Renal Histopathology and Transcriptomic Analysis in Hepatorenal Syndrome during Liver Transplantation

STUDY PROTOCOL

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Background

Hepatorenal Syndrome (HRS) is a severe complication of cirrhosis characterised by functional renal impairment in the absence of structural kidney disease. The diagnostic criteria for HRS have evolved significantly over time, with the International Club of Ascites (ICA) revising the criteria in 2019 and harmonizing with the definition of acute kidney injury (AKI) established by the Kidney Disease: Improving Global Outcomes (KDIGO). Type 1 HRS was renamed as HRS AKI.

There are two key notions about HRS AKI in the literature: (1) it is a functional disorder that typically occurs in the context of structurally normal kidneys, and (2) it is reversible with timely liver transplantation (LT). However, no one has ever documented renal histology in HRS AKI patients. The conclusion was only supported by post-mortem histological examination showing near-normal renal histology despite severe renal impairment in cirrhotic patients. Post-mortem studies have significant limitations, as they only provide a snapshot of the kidney's condition after death.

Our preliminary study from December 2017 to December 2023 recruited 54 liver transplant recipients for renal biopsy at the time of LT. We found a high prevalence of structural abnormalities in LT recipients, with up to 96.2% having abnormal renal histology. This challenges the conventional understanding that HRS AKI occurs in structurally normal kidneys. Hence, we would like to examine the actual structural changes in the kidneys of patients with HRS through tissue biopsy and advanced molecular analysis, and hope that may help us better understand the disease mechanism and potentially improve treatment approaches. We believe that this study will provide valuable insights into the pathophysiology of HRS and may lead to improved diagnostic and treatment strategies in the future.

Objectives

This study aims to examine the actual structural changes in kidneys of patients with HRS through tissue biopsy and advanced molecular analysis, and hope that may help us better understand the disease mechanism and potentially improve treatment approaches.

Primary Objective

To refute the null hypothesis that HRS AKI typically develops in the context of structurally normal kidneys.

Secondary Objectives

1. To assess if the histological findings on renal biopsy in HRS AKI patients are reversible following liver transplantation

2. To define the single-cell spatial transcriptomic landscape in HRS AKI

3. To compare and delineate the differential alterations in transcriptomic profiles between HRS AKI and non-HRS AKI cohorts

Study Design

This is a 3-year single center prospective, non-randomised, open label study at Queen Mary Hospital, The University of Hong Kong. All consecutive patients accepted on the liver transplant waiting list will be invited to participate. Patient will undergo several procedures related to liver transplant and kidney assessment, and receive liver tranplantation and renal biopsy.

Procedures

1. Before Liver Transplantation

   Patients will receive standard evaluation and preparation for liver transplant. Blood and urine samples will be collected to measure kidney function markers, and kidney function will be monitored regularly through blood tests.

2. During Liver Transplantation

   All transplant procedures will be performed in standard manner. Intraoperative renal biopsy will be performed by operating surgeons following liver graft implantation and reperfusion. Two cores of renal tissue with minimal length of 8mm will be obtained using a 14-gauge automated biopsy needle. This additional procedure will be performed as part of the transplantation and is expected to add minimal extra risk.

3. After Liver Transplantation

   Following transplantation, patients will receive standard post-operative care and monitoring. Regular blood test will be performed to monitor liver and renal function. Blood and urine samples will also be collected periodically for research purposes. Standardized immunosuppression protocol including induction agent (Basiliximab), perioperative hydrocortisone, and maintenance regimen with mycophenolate mofetil and calcineurin inhibitors will be applied and montior closely.

4. 3 Months After Liver Transplantation

   a. Second Renal biopsy

      Ultrasound-guided renal biopsy will be performed by radiologists using a 14-gauge automated needle to obtain two cores of renal tissue. Two small tissue samples will be taken for comparison with the first biopsy.

   b. Laboratory Analysis

      The kidney tissue samples will be examined under a microscope to assess structural changes. Advanced molecular analyses will be performed to understand cellular changes, and blood and urine samples will be analysed for renal function markers.

      <u>Histopathological and Transcriptomic Analysis</u>

      Histopathological Assessment

      - Quantification of glomeruli and scoring of scarring and fibrosis
      - Assessment of glomerular, tubular, and interstitial changes
      - Specialized stains including PAS, silver stain, trichrome stains
      - Immunohistochemical and immunofluorescence studies
      - Electron microscopic examination

      Transcriptomic Analysis

      High-resolution single-cell spatial transcriptomic analysis using 10x Visium HD technology will be performed on formalin-fixed paraffin-embedded tissue samples. Sequential Fluorescence In Situ Hybridization (seqFISH) and uniform manifold approximation and projection (UMAP) will be used to identify distinct cellular populations and spatial gene expression patterns.

      <u>Biomarker Monitoring</u>

      - Serum Cystatin C for precise measurement of renal function
      - Neutrophil Gelatinase-Associated Lipocalin (NGAL) - elevated in acute tubular necrosis but lower in HRS AKI
      - Interleukin-18 (IL-18) - elevated in acute tubular necrosis but lower in HRS AKI

Outcome

Primary Outcome

- Proportion of patients with HRS AKI who have normal, reversible or permanent injury on renal histopathology
- Histopathological changes and their association with clinical parameters

Secondary Outcomes

- Incidence and duration of HRS AKI
- Response to standard treatment (Terlipressin and albumin)
- Perioperative morbidity including need and duration for renal replacement therapy
- Post-transplant renal recovery rates
- Transcriptomic signature differences between HRS AKI and non-HRS AKI patients

Selection and Withdrawal of Subjects

42 patients will be recruited to this study over a 2-year period.

According to our preliminary study, 50 out of 54 patients (92.6%) had abnormal renal biopsy results at the time of LT. The difference in proportion between abnormal and normal histology was 85.2% (= 92.6% – 7.4%). To ensure our study will be adequately powered, we will assume the difference to be 80%, with alpha (α) at 0.05 and power (1-β) at 0.9, based on:

$$n = \frac{\left(Z_{\frac{\alpha}{2}} + Z_{\beta}\right)^2 [p_1(1-p_1) + p_2(1-p_2)]}{(p_1 - p_2)^2}$$

The sample size is around 31.7 i.e. 32 patients will be needed. Assuming 30% dropout rate, 42 patients will be recruited in the study.

Subject enrollment criteria

Inclusion Criteria
- Patient must be able to understand and provide informed consent
- Age ≥ 18 years at time of study entry
- Accepted for LT
- Fulfill the diagnostic criteria of HRS AKI according to International Club of Ascites (ICA) guidelines

Exclusion Criteria
- Inability or unwillingness to give written informed consent
- Patient with known pre-existing renal disease
- Patient with solitary kidney
- Re-transplantation
- ABO-incompatible LT
- Fail to provide 3-month post transplant renal biopsy

3-month Post-transplant Renal Biopsy

Inclusion Criteria
- Patient with adequate intraoperative renal biopsy
- Patient must be able to understand and provide informed consent

Exclusion Criteria
- Graft failure after LT
- Any condition deemed inappropriate by the Principal Investigator

Diagnosis and Definition of HRS AKI

The definition of HRS AKI is adopted from the latest diagnostic criteria by the International Club of Ascites (ICA):

Diagnostic Criteria

1. Presence of cirrhosis with ascites
2. Diagnosis of AKI: increase in serum creatinine by ≥ 0.3 mg/dL (≥ 26.5 μmol/L) within 48 hours, or percentage increase in serum creatinine of ≥ 50% from baseline within prior 7 days, or urine output < 0.5ml/kg/hr in 6 hours
3. Absence of shock
4. No current or recent nephrotoxic drug use
5. No improvement after volume expansion with albumin (1g/kg body weight per day up to maximum 100g/day for 2 consecutive days)
6. Exclusion of other renal disease through urinary analysis and kidney imaging

Assessment of Safety and potential risks

Standard liver transplantation procedures are employed. Intraoperative renal biopsy is a well-established procedure with minimal additional risk. Post-transplant renal biopsy carries standard procedural risks including bleeding, infection, and pain, with overall complication rates < 1%.

Close monitoring for complications will be performed, including assessment using the Clavien-Dindo classification system. All adverse events will be documented and reported according to institutional guidelines.

Liver Transplantation

Liver transplantation is a standard, life-saving procedure with a success rate over 90%. Common risks include surgical complications, infection, rejection, and side effects of immunosuppressive medications etc. These risks will be minimized through experienced surgical teams, sterile techniques, and close monitoring.

Intraoperative Renal Biopsy

Obtaining kidney tissue during liver transplantation carries minimal additional risk beyond the standard transplant procedure. Potential complications include pain at the biopsy site, minor bleeding (which usually stops on its own), and infection (rare). These will be minimised by performing the biopsy with sterile equipment and experienced surgeons.

Post-transplant Renal Biopsy

The renal biopsy under ultrasound guidance may cause risks such as pain at the biopsy site, bleeding requiring hospitalisation (less than 1%), infection (rare), or very rarely, the need for blood transfusion etc. These risks will be reduced by using experienced radiologists and proper monitoring during and after the procedure.

Blood and Urine Collection

Routine blood draws may cause temporary discomfort, bruising, or rarely, infection at the needle site. These risks will be minimised by using sterile techniques and skilled personnel.

Statistical Analysis

Descriptive characteristics of all patients will be described using median (IQR) and/or proportions. Categorical data will be analyzed with Chi-square or Fisher's exact test where appropriate. Continuous variables will be analyzed with Mann Whitney-U test. All P values are two-sided, and statistical significance is defined as P value <0.05. All analyses are performed by R version 4.4.1. Proportion of different renal histology findings and comparison will be made at time of LT and 3 months post-LT between HRS AKI and non-HRS AKI cohorts, and their correlation with pre-LT, intraoperative and post-LT variables. Incidence of functional and histological renal recovery will be described, and their correlation with clinical parameters will be made between patients who have renal recovery and who do not

Duration of Study and Data/Samples Handling

The study duration is approximately 3 years, with 2 years for recruitment and 1 year for follow-up and analysis. Collected personal data and study data will be stored on password protected, encrypted database in the Department of Surgery, with the study team permanently as it is possible to be reviewed again in future. Collected samples will also be stored permanently in the department for future use. Unused tissue samples will be destroyed and discarded upon request, however, the results will remain as a part of the overall research data if part of the tissue has been proceeded.

Consent

Written voluntary consent will be obtained after full explanation of the study to patients before liver transplantation. Additional consent will be obtained for the 3-month post-transplant renal biopsy.

Conflicts of Interest

All investigators have no financial interests in the materials used in the study. This study will not receive any financial support from organizations that have any relationship with the materials used in this study.

Ethics

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki, and applicable regulatory requirements Patient data protection.