Therapeutic effects of Compound Zhenzhu Tiaozhi capsules in nonalcoholic fatty liver disease-a randomized controlled study

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I. Background

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in China now. It comprises a wide spectrum of disorders that range from simple steatosis to nonalcoholic steatohepatitis (NASH), advanced fibrosis, related cirrhosis, and even hepatocellular carcinoma (HCC). Currently there are no effective medications to cure NAFLD. Simple steatosis can progress into NASH, irreversible cirrhosis, and even HCC. Recently, extensive studies have emphasized that NAFLD is closely associated with metabolic syndrome, type 2 diabetes mellitus, dyslipidemia, insulin resistance, and oxidative stress/lipid peroxidation injury. In addition to the liver pathogenesis, both simple steatosis and NASH are well-established risk factors for many diseases, including diabetes, arteriosclerosis, hypertension, and colorectal cancer. Therefore, it is urgent to develop effective therapies for the early stages of NALFD and thereby reduce the risk and morbidity of cirrhosis, HCC, diabetes, hypertension, colorectal cancer, and other serious diseases. Current treatments for NAFLD include weight reduction through lifestyle modification and exercise, insulin-sensitizing agents, lipid-lowering agents, and hepatoprotective drugs. (Cited from: Hu Yinxia, Li Lan, Yuan Yu, Wu Lihao, He Xingxiang. Therapeutic effect of teduglutide on non-alcoholic fatty liver disease in rats[J]. World Chinese Journal of Digestology, 2016 (7): 1009-1016.). However, the efficacy of these therapies is not satisfactory, some drugs may even induce liver toxicity. Thus, it is necessary to develop novel therapies that are effective and safe for the treatment of NAFLD.

The pathogenesis of NAFLD is complicated, which is mainly based on a 2-hit hypothesis, first proposed by Day and James in 1998. Essentially, the “first hit” refers to the development of hepatic steatosis via hepatic triglyceride accumulation and insulin resistance. The “second hit” is the shift from hepatic steatosis to NASH, including oxidative stress, inflammatory cytokines, and chronic intermittent hypoxia. Insulin resistance is the critical factor for hepatic steatosis. Insulin resistance is an abnormal metabolic state, in which peripheral tissue has impaired sensitivity to insulin

and attenuated biological response. In the stage of simple steatosis, improvement of insulin resistance could prevent the development of hepatic steatosis, reduce the accumulation of hepatic lipid, and effectively preclude the emergence of second hits.

The liver and intestine are functionally connected in several ways. The interactions between the liver and the gut, called the "gut-liver axis," has been a gradually accepted theory and provides a powerful rationale for the treatment of many chronic liver diseases. Both animal experiments and clinical studies have demonstrated that the intestinal microflora are closely associated with the onset and progression of chronic liver diseases. Liver diseases can cause increased intestinal permeability, in which widened tight gap junctions lead to microbial translocation. On the other hand, microbial translocation and dysbiosis in the gut can further promote the development and progression of liver diseases. (Cited from: Chen Meihui, He Xingxiang, CaiJiyi. Progress on liver dysfunction and small intestinal diseases[J].Journal of Guangdong Pharmaceutical University, 2015, 31 (4): 558-560). A previous study found a NASH-like pattern of liver injury in a mouse model of small intestinal bacterial overgrowth (SIBO). Furthermore, the authors observed that SIBO mice had a higher incidence of NAFLD, which was related to hepatic steatosis. In the congenitally obese mice model, the intestinal mucosal barrier was damaged, which caused endotoxemia and led to the development of NASH via TNF-alpha secretion (cited from: Vanni E, Bugianesi E. The gut-liver axis in nonalcoholic fatty liver disease: Another pathway to insulin resistance, Hepatology, 2009, 49 (6): 1790-1792). Intestinal flora disorders and increased intestinal permeability were induced through immune responses, which furtherly promoted the development and progression of NAFLD. In contrast, the application of antibiotics, prebiotics and probiotics that regulate intestinal microbiota can prevent the progress of NAFLD (Cited from: Wang Junyao, Liu Yulan. The roles of gut-liver axison the development and progression of nonalcoholic fatty liver disease. Journal of Clinical Hepatology, 2012,15 (4): 276 -278).In addition, some studies reported that the changes in intestinal microbiota were significantly related to insulin resistance. Alterations in intestinal microbiota not only induced elevated lipopolysaccharides (LPS) in the intestinal tract, but also damaged the tight junctions

of the intestinal epithelium, leading to increased flow of LPS into the blood circulation, causing a series of non-specific inflammatory responses. In consequence, the alterations in intestinal microbiota interfered with insulin signaling and induced insulin resistance.

Some studies have shown that traditional Chinese medicine (TCM) has potentially effective therapies for chronic diseases. Additionally, TCM has unique therapeutic effects on some diseases that are particularly related to metabolic syndromes like hyperlipidemia, diabetes and fatty liver. For example, berberine was reported to improve insulin resistance, have glucose-and lipid-lowering effects, and was even used for the treatment of NAFLD. Researchers found in a NASH mice model that oral administration of berberine can change the intestinal microbiota, improve biochemical markers, attenuate liver histological changes, and reduce the intrahepatic inflammatory responses. The functions of berberine on whole-body energy metabolism and NAFLD were exerted by adjusting the ecological structure and microenvironment of the intestinal flora(Cited from: Cao Yi, Xu Leiming, PanQin, Wang Xiaoying, Shen Feng, Chen Guangyu, Fang Jiangao. Modulation of gut microbiota with berberine improves nonalcoholic steatohepatitis in mice[J]. Journal of Clinical Hepatology, 2013, 16 (2): 137-140).Compound Zhenzhu Tiaozhi capsules consist of eight Chinese herbal medications, such as Ligustrum lucidum, Atractylodes macrocephala, Radix Salviae Miltiorrhizae, pseudo-ginseng, Astragalus membranaceus. Previous studies found that Compound Zhenzhu Tiaozhi capsules can reduce serum triglyceride (TG), total cholesterol (TC), fasting blood glucose, insulin, and the insulin resistance index. In addition, it can increase serum high-density lipoprotein cholesterol (HDL-C) levels, reduce weight, and improve obesity in a rat model, which means that it can be used to treat metabolic syndrome. It was also reported that Compound Zhenzhu Tiaozhi capsules upregulated PI-3Kp85 mRNA expression in rat adipose tissue, which might be one of the mechanisms involved in insulin resistance (cited from: Hu Xuguang, Guo Jiao, Bai Weijian, He Wei, Wang Shou, Liu Shasha. Effect of Fufang Zhengzhu Tiaozhi Capsules on Insulin Resistance and Insulin Signal PI-3K in Metabolic Syndrome Rats [J].Traditional Chinese Drug

Research and Clinical Pharmacology, 2012, 23 (2): 140-143).Additionally, Compound Zhenzhu Tiaozhi capsules can reduce serum TG, TC, and low-density lipoprotein cholesterol (LDL-C) and increase serum HDL-C levels in a hyperlipidemic rat model (Cited from: Tang Chunping, Gong Mengjuan, Guo Jiao, Yang Chaoyan, Chen Fangchao, Yao Hongxia, Chen Yuefeng. The effects of Fufang Zhenzhu Tiaozhi capsules on blood lipid level and hemorheology of rats with hyperlipemia [J]. Journal of Guangdong Pharmacy College, 2010, 26 (6): 612-616).

Based on the above studies, the investigators hypothesize that Compound Zhenzhu Tiaozhi capsules have the potential to effectively improve NAFLD and related biomedical markers. The possible mechanisms include the improvement of SIBO and the function of the intestinal mucosal barrier. The investigators thus designed the following clinical study to test this hypothesis.

II. Objectives

To evaluate the efficacy of Compound Zhenzhu Tiaozhi capsules in the treatment of NAFLD and analyze the relationship between improvement in NAFLD parameters and changes in intestinal functions.

III. Content of the study

1. To evaluate the relationship between the function of intestinal mucosal barrier, SIBO, and NAFLD;

2. To assess the effects of Compound Zhenzhu Tiaozhi capsules on intrahepatic fat content, hepatic noninvasive score, liver biochemical parameters, blood lipid, and insulin resistance. To evaluate the therapeutic effects of Compound Zhenzhu Tiaozhi capsules on the treatment of NAFLD, and determine whether the risk stratification of atherosclerotic cardiovascular disease (ASCVD) and quality of life (using the 36-Item Short Form Health Survey [SF-36]) can be improved in NAFLD patients with Compound Zhenzhu Tiaozhi capsules.

3. To compare the therapeutic effects of Compound Zhenzhu Tiaozhi capsules with conventional medications in the treatment of NAFLD, and to compare their efficacies in improving the risk stratification of ASCVD and quality of life, as

assessed by the SF-36.

4. Finally, to analyze the relationship of Compound Zhenzhu Tiaozhi capsules in improving NAFLD and parameters related to the function of the intestinal mucosal barrier and SIBO.

IV. Methods

This is an interventional and randomized parallel control study. The study will be conducted from December 31 2017 to December 31 2019. The recruitment and observation period will start in December, 2017 and continue until the required sample size is reached. Research venue: First Affiliated Hospital, Guangdong Pharmaceutical University, Guangdong province, China. Institute level: a third-grade-A-class hospital.

1. Ethical approval: The study will be conducted after obtaining ethical approval from Ethics Committee of the First Affiliated Hospital, Guangdong Pharmaceutical University. The investigators will inform all prospective subjects of the study background, purpose, design, and medication contents. If a subject is willing to participate in the study, he or she will sign the consent form (Supplementary 1).

2. Enrollment:

The subjects will be enrolled following the design of a prospective study. The inclusion criteria will be strictly in accordance with the diagnostic criteria of simple steatosis and NASH, as stipulated in the *Chinese Guidelines for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease (Revised version 2010)*. The subjects should have a diagnosis of simple steatosis or NASH (age distribution: 18 to 75 years old), with serum alanine transaminase (ALT)≤2 times the upper limit of normal, with no other chronic liver diseases or serious complications in other organs, with on malignant tumors.

2.1. Diagnostic Criteria of NAFLD (2010): NAFLD can be diagnosed if the following items 1–4,plus item 5 or item 6 are present:

i. No alcoholic history (alcohol consumption <140 g/week for male;or <70

g/week for female);

ii. No other diseases that can lead to fatty liver, such as viral hepatitis, drug-induced liver disease, total parenteral nutrition, hepatolenticular degeneration diseases, or autoimmune liver diseases;

iii. Overweight and/or visceral obesity, hyperglycemia, dyslipidemia, hypertension and other metabolic syndrome;

Serum transaminase and GGT levels are normal or elevated to a mild to moderate degree;

iv. Liver imaging findings show diffuse hepatic steatosis;

v. A liver biopsy meets the histologic diagnostic criteria of fatty liver diseases;

2.2. Diagnostic criteria of simple steatosis: Simple steatosis can be diagnosed if the following item 1-2, plus item 3 or item 4 are present.

i. Presence of item1-3 of the clinical diagnosis criteria of NAFLD;

ii. Liver biochemical tests are normal (blood biochemical analysis);

iii. Liver imaging findings meet the diagnostic criteria for hepatic steatosis (Fibro touch test);

iv. A liver biopsy meets the histologic diagnostic criteria for simple hepatic steatosis.

2.3. The diagnostic criteria of NASH: NASH can be diagnosed with the following items 1 to 3 or item 4.

i. Presence of items 1–3 of the clinical diagnosis criteria of NAFLD;

ii. Serum ALT levels are persistently elevated for more than 4 weeks;

iii. Liver imaging findings show diffuse hepatic steatosis;

iv. A liver biopsy meets the histologic diagnostic criteria for NASH.

2.4. The following individuals will be considered as lost to follow-up: first, patients who suddenly present serious physical or mental diseases (not related to the study) during the study period, such as malignant tumors, severe mental illness, traffic accidents, etc.; second, patients who have serious side effects in which the symptoms can be relieved by discontinuing the study medication; third, patients who actively ask to stop using the study medication or refuse to come to follow up visits. Fourth,

patients who need to take other medications due to the development of other new diseases, where the new medications may interfere with the study results, such as hypoglycemic agents, including insulin.

3. Assignment and intervention:

3.1. Calculation of the sample size and the method of assignment:

Sample size calculation: A total of 196 patients will be enrolled in this study. The sample size is estimated as follows:

Previous studies have reported that the efficiency rate of metformin and simvastatin in the treatment of NAFLD is 36-52%. The investigators hypothesize that the efficiency rate of Compound Zhenshu Tiaozhi capsules will increase by 25% compared to standard drugs.

Statistical calculation: P1 = 69%, P2 = P3 = 44%, P4 = 10%, δ = P1-P2 = 25%, with α = 0.05, power = 0.80, and a bilateral test, of which $w = \sqrt{\sum_{i=1}^{m} \frac{(P_{t,i}-P_{c,i})^2}{P_{c,i}}}$, thus n will be equal to 44. Assuming that the rate of loss to follow up is 10%, each group will require 49 cases. Therefore, a total of 196 cases will be required for the four groups.

3.2 The method of assignment: Zhang Min, a staff member working in the Department of Statistics in Guangdong Pharmaceutical University, is responsible for generating random sequences using the SAS software. A total of 196 numbers from 1 to 196 will be randomly generated (Supplement 2). Newly diagnosed patients who meet the inclusion criteria will have a case number according to their order of enrollment (for example: case number of the first patient: 001, second patient: 002). They will then obtain the corresponding therapeutic regimens based on their case number with 1:1:1:1 ratio randomized tables. The first group is the TLC group; the second group is the TLC + metformin group (0.5 g, PO tid); the third group is the TLC + Compound Zhenzhu Tiaozhi capsules group (2.52 (four tablets), PO tid); the fourth group is the TLC + simvastatin group (20 mg, PO qn).

4. Endpoints:

4.1 Primary endpoints

Fat attenuation index: The fat attenuation index will be used to assess the therapeutic efficacy. A normal fat attenuation index is defined as < 240 db/m, mild is 240-264 db/m, moderate is 265-294 db/m, and severity is > 295 db/m. The investigators will detect the fat attenuation index at 0, 1, 3, 6 months following treatment. Effective: fat attenuation index is reduced by a level or more (example: moderate to mild). Invalid: fails to meet the effective standard.

4.2 Secondary endpoints:

(1) *Serum triglyceride*

(2) *Serum cholesterol*

(3) *Serum lipoproteins:* chylomicron (CM), very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL).

(4) *Hydrogen/methane breath testing*: breath testing for SIBO.

(5) *Intestinal mucosal barrier*: detect levels of diamine oxidase (DAO), D-lactic acid (D-lac), and lipopolysaccharide (LPS), and combine with the clinical manifestations to evaluate the function of the intestinal mucosal barrier.

(6) *Quality of life (SF-36 Scores)*: The quality of life of the patients before and after treatment will be assessed using SF-36 scores and compared to assess the improvement.

4.3. Analysis of the relationships among the function of intestinal mucosal barrier, SIBO, and NAFLD.

1) To analyze the relationship of DAO, D-lac, LPS, intestinal mucosal barrier dysfunction grade I, II, III, IV within trahepatic fat content in NAFLD patients(possible results: unrelated, positively related, or negatively related).

2) To evaluate the relationship between SIBO and intrahepatic fat content in NAFLD patients (possible results: unrelated, positively related, or negatively related).

3) To assess the relationship of DAO, D-lac, LPS, intestinal barrier function dysfunction grade I, II, III, IV with insulin resistance in NAFLD patients (possible results: unrelated, positively related, or negatively related).

4) To detect the relationship between SIBO and insulin resistance in NAFLD patients (possible results: unrelated, positively related, or negatively related).

5) To explore the relationship of DAO, D-lac, LPS, intestinal barrier dysfunction grade I, II, III, IV and peripheral blood lipidsprofile and liver biochemical parameters (ALT, AST, GGT) (possible results: unrelated, positively related, or negatively related).

6) To investigate the relationship of SIBO and lipids profile and liver biochemical parameters (ALT, AST, GGT) in NAFLD patients (possible results: unrelated, positively related, or negatively related).

7) To estimate the relationship of DAO, D-lac, LPS, intestinal barrier dysfunction grade I, II, III, IV and NAFLD-FS in NAFLD patients (possible results: unrelated, positively related, or negatively related).

8) To determine the relationship between SIBO and NAFLD-FS in NAFLD patients (possible results: unrelated, positively related, or negatively related).

4.4. To evaluate the therapeutic effects of Compound Zhenzhu Tiaozhi capsules on NAFLD

1)To assess whether the content of intrahepatic fat will decrease after 1 month, 3 months, and 6 months of Compound Zhenzhu Tiaozhi capsule therapy.

i. Compare the therapeutic effects of Compound Zhenzhu Tiaozhi capsules at 1 month, 3 months, and 6 months with the baseline (pre-vs.post-therapeutic effects of Compound Zhenzhu Tiaozhi capsules).
ii. Compare the therapeutic effects in the Compound Zhenzhu Tiaozhi capsulegroup with that of the other three groups atparallel time points (Compound Zhenzhu Tiaozhi capsules vs.Conventional drugs).

2) To assess whether NAFLD-FS will be alleviated after 1 month, 3 months, and 6 months of Compound Zhenzhu Tiaozhi capsuletherapy.

i. Compare the therapeutic effects of Compound Zhenzhu Tiaozhi capsules at 1 month, 3 months, and 6 months with the baseline (pre- *vs.* post-therapeutic effects of Compound Zhenzhu Tiaozhi capsules).
ii. Compare the therapeutic effects in the Compound Zhenzhu Tiaozhi capsule group with those of the other three groups at parallel time points

(Compound Zhenzhu Tiaozhi capsules vs.Conventional drugs).

3) To assess whether insulin resistance will be improved after 1 month, 3 months, 6 months of Compound Zhenzhu Tiaozhi capsule therapy.

i. Compare the therapeutic effects of Compound Zhenzhu Tiaozhi capsules at 1 month, 3 months, and 6 months with the baseline (pre- *vs.* post-therapeutic effects of Compound Zhenzhu Tiaozhi capsules).
ii. Compare the therapeutic effects in the Compound Zhenzhu Tiaozhi capsule group with those of the other three groups at parallel time points (Compound Zhenzhu Tiaozhi capsules *vs.* Conventional drugs).

4) To assess whether the lipids profile in peripheral blood and liver biochemical parameters(ALT, AST, GGT) will be reduced after 1month, 3months, and6months of Compound Zhenzhu Tiaozhi capsule therapy.

i. Compare the therapeutic effects of Compound Zhenzhu Tiaozhi capsules at 1 month, 3 months, and 6 months with the baseline (pre- *vs.* post-therapeutic effects of Compound Zhenzhu Tiaozhi capsules).
ii. Compare the therapeutic effects in the Compound Zhenzhu Tiaozhi capsule group with those of the other three groups at parallel time points (Compound Zhenzhu Tiaozhi capsules *vs.* Conventional drugs).

4.5 In the Compound Zhenzhu Tiaozhi capsules group, firstly calculate the changes in the related parameters of NALFD and the changes in the function of intestine microbiota, then evaluate whether the two changes are related to each other.

1)Analyze the relationship between the changes in DAO, D-lac, LPS, intestinal barrier dysfunction grade I, II, III, IV and the reduction in intrahepatic fat at 1 month, 3 months, and 6 months of Compound Zhenzhu Tiaozhi capsuletherapy (possible results: unrelated,positively related, or negatively related).

2)Analyze the relationship between the change in SIBO and the reduction in intrahepatic fat at 1 month, 3 months, and 6 months of Compound Zhenzhu Tiaozhi

capsule therapy (possible results: unrelated,positively related, or negatively related).

3)Analyze the relationship between the changes in DAO, D-lac, LPS, intestinal mucosal barrier dysfunction I, II, III, IV and the improvement of insulin resistance at1month, 3months, and 6months of Compound Zhenzhu Tiaozhi capsule therapy (possible results: unrelated, positively related, or negatively related).

4)Analyze the relationship between the change in SIBO and the improvement of insulin resistance at 1 month, 3 months, and 6 months of Compound Zhenzhu Tiaozhi capsule therapy (possible results: unrelated, positively related, or negatively related).

5) Analyze the relationship between changes in DAO, D-lac, LPS, intestinal mucosal barrier dysfunction I, II, III, IV and the changes in the lipids profile in peripheral blood and liver biochemical parameters(ALT, AST, GGT) at 1 month, 3 months, and 6 months of Compound Zhenzhu Tiaozhi capsule therapy (possible results: unrelated, positively related, or negatively related).

6) Analyze the relationship between the change in SIBO and the changes in the lipids profile in peripheral blood and liver biochemical parameters(ALT, AST, GGT) at 1month, 3months, and 6months of Compound Zhenzhu Tiaozhi capsule therapy (possible results: unrelated, positively related, or negatively related).

7) Analyze the relationship between changes in DAO, D-lac, LPS, intestinal mucosal barrier dysfunction I, II, III, IV and the change in NAFLD-FS at 1month, 3months, and 6months of Compound Zhenzhu Tiaozhi capsuletherapy (possible results: unrelated, positively related, or negatively related).

8)Analyze the relationship between the change in SIBO and the change in NAFLD-FS at 1 month, 3 months, and 6 months of Compound Zhenzhu Tiaozhi capsule therapy (possible results: unrelated, positively related, or negatively related).

4.6. To estimate the ASCVD risk for all NAFLD patients, and to evaluate whether the therapy of Compound Zhenzhu Tiaozhi capsules improves the risk stratification of ASCVD for NALFD patients, as well as to compare the therapeutic effects of the study capsules and other conventional drugs. The investigators will estimate the ASCVD risk for NAFLD patients after 1month, 3 months, and 6 months of

Compound Zhenzhu Tiaozhi capsule therapy, according to *Guidelines for Prevention and Treatment of Dyslipidemia in Adults*.

ASCVD populations with different risk stratifications have different target values of lowering LDL-C / non-HDL-C, as shown in the table below.

Risk Stratification	LDL-C	Non-HDL-C
High-risk	<2.6mmol/L(100mg/dl)	<3.4mmol/L(130mg/dl)
Very high-risk	<1.8mmol/L(70mg/dl)	<2.6mmol/L(100mg/dl)
Note: ASCVD: atherosclerotic cardiovascular disease; LDL-C: low density lipoprotein cholesterol; Non-HDL-C: non-high density lipoprotein cholesterol		

If the baseline of LDL-C is high, as it is difficult to reduce LDL-C level to the basic target value after 3 months of standard treatment with existing lipid-lowering drugs, we propose to lower LDL-C level to at least 50% of baseline as the surrogate target value(IIa recommendation, Level B evidence). There are also some patients at very high-risk who have a baseline LDL-C within the basic target value; for these patients the investigators propose to reduce LDL-C levels to about 30% of baseline (I recommendation, A-level evidence).

Those who meet any of the following criteria can be directly classified as high-risk or very high-risk populations, as indicated:

1. Very high-risk: ASCVD patients (acute coronary syndrome, stable coronary heart disease, revascularization, ischemic cardiomyopathy, ischemic stroke, transient ischemic attack, peripheral atherosclerosis, etc.);

2. High-risk: (1) LDL-C ≥4.9 mmol/L or TC ≥7.2 mmol/L, (2) Diabetics:1.8 mmol/L≤ LDL-C <4.9 mmol/L (or) 3.1 mmol/L≤ TC <7.2 mmol/L, and age ≥ 40 years old.

3. For those who do meet the above criteria, the investigators will evaluate their 10-years ASCVD risk as follows

Number of risk	Stratification of Serum Cholesterol (mmol/L)

factors	3.1oTC<4.1(or) 1.8≤LDL-C<2.6	4.1.TC<5.2(or) 2.62LDL-C<3.4	5.2≤TC<7.2(or)3 .42LDL-C<4.9
Without hypertension0–1	Low-risk (<5%)	Low-risk (<5%)	Low-risk (<5%)
2	Low-risk (<5%)	Low-risk (<5%)	Moderate risk (5%–9%)
3	Low-risk (<5%)	Moderate risk (5%–9%)	Moderate risk (5%–9%)
hypertension 0	Low-risk (<5%)	Low-risk (<5%)	Low-risk (<5%)
1	Low-risk (<5%)	Moderate risk (5%–9%)	Moderate risk (5%–9%)
2	Moderate risk (5%–9%)	High-risk (≥10%)	High-risk (≥10%)
3	High-risk (≥10%)	High-risk (≥10%)	High-risk (≥10%)

4. If the 10-year ASCVD risk is moderate and the age is less than 55 years old, then the investigators propose to do the remaining life assessment. Those who meet any two of the following items can be classified as high-risk: 1. ≥160 mmHg or diastolic blood pressure≥100mmHg; 2. non-HDL-C ≥5.2 mmol/L (200 mg/dl); 3. HDL-C <1.0 mmol/L (40mg/dl); 4. BMI≥28kg/m^2; 5. history of smoking.

3.5. To assess the quality of life using the SF-36 questionnaire for all NAFLD patients, and to evaluate whether the therapy of Compound Zhenzhu Tiaozhi capsules improves the quality of life for NALFD patients, as well as to compare its therapeutic effects with other conventional drugs.

Patients will be required to fill in the SF-36 questionnaire at the time of enrollment, and after 1 month, 3 months, and 6 months of treatment. SF-36 is one of the most widely used and standardized tools for quality of life assessment. SF-36, as a simple health questionnaire, recapitulates the quality of life of the respondents based on eight categories: physiological function, physical pain, general health status, energy, social function, emotional function, and mental health. The first four categories describe physical health, the latter four describe mental health.

The early symptoms of NAFLD are not obvious and almost have no impact on daily life. As it is difficult to effectively assess whether patients have benefited from treatment by evaluating symptoms, the investigators will use the SF-36 questionnaire. The questionnaire will be administered at the time of patient enrollment, and at 1month, 3months, and 6 months after treatment. To determine whether drug intervention during the early stage of NAFLD can improve symptoms, the scores of theSF-36 before and after treatment will be compared in the following groups: TLC + metformin, TLC +Compound Zhenzhu Tiaozhi capsules, TLC + simvastatin, and TLC. The four groups will also be compared to one another.

4. Prescriptions:

4.1: Medications: the dose of Compound Zhenzhu Tiaozhi capsules is 2.52 (4 tablets) tid; metformin is 0.5, tid, and simvastatin is 20mgqn. In general, subjects will be in the hospital for approximately 1 week and continue to receive treatment after discharge from the hospital. The total period of treatment will be 6 months.

4.2:Biochemical assessments: Patients will undergo all the following tests upon admission:

Routine blood test, tumor biomarker tests (alpha-fetoprotein [AFP], carcinoembryonic antigen [CEA], carbohydrate antigen 125 [CA125], and carbohydrate antigen 19-9 [CA19]), uric acid, liver function (representing hepatocyte injury: alanine aminotransferase, aspartate aminotransferase, leucyl aminopeptidase, glutamyl transpeptidase; representing liver secretory function: total bilirubin (TBS), direct bilirubin (DBIL),indirect bilirubin (IBIL); representing liver reserve function: total protein (TP), albumin (ALB), globulin (GLB), and the albumin to globulin ratio (A/G), serum lipid profile (triglyceride, very low density lipoprotein, total cholesterol, low density lipoprotein, high density lipoprotein), FINS (fasting insulin), fasting blood glucose, serum biomarkers of HBV ([i]Hepatitis B surface antigen [HBsAg]; [ii]Hepatitis B surface antibody [anti-HBs]; [iii]hepatitis B e antigen [HBeAg]; [iv]Hepatitis B e antigen antibody[anti-HBe]; [v]Hepatitis B core antibody [anti-HBc]), TP-Ab, syphilis serological test (TRUST), human immunodeficiency virus(HIV), hepatitis C virus antibody,

glycosylated hemoglobin, intestinal mucosal barrier function, coagulation test (prothrombin time [PT], activated partial thromboplastin time [APTT], thrombin time [TT], and fibrinogen [FIB]), Fibrotouch, hydrogen and methane breath test.

4.3 Medical advice on discharge: Physicians need to inform patients regarding the severity of complications in the late stage of NALFD and the importance of early treatment. All the patients should continue treatment after discharge and follow-up in the clinic.They will be required to come back to hospital one month later for re-assessment of the above mentioned items. The appointment telephone number is 15819544433. The contact doctor is Dr. Lin.

4.4 All patients will be treated with TLC.

4.4.1 Dietary advice: On the basis of daily nutrient and energy requirements, if the total amount of saturated fatty and trans fatty acids exceeds the recommended upper limit, unsaturated fats can be used to replace saturated fats. Recommended daily intake of cholesterol is less than 300 mg, especially for ASCVD high-risk patients.The total fat caloric intake should be limited to 20%–30%of the total caloric intake.

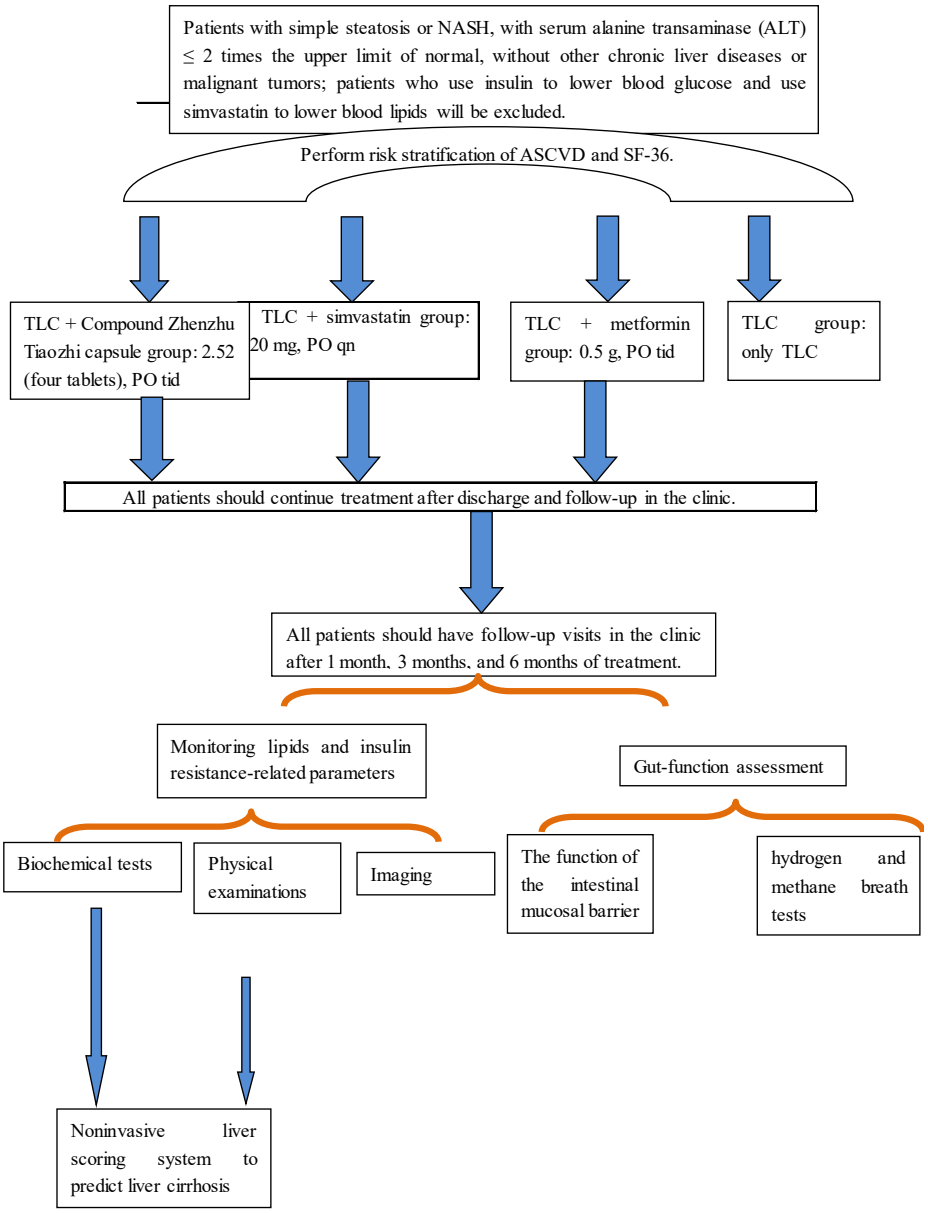
4.4.2 Controlling weight: Obesity is an important risk factor for dyslipidemia. Energy intake in overweight or obese people with dyslipidemia should be lower than energy consumption to prevent weight increase.The goal is to reduce weight gradually until a normal state is reached. Reducing the total amount of daily caloric intake(daily reduction of 300–500 kcal), improving the diet style, and increasing physical activities can reduce weight by more than 10%for overweight and obese people. It is recommended that patients maintain a healthy weight.

4.4.3 Physical activity advice: It is recommended that patients do moderate-intensity exercises, at least 30 minutes per day for 5 days per week. ASCVD patients should do loading activities first to assess whether they are tolerable, and then do physical activities.

4.4.4 Smoking cessation: Smoking cessation and effective reduction of exposure to second-hand smoke are beneficial to prevent ASCVD and increase serum HDL-C levels.

All smokers can be assisted in the clinics, inquire about smoking cessation through hotlines, or take medication to help with smoking cessation.

4.4.5 Restrictions on drinking: Moderate drinking (male: 20-30 g of ethanol per day, female:10 - 20 g of ethanol per day) can increases serum HDL-C level. However, even a small amount of drinking can increase TG in patients with high TG. There is no convincing evidence that alcohol can increase cardiovascular disease. It is recommended that patients limit drinking.



Biochemical tests: routine blood test, liver function tests including transpeptidases, uric acid, FBS,FINS, lipid profile.

Physical examinations: waist circumference, hip circumference, blood pressure, heart rate, BMI

Imaging: abdominal color doppler ultrasound, Fibrotouch

5. Expected results:

1) The liver biochemical parameters and other clinical parameters in NAFLD patients will positively correlate with SIBO and intestinal mucosal barrier function.

2) Compound Zhenzhu Tiaozhi capsules will effectively improve liver function (intrahepatic fat content, liver biochemical markers, NAFLD-FS) and related serological parameters(improvement of dyslipidemia and insulin resistance) in NAFLD patients;Compound Zhenzhu Tiaozhi capsules will effectively improve the SF-36 score and reduce the risk of ASCVD in patients with NAFLD.

3) Compound Zhenzhu Tiaozhi capsules will be superior to conventional drugs (simvastatin and metformin) in terms of improving liver biochemical markers and related serological parameters, SF-36 score, and risk stratification of ASCVD.

4) All assessed items of NAFLD patients (liver: intrahepatic fat content, liver biochemical parameters, NAFLD-FS; and its related serological indicators: dyslipidemia, insulin resistance; SF-36 score; and risk stratification of ASCVD) will be positively associated with SIBO and improved function of the intestinal mucosal barrier.