Title: To explore the humanin's Value for Early Diagnosis and Short-term Prognosis in Patients With AKI After Heart Transplantation

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Research protocols

1、To explore the humanin's Value for Early Diagnosis and Short-term Prognosis in Patients With AKI After Heart Transplantation

1.1 Study design

Prospective cohort study. Observation and analysis of patients who underwent heart transplantation from Guangdong Provincial People's Hospital from September 2022 to May 2024 were selected according to the following criteria: Guangdong Provincial People's Hospital underwent heart transplantation; healthy control group: from the healthy physical examination population, the physical examination blood routine, urine routine, liver function, kidney function, glycosylated hemoglobin and other indicators were normal; adult patients aged ≥ 18 years old, male and female; the baseline medical records of the patients were complete, including underlying cardiac diseases, comorbidities, hemoglobin, renal function, urine protein quantification, Serum albumin, lactate, C-reactive protein, heart transplantation operation time, preoperative and postoperative medication and blood pressure, blood transfusion history, ICU hospital stay, cardiac ultrasound (including LVEF), etc., were excluded according to the following criteria: patients were younger than 18 years old, and baseline data were missing;

1.2 Data Collection

Preoperative and postoperative plasma (on the 1st, 2nd, 3rd, 5th, and 7th postoperative days) and urine (within 48 hours after surgery) of patients undergoing heart transplantation from Guangdong Provincial People's Hospital from September 2022 to May 2024 were collected (the supernatant was collected after centrifugation at 3000r/min for 10 min, and the supernatant was divided into -80° C and stored)

The clinical baseline data of patients who met the inclusion criteria were collected, including gender, age, blood pressure, underlying diseases (hypertension, diabetes, hyperuricemia), serum creatinine, eGFR, serum albumin, hemoglobin concentration, glycosylated hemoglobin, cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, urine protein, urine red blood cells, urine white blood cells, cardiac surgery time, and length of hospital stay in ICU. Endpoints: AKI within 7 days, death during hospitalization, survival status at days 28 and 90, and need for dialysis

2. Plasma/urine Humanin concentration detection

2.1 Specimen retention

Patients who met the inclusion criteria were randomly urinated with 4ml of blood using an EDTA catheter or sterile urinary catheter during hospitalization After centrifugation (3000 r/min, 10min), 300-600 μ of the supernatant was stored in a -80° C freezer after the specimen was kept for 2 h.

2.2 Experimental reagents, consumables and instruments

Human humanin peptide (MT-RNR2) ELISA kit, centrifuge, EP tube, different specifications of enzyme-free pipette tips, different specifications of pipettes and guns, plasma water, microplate

reader, 4 ° C freezer, -20 ° C freezer, -80 ° C freezer, 37 ° C constant temperature incubator, etc

2.3 Experimental methods

（1）Reagent rewarming: Equilibrate the various reagents to room temperature (18-25C) for at least 30 minutes

（2）Reagent Equipment:

(1) Standard: Take out a standard from the kit and raise the center at 6000-10000rpm for 30 seconds. Dissolve with 1ml of sample diluent, and use the pipette tip to align the bottom of the cryovial with 5 times of repeated suction to dissolve, fully mix to obtain standard S7, and place it for later use, take 7 1.5ml centrifuge tubes to configure different concentrations of standards (the following concentrations are used for the standard vertebral curve: 1800, 900, 450, 225, 112.5, 56.25, 28, 0pg/ml);

(2) Lotion working solution: the concentrated washing solution is diluted with deionized water at 1:25 times, and it is prepared before use;

(3) Biotin labeled antibody working solution: biotin labeled antibody solution should be diluted with biotin labeled antibody diluent at 1:100 times, and it should be prepared within 10 minutes before use;

(4) Horseradish peroxidase labeling avidin working solution: horseradish peroxidase labeling avidin press 1; 100 times diluted with horseradish peroxidase-labeled avidin dilution, and prepared within 10 minutes before use;

(5) Sample dilution: The sample is diluted 1:5 times with the sample diluent for testing;

（3）Sample addition: Standard wells and sample wells to be tested are set respectively. Each well was supplemented with 100 u1 of standard or sample to be tested, mixed with light shaking, covered with a plate sticker, and incubated at 37C for 2 hours.

（4）Discard the liquid, spin dry, and do not wash.

（5）Each well was filled with 100 u1 of biotin-labeled antibody working solution, covered with a new plate patch, and incubated at 37C for 1 hour.

（6）Discard the liquid in the well, shake it in, and wash the plate 3 times. Soak for 2 minutes each time, 200U/well, spin dry.

（7）Each well was supplemented with horseradish peroxidase-labeled avidin 100 u1, covered with a new plate sticker, and incubated at 37° C for 1 hour.

（8）Discard the liquid from the wells, spin dry, and wash the plate 5 times. Soak for 2 minutes each time, 200ul/well, and spin dry.

（9）Add substrate solution 90u1 and 37C for 15-30 minutes in each well.

（10）Stop the reaction by adding 50 u1 of stop solution to each well in order.

（11）The optical density (OD value) of each well was measured sequentially at 450 nm with a microplate reader within 5 minutes after the reaction was terminated

（12）Calculate the concentration: According to the concentration of the standard and the corresponding OD value, the standard curve graph of Humanin is analyzed by Curve Expert 1.4 software, and the concentration of Humanin in the sample to be measured is calculated by using the equation of the standard curve.

3. Statistical methods

SPSS software was used for data processing. Normally distributed continuous data were statistically described as ($x \pm s$), and t-test was used for comparison between groups. Counting data are expressed in terms of frequency, percentage (%), and chi-square test. The receiver operating characteristic curve (ROC) was plotted to analyze the diagnostic value of Humanin for AKI after heart transplantation. The Kaplan-Meier method estimated the survival curve, and the Cox regression model evaluated the influencing factors affecting the survival process. The difference was statistically significant, with $P<0.05$ as the difference.