Informed Consent Form & Statistical Analysis Plan

Project Title	**Alterations of Gut Microbiota and Metabolites in Asian Patients with Alcohol-associated Liver Disease**
Scheme number	**XH2021.12.30**
Version number	**V2.1**
Version date	**2022.5.25**
Principal Investigator	**Huikuan Chu**
Department	**Division of Gastroenterology** **Union Hospital, Tongji Medical College** **Huazhong University of Science and Technology**

Dear Madam/Sir:

You will be invited to participate in "**Alterations of Gut Microbiota and Metabolites in Asian Patients with Alcohol-associated Liver Disease**", the research is initiated by Division of gastroenterology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. The following items describe the research background, purpose, methods, benefits and possible risks or inconveniences as well as your rights and interests during the research process of this clinical study. Please read it carefully before participating in the clinical study. The information provided to you in this informed consent form can help you decide whether to participate in this clinical study. If you have any questions, please ask the investigator in charge of the study to ensure that you fully understand the relevant content. Your participation in this study is voluntary. If you agree to participate in this clinical study, please sign the statement of informed consent.

This study has been approved by the Ethics Committee of the Medical Ethics Committee of the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology.

1. Research Background

Alcohol-associated liver disease (ALD) is a common disease caused by alcohol use disorder (AUD), ranging from asymptomatic liver steatosis to alcohol-associated hepatitis (AH), alcoholic cirrhosis and potentially, hepatocellular carcinoma (HCC). ALD is the most common reason for liver transplantation in the United States. Globally, about 2 million people die from liver disease each year and up to 50% of the death with cirrhosis can be attributed to alcohol consumption. In Europe, it has been estimated that 60%-80% of liver-related deaths can be attributed to alcohol consumption. Currently, the pathogenetic mechanisms have not been fully elucidated, but they might be related to oxidative stress, acetaldehyde-induced toxicity, cytokine and chemokine-induced inflammation. There is no effective therapeutic method for ALD till now except for liver transplantation. Recent studies have reported that gut microbiota has an intimate relationship with ALD, which provides broader insights and opportunities for understanding and treating this disease.

2. Research purpose

In this study, we aim to map the alterations of gut microbiota and metabolites in patients with different levels of ALD, and to investigate the effects and mechanisms of key strains and their metabolites on the development of ALD, providing a theoretical basis and potential targets for its treatment.

3. Who can participate in the study

Inclusion criteria:

1. The group of ALD:

1) aged >18 years;

2) patients who meet the diagnostic criteria of ALD in Chinese Guideline for the Prevention and Management of Alcoholic Liver Disease (2018 Update);

3) history of chronic heavy alcohol consumption;

4) with relatively complete clinical data and good compliance.

2. The group of purely drinking:

1) aged >18 years;

2) history of chronic alcohol consumption;

3) no evidence of fatty liver, hepatitis or liver injury.

3. The group of healthy control:

1) aged >18 years;

2) without history of alcohol consumption;

3) no evidence of fatty liver, hepatitis or liver injury.

Exclusion criteria:

1) with hepatocelluar carcinoma (HCC) or hepatic metastases;
2) combined with infectious liver diseases, such as hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus and human immunodeficiency virus (HIV);
3) combined with non-infectious liver diseases, such as non-alcoholic fatty liver disease, drug-induced hepatitis, autoimmune liver disease, IgG4-related liver disease, Wilson's disease, alpha 1-antitrypsin deficiency, Budd-Chiari syndrome, and other congenital liver diseases;
4) combined with severe organic lesions of other organs;
5) pregnant and lactating women.

Location of specimen collection

Subjects with ALD will be recruited at the inpatient department of gastroenterology, while subjects in purely drinking group and healthy control group will be recruited at the outpatient department and physical examination center. The above locations are all in Union hospital, Tongji Medical College, Huazhong University of Science and Technology. The Department of Gastroenterology of Yichang Central People's Hospital, and the Department of Gastroenterology of Songzi People's Hospital will assist in recruiting subjects and collecting samples locally.

4. Research Introduction

This is a prospective cohort study enrolling about 200 subjects, who meet the inclusion criteria. They will be divided into alcohol-associated fatty liver group (group A1, about 40 patients), alcohol-associated hepatitis group (group A2, about 40 patients), alcohol-associated cirrhosis group (group A3, about 40 patients), purely drinking group (group B, about 40 patients), and healthy control group (group C, about 40 patients) according to their baseline characteristics.

This study will have no adverse effects on your health. Investigators will analyze your blood and stool specimens, combined with your clinical data at baseline, such as blood biochemistry, ultrasound of liver, gallbladder, pancreas and spleen, and ascites, FibroTouch, etc. Blood specimens are collected in a small tube during the normal course of the procedure without additional burden to you. Stool specimens are collected at your convenience, which are collected through our prepared stool collection tubes.

5. Statistical Analysis Plan:

All statistical analyses were performed using SPSS 26.0 and R statistical language. Normally distributed continuous variables were reported as mean±standard deviation (SD) and were analyzed by student t test. Abnormally distributed continuous variables were expressed with median and interquartile range (IQR), and were compared by non-parametric tests. The categorical variables were demonstrated with proportion (%) and were analyzed by Chi-squared test or Fisher’s exact test.

6. Obligations of Subjects

Follow general diagnostic and treatment procedures in order to get a true picture of your physical health. Once enrolled in this study, you need sign the informed consent form.

7. Detection, preservation and destruction of biological samples

This study intends to collect whole blood samples and stool samples for gene sequencing, which will be stored in the Department of Gastroenterology, Union Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology, until the end of the study. Once investigators finish the study, those samples will be packed in special containers or bags according to the Regulations on Medical Waste Management , and then be sent to the designated disinfection site by a special staff, and finally be incinerated by a special organization.

8. Possible risks of participating in this study

Blood specimens are collected during the normal course of the procedure without additional burden to you. Skin damage and redness may occur during the blood collection process,

9. Possible benefits of participating in this study

Because of the complexity and long duration of this study, it is highly unlikely that the results of the blood and stool

tests will be available to you, but the results of the study will help improve treatment strategies for you and those of patients in the same category.

10. Can I be compensated for participating in clinical trials?

The examination items are all necessary for the diagnoses and treatment, so there is no additional compensation.

11. How much do I need to pay?

You will not be charged in any way for participating in this study.

12. How will new clinical research information be handled?

When there is new information that may affect the subject's continued participation in the trial, the procedures for informing the subjects or their guardians in a clear and timely manner.

13. Under what circumstances may the clinical study be terminated?

You can discontinue the study if you do not wish or feel uncomfortable with collecting blood and stool specimens.

14. How long is participation in this study likely to last?

About 1 to 2 days. Sample collection will follow the physical examination or normal medical process and will not take up additional time for you.

15. How many people will be involved in this study?

About 200 patients will be collected in this study.

16. Privacy and Confidentiality

If you decide to participate in this study, your participation in the trial and your personal data in the trial will be kept confidential. For you, all information will be kept confidential. Information that could identify you will not be disclosed to members outside the research team unless you have given your permission. All study members and study sponsors are required to keep your identity confidential. Your information is only stored in the Department of Gastroenterology of Union Hospital and is only accessible to researchers. To ensure that research is conducted in accordance with regulations, when necessary, and without violating the principle of confidentiality and relevant regulations, supervisors, auditors, ethics committees and inspectors of drug regulatory authorities can access your original medical records to verify clinical trials, process and data. When research results are published, your personal data will also be kept confidential.

17. The right to voluntarily choose to participate in and withdraw from research

You may choose not to participate in this study, or withdraw from the study at any time after notifying the investigator without discrimination or retaliation, and any of your medical treatment and rights will not be affected. The investigator may terminate your continued participation in this study if you require additional diagnosis/treatment, or if you do not comply with the study plan, or for any other reasonable reason.

18. How to get help in research?

When there are questions about trial information, research progress and rights of subjects, as well as any discomfort and damage related to the trial, you can directly contact researchers or contact the ethics committee of our center.

Subject Statement

I have read this informed consent form carefully. I have opportunity to ask questions and all questions have been answered. I understand that participation in this study is voluntary, and I can choose not to participate in this study, or withdraw at any time after notifying the investigator without discrimination or retaliation, and my medical treatment and rights will not be affected by this.

The investigator may terminate my continued participation in this clinical study if I require additional diagnosis/treatment, or if I do not comply with the study plan, or for other reasonable reasons.

I voluntarily agree to participate in this clinical study and I will receive a signed copy of the Informed Consent Form.

Subject's Name (Print):	Contact Number:
Subject Signature:	Date:

If the subject cannot sign informed consent due to incapacity or other reasons, or if the subject is a minor, the guardian shall sign it.

Guardian Name (Print):	Contact Number:
Guardian's Signature:	Date:
Relationship with the subject:	
Reasons why subjects cannot sign informed consent:	

Signed by an impartial witness when the subject or his guardian is incapable of reading.

Name of impartial witness (print):	Contact number:
Signature of an impartial witness:	Date:

Investigator Statement

I have accurately informed the subject of the informed consent form and answered the subject's questions, and the subject voluntarily participated in this clinical study.

Investigator's name (print): Huikuan Chu	Contact number: +86 13554105386
Investigator Signature:	Date: