Research Article

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Cirrhosis

Submassive hepatic necrosis distinguishes HBV-associated acute on chronic liver failure from cirrhotic patients with acute decompensation

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Background & Aims: Distinguishing between acute on chronic liver failure (ACLF) and decompensated liver cirrhosis is difficult due to a lack of pathological evidence.

Methods: A prospective single-center study investigated 174 patients undergoing liver transplantation due to acute decompensation of hepatitis B virus (HBV)-associated liver cirrhosis. Two groups were distinguished by the presence or absence of submassive hepatic necrosis (SMHN, defined as necrosis of 15–90% of the entire liver on explant). Core clinical features of ACLF were compared between these groups. Disease severity scoring systems were applied to describe liver function and organ failure. Serum cytokine profile assays, gene expression microarrays and immunohistochemical analyzes were used to study systemic and local inflammatory responses.

Results: SMHN was identified in 69 of 174 patients proven to have cirrhosis by histological means. Characteristic features of SMHN were extensive necrosis along terminal hepatic veins and spanning multiple adjacent cirrhotic nodules accompanied by various degrees of liver progenitor cell-derived regeneration, cholestasis, and ductular bilirubinostasis. Patients with SMHN presented with more severely impaired hepatic function, a higher prevalence of multiple organ failure (as indicated by higher CLIF-SOFA and SOFA scores) and a shorter interval between acute decompensation and liver transplantation than those without SMHN (p <0.01 for all parameters). Further analyzes based on serum cytokine profile assays, gene expression microarrays and

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Abbreviations: ACLF, acute on chronic liver failure; ALF, acute liver failure; AD, acute decompensation; MHN, massive hepatic necrosis; SMHN, submassive hepatic necrosis; HBV, hepatitis B virus; LT, liver transplantation; SIRS, systemic inflammatory response syndrome; TB, total bilirubin; CBA, Cytometric Bead Array; MELD, model for end-stage liver disease; SOFA, sequential organ failure assessment score; CLIF-SOFA, chronic liver failure-sequential organ failure assessment score; APACHE, acute physiology score, age points, chronic health points; H&E, hematoxylin & eosin; IHC, immunohistochemistry; LPC, liver progenitor cells; DR, ductular reaction; IH, intermediate hepatocyte; SD, standard deviation; IQR, interquartile ranges; AIH, autoimmune hepatitis; ALD, alcoholic liver disease; ALT, alanine aminotransferase; AUC, area under curve; CK7, cytokeratin 7; EASL-CLIF, European Association for the Study of the Liver-chronic liver failure; INR, international normalized ratio; PBC, primary biliary cirrhosis; ROC, response operating characteristic; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

immunohistochemical analyzes revealed higher levels of anti-inflammatory cytokines in patients with SMHN.

Conclusions: SMHN is a critical histological feature of HBV-associated ACLF. Identification of a characteristic pathological feature strongly supports that ACLF is a separate entity in end-stage liver disease.

Introduction

The term "acute on chronic liver failure" (ACLF) was established in consideration of another deadly liver disease, acute liver failure (ALF), by Ohnishi and colleagues in 1995 [1]. One decade later, the Asian-Pacific Association for the Study of the Liver, the American Association for the Study of the Liver and the European Association for the Study of the Liver proposed definitions for ACLF [2,3]. Given that both definitions were based on clinical manifestation and biochemical parameters (e.g., precipitating event, high serum bilirubin and hepatic encephalopathy), consequent diagnostic criteria overlapping with end-stage decompensated cirrhosis elicited a debate as to whether ACLF really exists as a separate entity [4]. Recently, an elegant multicenter study based on 1343 patients with cirrhosis hospitalized for acute decompensation (AD) provided solid evidence that ACLF is a distinct clinical entity derived from AD of cirrhosis [5]. That study established new diagnostic criteria for ACLF, including organ failure and high mortality. However, the controversial issue of "overlap" between patients with ACLF and decompensated cirrhosis in clinical practice has not been resolved. The unique pathophysiology underlying ACLF is not known [6].

ALF is characterized by hepatic encephalopathy and a bleeding tendency due to severe impairment of liver function [7]. The definition, classification and prediction of outcome in ALF are based on clinical manifestations, but also on histological characteristics. It has been confirmed that massive or submassive hepatic necrosis (MHN/SMHN) causes ALF [7].

In the present study, we describe SMHN in more than one-third of cirrhotic livers of patients with chronic hepatitis B virus (HBV) infection (69 out of 174) undergoing liver transplantation (LT). Therefore, we hypothesized that SMHN is the critical histological hallmark of ACLF and patients were divided into two groups according to the presence or absence of SMHN. Histological, immunological and clinical characteristics were compared between both groups and correlated with the critical parameters of ACLF.

Patients and methods

Enrollment of patients

This was a prospective cohort study conducted at a Liver Transplantation Center (Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine) from 2008 to 2011. We screened hospitalized patients with chronic liver diseases (defined as "with impaired liver function of any etiology for >6 months") on a waiting list who had experienced AD within the last two months.

AD was defined by the development of one or more major complications of liver diseases: (i) development of grade 2 to 3 ascites [8] within <2 weeks; (ii) hepatic encephalopathy [9]; (iii) gastrointestinal hemorrhage [10]; (iv) bacterial infections (spontaneous bacterial peritonitis, spontaneous bacteremia, urinary tract infection, pneumonia, cellulitis).

This study focused on patients with chronic HBV infection. Thus, patients with another single etiology (e.g., alcohol, drug, autoimmune) and those co-infected with HBV and the human immunodeficiency virus were excluded. The diagnosis of liver cirrhosis based on histological means was defined according to three critical features: (i) the entire liver is disrupted by interconnecting fibrous scars; (ii) fibrous septa are present in the form of delicate or broad bands obliterating multiple adjacent lobules; (iii) parenchymal nodules are created by fibrotic isolation of "islands" of hepatic parenchyma [11,12]. Diagnosis of cirrhosis was made after two pathologists had assessed the transplanted liver tissues. Patients were divided into two groups according to the presence or absence of MHN/SMHN.

The study protocol was approved by the Ethics Committee of Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine (Shanghai, China). Written informed consent was obtained from patients or their representatives.

Data collection

We collected data from patients with acute decompensation with chronic HBV infection based on medical history, physical examination (temperature, blood pressure, etc.), laboratory measurements (urine, blood, ascites examination), infectious parameters, systemic inflammatory response syndrome (SIRS) and potential precipitating events: HBV reactivation; superimposed viral hepatitis; bacterial infection or sepsis; alcohol intake; surgery; hepatotoxic drugs or herbs; variceal bleeding; portal vein thrombosis; physiological exhaustion (defined as "a period of excessive physical activity such as excessive work, exercise or trips").

Clinical/biochemical parameters (including total bilirubin (TB), INR and creatinine) were registered systematically during hospitalization. When patients on the waiting list were in the Liver Transplantation Unit, this time was defined as "time zero" (day 1) for dynamic analyzes of clinical and biochemical data. The selected time points for analyzing were day 1, day 3 and day 7 after admission to the Liver Transplantation Unit and 24 h before LT.

Levels of cytokines in peripheral blood were detected using a Cytometric Bead Array (CBA) assay (Becton Dickinson, San Jose, CA, USA). Considering that during the transplantation, especially before the hepatectomy of the native liver, transfusion of packed red blood cells, frozen plasma, or albumin were usually inevitable. These allogenic blood products could have an unpredictable influence on the cytokine profiles. Thus, blood samples were collected 24 h before LT. In addition, five scoring systems associated with the clinical prognosis were calculated: Child–Pugh [13], model for end-stage liver disease (MELD) [14], Sequential Organ Failure Assessment Score (SOFA) [15], Chronic Liver Failure-Sequential Organ Failure Assessment Score (CLIF-SOFA) [5] and Acute Physiology Score, Age Points, Chronic Health Points (APACHE) III [16].

Histology

After total hepatectomy for subsequent LT, liver tissue specimens from different liver lobes were collected for histological examination. Histological evaluation was performed in every collected specimen by two experienced pathologists (T.L.W., L.Q.). Sections from one liver tissue block ($2.5 \times 2.5 \times 1.0\ cm^3$, from the right lobule) were used to define MHN/SMHN. After two pathologists evaluated liver samples from transplantation, patients were divided into groups with or without SMHN. Tissues were fixed in 4% neutralized formaldehyde and embedded in paraffin. Sections (4 μm) were stained with hematoxylin & eosin (H&E), Masson trichrome, Picro-Sirius red, orcein and immunohistochemistry (IHC). Besides histological assessment, parts of fresh tissues were collected for microarray analysis and the remainder stored in liquid nitrogen.

In the present study, the definition of MHN/SMHN was in accordance with previous descriptions [11,12], with minor modifications. Briefly, patients with MHN/SMHN presented with extensive and diffuse necrosis spanning multiple adjacent regenerative nodules. MHN was defined as necrosis of >90% as noted upon examination of the entire liver on explant. SMHN was defined as necrosis of 15–90% of the entire liver on explant. For semi-quantitative analyzes, the area of necrosis in the detected area was scored as: +1, <33%; +2, 33–66%; +3, >66%. Immunohistochemical staining for CK7 (dilution 1:200) was used to identify liver progenitor cells (LPCs), ductular reaction (DR) and intermediate hepatocytes (IHs) [13]. The definition of LPC, DR and IH was according to a previous description [14].

Statistical analyzes

Continuous normally distributed variables and continuous non-normally distributed variables are the mean ± standard deviation (SD) and median values with interquartile ranges (IQR), respectively. *p* values were calculated using a two-sided (unpaired) Student's *t* test on normalized variables or log-transformed normalized