Clinical Study Protocol

Natural history of advanced chronic liver diseases and factors associated with hepatic events – a registry study

Short title: ACLD_REG study

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1. BACKGROUND

Chronic liver diseases (CLD) has burdened the global healthcare system throughout the years. Among all causes of CLD, chronic hepatitis B (CHB) is generally the commonest cause of CLD in the Asia-Pacific region but the prevalence is expected to decline due to effective antiviral treatment.(1) Similarly, with the introduction of direct-acting antiviral (DAA), chronic hepatitis C (CHC) is now readily curable. On the other hand, an increasing trend is observed in metabolic dysfunction-associated steatotic liver disease (MASLD) and alcohol-related liver disease (ARLD) which is likely to change the landscape of CLD both in the region and worldwide. (2, 3)

Advanced chronic liver disease (ACLD) or cirrhosis is a final common pathway of all CLD and was the 9th and 15th leading cause of death in Southeast Asia (0.42 million deaths) in 2019 as reported by the World Health Organization (WHO) Global Health Estimates.(4) It also significantly increases the risk of hepatocellular carcinoma (HCC). With its significant impact on morbidity and mortality, the prognosis of compensated and decompensated states differ drastically and the field is pushing forward ways to prevent hepatic decompensation in order to improve liver-related outcomes. Non-selective beta-blockers (NSBB) has been shown to reduce risk of hepatic decompensation in patients with compensated advanced chronic liver disease (cACLD) and concomitant clinically significant portal hypertension (CSPH).(5) Some other drugs including angiotensin converting enzyme inhibitor (ACEI)/angiotensin-receptor blocker (ARB), statin *etc*.(6, 7) have been shown in retrospective studies to reduce risk of hepatic decompensation but more evidence is required to draw conclusive interpretation.

Apart from medications, non-invasive tests (NIT), including liver stiffness measurement (LSM) and spleen stiffness measurement (SSM) from vibration-controlled transient elastography

(VCTE) help prognosticate chronic liver diseases.(8) Yet more validation is required on certain conditions such as in patients with obesity as well as HCC. Biomarkers are also under the spotlights for risk prediction but are yet to reach the stage for widespread clinical practice. Hence these areas deserve further studies.

Hong Kong is an area endemic for chronic hepatitis B as well as expected for an increase with MASLD and ARLD. There has not been an established registry to capture these ACLD patients for systematic monitoring and analysis. Thus, a registry for ACLD is imperative.

2. STUDY OBJECTIVES

2.1 To study the secular trend and natural history of ACLD in our locality over the past decades

2.2 To identify factors associated with hepatic events, including liver-related death

2.3 To validate and explore NIT and biomarkers in risk prediction and prognostication of ACLD

3. STUDY DESIGN OVERVIEW

This is a retrospective-prospective registry cohort study. Patients with ACLD will be prospectively invited to this study. The study follow-up duration will be 10 years. The study consists of two parts.

Part 1: We will perform retrospective analysis of the secular trend of ACLD from the past decades and to explore factors associated with hepatic decompensation. The Clinical Data Analysis and Reporting System (CDARS) will be used to capture relevant information from 01 January 2000 until 30 June 2024. Further clinical information will be prospectively captured for 10 years from the CDRAS during the whole study period.

Part 2: We will prospectively recruit patients with ACLD to receive baseline VCTE for LSM and SSM, and have blood tests for storage and analysis of novel biomarkers in our own research laboratory. Yearly VCTE assessment to monitor the changes in LSM and SSM, as well as yearly blood tests to look into changes of Child-Pugh and MELD scores, as well as for storage and analysis of novel biomarkers will be performed until the end of the study. This part aims to establish baseline characteristics of the cohort and to establish the change in these parameters in correlation to clinical outcomes, as well as to explore any biomarkers associated with clinical outcomes.

4. PATIENTS

The territory-wide retrospective cohort will be identified through CDARS. The prospective cohort will comprise of consecutive patients with ACLD attending hepatology clinics or in-patient care in Prince of Wales Hospital in Hong Kong. All patients will be screened for the following eligibility criteria:

4.1 Inclusion criteria

- Aged 18 or above
- Known ACLD, defined by:
    - LSM > 10kPa or
    - Clinical cirrhosis, suggested by 1. ultrasonography of the hepatobiliary system shows features of cirrhosis (*e.g.* shrunken and nodular liver) and portal hypertension (*e.g.* dilated portal vein, portal-systemic collaterals or varices, splenomegaly, ascites). 2. Oesophagogastroduodenoscopy (OGD) shows presence of oesophageal varices (OV) and/or gastric varices (GV) and/or portal hypertensive gastropathy.

4.2 Exclusion criteria

- Current or past history of HCC

- History of liver transplantation
- Asplenism or history of splenectomy
- Serious medical illness with limited life expectancy of less than 6 months
- Pregnancy
- Unable to obtain or refusal of informed consent from patient

5. CLINIC VISITS AND ASSESSMENTS

5.1 Clinical assessments

The following procedures will be performed at baseline and at least yearly until year 10.

- Documentation of medical history and new symptoms
- Documentation of high-risk varices, hepatic events and their dates of occurrence (i.e., ascites, spontaneous bacterial peritonitis, variceal haemorrhage, hepatic encephalopathy, hepatorenal syndrome-acute kidney injury, HCC, liver transplantation)
- Dates and causes of death for patients who pass away between visits will be recorded
- Physical examination
- Anthropometric measurements including body weight, height, waist and hip circumferences, will be collected. Body mass index will be calculated as body weight (kg) divided by height (m) squared
- Body composition measurement using the MC780 machine (Tanita, Tokyo, Japan)
- Blood pressure and pulse rate measured after a 15-minute rest
- Blood tests will include complete blood count, prothrombin time, renal function test, liver blood test (including alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transpeptidase), and alpha-fetoprotein
- Store 10 mL of clotted blood and 10 mL of EDTA blood for exploration or novel biomarkers, future genetic and biochemical research

5.2 Vibration-controlled transient elastography (VCTE)

LSM and SSM assessments will be carried out using VCTE (FibroScan 630 Expert, Echosens, Paris, France). Operators with a minimum of 500 examinations completed and trained according to the manufacturer's guidelines will conduct the tests.(9) Examinations will be performed at baseline (unless there is a recent VCTE examination within 6 months available, with LSM and SSM measured), and then yearly until year 10. Ten measurements will be

recorded each for the liver and spleen, and their median value will be used to derive the corresponding stiffness. The machine's automated probe selection tool will determine the use of M and XL probes for LSM, while SSM will be measured by both the M or XL probes (50Hz@LSM) as well as the dedicated 100Hz probe.

LSM is considered reliable if there are ten or more valid measurements, and the interquartile range-to-median ratio of the measurements is ≤0.3. While no standardised reliability criteria exist for SSM, variability of SSM will be tracked in this study.

5.3 Follow-up

Patients in the prospective cohort will be followed up in hepatology clinics in Prince of Wales Hospital at least once per year. Other follow-up procedures will be considered as part of the standard of care and will be covered by local resources. These include but are unlimited to the treatment of chronic liver disease and co-morbid conditions as well as surveillance for HCC through periodic imaging and/or alpha-fetoprotein testing. At each visit, incident hepatic events will be documented.

6. DATA PROCESSING AND ANALYSIS

6.1 Analysis datasets

Part 1: This retrospective analysis will include data from CDARS to study on the secular trend and factors associated with hepatic decompensation.

Part 2: The analysis will include all patients who receive serial VCTE and blood tests per study timeline.

6.2 Clinical and laboratory parameters

Relevant clinical and laboratory parameters were captured via CDARS from 1 January, 2000 till latest available date. The required data for this study are listed below in detail.

- **Data requirements**
  - Demographics
  - Diagnosis
  - Procedures
  - Medications

- Laboratory tests & results
- VCTE results
- Invasive test & results

- **Detailed data information**
  - Demographic data
    - Age
    - Date of birth
    - Gender
    - Clinics follow up

  - Laboratory parameters
    - Complete blood picture
    - Clotting profile (prothrombin time, international normalized ratio)
    - Liver function test
    - Renal function test
    - Alpha-fetoprotein
    - Hepatitis B surface antigent (HBsAg), antibody to hepatitis B surface antigen (anti-HBs)
    - Hepatitis B e antigen (HBeAg), antibody to hepatitis B e antigen (anti-HBe)
    - Hepatitis B virus DNA (HBV DNA)
    - Antibody to hepatitis C virus (anti-HCV)
    - Hepatitis C virus (HCV RNA)

  - All clinical diagnosis codes and procedure codes

  - Medications
    - Antiviral agents to CHB (such as peginterferon, entecavir, tenofovir) and CHC (including peginterferon, direct-activing antivirals)
    - Anti-hypertensives, including beta-blockers, calcium channel blockers, nitrats, ACEI/ARB *etc.*
    - Medications for diabetes mellitus, including insulin, metformin, sodium/glucose cotransporter-2 (SGLT-2) inhibitor *etc.*

- Anti-platelet and anti-coagulant agents
- Non-steroidal anti-inflammatory agents (NSAID)
- Statin

- VCTE results
  - LSM and SSM values

- Invasive test & results
  - Liver biopsy results
  - OGD results, including documentation of OV and/or GV, and its corresponding endoscopic therapy

6.3 Primary endpoint

The primary endpoint of this study is the incident hepatic events and liver-related mortality, for both compensated or decompensated ACLD. Hepatic events is defined by the presence of ascites, variceal bleeding, overt hepatic encephalopathy. Ascites is defined by compatible clinical signs and confirmed with ultrasound or paracentesis. Intraperitoneal fluid detected only by ultrasound is not considered an endpoint. Variceal bleeding will be suspected if patients are presented with haematemesis or melaena or a decrease in the haemoglobin level of at least 2 g/dL. The diagnosis is made when OGD shows one of the following features: active bleeding from a varix, a “white nipple” overlying a varix, clots overlying a varix, or varices with no other potential source of bleeding. Overt hepatic encephalopathy is evidenced if there is symptom or sign compatible with West Haven criteria grade II or above.

6.4 Secondary endpoints

Secondary endpoints include:

- Development of each hepatic event
- Development of HCC
- Change in hepatic function in terms of Child-Pugh and model for end-stage liver disease (MELD) scores
- Change in LSM and SSM
- Change in blood parameters including novel biomarkers

6.5 Sample size estimation

From 01 January 2000 till 31 December 2020, **31,542 subjects** with cirrhosis were identified on CDARS, and will be recruited for subsequent analysis from this **retrospective cohort**. Since this is a registry study, consecutive patients shall be recruited. In 2020, around 1,000 patients with cirrhosis were identified in the whole territory. Given it is a single-centre registry with **prospective recruitment**, the target prospective enrolment would be **500 subjects**.

6.6 Data analysis

Continuous variables were expressed in mean ± standard deviation or median (interquartile range [IQR]), as appropriate, while categorical variables were presented as number (percentage). Qualitative and quantitative differences between subgroups were analysed by chi-square or Fisher's exact tests for categorical parameters and Student's t test or Mann-Whitney test for continuous parameters, as appropriate. Qualitative and quantitative differences between ordinal subgroups were analysed by chi-squared test for linear trend or Fisher's exact tests for categorical parameters and one-way ANOVA or Kruskal-Wallis test for continuous parameters, as appropriate. Patients were followed till the end of study or death, and censored upon the time of liver transplantation, whichever occurs earlier. Patients who withdraw consent will be censored assuming no endpoints developed after the last documented clinic visit. The primary and secondary endpoints will be analysed as time-to-event variables with hazard ratio and 95% confidence interval (CI) calculated. Event rates of endpoints will be compared with stratified log-rank test. Survival will be estimated by Kaplan-Meier method. Non-liver related mortality is considered a competing risk for the primary endpoint.

The accuracy of LSM and/or SSM models in predicting hepatic events will be evaluated using the area under the receiver-operating characteristic curve with corresponding 95% CI. The corresponding sensitivity, specificity, positive predictive value and negative predictive value will be calculated. Multivariable logistic regression model will be performed to identify independent factors associated with hepatic events and liver-related death. All statistical tests are two-sided with p-value <0.05 indicating statistical significance.

7. ETHICS

7.1 Ethics Review

The final study protocol, including the final version of the Informed Consent Form, must be approved or given a favourable opinion in writing by our Joint CUHK-NTEC CREC. The protocol must be re-approved by our Joint CUHK-NTEC CREC annually. All subsequent protocol amendments must be approved by the CREC.

7.2 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/GCP, applicable regulatory requirements and our Joint CUHK-NTEC CREC. This study is designed based on a thorough knowledge of the scientific background, a careful assessment of risks and benefits, have a reasonable likelihood of benefit to the population studied and will be conducted by suitably trained investigators using approved protocol.

7.3 Written informed consent

The confidentiality of participants in the retrospective cohort will be protected as their personal identifiers, including name, address, identification numbers (e.g. hospital number), electronic contact details (e.g. telephone number, fax number, email address) will be separated from data files during the data acquisition process. Project-specific serial number will be used to represent each subject. Data involved in this study will be de-identified and released for public academic use to facilitate the research in this area. Information about the data from certain patient cohort will be given on publicly accessible webpage.

For the prospectively recruited subjects, the investigator(s) will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. The informed consent forms are available in both English and Chinese. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allow time to consider the information provided. The subject’s signed and dated informed consent must be obtained before conducting any procedure specific for the study. The Principal Investigator must store the original, signed Informed Consent Form. One copy of the signed Informed Consent Form must be given to the subject. If modifications are made according to local requirements, the new version has to be approved by our Joint CUHK-NTEC CREC.

7.4 Data sharing

In line with current international standard of accountability and transparent reporting, data from this study may be shared with outside parties upon reasonable request. Care will be taken to remove sensitive data (name, identity numbers and dates of birth) before data sharing.

8. POSSIBLE RISKS AND ADVERSE EVENT REPORTING

8.1 Possible risks and discomfort

There is minimal discomfort in blood taking and undergoing VCTE. The adverse event reporting period for this study is from signing the informed consent through 30 days after the last follow-up visit.

8.2 Adverse event

An adverse event is any undesirable medical event occurring in the subject within the trial period, whether or not it is related to the study intervention.

The severity of an adverse event is defined as:
Mild: Transient symptoms, no interference with the subject's daily activities
Moderate: Marked symptoms, moderate interference with the subject's daily activities
Severe: Severe interference with the subject's daily activities.

The relationship of an adverse event to the study intervention is defined as:
Probable: Good reasons and sufficient documentation to assume a causal relationship
Possible: A causal relationship is conceivable and cannot be dismissed
Unlikely: The event is unlikely related to the study intervention

A telephone enquiry hotline for reporting subject's adverse events is available. The investigator will record all relevant information including signs and symptoms of the event, the onset time, the date of the event, the laboratory findings, concomitant drugs, and the outcome. All adverse events will be followed up until we have reached a defined outcome of the event, which can be one of the followings: (1) recovered with sequelae (for chronic conditions), (2) recovered, or (3) the management of the adverse event is taken over by another physician when the study ends.

A clinical laboratory adverse event is any clinical laboratory abnormality that suggests a disease and/or organ toxicity is of sufficient severity that requires active intervention (i.e. change of dose, discontinuation of drug, more frequent follow-up or further investigation). An unscheduled visit will be performed if subjects have to withdraw early from the study.

8.3 Serious adverse events

A serious adverse event is an adverse event that results in one of the following outcomes:

- Death
- Life-threatening
- In-patient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability or incapacity
- A congenital anomaly or birth defect

The definitions of causal relationship to study intervention are the same as those for adverse events. We have a 24-hour on-call system to handle serious adverse events. The investigator will assess and treat the subjects as soon as possible. A standard serious adverse event form will be used (provided by the CREC at http://intranet.ccter.cuhk.edu.hk/sae/) to report the events within 24 hours after acknowledgement. We will arrange unscheduled follow-up visits immediately or within 24 hours on receiving the subject's self-report of serious adverse events after the subjects have been discharged or if the subject has already been admitted to the hospital. Our investigators will assess the subject within 24 hours. The study team will record all relevant information including signs and symptoms of the event, the onset time, the date of the event, the laboratory findings, concomitant drugs, and the final outcome. A follow-up serious adverse event form will be sent within 14 days after submitting the initial serious adverse event form. Serious adverse events related to the study drug will be followed up until the subject has "recovered", "recovered with sequelae" or "died". SAE reports will be sent to our ethics committee. An unscheduled visit will be performed if subjects have to withdraw early from the study.

8.4 Emergency contact person

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9. REFERENCES

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