Informed Consent Form & Statistical Analysis Plan

Project Title	**Clinical study on the alterations of gut microbiota and serum biochemical markers in Asian patients with drug-induced liver injury**
Scheme number	**XHGA2022.5.17**
Version number	**V2.0**
Version date	**2022.6.29**
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 the "**Clinical study on the alterations of gut microbiota and serum biochemical markers in Asian patients with drug-induced liver injury** ", the research is initiated by the 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

Drug-induced liver injury(DILI) refers to the liver injury induced by all kinds of prescription drugs or over-the-counter drugs, biological agents, traditional Chinese medicine, and so on. Acute liver failure may occur in serious cases. China has a large population base and a wide variety of clinical drugs, and it is common for the population to use drugs irregularly. Therefore, the incidence of DILI is increasing year by year. The pathogenesis of DILI is complicated, and there are often multiple mechanisms successively or altogether. As a result of the same effect, it is particularly important to study the pathogenesis of DILI and find its therapeutic target. More and more evidence shows that DILI is related to the intestinal microbiota. It is reported that intestinal microbes can participate in drug metabolism, and intestinal microbial products may compete with drugs in the metabolic process, thus affecting the efficacy and toxicity of drugs. At present, our research group found that there is a close relationship between the disease severity of the DILI mouse model and intestinal microbiota, in addition, Escherichia coli infection can aggravate DILI. Therefore, our group intends to collect clinical test data, and serum and fecal samples of patients with DILI to study the changes in intestinal microbiota and the effect of Escherichia coli abundance on the prognosis of patients. At the same time, through the animal model, we further explored the mechanism of the influence of different strains of the gastrointestinal tract and their metabolites on the occurrence and development of DILI, to seek a theoretical basis and potential targets for DILI therapy.

2. Research purpose

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

3. Who can participate in the study

Inclusion criteria:

1. The group of DILI:

1) aged >18 years;

2) patients who meet the diagnostic criteria of DILI in Guidelines for Diagnosis and Treatment of Drug-induced Liver Injury;

3) history of taking hepatotoxic drugs;

4) with relatively complete clinical data and good compliance.

2. The group of healthy control:

1) aged >18 years;

2) no history of liver disease and other diseases.

Exclusion criteria:

1) with hepatocellular 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, alcoholic liver disease, 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 DILI will be recruited at the inpatient department of gastroenterology, while the 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.

4. Research Introduction

This is a prospective cohort study enrolling about 90 subjects, who meet the inclusion criteria. They will be divided into a drug-induced liver injury group (group A, about 60 patients), and a healthy control group (group B, about 30 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, spleen, 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 to 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, 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 90 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 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 the 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 the 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: