed consent of this trial or participating in another trial involving an investigational drug and/or follow-up
- Any clinical condition that would jeopardize patient safety while participating in this clinical trial

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Table 2. Details of Blood/urine Sampling

Blood test (on-site)	
Complete blood count	WBC, RBC, Hb, Hct, Plt
Blood chemistry/enzyme tests	AST, ALT, ALP, γ-GTP, LDH, BUN, Cr, CPK, T-Bil, TC, HDL-c, TG, FBS, HbA1c, UA
Blood test (central)	
Blood chemistry/enzyme tests	HbA1c, IRI, hsCRP, cathecolamines, BNP, NT-proBNP, proBNP, ANP, TSH, fT4, fT3, ketones
Urine test	
Dipstick test	U-prot, U-glu, U-ketone, pregnancy test
Urine chemistry	Alb, Cr

Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANP, atrial natriuretic peptide; AST, aspartate aminotransferase; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; CPK, creatine phosphokinase; Cr, creatinine; FBS, fasting blood sugar; fT4, free thyroxine; fT3, free triiodothyronine; Hb, hemoglobin; HbA1c, hemoglobin A1c; Hct, hematocrit; HDL-c, high-density lipoprotein cholesterol; hsCRP, high-sensitive c-reactive protein; IRI, insulin; LDH, lactate dehydrogenase; NT-proBNP, N-terminal-prohormone for brain natriuretic peptide; Plt, platelet; proBNP, prohormone for brain natriuretic peptide; RBC, red blood cell; T-Bil, total bilirubin; TC, total cholesterol; TG, triglycerides; TSH, thyroid-stimulating hormone; UA, uric acid; U-glu, urine glucose; U-prot, urine protein; WBC, white blood cell; γ-GTP, gamma-glutamyl transferase

Table 3. Scheduled Visits and Assessments in the EMPYREAN Study

	Screening			Randomization		
	Step 1	Step 2	Step 3	Step 4		
	4 weeks prior to randomization			0 weeks	12 weeks	24 weeks
Eligibility	○		○	○		
Informed consent	○					
Patient characteristics	○					
Physical examination	○			○	○	○
Blood test (on-site)		○			○	○
Schellong test		○				
ECG		○				
Holter ECG			○		○	○
Blood test (central)			○		○	○
Medication adherence				○	○	○
Safety	←					→

Table 4. HRV Indices Assessed in the EMPYREAN Study

Time domain indices	
AVNN, ms	Average of all N-N intervals
SDNN, ms	Standard deviation of all N-N intervals
SDANN, ms	Standard deviation of the averages of N-N intervals for all 5-minute segments of a 24-hour recording
SDNNIDX	Mean of the standard deviations of N-N intervals for all 5-minute segments of a 24-hour recording
rMSSD, ms	Root mean square of successive differences between adjacent N-N intervals
pNN50, %	Percentage of differences between adjacent N-N intervals that are greater than 50 ms
Frequency-domain indices	
LF	Total spectral power of all N-N intervals between 0.04 and 0.15 Hz
HF	Total spectral power of all N-N intervals between 0.15 and 0.4 Hz
LF/HF ratio	Ratio of low to high frequency power

Table 5. Study Endpoints

Primary endpoint
Change in LF/HF ratio (from baseline to end of study)
Secondary endpoints
Changes in LF, HF, and LV/HF ratio (from baseline to 12 weeks)
Changes in LF and HF (from baseline to 24 weeks)
Changes in the following variables (from baseline to 12 and 24 weeks):
HRV indices (AVNN, SDNN, SDANN, SDNNIDX, rMSSD, and pNN50), incidence of premature heartbeat and arrhythmia, body weight, body mass index, and HbA1c
Exploratory endpoints
Changes in the following variables (from baseline to 12 and 24 weeks):
Waist circumference, catecholamines, thyroid hormones (TSH, fT4, and fT3), plasma BNP, NT-proBNP, proBNP, and ANP

Abbreviations are listed in Tables 2 and 4.

Figures

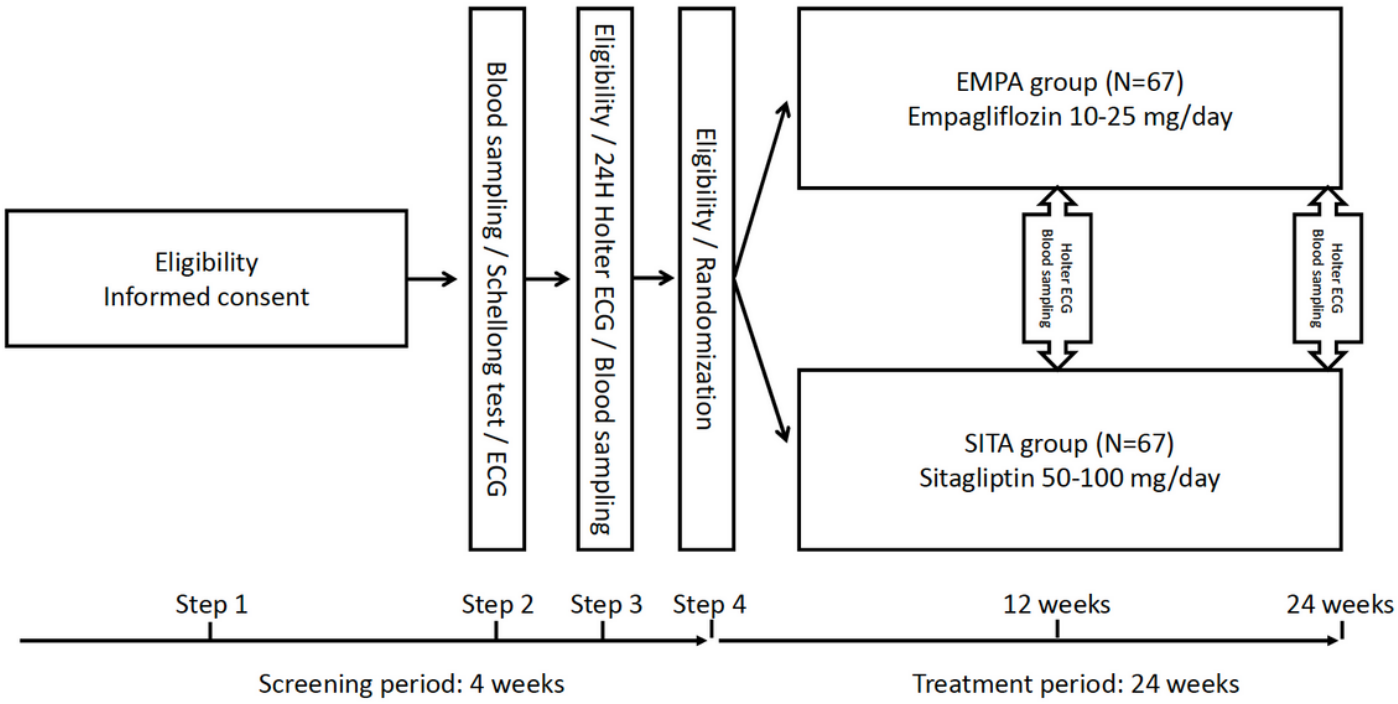

Figure 2

EMPYREAN study protocol Eligible patients undergo a 4-week screening period to evaluate baseline clinical characteristics. Blood/urine sampling, ECG recording, the Schellong test, and 24-hour Holter ECG are performed. Following the screening period, patients still meeting the inclusion/exclusion criteria are randomized (1:1) to receive either empagliflozin (EMPA) 10 mg or sitagliptin (SITA) 50 mg once daily in addition to their background therapy. Twenty-four hour Holter ECG is performed at 12 and 24 weeks of treatment. HRV is analyzed in the time-frequency domain as shown in Table 4.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- EMPYREANProtocolAppendix20200208.doc
- IRBdecideEMPYREAN.pdf
- ContractJapanese.pdf
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