CHINESE CLINICAL TRIAL PROTOCOL

FibroTouch Non-invasive Evaluation of Liver Fibrosis and Cirrhosis

Principal Center:

01 Beijing Friendship Hospital, Capital Medical University

Other Participant Center:

02 The Military General Hospital of Beijing, PLA

03 The First Affiliated Hospital, Third Military University

04 The First Affiliated Hospital, Fourth Military University

05 The First Bethune Hospital, Jilin University

06 The Third Hospital, Hebei Medical University

07 Henan Provincial People Hospital

08 Jiangsu Provincial People Hospital

09 Ruijin Hospital, Shanghai Jiaotong University School of Medicine

10 West China Hospital, Sichuan University

11 Tianjin Third Central Hospital

12 Prince of Wales Hospital, Chinese University of Hong Kong

13 No.85 Hospital of the PLA

14 The Third Hospital, Sun Yat-sen University

15 Beijing Youan Hospital, Capital Medical University

16 Xinjiang Uygur Autonomous Region Hospital of Traditional Chinese Medicine Hospital

17 China-Japan Friendship Hospital

Sponsor: Wuxi Hisky Medical Technology Co., Ltd.

April 17, 2014

Protocol version: 1.1

This protocol is unanimously passed after the discussion among investigators from the principal center and other participant centers, sponsor and the principal statistician from statistical group.

1. Sponsor

I will, according to the relevant regulations (e.g. Chinese Good Clinical Practice, and Regulations for the Clinical Study on Medical Device), carefully perform the duties of the sponsor to initiate, apply, organize and aid this study, afford the relevant medical expenses, legal liability and proper economic compensation for the incurred damage related to the study device during this study, and offer the legal guarantee for the investigators. I also agree to carry out this study strictly according to the design and specifications of this protocol.

Sponsor: Wuxi Hisky Medical Technology Co., Ltd.

Legal representative (signature): Shao Jinhua Date:

2. Principal investigator

I will, according to the relevant regulations (e.g. Chinese Good Clinical Practice, Regulations for the Clinical Study on Medical Device and Declaration of Helsinki), carefully perform the duties of the principal investigator. I agree to carry out this study strictly according to the design and specifications of this protocol. Before the starting, during the progress and at the end of this study, I will carefully coordinate in time each participant on the relevant matters in this study, so as to guarantee the smooth progress of this study at this clinical trial phase.

Responsible institution: Beijing Friendship Hospital, Capital Medical University

Principal investigator (signature): Jia Jidong Date:

3. Statisticians:

Peking University Clinical Research Institute

Principal statistician (signature): Yao Chen Date:

Abbreviation

Abbreviation	Term
AE	Adverse event
CRF	Case Report Form
EC	Ethic Committee
ECG	Electrocardiography
FAS	Full analytical set
GCP	Good Clinical Practice
PPS	Per-protocol set
SAE	Serious adverse event
SAP	Statistical analytical plan
SD	Source data
CFDA	China Food and Drug Administration
SS	Safety set
PTA	Prothrombin activity
TP	Total protein
ALB	Albumin
AST	Aspartic transaminase
ALT	Alanine transaminase
ALP	Alkaline phosphatase
GGT	Glutamyl transpeptidase
TBIL	Total bilirubin
DBIL	Direct bilirubin
TC	Total cholesterol
TG	Triglyceride
HDL	Blood lipid – high-density lipoprotein
LDL	Blood lipid – low-density lipoprotein
AFP	Serum alpha-fetoprotein
GHb	Glycosylated hemoglobin
FPG	Fasting plasma glucose
INS	Serum insulin

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I. Study Background

The liver diseases frequently occur in China. For various reasons, the chronic liver diseases are not controlled in time, and then develop gradually into liver fibrosis and cirrhosis. Without effective treatment, the advanced liver cirrhosis seriously influences the quality of life of patients, and places an intolerable burden on family and society. At present, the scholar generally thought that the liver fibrosis at early stage is reversible. Therefore, if the liver fibrosis in patients with chronic liver diseases can be accurately evaluated at early stage and be treated in time, so we can stop the progress of diseases and reduce the occurrence of liver cirrhosis and liver cancer.

For many years, liver biopsy is still as “golden standard” for diagnosis of liver inflammation and fibrosis. However, with its invasiveness, potential risks and some complications, liver biopsy is limited in clinical application due to the poor acceptability and repeatability. In recent years, the liver fibrosis cannot directly and accurately diagnose via the various diagnostic model using serological biomarkers (e.g. FIBROTEST and FIBROSIS PREDICTIVE APRI) and medical imaging technology (e.g. ultrasonic B, CT and MRI).

Transient elastography is a new technology in the field of ultrasonic imaging. Liver stiffness measurement (LSM) is based on the relationship between speed of the spread of acoustic wave and stiffness of tissues. It utilizes specific probes to send out controlled low-frequency shear waves, the waves signals transmit through liver tissues, a high-frequency signals to track the transmitting process of shear wave in the liver and the value of liver stiffness (KPa) is quickly calculated with reference to a built-in liver histological model, which provides a quantitative standard for diagnosis of liver fibrosis of chronic liver disease. The bigger LSM value means the faster transmission of shear wave, and the harder of determined tissue.

At present, FibroScan (France, Echosens) as a transient elastography product, has been widely applied in clinical practice. Many studies both at home and abroad on chronic hepatitis B (CHB) and chronic hepatitis C have demonstrated that transient elastography is well correlated to liver histology by liver biopsy in diagnosis of liver fibrosis and cirrhosis. Recently, FibroScan is well recognized at various meetings and recommended by many

medical guidelines.

Marianne *et al* studied on 327 patients with chronic hepatitis C, each patient was scoring by METAVIR system, and the median value of LSM for F0-1, F2, F3 and F4 was 5.5 (4.1-7.1) kPa, 6.6 (4.8-9.6) kPa, 10.3 (7.6-12.9) kPa and 30.8 (16.3-48) kPa respectively; and the Area Under ROC (AUR) for F≥2, F≥3 and F=4 (METAVIR scoring system) was 0.79, 0.91 and 0.97 respectively. Therefore, the LSM varied significantly ($P<0.0001$) and was significantly related to the liver fibrosis (tau beta of Kendall, 0.55; $P<0.0001$).

A multi-center and large-sample comparison study between FibroScan and liver biopsy in the chronic hepatitis B patients in China also demonstrated that LSM had good diagnostic accordance rate to the pathological stages. The cut-off value of pathological stages ⩾F4 was 17.5 kPa as, the sensitivity and specificity were 0.6 and 0.93 respectively; the cut-off value of pathological stages ⩾ F3 was 12.4kPa, the sensitivity and specificity were 0.58 and 0.95 respectively; the cut-off value of pathological stages=F2-F3 was 12.4kPa, the sensitivity and specificity were 0.73 and 0.90 respectively; and the cut-off value of pathological stages ⩾F2 was 7.3kPa, the sensitivity and specificity were 0.66 and 0.83 respectively.

As stated in the "12th Five-year Plan for Medical Device Technological Industry" (the Ministry of Science and Technology), China will greatly support the research and development of new medical devices and promote the application of Chinese transient elastography system, FibroTouch, which was R&D by Tsing-Hua University independently with new algorithm. FibroTouch can rapidly determine the LSM in a non-invasive way and provide useful information for liver fibrosis staging.

As reported by a recent study on 214 CHB patients in Beijing Friendship Hospital underwent FibroScan testing and FibroTouch testing at the same time. FibroTouch and FibmScan have good consistency in the evaluation of the degree of liver fibrosis. FibroTouch has a significantly higher rate of effective detection (99% vs. 72%) and higher rate of successful detection than FibroScan (100% vs. 97%).

Due to FibroTouch is a new transient elastography system in marketing, there is no too much study on the correlation between FibroTouch and liver biopsy in diagnosis of liver fibrosis and cirrhosis.

II. Study Objective

(I) Primary Objective

Use liver biopsy as “golden standard” for pathological staging, to evaluate the clinical values of FibroTouch for the non-invasive and quantitative diagnosis of liver fibrosis and cirrhosis in CHB patients.

(II) Secondary Objective

- ◆ Difference between FibroTouch and FibroScan in the rate of d successful detection
- ◆ Difference between FibroTouch and FibroScan in the determined values
- ◆ Correlation between determined values of FibroTouch and ultrasonic semi-quantitative scores
- ◆ Performance of FibroTouch.

- Positioning accuracy evaluated by investigators
- Image displaying ability evaluated by investigators
- Scanning speed evaluated by investigators
- Detecting and calculating speed evaluated by investigators

III. Study Goal

To determine the diagnostic accuracy, specificity and sensitivity of FibroTouch for liver fibrosis assessment in CHB patients.

IV. Study Type

It’s a prospective and multicenter study.

V. Anticipated Total Cases

According to publications on FibroScan and liver biopsy examination, the mean sensitivity and specificity are 0.61 and 0.90 respectively for different stages (four pathological stages: ≥F4, ≥F3, ≥F2 and F0-F1). Assuming a similar sensitivity and specificity of FibroScan and FibroTouch, the lowest clinically acceptable sensitivity and specificity is 0.55

and 0.8 respectively, when α=0.05 and power=80%, 423 cases are required based on sensitivity; total 529 cases are required based on the determination effectiveness=80%; 328 cases are required based on specificity; total 410 cases are required based on the determination effectiveness=80%. By considering the center assignment, ≥600 qualified cases are included to guarantee 500 final statistical cases; and ≥100 cases are required for S0+S1, S2, S3, S4 and liver cirrhosis (compensatory stage) respectively. For each stage, the case is assigned as equally as possible.

VI. Study Time

One year (from May 2014 to May 2015).

VII. Case Assignment System (via central registration system)

Subject No. is assigned through IWRS system. After the investigator logs in the system and fills in the pathological stages, this system will, according to the completed contents, automatically assign the sole subject No. for this subject.

VIII. Statistical Analysis

Peking University Clinical Research Institute

IX. Study Design

(I) Inclusion Criteria

1. Age: 18－65 years, both gender.
2. History of HBV or HBsAg positive > 6 months up to now.
3. With qualified liver biopsy within three months before or after Fibrotouch examination for pathological staging.
4. Without chemical therapy history of powerful medicine to lower enzyme in the two weeks before blood biochemistry tests (e.g. dimethyl diphenyl bicarboxylate and bicyclol).
5. Participant agreed and signed the informed consent form.

(II) Exclusion Criteria

1. The people who are unable or unwilling to sign informed consent form.

2. The people who have merger of hepatitis c, alcohol and non-alcoholic fatty liver disease, autoimmune liver disease, inherited metabolic liver disease, biliary systemic disease or liver and gall parasitic diseases.
3. The people who have other serious chronic disorders or history of malignancy.
4. The people with ALT ≥5 ULN in the past 1 month.
5. The people with WBC＜3.5×10^9/L, PLT＜60×10^9/L, PTA＜60%.
6. The people with DBIL≥1.5 ULN.
7. The people with decompensated cirrhosis (especially the people with ascites).
8. Pregnant or lactating women, or women who has a pregnant plan and don't want to birth control in the study period.
9. The people who have wound on the right upper abdomen recently.
10. The people who have various space-occupying tumor or cyst in right liver.
11. The people who have none or limited legal capacity.

(III) Study method

1. Outpatients and inpatients with chronic hepatitis B and compensatory liver cirrhosis, conforming to the inclusion criteria and signing the ICF.

2. Collection of materials and specimen:

◆ Collection of clinical materials: Fill in the uniformly-printed CRF and keep or duplicate the original medical record.

(1) Name, sex, age, illness course, family history of infectious diseases and hereditary diseases, drinking history, pharmacotherapy history, and history of chronic diseases (e.g. essential hypertension, heart disease and diabetes).

(2) Physical examination: Height (cm), weight (kg), body mass index (BMI=weight/height^2), heart rate (bpm), respiratory rate (times/min), blood pressure (mmHg).

◆ Retaining of hematological specimen: Within one week before the FibroTouch examination, the venous blood is drawn at an empty stomach, and the different portions of

blood are kept: 2ml whole blood (freeze in two 2ml tubes); 8ml completely-separated serum (freeze in four 2ml tubes); and 3ml plasma (freeze in two 2ml tubes). Keep the tube at -80℃, and uniformly send it to the central laboratory.

◆ Retaining of liver puncture specimen: Fix with 10% neutral formalin, retain additionally 6 white films at every unit, and send it to the pathological examination center (the quality control is shown in Appendix 1).

3. Hematological examination: (the quality control is shown in Appendix 1)

(1) Routine blood tests;

(2) PT, PTA, INR;

(3) TP, ALB, AST, ALT, CHE, GGT, ALP, TBA, TBIL, DBIL, GLU, TC, TG, HDL, LDL, BUN, Cr;

(4) AFP;

(5) Serum virological indices: hepatitis B test (five-item), HBV-DNA assay, HCV antibody test, HIV antibody test; HDV antigen test.

4. Ultrasonic examination:

Within three months after the liver biopsy, the ultrasonic examination is made at an empty stomach for ≥8h. The inner diameter of liver, spleen, gallbladder, portal vein and splenoportal vein is measured through the ordinary two-dimensional ultrasonic examination. The echo data of liver surface/edge/parenchyma and gallbladder wall is scored to evaluate the severity of fibrosis. At every center, the operation should be made via the fixed machine by the fixed person, so as to prevent the artificial error; and the ultrasonic examination, the elasticity determination of liver tissues and the collection of medical history should be made in the same day.

Semi-quantitative Scoring Criteria at Liver Ultrasonic Examination

Score Item	**1 score**	**2 scores**	**3 scores**
Liver surface (Ls)	Smooth, flat	Unsmooth, fine water ripple	Wave, saw-shaped or discontinuous
Liver parenchyma (Lp)	Fine spot, uniform distribution	Slightly enhanced, coarse particle of non-uniform	Obviously echo enhanced, particulate or nodular echo group

		distribution	
Gallbladder wall (G)	Smooth	Coarse, slightly thickened	Obviously thickened or two-side sign

5. Liver Stiffness Measurement (LSM)

Within 3 months before and after the liver biopsy, the liver stiffness measurement (LSM) values are determined via FibroTouch.

◆ Investigators will operate the FibroTouch system to measure the patients' values of liver stiffness and fat attenuation parameters according to the SOP of FibroTouch.

<1>. Subjects lie in face-up position, and stretch his or her right arm to the maximum extent or cross behind his or her head. Enter the name, gender, birth date, medical card No., height, weight and other information into patient information log in FibroTouch system.

<2>. In order to determine accurately, image-guided positioning with ultrasonic B probe should be performed prior to the scanning with liver fibrosis probe. Apply ultrasonic coupling agent to the patient's skin at the position of right side, interspace between the Seventh and eighth rib along the midaxillary line, place the ultrasonic B probe perpendicularly on the skin, and then turn on the FibroTouch image-guided scanning model. Investigator can choose the proper position for liver examination with reference to the ultrasonic B image on the video display screen.

The testing position (at the center line of ultrasonic B image) is selected as following roles:

1. Be away from the heterogeneous liver tissues, such as great vessels, cysts and nodes.
2. Be away from the edge of liver.
3. The position where liver tissue are homogeneous, well-proportioned and with thickness no less than 7cm.

<3>. Save the ultrasonic B image, hang off ultrasonic B probe from the selected position, and change to liver fibrosis scanning model. Hold the fibrosis scanning probe in right hand, bend forefinger to keep probe perpendicularly at the selected position on the skin, lie right elbow on the bed (to avoid the right arm from shaking and moving), and left hand hold the tail of the probe to control the direction. Press the probe slightly on patients' skin to make the pressure indicating strip turn green.

<4>. With subject holding his or her breath slightly, operator observes the liver tissue displayed by ultrasonic-M signal. When the tissue is dark, more homogeneous and laminar distribution, the operators can press down the foot switch, and the fibrosis scanning probe sent out low-frequency shear waves for diagnosing and ultrasonic waves for monitoring, the probe is activated and complete once examination of LSM.

<5>. Ten times of successful detection are performed at the same position. When the inter

quartile range (IQR) value is qualified, the median value of these detections will be considered as the examination result of LSM.

<6>. Save and print the test result.

◆ The operator of FibroTouch must be trained by Hisky and obtain an operating license or certificate.

◆ A successful and effective test by FibroTouch must meet the following requirements: ultrasonic-A signal does not have intensive and rapid shaking, and does not fluctuate significantly in different depth; ultrasonic-M signal does not have big difference of lightness and displays a laminar distribution pattern in different depth; ultrasonic-E signal presents a routine white-and-black twill diagram.

◆ Each subject will obtain 10 successful acquisitions and the median value of these acquisitions will be considered as the examination result of LSM and FAP. The IQR-to-median ratio of the 10 acquisitions should be ≤0.3 (the smaller the IQR value, the reliable the result is). If the result is not reliable, it should be detected again until the result is reliable.

◆ To avoid artificial errors, the instrument needs to be operated by a fixed professional in each site, and all information will be documented in a uniform model and stored in U-disk, keep by a designated personnel.

6. FibroScan Examination

6.1 At the same site as that for FibroTouch, the stiffness of liver is determined via Fibroscan at the same day.

6.2 Operation procedure

(1) Keep in a supine position, lift up both hands to expose the intercostal gap in the area of right liver lobe of chest, and take a deep breath to make the intercostal gap as large as possible at the time of determination.

(2) Vertically stick the probe smeared with coupling agent to the intercostal gap.

(3) Determination site: Select the intercostal gap at the anterior or middle axillary line between the Rib 8~10 for routine determination, or adjust the determination site until the optimal one according to the real-time audiovisual diagram if the determination fails at the routine site.

(4) Observe the audiovisual diagram displayed in the instrument, and press the emission button for determination when there is a laminar liver image.

(5) Determine effectively for 10 times, and regard the mean of these 10 determinations as the determination result.

7. Liver biopsy

Within 3 months before the FibroTouch examination, puncture the 16G liver biopsy needle into 1.5~2.0cm of liver tissues (≥8 complete portal areas), fix with 10% neutral formalin, embed with paraffin wax, make the continuous section for HE, Masson and reticular fiber staining (make the HE staining at every study unit, and send another 6 white films to the central laboratory). Then, three pathologists interpret the films independently according to the standard and modified Chevallier semi-quantitative scoring method in the "Protocol for Prevention and Treatment of Viral Hepatitis (2001)". The interpretation result of these three pathologists should be verified as well consistent through the Kappa test, and otherwise the specimen of inconsistent interpretation result is sent for central interpretation and evaluation.

(1) The liver fibrosis is staged and scored through the following protocol:

① National Protocol for Prevention and Treatment of Viral Hepatitis (2000): staging of liver fibrosis

② Chevallier System (SSS scoring)

③ Metavir System

④ Ishak System

(2) Evaluation protocol for inflammatory activity level and histological activity index (HAI):

① National Protocol for Prevention and Treatment of Viral Hepatitis (2000): grading of inflammation severity

② Ishak Modified HAI: ____/18 (maximum score)

③ METAVIR System: histological activity score

(IV) Safety Evaluation

1. Clinical Safety Evaluation

The clinical safety is evaluated through the adverse events reported by the patients or observed by the doctors.

2. AE

Definition: Any adverse medical event reported by the patients or observed by the doctors from the signing of ICF and recruitment into study to the final follow-up, either at a causality correlation with the study device or not.

(1) AE record

During the study, the occurrence time of AE, its correlation with study device, its severity, duration, countermeasures and prognosis should be recorded strictly by the facts. The AEs should be recorded into the assigned CRF of adverse events.

(2) Judgment criteria for AE severity

◆ While filling in the CRF, the investigators will describe the intensity of AE in the following grading criteria:

Mild: No influence on the normal function of subjects;

Moderate: A certain influence on the normal function of subjects;

Serious: Obvious influence on the normal function of subjects.

(3) Judgment criteria for correlation between AE and study device

The investigators should evaluate the possible correlation between AE and study device in the following principles:

① Whether there is a rational precedence relation between the starting time of FibroTouch examination and the occurrence time of AE;

② Whether the suspicious AE conforms to the known type of AE for this device;

③ Whether the suspicious AE can be interpreted by the clinical state of patients or the influence of other therapies;

④ Whether the AE disappears or is relieved after the suspension of examination.

According to the above principles, the correlation is classified into 5 grades: definitely relevant, probably relevant, possibly relevant, possibly irrelevant and irrelevant.

(4) SAE:

◆ Judgment of SAE: Under one or more following conditions, the AE is regarded as a SAE: ① Death; ② Life-jeopardizing (e.g. risk of immediate death); ③ Induced or prolonged hospitalization; ④ Influenced working ability; ⑤ Permanent or serious

disability; ⑥ Congenital malformation or defect. If a certain medical event of no death, life-jeopardization or hospitalization may harm the patient or require the medicines or surgery to prevent the occurrence of above conditions in the viewpoint of study doctor, such event should also be regarded as SAE.

◆ Treatment of SAE: If the AE is irrelevant to study device and does not seriously influence the patient and the patient can be followed up as scheduled, the study should be continued. Under the other conditions, the patient should drop out.

◆ Recording and reporting of SAE: Any SAE during this study should immediately be reported to the supervisor, sponsor and national medicine clinical study institution (i.e. leading unit), which should report to the Ethic Committee and then within 24h to the CFDA and health administrative departure Meanwhile, the investigators must fill in the “Reporting Form of Serious Adverse Events”, and record the occurrence time, severity, duration, countermeasures and prognosis of SAE. If a patient suddenly dies during the study, such event should be reported as SAE, and the autopsy report should be enclosed if any.

(V) Quality Control and Quality Assurance of Clinical Study

◆ The participants must pass the uniform training on the uniform recording method and judgment criteria.

◆ The investigators must work strictly according to this protocol, fill in all study materials in an accurate timely complete neat way, so as to ensure the true reliable contents of report.

◆ Any observation result and finding during this study should be verified so as to ensure the reliability of data and guarantee that every conclusion of clinical study stems from raw data. At the stage of clinical study and data processing, there should be corresponding measures for data management.

◆ During this study, the clinical supervisor will regularly or irregularly make the field supervisory visit to the study center. According to the relevant regulations, the supervisor has the right to check the original record and remind/urge the investigators to carefully normatively complete the study work; and the investigators are liable to coordinate with such work.

(VI) Ethical Requirements and Informed Consent

◆No subject can be enrolled in the study before he or she express his/her willingness to participate in this study in written statement. To make this decision, subjects should be informed of content of this study and the potential risks and benefits involved.

The investigator must inform this information in both spoken and written form. Informed consent should be obtained from the participants (or legally authorized representative or Witness, if applicable) prior to any other study assessment. A copy of the signed Informed consent form (ICF) will be given to patients.

After signed ICF, the subject's investigational data can be collected. The information in ICF involved in clinical study may be released and monitored by the Sponsor, device regulatory authorities, designated auditors and monitors according to the confidentiality statement.

◆ The following information should be told to the patient: objective and procedure of study; benefits and possible side effects for participation into the clinical study; the privacy of each patient should be protected; and at any study-related injury during this study, the investigators will give the active treatment and the sponsor will make the proper compensation.

◆ Before the initiation of this study, the permission of Ethic Committee should be obtained, and the written certificate for such permission should also be obtained. According to the guideline of GCP, this protocol can be modified only after the permission of Ethic Committee. If such modification may endanger the patient, the contents of ICF should be updated, and the signed consent should be obtained from the participating patients. This study should observe the Helsinki's Declaration and national specifications/regulations on clinical study, and can be made only after the permission of relevant institutions.

(VII) Statistical Analysis of Data

After the determination of study protocol, the statisticians should discuss with the principal investigator on preparing the statistical analytical plan. The data is analyzed through the statistical software SAS 9.3 (authorization No.: 11202165). The sample size is calculated through the software PASS 11. The contents of statistical analysis are classified into the

following parts:

1. Basic description: Age, sex, medical history and relevant indices of laboratory examination (e.g. ALT and AST). For the quantitative indices, the mean, standard deviation, median, minimum value, maximum value, lower quartile (Q1) and upper quartile (Q3) will be described; and for the classification indices, the case amount and percentage in each category will be described.

2. Evaluation of diagnostic ability via elasticity determination result

◆ By grouping according to the grading result via golden standard, the inter-group comparison is made on the FibroTouch determination results through the variance analysis or Wilcoxon rank-sum test, the correlation between two groups is tested through the Spearman correlation analysis, and the correlation coefficient (r) and 95% CI are calculated for two groups.

◆ By regarding the different grading results of golden standard as the judgment critical point, the curve of receiver operating characteristic (ROC) for elasticity value is constructed. The subjects are grouped by regarding the different determination results of elasticity value as the judgment critical point, the sensitivity/specificity is calculated according to the grading result of golden standard, the ROC curve is plotted through the X-axis of specificity, the Y-axis of sensitivity and the calculated result, and the area under curve and its 95% confidence interval are calculated. The critical value corresponding to whichever is greater for the result of sensitivity and specificity is regarded as the judgment critical point for FibroTouch determination value, and then the corresponding sensitivity, specificity, positive predicted value and negative predicted value are calculated.

Comparison of secondary objective: by statistically assuming the success rate of FibroTouch is non-inferior to that of Fibroscan, the critical value of non-inferiority is 10%; and by statistically assuming the determination result of FibroTouch is equivalent to that of Fibroscan, the critical value of equivalence is 1.5KPa.

In the above analysis, $P<0.05$ indicates a significant difference.

(VIII) Data Management and Data Traceability

8.1 Data center

The Data Management Dept of Peking University Clinical Research Institute (PUCRI-DM) undertakes the work of project data management.

8.2 CRF design and electronic database construction

According to the CRF template of PUCRI, the investigators draft the study CRF, which is sent to PUCRI-DM for revising the data management draft. After the confirmation of investigators and the signing of investigators, PUCRI-DM and statisticians, the final version is prepared into CRF 1.0.

Based on CRF 1.0, PUCRI-DM constructs the electronic data collection system (Real-data RDDM v1.0), which supports the electronic signature, authority control and trace management.

8.3 Data management plan and data verification plan

In this study, PUCRI-DM drafts the “Data Management Plan” and “Data Verification Plan”.

The Data Management Plan describes the detail/schedule of data management work and the duty division of each party concerned for the whole course of this project. After the finalization of study protocol by the PUCRI-DM, investigators and supervisors, this plan is finalized by signing before the project initiation.

The Data Verification Plan describes the detail of project data verification. It is drafted by the PUCRI-DM after the finalization of study protocol and study CRF, and reviewed by the investigators. In this plan, the point and method of data verification are determined one by one according to the study protocol and the specific contents of CRF.

Based on the Data Verification Plan, the data verification is constructed through the electronic data collection system.

8.4 Input and modification of data

The investigator is responsible to input and modify the data, and answer the data question in field. Through the Real-data RDDM v1.0, the investigator inputs the electronic data of personal system account, and answers via the system the data questions raised by the data administrator or supervisors. The input data and question answer of investigators are traceable and kept in the system.

8.5 Data Monitoring

Through the account of project supervisor, the supervisors clinically supervise the study data through the following procedures: check the complete and correct filling of electronic data through the EDC system; compare the electronic data with materials in original medical history; and query the investigators on the data point of inconsistency (if any) which is submitted for modification or confirmation. All data questions and checking records are traceable and kept in the system.

8.6 Data Verification And Data Management

Through the account of administrator, the data administrator checks the completeness and logic question of input data. At any doubt on data, the data question is raised to the investigators.

8.7 Database Locking and Data Review

After the inputting of data of all subjects, the investigators coordinate in clearing all residual data questions, the supervisors completes the checking of original medical history, then some authorities are closed (i.e. data input/modification of investigators and data query of supervisors and data administrator), and the study data is frozen so as to guarantee the stability of study data.

After the freezing of database, the “Data Management Report” is drafted by the PUCRI-DM and submitted to the data review meeting, and then the possible data in this report is solved at the simultaneous presence of investigators, statisticians and PUCRI-DM, and the statistical population is divided.

If the frozen database is confirmed as no data question by the investigators, the database is locked and unblended after the division of statistical population is confirmed.

X. Anticipated Schedule of Clinical Study

Conference on the FibroTouch clinical protocol: December 2013 ~ January 2014.

Training on the operation of FibroTouch ultrasonic diagnostic device: February ~ March 2014

Clinical study on FibroTouch ultrasonic diagnostic device: May 2014 ~ May 2015

Appendix 1: Quality Control of Central Laboratory

I. Hematological Determination

1. Collection of specimen

2. Determination of specimen

Each laboratory should complete the hematological, coagulation and clinical chemical examinations within 2h, and complete the virological examination within 2 weeks.

(1) Hematological examination (whole blood, 18-item)

(2) Coagulation examination (PT, PTA and INR)

(3) Clinical chemical examination (TP, ALB, TBIL, DBIL, AST, ALT, TBA, ALP, GGT, CHE, GLU, TC, TG, HDL, LDL, BUN, CR and AFP).

For the above examination items, the fully-automatic blood analyzer and matching reagents of Beckman Culter and Sysmex Co. are recommended. The requirements of quality control are shown in the quality control of Clinical Examination Center, the Ministry of Health.

(4) Serum virological examination (HBV-DNA, hepatitis B test (5-item), HCV antibody test, HDV antigen test, HDV antibody test and HIV antibody test).

The hepatitis B test (5-item) is made through the fully-automatic chemiluminescent device and its matching reagents (e.g. Roche and Abbort Co.); the HBV-DNA assay is made through the real-time fluorimetric reagents with the registration certificate of China Food and Drug Administration and the quality control qualification certificate of Clinical Examination Center, the Ministry of Health and through the fully-automatic real-time fluorometric PCR analyzer with the registration certificate (e.g. Roche and AB Co.); the hepatitis C antibody test is made through the fully-automatic chemiluminescent device and its matching reagents (e.g. Ortho Johnson ECI and Abbort); and the hepatitis D antibody test and HIV antibody test are made primarily through the imported reagents and secondarily through the China-made ones according to the conditions of each unit. The requirements of quality control are shown in the quality control of Clinical Examination Center, the Ministry of Health.

2. Treatment, storage and transportation of specimen

Treatment: The collected specimen should be immediately sent to the laboratory of each

unit, and treated in time by special person after the receipt of specimen, including: specimen No. and centrifuged or separated serum.

Storage: Before the test operation, the serum should be retained at each laboratory, i.e. fill 2ml serum in the air-tight tube, and then keep at -80℃ ready for random inspection. The specimen sent to the central laboratory for uniform examination (relevant indices for liver fibrosis) should be numbered via the uniformly-colored symbol by the special person, and the different portions of blood are kept: 2ml whole blood (freeze in two 2ml tubes); 8ml completely-separated serum (freeze in four 2ml tubes); and 3ml plasma (freeze in two 2ml tubes). Keep the tube at -80℃, and record properly (basic information of patients).

Transportation: The specimen sent to the central laboratory for uniform examination should be sent as centrally as possible (monthly) at the frozen and sealed state (i.e. keep in the freezing tube, and transport via the dry-ice storage box) by avoiding the repeated freezing/melting and the specimen sprinkling/leakage.

II. Liver Biopsy

1. Selection criteria for liver puncture specimen

The specimen of liver tissues should be punctured generally at more than 2.0~2.5cm (including >8 intact duct convergence areas) and at least 1cm.

2. Treatment specifications or procedures for liver puncture specimen

After the fixation, dehydration, transparency, paraffin soaking/embedding and section, the specimen of liver puncture tissues undergo the routine HE staining, Gordon-Sweet reticular staining and Massson staining.

Specifications and procedures:

Rapidly put the puncture liver tissues into the specimen bottle full of 10% neutral formalin buffer solution, fix at the ordinary temperature for 3h, and mark the name abbreviation, department and case No. of patients on the specimen bottle.

Notes: Do not compress the specimen while clamping the specimen with the nippers so as to prevent the mechanical specimen damage. Do not adopt the toothed nippers. Collect the tissue specimen at an intact state as far as possible.

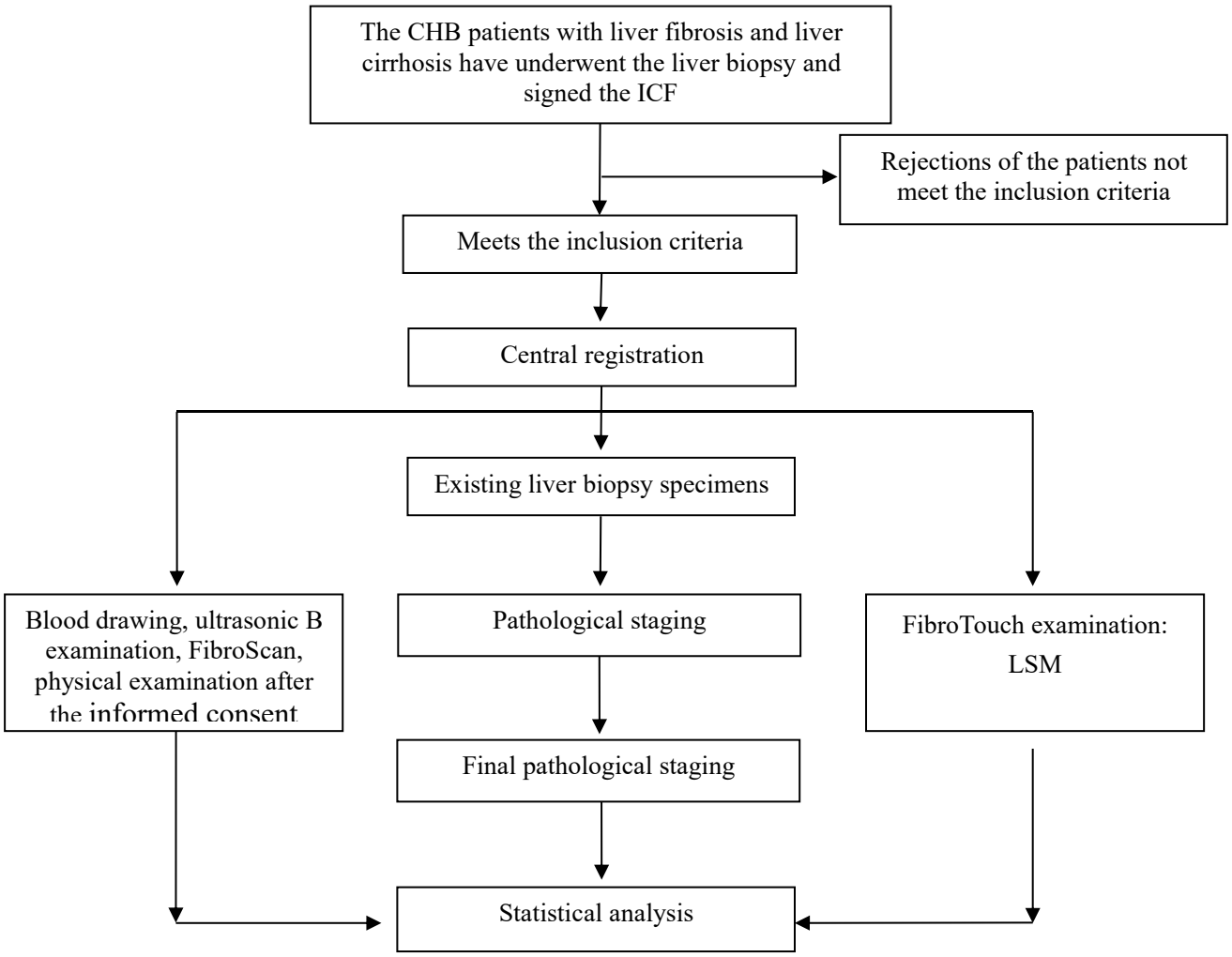
3. Histological evaluation criteria

(1) Pathological diagnostic criteria for chronic hepatitis specified in the “Protocol of Prevention and Treatment of Viral Hepatitis (2001)” jointly revised by the Society of Infectious and Parasitic Diseases and the Society of Liver Diseases under the Chinese Medical Association in September 2000.

(2) Scoring of inflammatory activity level of chronic hepatitis and severity of liver fibrosis through the international scoring criteria for chronic hepatitis (Ishak).

(3) Final histological evaluation by 3 experienced pathological experts.

Appendix 2: Flow Chart of Study Design



Appendix 3: Knodell System: a scoring system for liver histological activity index (HAI)

Necrosis around the convergence duct	Score	Degeneration and focal necrosis in the liver lobule	Score	Inflammation in the duct convergence area	Score	Liver fibrosis	Score
No	0	No	0	No	0	No	0
Mild flaky necrosis	1	Mild (scattered necrosis foci of liver cells in the eosinophilic corpuscle, ballooning degeneration and/or 1/3 of nodes)	1	Mild (inflammatory cells in 1/3 of duct convergence area)	1	Fibrous expansion of duct convergence area	1
Moderate flaky necrosis (involving <50% of area around the duct convergence)	3	Moderate (involving Involve 1/3~2/3 of liver lobules or nodes)	3	Moderate (increase of inflammatory cells in 1/3~2/3 of duct convergence area)	3	Bridged fibrous connection (between the duct convergence areas or between the duct convergence area and central vein)	3
Obvious flaky necrosis (involving >50% of area around the duct convergence)	4	Obvious (involving >2/3 of liver lobules or nodes)	4	Obvious (increase of inflammatory cells in >2/3 of duct convergence area)	4	Liver cirrhosis	4
Moderate flaky necrosis + bridged necrosis	5						
Obvious flaky necrosis + bridged necrosis	6						
Multi-lobular necrosis	10						

Appendix 4: Evaluation Protocol for Staging and Scoring of Liver Fibrosis

Stage	Knodell	Ishak	Scheuer	METAVIR
0	**No fibrosis**	**No fibrosis**	**No fibrosis**	**No fibrosis**
1	Expansion of duct convergence area	Some PFs ± short fibrous gaps	Expansion of duct convergence area	PF, but no fibrous gap
2	Most PFs ± short fibrous gaps	PF, formation of fibrous gap	PF, a few gaps	PF, a few gap fibrosis
3	Bridged fibrosis **P-P/P-C**	Most PFs, occasional P-P	Fibrous gap with nodular structural disorder	Gap fibrosis
4	Liver cirrhosis	PF with obvious P-P and P-C	Possible or definite liver cirrhosis	Liver cirrhosis
5		Obvious P-P/P-C, occasional node		
6		Possible or definite liver cirrhosis		