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Research

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Study Protocol: Traditional Chinese Medicine (TCM) Syndrome Differentiation for Heart Failure patients and its Implication for Long-term Therapeutic Outcomes of the Qiliqiangxin Capsules

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Abstract

Background: Syndrome differentiation is a commonly used methodology and practice in Traditional Chinese Medicine (TCM) guiding the diagnosis and treatment of diseases including heart failure (HF). However, previous clinical trials seldom consider the impact of syndrome patterns on the outcome evaluation of TCM formulae. Qiliqiangxin (QLQX) capsule is a TCM formula with cardiotonic effect to improve the cardiovascular function for heart failure with proven efficacy from well-designed clinical trials. Though, there is no clinical trial with a large sample size and long assessment period that considers the relationship between TCM syndrome differentiation and the treatment efficacy of

28 QLQX. In the present study, we design a study protocol to evaluate the relationship between TCM
29 syndrome differentiation and the severity of heart failure as well as its progression. Furthermore, we
30 will evaluate the impact of the TCM syndrome patterns on the efficacy of QLQX in the outcome of
31 heart failure.

32 **Methods:** This is a clinical study conducted in conjunction with an ongoing clinical trial (QUEST
33 Study¹) to evaluate the efficacy of QLQX to improve the outcome of HF treatment through the
34 assessment of morbidity and re-hospitalization, which involves over 100 hospitals in mainland China
35 and Hong Kong SAR with 3,080 HF patients. Firstly, we will apply a modified TCM Questionnaire to
36 collect the clinical manifestations of HF and acquire the tongue images of the patients to facilitate the
37 syndrome differentiation. We will base on the “2014 Consensus from TCM experts on diagnosis and
38 treatment of chronic heart failure” to evaluate the TCM syndromes for the patients. Secondly, we will
39 investigate the characteristic of TCM syndrome distribution of HF patients and its correlation with the
40 functional and biochemical data. Furthermore, we will evaluate the relationship between the TCM
41 syndrome patterns and the efficacy of QLQX in the treatment of heart failure. Lastly, we will
42 investigate the implication of tongue diagnosis in the severity and therapeutic outcome of HF.

43 **Expect Outcomes:** To our knowledge, this will be the latest and well-designed clinical trial to evaluate
44 the rules of TCM syndrome differentiation in the progression and therapeutic outcome of HF patients.

45 We expect to obtain direct clinical evidence to verify the importance of TCM syndrome differentiation
46 for the diagnosis and treatment of HF.

47

48 **Trial Registration:** ¹The trial was registered at Chinese Clinical Trial Registry,
49 <http://www.chictr.org.cn>. (Registration No.: ChiCTR1900021929); Date: 2019-03-16.

50

51 **Keywords:** Qiliqiangxin (QLQX) capsule, traditional Chinese medicine (TCM), TCM syndrome
52 differentiation, syndrome, syndrome element, tongue diagnosis, syndrome questionnaire, chronic heart
53 failure, treatment efficacy

54

55 Background

56

57 Heart Failure (HF) is an end-stage cardiovascular condition characterized by structural or functional
58 cardiac impairments of ventricular filling or blood ejection, leading to abnormal hemodynamics,
59 activating neuro-hormones, and developing myocardial remodeling(1, 2). The typical clinical
60 manifestations include shortage of breath, fatigue and ankle swelling, accompanied by peripheral
61 edema and elevated jugular venous pressure, etc. (3, 4). HF is one of the leading causes of death
62 globally (5). There are approximately 64.3 million patients with heart failure worldwide (6). In the US,
63 there are around 5.7 million HF patients and it is estimated to reach 8 million HF patients in 2030,
64 accounting for a 46% increase in prevalence(7). Around 30-40% of HF patients have a history of
65 hospitalization due to HF, and the reported 5-year all-cause readmission, readmission for HF, and
66 mortality rates are 80.4%, 42.3%, and 75.4% respectively (8, 9). Thus, HF becomes a major public
67 health burden in human diseases.

68

69 Current therapeutic strategies for HF include the following catalogs: Using nitrates and hydralazine to
70 resolve vasoconstriction; Using angiotensin-converting enzyme (ACE) inhibitors, angiotensin II
71 receptor blockers (ARBs), beta-blockers, and mineralocorticoid antagonists (MRAs) to target neuro-

72 hormonal systems; Using Invabradine to target the sinus nodes discharge and slow down the heart
73 rates for the HF patients with unsatisfactory results with beta-blocker; Using cardiac resynchronization
74 therapy (CRT) targeted the sinus rhythm and implantable cardioverter-defibrillator (ICD) targeted
75 patients with cardiac arrhythmia, etc. (5). Even with the advanced treatment approaches developed,
76 the outcome of HF treatments is still unsatisfactory as the re-hospitalization of worsening heart failure
77 and mortality remain high.

78

79 Traditional Chinese Medicine (TCM) formulae, including Qiliqiangxin, Nuanxin, Shencaotongmai,
80 Yangxinkang, appears to be the effective adjunct therapies with conventional drugs to improve cardiac
81 function of HF patients (10). According to the TCM theory of Collateral Disease, the heart-qi and/or
82 heart-yang deficiency could induce blood stagnation and the disruption of body fluid metabolism (11).
83 The HF-induced hemodynamic dysfunctions and edema could be attributed to the pathological status
84 of heart-qi deficiency accompanied with blood stagnation and body fluid accumulation (12). Syndrome
85 differentiation is a unique TCM diagnosis in guiding therapeutic strategies for heart failure. However,
86 there is no standardized methodology for syndrome differentiation of heart failure (13-15). The
87 diversities of syndromes lead to difficulties in drawing conclusive outcomes and therefore, hindering
88 the development of TCM treatment for heart failure (16, 17). On the other hand, Tongue Diagnosis is

89 crucial for identifying syndromes and evaluating the therapeutic responses in TCM practice. Tongue
90 Diagnosis has been applied for different diseases such as metabolic syndrome, cancer, menstrual
91 clinical symptoms, etc. (18-21). Recent studies observed the correlation between tongue patterns and
92 risk factors of heart failure in dialysis patients (22-24). The tongue changes were also observed in heart
93 failure patients with normal ejection fraction (22-24). Whether heart failure patients have specific
94 tongue patterns remains unclear. Therefore, we design this study to explore the impacts of TCM
95 syndrome patterns and tongue features on cardiovascular functions and the therapeutic outcome of
96 heart failure.

97

98 Qiliqiangxin (QLQX) capsule is a patent TCM formula approved by China Food and Drug
99 Administration for chronic heart failure (25). QLQX capsule is extracted from 11 TCM herbs including
100 Astragali Radix, ginseng radix at rhizoma, aconite lateralis radix preparata, salvia miltiorrhizae radix
101 et rhizome, semen descurainiae lepidii, alismatis rhizome, polygonati odorati rhizome, cinnamomi
102 ramulus, carthami flos, periploca cortex, and citri reticulatae periarpium. Previous studies indicated
103 that: (1) QLQX could attenuate cardiac remodeling via activating peroxisome proliferator-activated
104 receptor γ (PPAR- γ) and mTOR; (2) QLQX could prevent cardiomyocytes hypertrophy via activating
105 PGC-1 α and downregulating MiR-199a-5p, etc. (26-30); 3) QLQX could improve ejection fraction

106 and attenuates the left ventricular remodeling via inhibitory effect of the renin-angiotensin-aldosterone
107 system (RAAS) (31, 32); (4) QLQX could inhibit the myocardial inflammation and cardiomyocyte
108 apoptosis to promote the proliferation of cardiomyocyte (33). A multi-center, randomized, double-
109 blind and placebo-controlled clinical trial reported that QLQX, as an adjunct therapy, reduced the level
110 of NT-proB-type natriuretic peptide (NT-proBNP) and improved the NYHA functions, left ventricular
111 ejection fraction (LVEF), 6-min walking distance, quality of life and composite cardiac events in HF
112 patients (34). Those studies suggest that QLQX is a promising TCM formula for HF patients. However,
113 the efficacy of QLQX on hard endpoint incidents such as re-hospitalization of worsening HF and
114 mortality is yet to be proven. Particularly, whether different TCM syndrome patterns impact the
115 therapeutic outcomes of QLQX is unknown. As such, we have designed QUEST study (35) to explore
116 the long-term efficacy of QLQX in addition to conventional heart failure treatment of CHF. The
117 primary outcome will be the occurrence of the composite endpoint which is defined as either
118 cardiovascular (CV) death or re-hospitalization due to the exacerbation of HF. The secondary outcome
119 measures include the all-cause mortality, secondary endpoint events (treatment terminated due to
120 worsening heart failure, successful resuscitation after cardiac arrest, malignant arrhythmia, non-fatal
121 stroke), CV death and re-hospitalization due to worsening heart failure in patients with ischemic heart
122 disease, and the level of Serum NT-proBNP. In the present study, we target to evaluate the impacts of

TCM syndrome differentiation on the disease progress of HF and the therapeutic outcome of QLQX. The study is coached by three specific objectives to answer the following questions: 1) Whether TCM syndromes are associated with the severity of heart failure; 2) Whether different TCM syndrome patterns have different therapeutic outcomes in QLQX treatment for heart failure; and 3) Whether TCM tongue diagnostic characteristics could be an effective index for the severity of heart failure and the indication of the progression as well as the therapeutic outcomes.

129

130 Methods/Design

131 1. Patient Recruitment and Selection Criteria

Our study is conducted in conjunction with a multicenter, randomized, double-blind, placebo-controlled, parallel-group, event-driven clinical study named the QUEST on chronic heart failure patients. This study is carried out in approximately 100 centers located in China and Hong Kong SAR. These centers are eligible hospitals or affiliated hospitals of the medical universities. The target number of patient recruitments will be 3,080, which is calculated according to the PARADIGM-HF study based on the cardiovascular death or hospitalization rate for heart failure (36). This is an event-driven study and all the recruited patients will remain in the study (whether taking the study drug or not) until the number of the primary endpoint events reaches the predicted target of 620 cases, or the study meets

140 the pre-defined efficacy or safety criteria assessed by the Clinical Event Adjudication Committee
141 (CEAC).

142

143 Recruitment of patients is assessed according to the pre-defined inclusion and exclusion criteria of the
144 QUEST study (Table 1). Recruited patients must be at least 18 years old, can fulfill all the inclusion
145 criteria including the NT-proBNP, LVEF, NYHA cardiac functional grading, and the standardized
146 drug treatment for at least 2 weeks prior to enrollment, etc., and not meeting any exclusion criterion.

147

148 **Table 1. Inclusion and Exclusion Criteria**

Inclusion criteria:

- 1) Provision of signed informed consent prior to any study specific procedures;
- 2) Male or female, aged ≥ 18 years at the time of consent;
- 3) Established documented diagnosis of heart failure for at least three months ago according to “Chinese Heart Failure Diagnosis and Treatment Guideline” issued by the Chinese Medical Association Cardiovascular Branch.
- 4) Left ventricular ejection fraction (LVEF) $\leq 40\%$ (echocardiogram, radionuclide, ventriculogram, contrast angiography or cardiac MRI);
- 5) NYHA cardiac functional grading II to III, with stable clinical symptoms; including those diagnosed as grade IV within 2 weeks before enrollment;
- 6) Serum NT-proBNP $\geq 450\text{pg/ml}$;
- 7) Those who have received standardized baseline treatment regimens without doses adjusted and given intravenously for at least two weeks prior to enrollment; standardized drug treatment includes: angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) or angiotensin receptor neprilysin inhibitor (ARNI), beta blocker, and aldosterone receptor antagonist (the optimal therapeutic dose should be achieved, except for contraindications or intolerance);

Exclusion criteria:

Patients should not enter the study if any of the following exclusion criteria are fulfilled

- 1) Heart failure caused by valvular disease, congenital heart disease, pericardial disease, arrhythmia or non-cardiogenic disease, or caused by vital organ failure (such as renal, hepatic failure, etc.); and right heart failure caused by pulmonary or other definite causes; and acute heart failure;
- 2) Coronary revascularization (percutaneous coronary intervention [PCI] or coronary artery bypass grafting [CABG]) or cardiac synchronization therapy planned to undergo after randomization, or had received cardiac resynchronization therapy prior to enrolment;
- 3) Any condition outside the CV diseases such as but not limited to malignant tumor, severe mental illness, hematopoietic diseases, neuroendocrine system disease, liver transaminase and alkaline phosphatase $\geq 3 \times$ upper limit of normal (ULN), abnormal renal function serum creatinine > 2 mg/dl (176.82 μ mol/L), potassium > 5.5 mmol/L;
- 4) Patient with left ventricular outflow tract obstruction, myocarditis, aortic aneurysm, aortic dissection, or obvious hemodynamic changes caused by unrepaired valve;
- 5) Cardiogenic shock, uncontrollable malignant arrhythmia, sinus or atrioventricular block at second degree type II or above without pacemaker treatment, progressive unstable angina pectoris or acute myocardial infarction;
- 6) Uncontrolled hypertension systolic blood pressure (SBP) ≥ 180 mmHg and/or diastolic blood pressure (DBP) ≥ 110 mmHg; or SBP < 90 mmHg and/or DBP < 60 mmHg;
- 7) Participation in another clinical study with an IP during the last month prior to enrolment;
- 8) Women of child-bearing potential (i.e., those who are not chemically or surgically sterilized or who are not post-menopausal) who are not willing to use a medically accepted method of contraception that is considered reliable in the judgment of the investigator, from the time of signing the informed consent throughout the study and 4 weeks thereafter, OR women who have a positive pregnancy test at enrolment or randomization OR women who are breast-feeding;
- 9) Allergic constitution; known to be allergic to research drug;
- 10) Inability of the patient, in the opinion of the investigator, to understand and/or comply with study medications, procedures or any conditions may render the patient unable to complete the study.

149

150 Before the recruitment, patients must be “clinically stable” by receiving at least two weeks of
151 standardized medication treatment according to the local treatment guideline for heart failure with

152 fixed drug type and dosage, as well as have not taken any intravenous anti-HF drug nor oral TCM
153 medication with similar composition as the QLQX capsules. Once the patients are clinically stable,
154 they will be assigned to the QLQX group or the control group in a ratio of 1-to-1 through the double-
155 blind computerized randomize allocation. The patient will be given either QLQX capsules or placebo
156 capsules during the assessment period in addition to their standard treatment for heart failure following
157 the guidelines for diagnosis and treatment of HF in China 2018 (25) or local guideline. The QLQX
158 capsules and placebo are identical in size and shape. The dosage used in this study is 4 capsules of
159 QLQX or placebo 3 times daily. During the assessment period, patients should not take any TCM or
160 herbs which have similar contents to the QLQX capsule.

161

162 The entire study will last for approximately 36 months with the first 24 months as the recruitment
163 period. The study will be terminated once the last patient has finished the 12-months assessment. Once
164 recruited, the patient is required to visit the hospitals for efficacy and safety assessment according to
165 the study schedule. All patients are required to sign the Informed Consent Form before the recruitment.
166 For each visit, the patient will be assessed by the researchers/physician. A range of clinical information
167 and parameters will be collected and tests will be performed for the assessment of TCM syndrome, the
168 treatment efficacy, and safety including laboratory and ECG results, etc. The assessment of safety of

169 this study will base on the reporting of the adverse events, SAEs, and laboratory abnormalities, which
 170 are closely monitored by the Data Safety Monitoring Committee (DSMC). Two interim efficacy
 171 analyses will be conducted when 1/2 and 2/3 primary endpoint events (i.e. around 310 and 414 patients
 172 respectively) are gathered. Patients are allowed to withdraw from the study for any reason but the
 173 reason is required to be recorded in the case report form. If the patient has an intolerable adverse event
 174 that is related to the study drug based on the researcher's judgment, the patient should immediately
 175 discontinue the treatment with the study drug. The study flowchart is shown in Fig. 1.

176

177

178

Fig. 1 Study Flow Chart

179

180 2. TCM Syndrome Diagnosis Questionnaire for Heart Failure (SDQHF)

181 To collect clinical information for TCM syndrome differentiation, a specialized TCM Syndrome
182 Diagnosis Questionnaire for Heart Failure (SDQHF) is developed according to the TCM syndrome
183 scales for heart failure reported by previous studies (37-40). The clinical manifestation including the
184 signs and symptoms of heart failure diagnosis which are described according to the “2012 Guiding
185 Principles for Clinical Study on New Drug for Use in Traditional Chinese Medicine”, “2014
186 Consensus from TCM experts on diagnosis and treatment of chronic heart failure”, “2017 Guideline
187 for TCM Diagnosis and Treatment of Heart Failure (Chronic Heart Failure)” (16, 41, 42).

188

189 In the SDQHF, there are 36 closed-ended items and each item is given a ranked scale with simple
190 descriptions to indicate the features of the severity or frequency of signs or symptoms. These 36 items
191 are carefully considered to incorporate the major and concomitant signs/symptoms which are essential
192 for the diagnosis of HF and the TCM syndrome differentiation. The descriptive scale per item is clearly
193 defined and the scales were input by a group of experienced clinical experts and physicians. The
194 SDQHF has gone through the review and pre-pilot testing by 20 subject matter experts including
195 physicians and TCM practitioners.

196

197 The 36-items are divided into several dimensions including major signs and symptoms of heart failure,
198 cold and heat characteristics, perspiration, head and general body condition, diet, etc. to facilitate the
199 differentiation of heart failure and TCM syndrome. The terminologies used are specially considered
200 to avoid jargon so that the questionnaire can be easily understood by the researchers/physicians and
201 the patients. It will be an interviewer-administered questionnaire so that unclear questions can be
202 clarified to the patients and ensure patients answer all the questions. The first 31 items in the SDQHF
203 are required to be answered by the patients, and the last 5 items about the physical signs of the patients
204 should be determined by the physician/researcher based on their observation of the patients. The list
205 of 36-items in SDQHF is shown in Table 1.

206

207 **Table 1. The list of 36-items in the TCM Symptom Diagnosis Questionnaire for Heart Failure**
208 **(SDQHF)**

209

1. Palpitations	10. Coughing out of Phlegm	19. Cold Sweat	28. Constipation
2. Chest Tightness	11. Color of phlegm	20. Fear of cold	29. Diarrhea
3. Precordial pain	12. Texture of phlegm	21. Coldness feeling in upper or lower limbs	30. Frequency of urination
4. Dizziness	13. Feeling fullness in chest/abdomen	22. Feeling heat in 5 centers	31. Amount of urination
5. Shortness of breath	14. Hypochondriac pain or feeling of distension	23. Appetite	32. Distension of jugular vein
6. Gasp for breath	15. Edema	24. Sense of thirstiness	33. Cyanosis of face and lips
7. Posture	16. Tiredness	25. Loss of sleep/insomnia	34. Color of face/complexion
8. Coughing	17. Sweating at daytime	26. Dreaming	35. Color of lips

9. Phlegm in throat with sound	18. Sweating at night time	27. Somnolence	36. Location of edema
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210

211 All enrolled patients are required to complete this questionnaire in a total of 6 specific study visits to
212 the hospitals (i.e. Visit 1 at (Day 0), Visit 2(1M), Visit 3(3M), Visit 6(12M), Visit 10(24M) and Visit
213 14(36M)/EOS). The visit schedule and list of parameters are shown in Table 2. For each scheduled
214 visit, the physician/researcher will conduct a face-to-face interview with the patient to go through this
215 survey questionnaire. The entire interview process will last for approximately 8 to 10 minutes. The
216 data collected will be first recorded manually in the paper SDQHF and then input into the Epidata
217 software(43) which is the centralized database for the collection of research data from the QUEST
218 study.

219

220 **Table 2. Planned visits and parameters**

	Enrollment & Allocation	Postallocation														Closeout	
Visit Schedule	1	2	3	4	5	6	7	8	9	10	11	12	13	14	UNS	EOS	
Day/Month	Day0	1M	3M	6M	9M	12M	15M	18M	21M	24M	27M	30M	33M	36M			
	-14	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		=2wks	
Informed Consent	x																
Inclusion/Exclusion criteria	x																
Randomization	x																
General data and medical history	x																
Medical history of HF	x																
Medical history of CV Diseases	x																
Physical examination	x	x ¹	x ¹	x ¹	x ¹	x	x ¹	x ¹	x ¹	x	x ¹	x ¹	x ¹	x	(x)	x	

Heart failure medications	x	x	x	x	x	x	x	x	x	x	x	x	x	x	(x)	x
Medications of other CVD	x	x	x	x	x	x	x	x	x	x	x	x	x	x	(x)	x
Other medications	x	x	x	x	x	x	x	x	x	x	x	x	x	x	(x)	x
TCM Syndrome Diagnosis Questionnaire (SDQHF)	x	x	x			x				x				x		x
Tongue Image Acquisition	x	x	x			x				x				x		x
Echocardiogram	x*															
Pregnancy Tests	x					x				x				x	(x)	x
Blood/urine routine test	x	x				x				x				x	(x)	x
Biochemical test	x	x				x				x				x	(x)	x
12-lead ECG	x*	x				x				x				x	(x)	x
Serum NT-proBNP at local laboratory	x	x		x												
Dispensing IP	x		x	x	x	x	x	x	x	x	x	x	x	x		
Returning IP for accountability			x	x	x	x	x	x	x	x	x	x	x	x		x
Endpoint Event		x	x	x	x	x	x	x	x	x	x	x	x	x	(x)	x
Adverse Event		x	x	x	x	x	x	x	x	x	x	x	x	x	(x)	x
Serious Adverse Event		x	x	x	x	x	x	x	x	x	x	x	x	x	(x)	x

221 • X¹ Simplified physical examination

222 • x* Cardiac ultrasound and 12-lead ECG within the first 6 months of enrollment

223 • UNS (unplanned visit): (x) The marked item is optional and performed according to the judgment of researchers

224 • EOS (final visit): Make arrangement according to the study end time (if there is a visit within one month before the end of study, it is
225 regarded as a final visit, but needs to be supplemented with the items required completely)

226 • Pregnancy test is only applicable to women of childbearing age (if the urine pregnancy test is positive, it must be confirmed by serum
227 pregnancy test).

228

229 3. TCM syndrome differentiation

230 According to the “2014 Consensus from TCM experts on diagnosis and treatment of chronic heart
231 failure” (17), there are 3 major types of TCM syndrome for heart failure: 1) qi-deficiency with blood
232 stasis syndrome; 2) qi-yin deficiency with blood stasis syndrome; 3) yang-qi deficiency with blood

233 stasis syndrome, and each can be associated with the phlegm-retention syndrome. These syndromes
 234 are comprised of a combination of 2-4 syndrome elements including 1) qi-deficiency; 2) yin-deficiency,
 235 3) yang-deficiency; 4) blood stasis and 5) phlegm-retention. The list of clinical manifestations
 236 including signs and symptoms, pulse, and tongue features of the 3 TCM syndrome types are shown in
 237 Fig. 2. The 5 syndrome elements with 3 syndrome types indicated in this guideline are impeccably
 238 matched with the TCM Theory of Collateral Disease for heart failure.

239
 240

241
 242
 243
 244
 245

246 A scoring model for syndrome differentiation is developed with reference to the criteria listed in Fig.2
 247 and the TCM theories. The output from the scoring model will be the type of TCM syndrome and the

level of severity of heart failure patients at a specific stage of the disease. The output will be used for further analysis on the correlation of syndrome differentiation to the biomedical indicators, the severity of HF, and the therapeutic outcome of QLQX capsules.

251

4. Correlation analysis of TCM syndrome patterns and the therapeutic outcome of QLQX in CHF

Our primary objective is to explore the implication of TCM syndrome to the severity of heart failure and the therapeutic outcome of QLQX capsules. We hypothesize that the efficacy of QLQX Capsule on heart failure could be related to the clinical syndrome patterns in TCM diagnosis and likely be favorable to the patients with qi-deficiency with blood stasis and yang-qi deficiency with blood stasis.

258

The efficacy of QLQX capsule will be evaluated with reference to the “2012 Guiding Principles for Clinical Study on New Drug for Use in Traditional Chinese Medicine”. We will evaluate the correlation between TCM syndrome patterns and the severity of heart failure the re-hospitalization rates and mortality. The distribution of TCM syndrome will also be evaluated by NYHA classification, results from an electrocardiogram (ECG), cardiac ultrasound interpretation, and biochemical parameters from routine blood tesst such as NT-proBNP, urine test, etc. Other outcomes include: 1)

265 the distribution of TCM syndrome patterns in heart failure patients; 2) the correlation between TCM
266 syndrome patterns and the severity of heart failure and 3) the dynamic changes between biomedical
267 parameters and the TCM syndrome patterns of heart failure, etc.

268

269 **5. Correlation of tongue features and the severity of HF and therapeutic outcome of QLQX**

270 Tongue images of the patients will be acquired as part of the TCM Syndrome Diagnosis Questionnaire
271 for Heart Failure (SDQHF) during the study visit. To ensure the quality and consistency of tongue
272 photos from all clinical centers/hospitals, all researchers/physicians are properly trained according to
273 the Standard Operation Procedures (SOP), and are provided with a standardized image acquisition
274 device to capture the tongue image. The SOP includes the schedule of the study visit, the procedures
275 for completing the SDQHF, and the criteria for capturing the tongue image such as the lighting
276 requirement, camera mode (i.e. normal mode, no flashes, etc.), the posture of the tongue, and the
277 tongue area to be included in the image, as well as the prerequisite requirements such as no intake of
278 food or drinks that will stain the tongue coating on the day of the study visit. All tongue images should
279 be sent to the central tongue image database via email with an indication of the patient number and the
280 date of study visit.

281

282 We will set up a team of 4 TCM practitioners with 3 to 30 years of clinical experience to evaluate the
283 tongue images. The identifiers and possible values for tongue analysis include the tongue color, tongue
284 shape, tongue coating proper and its color, tongue coating distribution, appearance and location of
285 ecchymosis, etc. Tongue images that fail to meet the SOP requirement will be excluded from the
286 analysis. A 4-stage decision flow is designed for the review and analysis of tongue images: 1) direct
287 confirmation if consistent interpretation and diagnosis from the first three TCM practitioners; 2) if
288 consistent results are identical from 2 TCM practitioners but different diagnosis from the 3rd one, take
289 the result from the majority; 3) if the interpretation and diagnostic results from all 3 TCM practitioners
290 are different, will involve the 4th TCM practitioner with over 30 years of clinical experience to judge
291 the syndrome type with the clinical information collected from the SDQHF; and finally, 4) if the image
292 is determined as not in good quality for drawing the conclusion after reviewed by the separate TCM
293 practitioner and professor, that tongue image will be excluded from the study.

294 Outcomes of the tongue analysis include 1) determination of the TCM syndrome differentiation of
295 patients throughout the assessment period with the clinical information collected through the SDQHF;
296 2) tongue features in heart failure patients in accordance to the TCM syndrome, NYHA classification,
297 and other biomedical data; 3) dynamic patterns of tongue condition in relation to the treatment efficacy
298 and other biomedical indicators

299 **6. Data Management**

300 The patient's demographic data and all clinical data will be recorded and kept in the physical medical
301 record folder during each study visit. The data will then be input into the Epidata software for
302 centralization of all research data and all the tongue images will be kept in a separate centralized image
303 database. The data management process will be complied with the regulatory requirements of Clinical
304 Trial Quality Management Regulations and Clinical Trial Data Management Work Technical
305 Guidelines to ensure the authenticity, integrality, accuracy, and traceability of data.

306

307 **7. Sample size estimations**

308 This study is benchmarked with the QUEST study in which 3080 patients will be enrolled in over 100
309 centers (1540 patients per group) and followed up for at least 12 months. The sample size was
310 estimated referring to the PARADIGM-HF study (44). For QUEST study, the estimated incidence of
311 cardiovascular death and hospitalization for heart failure is 25% in all patients receiving basic
312 treatment + placebo group within 36 months of follow-up and 20% with those receiving basic
313 treatment + QLQX Capsule group. The random distribution ratio is 1:1 between the study group and
314 the control group. With the consumption of type I error in the interim analysis, α is adjusted to
315 unilateral 0.02314. The sample size is the number of cases with composite endpoints events. Thus, 620

316 composite endpoint events are expected for the QUEST study to provide 80% power of test ($\beta = 0.2$),
317 and the 20% risk can be reduced in the study group by the log-rank test.

318 For the current study, a pilot test with at least 342 patients will be conducted to evaluate the
319 acceptability, reliability, and validity of the questionnaire. The number of patients for pilot testing is
320 calculated based on the confidence level of 95%, population size of 3,080, and 5% margin of error
321 for this 36-item questionnaire.

322

323 **8. Data analysis and statistic methodology**

324 Analysis of the TCM Questionnaire will be performed with the SPSS statistical software package,
325 version SPSS 24.0 (Chicago, IL,USA). Data will be presented by Mean $\pm$ S.D. Principal Component
326 Analysis (PCA) will be applied to the questionnaire to examine the factor structure. Logistic regression
327 analysis will be used to evaluate the capability of SDQHF to diagnose the severity of HF from a
328 functional perspective. Pearson's correlation coefficient will be used to determine the existence of
329 correlations between TCM syndrome differentiation and various parameters such as treatment efficacy,
330 NT-proBNP, NYHA classification, etc. An independent t-test will be used to compare quantitative
331 variables for the two-group designed study. Fisher's Exact test and the chi-squared test will be applied

332 to compare categorical variables between groups. Multiple groups designed data will be analyzed by
333 two-way ANOVA.

334

335 **Discussion**

336 In TCM, the syndrome pattern is a vital component in both diagnosis and treatment of disease.
337 Syndrome elements, syndromes, phenotypic features, as well as disease, form an integral process in
338 the diagnostic path. However, due to the lack of standardization of syndromes, many diversified
339 syndrome types were found in previous studies, leading to difficulties in drawing sound conclusive
340 outcomes and thus, hindering research development (13-17). It is crucial to have an end-to-end
341 approach with a clear and definitive set of syndromes for heart failure study. The present study will
342 focus on the objective and quantitative syndrome diagnosis criteria for heart failure, the scoring model
343 of the SDQHF, and the establishment of the tongue image database. For the TCM syndrome pattern
344 recognition, we aim to develop a scoring system to catalog the syndrome differentiation from the signs
345 and symptoms in the questionnaire with reference to the guideline and consensus including the “2012
346 Guiding Principles for Clinical Study on New Drug for Use in Traditional Chinese Medicine”, “2014
347 Consensus from TCM experts on diagnosis and treatment of chronic heart failure”, and “2017
348 Guideline for TCM Diagnosis and Treatment of Heart Failure (Chronic Heart Failure)”. We expect the

development of SDQHF and the scoring model could establish a solid ground for syndrome differentiation of heart failure and to facilitate the evaluation of the efficacy of the QLQX Capsule. With the large-scale clinical trial and multiple level evaluations, we could provide an example to resolve the unanswered question of whether the evidence-based clinical trials should consider the potential influence on different TCM syndrome differentiation patterns on disease progress and therapeutic outcome. This may enable the paradigm shift from reactive to predictive, preventive, and personalized care through the application of the TCM syndrome differentiation.

In terms of operational issues, quality control is one of the major challenges for such large-scale clinical studies involving over 100 clinical centers and more than 300 researchers/physicians in China and Hong Kong. We have established standardized operating procedure (SOP) and provided extensive training to all researchers/physicians prior to the patient recruitments. With the onset, supplementary training and continuous review, we could ensure proper execution of SOP to secure the quality of data collection. Tongue image acquisition is one of the critical issues for quality control. As such, we provided an imaging capturing device of the same model to each clinical center and trained all the researchers/physicians on how to properly acquire the tongue image, including the camera settings, the lighting requirement, and other pre-requisite requirements for patients prior to the photo-taking, etc. To ensure the image quality, we have a constant quality check on all tongue photos so that feedback

366 for improvement could be provided to the researchers/physicians on a timely basis. Thus, the quality
367 of tongue images could meet our requirement for the analysis of tongue features and patterns of heart
368 failure. We will also put efforts into patient communication to ensure all patients have a thorough
369 understanding of the trial so that they can strictly follow through the requirement and attend all
370 scheduled visits. With the high standard and high-quality clinical trial, we expect to achieve following
371 outcomes: (1) To verify that QLQX could be an adjunct therapy with conventional treatment to reduce
372 the mortality and re-hospitalization with worsening heart failure in the QUEST study. (2) To confirm
373 whether different TCM syndrome patterns and tongue characteristics would be associated with the
374 severity of heart failure and the therapeutic efficacy of QLQX on chronic heart failure patients.

375

376 **Conclusion**

377 To our knowledge, this study is a high standard designed, multi-center, randomized, double-blind,
378 parallel-group, placebo-controlled clinical trial with large scale subjects to verify the impacts of TCM
379 syndrome patterns on the severity of heart failure and the therapeutic outcome of Chinese medicine
380 formula. Furthermore, it may very well be the largest cohort clinical trial with long follow-up period
381 and hard endpoints to evaluate the long-term efficacy and safety of TCM formula in the treatment of
382 chronic heart failure.

383

384 **Trial status**

385 At the time of manuscript submission, the patient recruitment for the trial has just finished and we are
386 now in the assessment period.

387

388 **List of abbreviations**

389 6MWD: 6-min Walking Distance, CHF: chronic heart failure, HF: heart failure, NYHA: New York
390 Heart Association; QLQX: Qiliqiangxin Capsule, LVMi: Left ventricular mass index, LVEF: Left
391 ventricular ejection fraction, TCM: Traditional Chinese Medicine; SDQHF: Syndrome Diagnosis
392 Questionnaire for heart failure, NT-proBNP: NT-proB-type Natriuetic Peptide; SOP: Standard
393 Operating Procedures

394

395

Declarations

Ethics approval and consent to participate

All participating centers must obtain approval from appropriate independent ethics committees or institutional review boards (Approved No. of ethic committee:2018-SR-275). The study is conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki. This study is conducted in conjunction with a current trial which is registered at <http://www.chictr.org.cn-ChiCTR1900021929>. All participants must give written informed consent which should be acquired after a sufficient and detailed explanation to the research situation (including confidentiality, risk and benefits, the purpose of the study, etc.). For Hong Kong, the clinical trial is approved by the Department of Health with Certificate For Clinical Trial and Medicinal Test (Certificate No. CTMT-00008).

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

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The funding sources have not involved in the study design, data collection, analysis, interpretation of data and writing the manuscript.

Competing interest

The design of the study, data collection, data analysis and interpretation are independent to the funding body.

All executive committee members of QUEST study have received consulting fees as well as travel and research support in the planning and conduct of the QUEST study. The authors declare that they have no other conflict of interest to disclose.

431

432 **Author's contribution**

433 YLL participated in the design of the research protocol and drafted the manuscript. JJS contributed to
434 the conception and design of the research protocols. JJS also performed a critical revision of the
435 manuscript for important intellectual content. HYC provided major revisions to the manuscript. ZHJ
436 contributed to obtaining funding and the contact with multi-center. LXX contributed to the conception,
437 design of the research protocols of the QUEST study. All authors read and approved the final
438 manuscript.

439

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