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ABSTRACT BOOK

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Oral Presentation

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❖OP-001

Clinical characteristics and prognosis of concomitant autoimmune hepatitis and nonalcoholic fatty liver disease

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Object/Background: Autoimmune hepatitis (AIH) and nonalcoholic fatty liver disease (NAFLD) frequently co-exist. The effect of NAFLD on AIH has not been widely studied. To report clinical characteristics and outcomes of patients with coincident AIH and NAFLD.

Methods: 139 patients with biopsy-confirmed AIH were included: 30 with NAFLD, and 109 without NAFLD. The clinical characteristics and risk factors of the AIH/NAFLD patients were described, and the impact on treatment response and liver-related events were also analyzed.

Results: AIH/NAFLD patients have higher hemoglobin, IgA, and albumin levels, but lower alkaline phosphatase (ALP), total bile acid, and total bilirubin levels ($P < 0.05$). Logistic regression analysis showed that overweight (OR 2.899; 95%CI 1.048-8.017; $P < 0.05$), ALP (OR 0.993; 95%CI 0.987-0.999; $P < 0.05$), and IgA (OR 1.780; 95% CI 1.183-2.678; $P < 0.01$) were associated with AIH/NAFLD. After six months of therapy, the rate of transaminase normalization in the AIH/NAFLD group was significantly lower than in the AIH group (10% vs 34%, $P = 0.009$). The cumulative liver-related event rate did not differ through the Log-rank test between the two groups ($P = 0.837$).

Conclusion: Overweight, decreased ALP levels, and elevated IgA levels are dependent risk factors for AIH/NAFLD. NAFLD is prevalent in AIH patients but has little impact on liver disease progression.

❖OP-002

Human umbilical cord blood mononuclear cells ameliorate CCl4-induced acute liver injury in mice via inhibiting inflammatory responses and up-regulating peripheral interleukin-22

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Object/Background: It is impossible for the liver to compensate in fulminant liver failure. The only feasible treatment option is liver transplantation. Thus, new therapeutic strategies for ALF in reversible stage need to be explored. Cell therapies provide unprecedented opportunities for intractable diseases and injury. Human umbilical cord blood mononuclear cells (hUCBMNCs) are isolated from cord blood and show significantly therapeutic effects on a variety of inflammatory diseases, such as renal tubulointerstitial fibrosis, inflammation-induced preterm brain injury, and LPS-induced acute kidney injury. The deterioration of acute liver injury attributes to excessive inflammatory responses triggered by damage-associated molecular patterns (DAMPs) released by necrotic cells and pathogen-associated molecular patterns (PAMPs) released by bacteria. Regeneration of hepatocytes was another essential factor for recovery of injured liver. Anti-inflammatory therapy and promoting regeneration in liver seems to be a promising strategy for acute liver injury. Therefore, the present study aims to investigate the application value and mechanisms of the immunomodulatoryfunctions of hUCBMNCs treatment in several DAMPs-/PAMPs-induced acute liver injury mouse models.

Methods: Human umbilical cord bloods were collected from healthy newborns. Liver injury mice induced by PAMPs, DAMPs or DAMPs plus PAMPs were developed. DAMPs included CCl4 (carbon tetrachloride), APAP (acetaminophen), and ConA (Concanavalin A). PAMPs included Klebsiella pneumoniae (K.P.) and Salmonella typhimurium (S. Typhimurium). DAMPs plus PAMPs-induced liver injury was developed by sequential CCl4 and K.P. administration. hUCBMNCs were injected intravenously before or post insults. IL-22 neutralizing antibody was applied for blocking peripheral interleukin-22.

Results: hUCBMNCs treatment significantly prolonged mice survival time in DAMPs plus PAMPs-induced liver failure but had no benefit in bacterial infected mice (including K.P. and S. Typhimurium induced bacterial infection mouse models). Moreover, hUCBMNCs significantly alleviated hepatic necrosis post CCl4/ConA insult but nearly showed no beneficial impacts on APAP-induced acute liver injury. Mechanistically,

hUCBMNCs treatment alleviated hepatic inflammatory response in CCl4-/ConA-induced liver injury by down-regulating inflammatory markers, such as IL-6, IL-1β, TNF-α, IFN-γ, IL-17A. In CCl4-induced acute liver injury, peripheral levels of pro-regenerative interleukin (IL)-22 were up-regulated and liver regeneration was enhanced after treating with hUCBMNCs at 48h and activation of caspase-3 protein was attenuated at 24h under hUCBMNCs treatment. The levels of oxidative stress in CCl4 treated mice were also attenuated under the treatment of hUCBMNCs at 48h. Autophagy-related genes and hypoxia-inducible factor 1α (Hif1α) were up-regulated at 24h. The levels of p62 and LC3B-II, autophagy markers, were also up-regulated in hUCBMNCs treated group at 48h. Besides, blocking peripheral interleukin-22 by neutralizing antibody could inhibit the promoted pro-regenerative effect and enhanced autophagy of hUCBMNCs treatment, which impaired the therapeutic effects of hUCBMNCs on CCl4-induced liver injury.

Conclusion: In our study, hUCBMNCs as a kind of cell therapeutic strategy exerted hepato-protective effects on CCl4/ConA-induced acute liver injury mice but could hardly improve the necrosis in APAP-induced mice. In addition, hUCBMNCs nearly had no beneficial effects on K.P. and S. Typhimurium infected mice. In terms of mechanics, the hepato-protective effects of hUCBMNCs in CCl4-induced liver injury were demonstrated as intensified pro-regeneration and enhanced autophagy in the liver via inhibiting inflammatory responses, oxidative stress and up-regulating peripheral IL-22.

❖OP-003

CMTM4 promotes the proliferation and metastasis of hepatocellular carcinoma through the regulation of AKT and PD-L1

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Object/Background: Hepatocellular carcinoma has the fifth highest incidence rate in the world and the second highest mortality rate in the world. Only a thorough understanding of the specific molecular mechanisms within the many processes of hepatocellular carcinogenesis, proliferation and metastasis, and the study of corresponding therapeutic options to address these molecular mechanisms, can make our clinical treatment more effective and lead to a better prognosis for patients. CMTM4 is a member of the chemokine superfamily and its expression The expression of CMTM4, a member of the chemokine superfamily, has a significant role in the development of many cancers, but its role in the development of hepatocellular carcinoma and its mechanism of action have not been clearly established.

Methods: The expression levels of CMTM4 in hepatocellular carcinoma tissues from patients were examined by immunohistochemical staining, q-PCR and Western Blotting, and correlated with the clinicopathological characteristics of the patients to investigate the correlation between the expression levels of CMTM4 and the clinical status and prognosis of the patients. Lentiviral infection was used to construct stable knockdown/over-expression cell lines, and CCK-8, Transwell and clone formation assays were performed to investigate the effect of different CMTM4 expression levels on the biological function of hepatocellular carcinoma cells. Western Blotting demonstrated that CMTM4 expression could regulate the expression level of phosphorylated AKT, and cellular functional assays were performed with cell lines incorporating AKT inhibitors to explore the molecular mechanism of CMTM4 action on hepatocellular carcinoma cells. The correlation between the expression levels of CMTM4 and PD-L1 was explored using immunohistochemical staining, and the regulatory effect of CMTM4 on PD-L1 was further investigated by Co-IP as well as IP experiments. The effect of CMTM4 on the growth of hepatocellular carcinoma in mice was investigated by animal experiments.

Results: We found that the expression of CMTM4 was higher in hepatocellular carcinoma than in paraneoplastic tissues, and CMTM4 could promote the proliferation and metastatic ability of hepatocellular carcinoma cells. In addition, we found that CMTM4 could promote

the proliferation and metastasis of hepatocellular carcinoma cells through the AKT signaling pathway, and could reduce the ubiquitination level of PD-L1 and stabilize the expression of PD-L1.

Conclusion: High expression of CMTM4 usually predicts poor clinical status and worse prognosis, and it plays an important role in promoting the proliferation and migration of hepatocellular carcinoma cells. CMTM4 can promote the progression of hepatocellular carcinoma by activating the AKT pathway and can also promote the progression of hepatocellular carcinoma by stabilizing PD-L1 expression.

❖OP-004

Soluble Fibrinogen-Like Protein 2 Promotes the Progression of Hepatocellular Carcinoma by Inducing the Expression of Immune Checkpoints

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Object/Background: Immune checkpoint molecules, such as programmed cell death protein 1 (PD1), programmed cell death ligand 1 (PD-L1), programmed cell death ligand 2 (PD-L2) and cytotoxic T lymphocyte-associated protein 4 (CTLA4), promote tumor cell escape from immunosurveillance. Here, we explored the effect of soluble fibrinogen-like protein 2 (sFGL2) on the expression of the above checkpoints, so as to explain the mechanism of sFGL2 on the progression of hepatocellular carcinoma (HCC).

Methods: Gene expression profiling interactive analysis (GEPIA) was used to analyze the correlation between the mRNA expression level of fgl2 and PD1, PD-L1, PD-L2, CTLA4 in liver cancer patients. In vitro experiment, the expression of FGL2, PD-L1 and PD-L2 was detected by RT-qPCR and Western Blot. Hepa1-6 cells were inoculated subcutaneously or orthotopically in the C57BL/6J wild type (WT) mice and fgl2 gene knockout (fgl2-/-) mice. After 14 days of tumor bearing, the mice were sacrificed and tumor tissues were separated. The concentration of sFGL2 in tumor tissue homogenate was detected by ELISA. The expression of PD-L1, PD-L2 on tumor cells and PD1, CTLA4 on CD4+T cells and CD8+T cells was detected by flow cytometry.

Results: The analysis of GEPIA database showed that fgl2 was significantly positively correlated with PD1, PD-L1, PD-L2 and CTLA4 ($R=0.32, 0.45, 0.39, 0.61$, respectively, $P<0.001$). In HepG2 and HCCLM3 cell lines, the expression of PD-L1 decreased after fgl2 was knocked down（$P<0.05$）. In subcutaneous HCC models, the concentration of sFGL2 in tumor tissue homogenate in fgl2-/- mice was significantly lower than that in WT mice（$P<0.01$）. Moreover, on the fifth, seventh, ninth, eleventh and thirteenth day after inoculation, the mean tumor volume in fgl2-/- mice was much smaller than that in the control group（$P<0.05$）. PD-L1, PD-L2 expression on tumor cells and PD1, CTLA4 expression on CD4+T cells and CD8+T cells was lower in fgl2-/- mice than that in WT mice. Similarly, in orthotopic HCC models, the tumor invasion area was smaller after fgl2

was knocked out. The expression of PD-L1 on tumor cells along with PD1, CTLA4 expression on CD4+T cells and CD8+T cells was lower in fgl2-/- mice.

Conclusion: sFGL2 mediates tumor immune escape by inducing the expression of PD-L1 on hepatoma cells and the expression of PD1, CTLA4 on CD4+T cells and CD8+T cells, which promotes the progression of hepatocellular carcinoma

❖OP-005

Human Adipose Mesenchymal Stem Cells-derived Exosomes Ameliorate Hepatic Fibrosis by Regulating Choline Metabolism and PI3K/Akt/mTOR Pathway: Based on Transcriptomics and Metabolomics

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Object/Background: Liver fibrosis is a chronic liver disease with presence of the progressive wound healing response caused by liver injury. Currently, there are no approved therapies for liver fibrosis. Exosomes derived from human adipose mesenchymal stem cells (hADMSCs-Exo) have displayed a prominent therapeutic effect on liver diseases. However, few studies have evaluated therapeutic effect of hADMSCs-Exo in liver fibrosis and cirrhosis, and its precise mechanisms of action remains unclear.

Methods: Herein, we investigated in vitro and in vivo antifibrotic efficacy of hADMSCs-Exo, and identified important metabolic changes and the detailed mechanism through transcriptomic and metabolomic profiling.
Frist, hADMSCs were isolated, cultured and identified, and hADMSCs-Exo were isolated and identified. Then exosomes were added to human hepatic stellate cells (HSCs) line LX-2 activated by TGF-β1. Then western-blot, immunofluorescence, CCK8 proliferation test, cell cycle and apoptosis were used to detect the level of anti-hepatic fibrosis, inhibition of proliferation, regulation of cell cycle and apoptosis of HSCs. After that, hADMSCs and hADMSCs-Exo were intravenous administrated by tail vein injection to the mouse model of liver cirrhosis. The ability of hADMSCs and hADMSCs-Exo to anti-hepatic fibrosis, improve liver function, promote liver regeneration and inhibit apoptosis were detected by real-time tissue elastography, pathological section staining, western-blot and serological detection. To explore the transcriptional regulation and metabolic mechanism of hADMSCs-Exo anti-hepatic fibrosis by detecting the expression level of transcriptome gene and metabolite in liver tissue. The results of transcriptome and metabonomics were verified by western-blot, immunofluorescence, qRT-PCR and ELISA.

Results: The isolated hADMSCs could differentiate into three lines and stably express CD73, CD44, CD90 and CD105 stem cell surface markers. The hADMSCs-Exo was a type of cell-derived extracellular vesicles with diameter ranging from 30 to 150 nm visa Transmission Electron Microscope. It could express CD63, CD81, TSG101 markers and is

ultimately internalized in LX-2 via endocytosis. In addition, we found hADMSCs-Exo could inhibit the proliferation of activated hepatic stellate cells, promote their apoptosis, arrest G1 phase, effectively inhibit the expression of profibrogenic proteins and epithelial-to-mesenchymal transition (EMT) in vitro. Moreover, it could significantly decrease the expression of collagen deposition and EMT progression, improve liver function and reduce liver inflammation in liver cirrhosis mice model. In addition, Transcriptome analysis revealed that the key mechanism of hADMSCs-Exo anti-hepatic fibrosis was the regulation of PI3K/AKT/mTOR signaling pathway. Metabolic analysis showed that hADMSCs-Exo mainly affects the changes of metabolites in lipid metabolism and down-regulates PI3K-AKT-mTOR pathway through choline metabolism. Thus, our study indicates that hADMSCs-Exo can attenuated hepatic stellate cell activation and suppressed the progression of liver fibrosis, which holds the significant potential of hADMSCs-Exo for use as extracellular nanovesicles-based therapeutics in the treatment of liver fibrosis and possibly other intractable chronic liver diseases.

Conclusion: In conclusion, our study identified the ability of exosomes isolated from hADMSCs in ameliorating liver fibrosis progression. Improvements in choline metabolism and inhibition of PI3K/AKT/mTOR signaling appeared to be the underlying mechanism that restored cell membrane, attenuated stellate cell activation and suppressed the progression of liver fibrosis. Furthermore, we also found the therapeutic effect of hADMSCs-Exo were dose-dependent. Our findings provide important insights into the molecular mechanisms underlying the antifibrotic effects of hADMSCs-Exo with a focus on metabolic homeostasis, and help inform the development of a safe and effective therapeutic. The current study is, therefore, an important step before the clinical usage of hADMSCs-Exo for chronic liver fibrosis.

❖OP-006

Small extracellular vesicles derived from induced pluripotent stem cells to alleviate lipopolysaccharide /D-galactosamine induced acute liver failure

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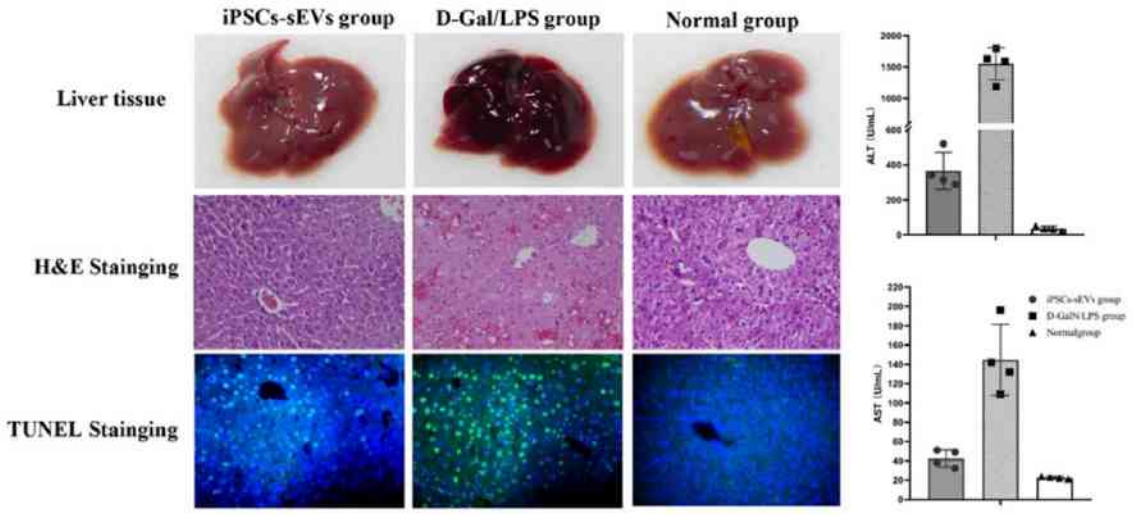
Object/Background: Acute liver failure is a type of fatal liver disease that has a high mortality rate and difficulty in treating. An important part of the body's metabolism, the liver has great potential for repairing and regenerating tissues. Previous experiments by our research team suggested that human iPSCs were induced to differentiate into hepatocyte-like cells in vitro. However, residual undifferentiated iPSCs pose perceived risks of tumorigenicity after transplantation. The paracrine theory of stem cells suggests that its paracrine active substances may play an important role in the regeneration and protection of hepatocytes. Recently, extracellular vesicles (EVs), such as exosomes and microvesicles, have been identified as paracrine mediators that influence intercellular communication associated with liver disease.The aim of this study was to investigate the therapeutic effect and underlying mechanisms of EVs in mouse with liver failure.

Methods: The mouse acute liver failure model with D-galactosamine and lipopolysaccharide was established to explore the effects of iPSCs derived sEVs. Liver function was valued by serum biochemical parameters and hematoxylin-eosin staining. Apoptosis was detected with TUNEL. Relevant RNA and protein levels were analyzed by qRT-PCR and Western blotting.

Results: Compared with the D-Gal/LPS group, the iPSCs-sEVs group could significantly reduce liver tissue bleeding and inflammatory cell exudation, reduce liver cell necrosis, and promote liver repair. After transplantation with sEVs, the levels of ALT and AST in serum of liver failure mice were reduced, and TUNEL staining showed a decrease in apoptotic cells. Western blotting and qRT-PCR were used to detect the expression of pyroptosis related genes such as Caspase1, Nlrp3, ASC and Nlrp1a. The results showed that the expression level of iPSCs-sEVs group was significantly lower than that of D-Gal/LPS group.

Conclusion: The EVs derived from iPSCs can alleviate the acute liver failure induced by lipopolysaccharide /D-galactosamine, which is expected to become a means of acute liver failure treatment.

Figure:



❖OP-007

Significant serum proteinic and metabolic alterations in hepatitis B cirrhosis patients treated with umbilical cord mesenchymal stem cells

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University of Electronic Science and Technology of China

Object/Background: Cirrhosis is the late stage of liver fibrosis and the initial stage of HCC, which is caused by multi-forms of liver diseases and conditions, such as viral hepatitis and chronic alcoholism. Anti-virus and symptomatic treatment are recommended in management of liver cirrhosis patients, while liver transplantation is considered in severe cases. To date, stem cells based-treatment has been investigated as an effective regenerative therapy for liver cirrhosis patients. Various Types of stem cells from embryonic, induced pluripotent, hematopoietic and mesenchymal stem cells (MSCs), have shown capacity of differentiation into hepatocyte-like cells, laying the foundation of their clinical application. Once homing to impaired liver tissue after infusion, repairment function of MSC is elicited by suppressing inflammatory response to recover damaged hepatic function and their immune-modulatory properties. Therapeutic efficacy of MSC or their bioactive derivates in patients with liver cirrhosis have also been confirmed in clinical trials. For example, intravenous injection of umbilical cord MSCs (uc-MSCs) was proved to be clinically safe, improved liver function and reduced ascites in patients with decompensated liver cirrhosis. However, data on the impact of stem cell transplantation on circulating metabolites in liver cirrhosis patients remains limited.

Methods: In this study, 7 eligible participants received 10E7 uc-MSCs injection through ultrasound-guided percutaneous liver puncture-based portal vein catheterization. Changes of serum proteins and metabolites at the timepoint of 72 hours after treatment were measured by proteomics and metabolomics respectively.
Tandem Mass Tag (TMT) based proteomic was performed using serum samples from liver cirrhosis patients prior to or 72 hours after UC-MSCs treatment. After removing high abundant proteins, each sample underwent reduction and alkylation, being digested and labeled with TMT for peptides preparation. 2ug of total peptides were separated and analyzed with a nano-UPLC (EASY-nLC1200) coupled to Q Exactive HF-X Orbitrap instrument (Thermo Fisher Scientific) with a nano-electrospray ion source. Files were processed for peptide identification and quantification.

❖OP-007

Untargeted metabolomics analysis was performed using ultra-high performance liquid chromatography systems with multiple reaction monitoring mass spectrometry (UHPLC-MRM-MS) coupled to identification with an internal library of authentic chemical standards.

Results: A total of 1209 proteins were identified and processed to taken logarithm modeled for principal component analysis (PCA). With the threshold of Fold change >1.2 and $p<0.05$, 35 proteins were identified as differentially expressed protein (DEP) between these two groups. 28 proteins were upregulated while 7 proteins were down-regulated in patients underwent uc-MSCs injection. Dramatic elevation in certain proteins associated with extracellular matrix (ECM) remodeling was observed, such as proteasome 20S subunits (PSMA/PSMB) and ECM components (LAMC1, POSTN). Gene ontology analysis also suggested these altered proteins were enriched in ECM remodeling and metabolic processes. A cluster of coregulated features strongly are relevant to the ECM components identified, which might be a potential symbol of recovered hepatic tissue.
Metabolomic data was assessed for clustering using PCA. 16 increased and 27 decreased metabolites were screened out. The altered metabolites in patients after uc-MSC treatment include a batch of phospholipid especially glyceryl phosphatide and their derivates. Several types of phosphatidylcholine (PC) were augmented after uc-MSC injection compared to baseline (PC14:0, PC16:1, PC18:1, PC18:2, PC20:1, PC20:4), whereas phosphatidylethanolamine (PE) was diminished (PE20:4). GO annotation was performed using 43 changed metabolites, leading with reduced glycerophospholipid metabolism and Nicotinate/Nicotinamide metabolism. Those altered metabolites were processed to demonstrate areas-under-the-curve (AUCs) for treatment status. 4 types of PCs (14:0, 18:1, 18:2, 20:1) are considerably valuable to reflect treatment status (AUC=1).

Conclusion: In conclusion, we illustrated changes of serum proteins and metabolites under uc-MSC treatment in cirrhotic patients, corroborating that uc-MSC alleviated dysregulation of metabolic function. Expression profiling of glyceryl phosphatides, especially PC and lysoPC, provides a mechanistic insight into the efficacy of uc-MSC in patients with hepatitis B cirrhosis.

❖OP-008

24-week combination treatment of TLR7 agonist TQ-A3334 and PD-L1 inhibitor TQ-B2450 enhanced HBsAg reduction in NAs-suppressed CHB patients: a preliminary analysis of a phase II study (OCEANcure05)

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Object/Background: Curing chronic hepatitis B (CHB) relies on the orchestration of innate and adaptive immunity. Toll-Like Receptor-7 (TLR-7) can induce the production of IFN-α and activate NK cells. TQ-A3334 is a selective orally TLR-7 agonist and potentially activates innate immune to hepatitis B virus (HBV). Upregulated PD1 on T cells and PD-L1 on liver cells cause exhaustion of virus-specific T cells and facilitate HBV chronicity. TQ-B2450, a humanized PD-L1 antibody, inhibits PD1/PD-L1 axis and rouses adaptive immune response to HBV. This ongoing study aimed to evaluate the safety and efficacy of nucleos(t)ide analogs (NAs) and TQ-A3334 combined with/without TQ-B2450 in CHB patients.

Methods: In this single-center, randomized, prospective, open-label Phase II clinical trial (NCT04202653), 24 NAs-suppressed CHB patients (250 ≤IU/ml HBsAg ≤ 5000 IU/ml, HBV DNA <100 IU/ml) were randomized to receive NAs alone (n=6), NAs + TQ-A3334 double combination therapy (n=9) or NAs + TQ-A3334 + TQ-B2450 triple combination therapy (n=9) for 24 weeks. In each cohort, the HBeAg positive and HBeAg negative patients were enrolled in a ratio of 1:2. TQ-A3334 (1.2mg: 1.5mg = 2:1) was administrated weekly in each combination therapy group, and TQ-B2450 (400mg) was administrated every 3 weeks.

Results: At week 24, compared to NAs monotherapy and double combination therapy, triple combination therapy was associated with greater HBsAg reduction (-0.04 vs -0.03 vs −0.37 log10 IU/ml, Figure 1A). Among the triple combination therapy， three patients (33%) had HBsAg reduction > 0.5 log10 IU/ml, and two of them (67%) experienced ALT elevation at week 12. Two patients (22%) had HBsAg declined to < 100 IU/ml, and one (50%) experienced ALT elevation at week 12. The maximum HBsAg reduction was -1.4 log10 IU/ml. The patients administrated with 1.5mg TQ-A3334 in triple combination group had a prominent HBsAg reduction compared with the 1.2mg TQ-A3334 administration

(-0.66 vs −0.25 log10 IU/ml, Figure 1B). However, this difference was not observed in double combination group. Adverse effects (AEs) were more common in TQ-B2450 including therapy. Grade 1 thyroiditis (n=5) was the most common immune-related AEs.

Conclusion: The combination of NAs, TQ-A3334 and TQ-B2450 induced sharper HBsAg decline in NAs-suppressed CHB patients. Moreover, the ALT elevation and high dose of TQ-A3334 were associated with greater HBsAg reduction in TQ-B2450 included triple combination.

Figure:

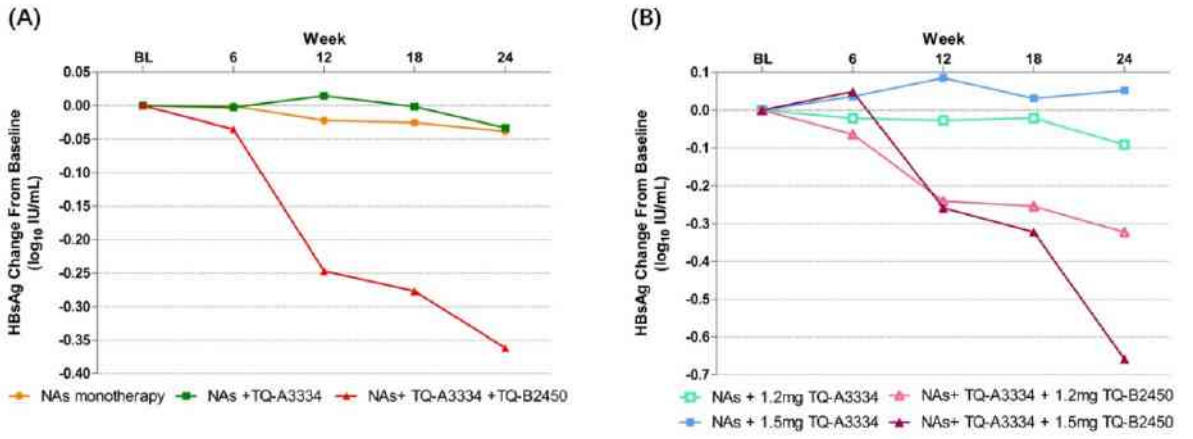

Figure1. (A) HBsAg change from baseline in NAs monotherapy, NAs+TQ-A3334 double combination therapy and NAs+TQ-A3334 +TQ-B2450 triple combination therapy. (B) HBsAg change from baseline in different TQ-A3334 dosage group.

❖OP-009

Immunogenicity of Inactivated COVID-19 Vaccines in Chronic Hepatitis B Patients with Antiviral Therapy

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Object/Background: To investigate the effect and mechanisms of different anti-hepatitis B drugs on the immunogenicity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines in patients with chronic hepatitis B (CHB).

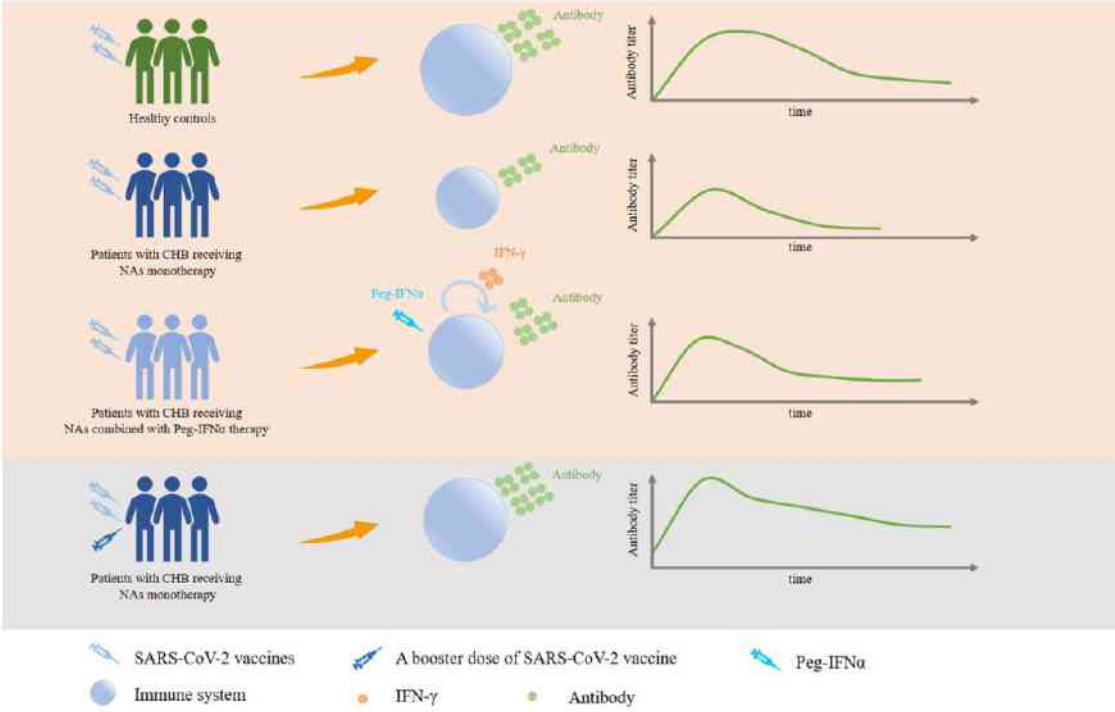
Methods: A total of 125 patients with CHB receiving nucleos(t)ide analogs (NAs) monotherapy or combined with Peg-interferon-alpha (Peg-IFNα) therapy and 29 healthy controls (HCs) were enrolled. Adverse reactions (ADRs) and levels of neutralizing antibody (NAb), IgG, IgM, and peripheral cytokines post-vaccination were analyzed.

Results: All ADRs were tolerable in CHB patients. Overall, there was no significant difference in the antibody levels between patients and HCs after two doses of vaccines. An inverse correlation between NAb and IgG titers and the days after two doses was observed in non-IFN group but not in IFN group. Correspondingly, peripheral interferon-γ levels were significantly higher in IFN group than in non-IFN group. After a booster dose, NAb and IgG antibodies were maintained at high levels in NA-treated patients.

Conclusion: Peg-IFNα-based therapy may help to maintain the immunogenicity of SARS-CoV-2 vaccines in CHB patients, which may be related to the high levels of IFN-γ induced by Peg-IFNα therapy. A booster dose can effectively recall the robust and long-lasting immunogenicity of SARS-CoV-2 vaccines.

❖OP-009

Figure:



❖OP-010

Lipid nanoparticle-mediated delivery of mRNA vaccine for IL-21 achieves clearance of hepatitis B virus (HBV) persistence in mouse models

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Object/Background: Covalently closed circular DNA (cccDNA) is the transcription template for hepatitis B virus (HBV) RNAs and not affected by current treatment options. Therefore, effective therapeutics with ability to remove cccDNA need to be developed. Previously, we established an HBV persistence mouse model via hydrodynamic injection of a clinical isolate (BPS) and identified IL-21 as a potent inducer of viral clearance.

Methods: We developed a therapeutic IL-21 mRNA vaccine based on lipid nanoparticle delivery system (LNP-IL-21) and analyzed its safety, expression, biodistribution and stability in vitro and in vivo. Next, to investigate the antiviral effects, LNP-IL-21 was injected into two HBV persistence mouse models based on BPS and recombinant cccDNA (rcccDNA) respectively.

Results: LNP-IL-21 administration successfully cleared HBV serum markers, and more importantly, BPS and rcccDNA in livers, which was associated with activation of viral specific immune responses. Notably, transfer of peripheral blood mononuclear cells from BPS persistence mice stimulated ex vivo by LNP-IL-21 and viral antigen could induce HBV clearance in recipient mice.

Conclusion: LNP-IL-21 has the ability to clear HBV both in serum and liver in persistent HBV infection mouse model, thus having great potential to be utilized as a therapeutic vaccine.

❖OP-011

Subcutaneous PD-L1 Antibody ASC22 (Envafolimab) plus Nucleos(t)ide Analogs Achieved Functional Cure of Chronic Hepatitis B in 42.9% of Patients with HBsAg ≤100 IU/mL: Interim Results from a Phase IIb Clinical Trial

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8. Nanjing Second Hospital
9. West China Hospital of Sichuan University
10. The Second Affiliated Hospital of Anhui Medical University
11. Shanghai Tongren Hospital
12. Xiangya Hospital of Central South University
13. The First Affiliated Hospital of Wenzhou Medical University
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16. Ascletis BioScience Co., Ltd

Object/Background: Patients with chronic hepatitis B (CHB) infection are characterized by a population of exhausted T cells, which have weak virus-specific T-cell responses, hindering the clearance of virus and recovery from hepatitis. Exhausted T cells have sustained expression of programmed cell death-1 (PD-1) and its ligand, PD-L1, is over-expressed in the liver of CHB patients, suggesting that PD-1/PD-L1 pathway might participate in T cell dysfunction, leading to the chronicity of hepatitis B virus (HBV) infection. This study aimed to evaluate the efficacy and safety of ASC22 for treating CHB patients on nucleos(t)ide analogs (NAs).

Methods: In this phase 2, single-blinded, placebo-controlled, multi-center study (NCT04465890), 149 eligible patients were enrolled, randomized to be treated with 1.0 mg/kg ASC22 (n=60), 2.5 mg/kg ASC22 (n=59), or PBO (n=30) once every 2 weeks (Q2W) plus NAs for 24 weeks, and then followed for another 24 weeks. As May 31 2022, 47, 19 and 21 in ASC22 1.0mg/kg, ASC22 2.5 mg/kg and PBO groups have completed the 24-week treatment and the 24-week follow-up. Since less than 50% of patients in ASC22 2.5 mg/kg group have completed the 48-week study, only data of patients in ASC22 1.0mg/kg and PBO groups who completed study is presented here.

Results: Baseline characteristics between ASC22 and PBO groups were comparable. Patients receiving ASC22 plus NAs showed more significant HBsAg decline. HBsAg reduction was correlated with patients' baseline (BL) HBsAg level. Patients with BL HBsAg ≤100 IU/ml had more significant HBsAg reduction upon ASC22 plus NAs treatment. Three out of 7 patients (42.9%) with BL HBsAg ≤100 IU/ml obtained HBsAg loss at Weeks 4, 16 and 16 as shown in Figure 1A, 1B and 1C, respectively. Two patient with HBsAg loss stopped to take NAs 3 days and 3 months later after completing the 24-week treatment, and their HBsAg maintained negative until the end of study (Week 48). A transient seroconversion of anti-HBs was even observed in one patient at Week 28 (Figure 1A). There was no study drug-related serious adverse event and most adverse events were mild (97%).

Conclusion: Subcutaneous administration of ASC22 Q2W for 24 weeks is shown to be safe and well-tolerated, and can induce HBsAg decline, even HBsAg loss, in CHB patients with HBsAg ≤100 IU/mL. A prospective study in a larger patient population will be soon initiated to verify our findings.

❖OP-011

Figure:

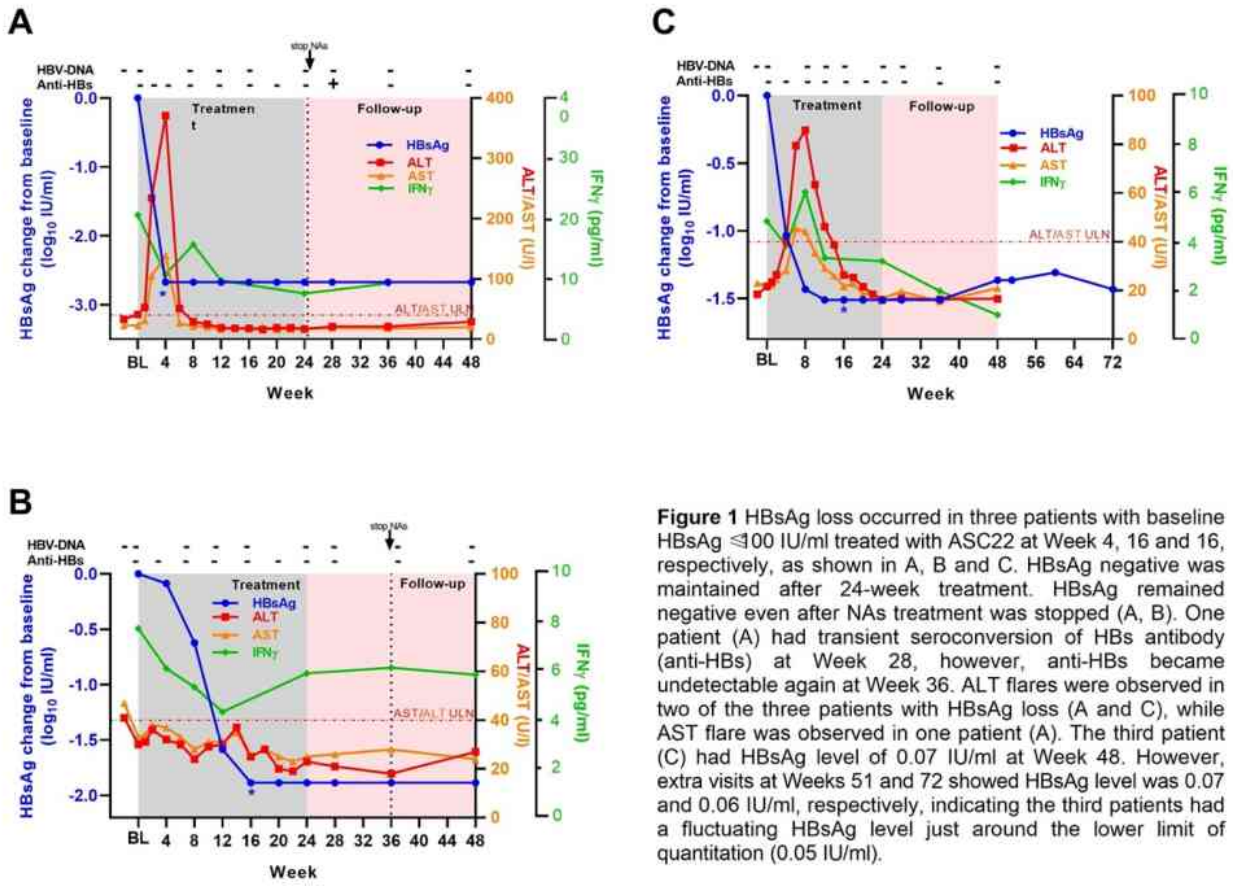

Figure 1 HBsAg loss occurred in three patients with baseline HBsAg ⩽100 IU/ml treated with ASC22 at Week 4, 16 and 16, respectively, as shown in A, B and C. HBsAg negative was maintained after 24-week treatment. HBsAg remained negative even after NAs treatment was stopped (A, B). One patient (A) had transient seroconversion of HBs antibody (anti-HBs) at Week 28, however, anti-HBs became undetectable again at Week 36. ALT flares were observed in two of the three patients with HBsAg loss (A and C), while AST flare was observed in one patient (A). The third patient (C) had HBsAg level of 0.07 IU/ml at Week 48. However, extra visits at Weeks 51 and 72 showed HBsAg level was 0.07 and 0.06 IU/ml, respectively, indicating the third patients had a fluctuating HBsAg level just around the lower limit of quantitation (0.05 IU/ml).

❖OP-012

Clinical evaluation of metagenomic next-generation sequencing method for the diagnosis of suspected ascitic infection in patients with liver cirrhosis in a clinical laboratory

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Object/Background: Bacterial infections, particularly spontaneous bacterial peritonitis, might play an important role in the progression of liver diseases. Etiological diagnosis is always the most important part in the diagnosis of infectious diseases. However, traditional culture has obvious deficiencies in practical application, such as low sensitivity and long period. Therefore, early and accurate identification of pathogens is essential for precision therapy and reducing the incidence of drug resistance. Metagenomics next-generation sequencing has emerged as an important method in detecting emerging, rare, co-infectious pathogens owing to its advantages of rapid and unbiased testing, a broad range of pathogen detection, and high accuracy. Currently, metagenomics next-generation sequencing detection is mostly carried out by the laboratory-developed test model in independent clinical laboratory at home and abroad. However, knowledge on this technological detection has improved with the use of this technology in clinical practice that the demand to detect pathogens in clinical laboratory affiliated with hospitals has gradually increased. We aimed to establish and evaluate a set of complete metagenomics next-generation sequencing assay in a clinical laboratory affiliated with a hospital. The sequencing was conducted to characterize the pathogenic spectrum of infectious ascites and analyze its clinical value and feasibility for infection diagnosis.

Methods: The study design is composed of laboratory and clinical studies. First, the metagenomics next-generation sequencing detection workflow was established in a clinical laboratory and the performance metrics were evaluated using two panels constructed with 12 common strains appearing in peritoneal infection. Additionally, 211 patients with cirrhosis were retrospectively enrolled from the Liver Disease Center, Beijing YouAn Hospital, Capital Medical University between May 2020 and September 2021. The secondary peritonitis and incomplete clinical data were excluded. The ascitic samples of the first baseline cohort from 205 patients were obtained. The metagenomics next-

generation sequencing was used to investigate the ascites microbiome and analyze the clinical diagnosis value compared with culture and composite standards (digital droplet PCR and clinical adjudication). Finally, we prospectively enrolled eight patients with suspected or confirmed peritoneal infection. The guiding significance of antibiotics treatment and clinical outcomes was further analyzed.

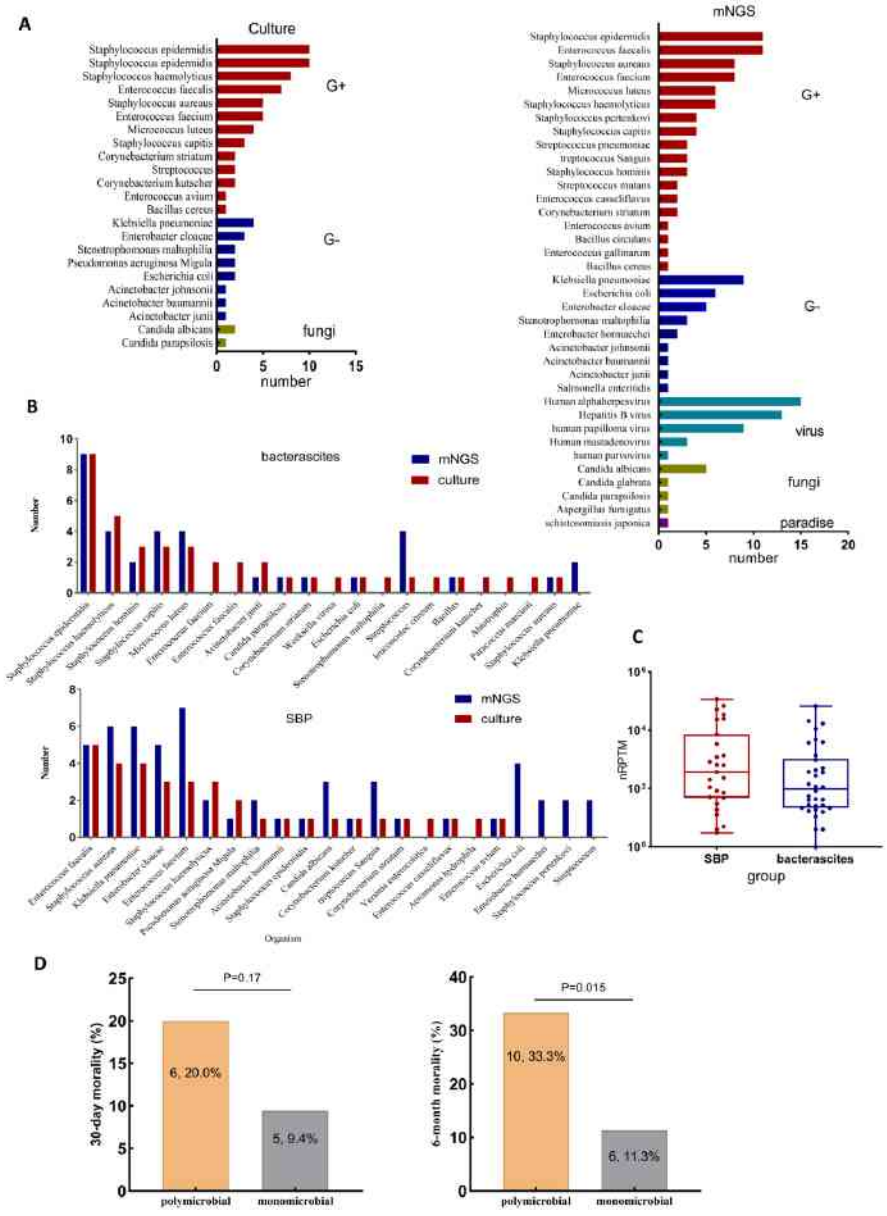
Results: The Threshold for Detected Pathogens and Test Accuracy
The analytical performance of metagenomics next-generation sequencing showed that the assay had great linearity, specificity, stability, interference and limits of detection of 33–828 colony forming units/ml and the workflow time is approximately 24 hours. The test sensitivity, specificity, positive predictive value, and negative predictive value for bacterial or fungal detection using culture standard were 84.2%, 82.0%, 64.0%, and 93.2%, respectively. After adjustment using digital PCR and clinical judgment, the sensitivity and specificity increased to 87.2% and 90.1%, respectively, and the positive predictive value increased to 82.0%.
Analytical Pathogens Characteristics in Patients with Infected Ascites
The ascitic pathogen analysis of 205 patients showed that the positive culture was 34.6% (71/205) and the positive metagenomics next-generation sequencing detection was 40.5% (83/205). For the microbial analysis, the detection accuracy of metagenomics next-generation sequencing (n=37 18 G+, 9 G-, 4 fungi, 5 viruses and 1 parasite) was higher than that of culture (n=23 13 G+, 8 G-, 2 fungi) in terms of pathogen numbers and spectrum (Figure 1A). According to the clinical composite diagnosis, 66 patients were diagnosed with SBP of the 103 patients with suspected peritoneal infection, among which the leading pathogens were E. faecalis, E. faecium, S. aureus, K. pneumoniae, E. coli, E. cloacae and among others (Figure 2B). These bacteria were also the common pathogens reported in previous ascitic studies. The main bacteria in 37 patients with bacterascites were S. epidermidis, S. haemolyticus, Micrococcus luteus, and Streptococcus (Figure 2B), with relatively weak pathogenicity. Additionally, the normalized reads per ten million count showed that the DNA concentration of patients with peritonitis was higher than that of patients with bacterascites, although the difference was not statistically significant (n=0.15, Fig 2C).
Diagnostic Efficiency of Metagenomics Next-generation Sequencing Testing in Patients with Spontaneous Bacterial Peritonitis

Of the 66 patients diagnosed with spontaneous bacterial peritonitis, pathogens were confirmed in 39 patients by culture and metagenomics next-generation sequencing and clinical adjudication. For Gram-positive bacteria, metagenomics next-generation sequencing results showed a sensitivity, specificity, positive predictive value and negative predictive value of 93.1%, 90%, 96.4%, and 81.8% (vs. 72.4%, 90%, 95.5%, and 53% of culture), respectively. For Gram-negative bacteria, metagenomics next-generation sequencing results showed a sensitivity of 93.8% and a negative predictive value of 95.8% (vs. 81.3% and 88.5% of culture). Microbial characterization showed that polymicrobial infection accounted for 49% (vs. 9% of culture, $P<0.05$). In addition, peritonitis caused by Gram-negative bacteria may be associated with mixed infection of multiple microorganisms ($P<0.05$, by Chi-square test). The 6-month mortality of polymicrobial infection was higher than that of monomicrobial infection ($P=0.15$, Figure 2D). Of eight patients prospectively enrolled in our study, three patients showed monomicrobial infection by culture, whereas metagenomics next-generation sequencing found polymicrobial infection of gram-positive bacteria combined with fungi and gram-positive bacteria combined with gram-negative bacteria. After the timely adjustment of antibiotics according to the results of sequencing, the patients were discharged with improved symptoms.

Conclusion: Metagenomics next-generation sequencing in clinical laboratories affiliated with a hospital reduced the risk of sample transfer and promoted the communication and determination of infections and pathogens for clinicians and laboratory inspectors. The pathogenic results were rapidly obtained within 24 h using this assay compared to culture, particularly in patients with polymicrobial infections. Metagenomics next-generation sequencing provided a basis for further antibiotic use. However, considering spectrum characteristics and test cost, pertinent pathogen panels should be developed in clinical practice.

❖OP-012

Figure:



❖OP-013

Metabolic Factors and Steatosis Increase the Risk of Adverse Outcomes in Treatment-Naïve Chronic Hepatitis B Patients with Normal Alanine Aminotransferase

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Object/Background: Recent studies found chronic hepatitis B (CHB) with normal alanine aminotransferase (ALT) still have hepatic inflammation and fibrosis. Some experts even believed that the ALT threshold for antiviral therapy should be lowered. But the increasingly common metabolic syndrome (MS) and hepatic steatosis have not received enough attention in this topic. We aimed to elucidate the impact of MS and non-alcoholic fatty liver disease (NAFLD) on treatment-naïve chronic hepatitis B patients with normal ALT.

Methods: The study included 733 treatment-naïve CHB patients with normal ALT who underwent transient elastography at the Department of Infectious Disease, Tongji Hospital, Wuhan, China from October 2018 to July 2021. In this study, metabolic disorder (MD) was defined as meeting one or more of the five components of MS. According to whether there was MD and/or NAFLD, patients were divided into None group, MD group and MD+NAFLD group. Then their clinical characteristics were analyzed, and the relationship among metabolic factors, steatosis and fibrosis in treatment-naïve CHB with normal ALT were studied. ALT ≤ 0.5 ULN was stratified as low-normal ALT (LNALT) and 0.5 ULN < ALT ≤ ULN as high-normal ALT (HNALT). Then their clinical characteristics were analyzed, and the roles of metabolic factors and steatosis in treatment-naïve CHB patients with normal ALT were explored. Multivariate analysis was used to identify risk factors associated with ALT elevation and liver fibrosis. Transient elastography was used to evaluate liver steatosis and fibrosis.

Results: Among 733 CHB patients enrolled, 23.1% of them had MS, 37.2% of them had NAFLD and 5.9% of them had significant fibrosis. With the emergence of MD and NAFLD, the obesity-related indicators including body mass index, waist circumference and hip circumference increased. The liver function, fasting blood glucose, blood lipids and blood pressure levels also increased. Controlled attenuation parameters (CAP) and liver stiffness measurement (LSM) were also significantly increased. No significant differences were

seen in age between the HNALT group and the LNALT group. The proportion of male patients was significantly higher in the HNALT group than in the LNALT group ($p < 0.001$).

The proportions of patients with MS, steatosis and significant fibrosis in the HNALT group were higher than those in the LNALT group ($p < 0.05$). In the study population, CAP values increased with the accumulation of metabolic syndrome components, and LSM values increased with the increasing steatosis grading. Multiple linear regression showed that age, male sex, aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), hepatitis B virus deoxyribonucleic acid (HBV DNA) load, steatosis and MS were independently related to ALT levels. When analyzed in the CHB with NAFLD subgroup and CHB without NAFLD subgroup, age, sex, AST, GGT and MS were significant in both groups, whereas the HBV DNA load was significant only in the CHB without NAFLD subgroup. Multivariate logistic regression showed that age (OR 1.049, 95% CI 1.012-1.087, $p = 0.010$), AST (OR 1.059, 95% CI 1.005-1.115, $p = 0.030$) and severe steatosis (OR 2.559, 95% CI 1.212-5.403, $p = 0.014$) were independently associated with significant fibrosis. When analyzed in the subgroup of CHB with NAFLD, age (OR 1.060, 95% CI 1.006-1.117, $p = 0.029$) and severe steatosis (OR 2.962, 95% CI 1.126-7.792, $p = 0.028$) were still statistically significant, whereas AST (OR 1.256, 95% CI 1.097-1.437, $p = 0.001$) and GGT (OR 1.044, 95% CI 1.011-1.078, $p = 0.008$) in the CHB without NAFLD subgroup.

Conclusion: The additive components of metabolic syndrome exacerbated hepatic steatosis, and severe steatosis was independently associated with significant fibrosis. In addition， AST levels may help predict the risk of liver fibrosis in this population. Our study highlights the importance of screening for metabolic factors and steatosis in CHB patients with normal ALT, which may require more aggressive intervention.

❖OP-014

High rates of HBV functional cure among HIV/HBV coinfected Chinese adults on antiretroviral therapy

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Object/Background: Achieving functional cure of chronic hepatitis B virus (HBV) infection (hepatitis B surface antigen [HBsAg] seroclearance/loss) in human immunodeficiency virus (HIV)/HBV coinfected individuals remains an elusive therapeutic target, particularly in China. As such, the aim of this study was to examine HBsAg loss and evaluate the predictors of HBsAg loss in a Chinese HIV/HBV coinfected cohort receiving long-term tenofovir-based antiretroviral therapy (ART).

Methods: In this retrospective, longitudinal observational cohort study, all HIV/HBV coinfected adults treated with tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF)-based therapy from January 2010 to March 2021 were recruited in Tianjin (China) following additional specific criteria. In addition, their demographic, clinical, and virologic data obtained before and during ART were collected. The gathered data were subsequently utilized to evaluate the rate of HBsAg loss and multivariable logistic regressions were used to assess the factors associated with HBV functional cure.

Results: A total of 240 HIV/HBV coinfected adults receiving TDF or TAF were selected. Their median baseline CD4+ T-cell count was 245 cells/μL and 41.2% were HBeAg positive. The findings indicate that 27 (11.3%) patients (median follow-up of 6.7 years) achieved HBV functional cure. Multivariable regression analysis revealed that patients with (i) longer duration on ART (p= 0.016), (ii) higher CD4+ T-cell gain 1 year after ART initiation (p= 0.005), and (iii) HBeAg positive at baseline (p= 0.012) were more likely to lose HBsAg.

Conclusion: This real-world study in China revealed that HIV/HBV coinfected patients receiving long-term TDF-/TAF-based ART achieve high rates of HBV functional cure. Furthermore, immune recovery seems to play a critical role in the substantial HBsAg-seroclearance rates. However, the mechanisms behind this phenomenon remain unknown. The high incidence of HBsAg loss in HIV/HBV coinfected adults may help in

paving the way toward development of potential strategies in the hunt for a functional HBV cure.

❖OP-015

Metagenomic Next-Generation Sequencing Outperforms Culture-Based Methods in the Diagnosis of Ascitic Fluid identifying pathogens in Patients with cirrhosis

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Object/Background: The effectiveness of metagenomic next-generation sequencing (mNGS) in identifying pathogens is being studied. Our aim was to compare the microbial recognition ability of mNGS and various methods in patients with cirrhotic ascites.

Methods: 78 patients with cirrhotic ascites were reviewed, the ascites fluid samples were examined by mNGS and conventional methods to assess the ability of mNGS and conventional methods in microbial identification. The clinical characteristics and the detected microbial conditions were analyzed.

Results: The NGS-based method showed a higher sensitivity on 58 (74.4%) samples than the culture-based method (23.1%).mNGS outperformed the conventional method in the positive detection rate of Bacteroides($P < 0.05$),Acinetobacter($P < 0.05$),Pseudomonas($P < 0.05$),Actinomyces($P < 0.05$),Mycobacterium($P < 0.001$),Candida($P < 0.01$),humancytomegalo virus(HCMV)($P < 0.001$),herpes simplex virus(HSV)($P < 0.001$).Analysis of clinical data showed a correlation between sequencing results and various clinical parameters such as Hemoglobin and red-cell count(RBC).

Conclusion: Our results indicate that the mNGS method has a higher sensitivity to the identification of clinically relevant pathogens than the standard microbial culture diagnosis in ascites infection. Patients with such infections may benefit from the use of mNGS methods, which may be earlier and better targeted treatment, which has the potential to reduce high morbidity and mortality in patients with cirrhotic ascites.

❖OP-016

A low albumin to globulin ratio is a prognostic marker for bacterial infection in alcohol-related liver cirrhosis: a retrospective study

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The First Hospital of China Medical University

Object/Background: and aims: Bacterial infections are an important reason for administration and prognostic factor in alcohol-related liver cirrhosis. Albumin to globulin ratio (AGR) has been proven related with mortality and survival in different population. Thus, the main aim of this study is to explore the role of albumin to globulin ratio on bacterial infection in patients with alcohol-related liver cirrhosis.

Methods: This is a retrospective study. Data of alcohol-related liver cirrhosis patients in the First Hospital of China Medical University were studied. According to the cut-off value of baseline AGR on the receiver operator characteristic curve, patients were divided into two groups: group 1 included patients with AGR<0.98, group 2 included patients with AGR>/=0.98.

Results: One-hundred-twenty-eight consecutive patients were enrolled in this study; 48 patients were in group 1 and 80 patients were in group 2. The mean follow-up was 34.7 months. A total of 46 patients experienced bacterial infection during follow-up, including 27 patients in AGR<0.98 group and 19 patients in AGR>/=0.98 group. Patients in AGR<0.98 group showed lower platelet count, hemoglobin, and albumin (p<0.000 for all). While serum globulin, bilirubin, bile acid, prothrombin time, and international normalized ratio were much higher in these patients (p<0.000 or p<0.05). Multivariate analysis showed that decreased AGR (hazard ratio=0.390, 95% CI 0.172–0.887, p=0.025) was an independent prognostic factor associated with increased risks of bacterial infection in this cohort.

Conclusion: A low AGR was associated with increased risks of bacterial infection in alcohol-related liver cirrhosis patients. AGR could be used as a simple tool to assess the on prognosis of bacterial infection in these patients.

Figure:

Parameter	AGR<0.98 n=48	AGR>/=0.98 n=80	*p*
Demographic characteristics			
Age (years)	51.0±8.9	55.0±10.0	<0.05
Smoking (n, %)	31 (64.6)	51 (63.8)	>0.05
Diabetes (n,%)	10 (20.8)	22 (27.5)	>0.05
Laboratory tests			
Neutrophil count (10^9/L)	3.98±3.58	3.31±2.09	>0.05
Platelet (10^9/L)	78.8±52.7	115.6±71.7	<0.000
Hemoglobin (g/L)	98.4±23.0	117.7±36.1	<0.000
ALT (U/L)	32.6±22.7	50.3±98.8	>0.05
AST (U/L)	66.4±56.2	63.3±85.9	>0.05
ALP (U/L)	145.5±96.1	112.6±85.8	>0.05
GGT (U/L)	182.6±235.1	238.1±319.2	>0.05
Albumin (g/L)	26.6±4.8	35.9±6.7	<0.000
Globulin (g/L)	36.8±6.7	27.4±4.8	<0.000
Total bilirubin (μmol/L)	74.4±88.4	37.1±56.7	<0.05
Total bile acid (μmol/L)	66.3±52.9	39.6±64.2	<0.05
Prothrombin time (s)	18.5±3.7	15.8±2.5	<0.000
International normalized ratio	1.56±0.41	1.27±0.27	<0.000
Creatinine (μmol/L)	82.0±69.3	69.1±28.4	>0.05
Alpha fetoprotein (ng/mL)	4.75±6.12	16.48±70.56	>0.05
Child-Pugh class (n,%)			
A	3 (62.5)	34 (42.5)	
B	28 (58.3)	43 (53.8)	
C	17 (35.4)	3 (3.7)	

Factors	No. of patients (%)	Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	*p*	HR (95% CI)	*p*
Neutrophils (10^9/L)	128 (100)	1.108 (1.023–1.201)	0.012	1.085 (0.996–1.182)	0.062
AGR	128 (100)	**0.355 (0.156–0.806)**	**0.013**	**0.390 (0.172–0.887)**	**0.025**
Diabetes					
No	96 (75.0)	reference			
Yes	32 (25.0)	0.618 (0.304–1.255)	0.183		
Smoking					
No	46 (35.9)	reference			
Yes	82 (64.1)	0.811 (0.438–1.502)	0.506		

❖OP-017

Clinical evaluation of bacterial DNA using an improved droplet digital PCR for the diagnosis of spontaneous bacterial peritonitis

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Object/Background: Spontaneous bacterial peritonitis is an infectious disease caused by pathogenic microorganisms that invade the abdominal cavity and cause obvious damage. The incidence rate of peritonitis has been shown to reach 40% to 70%. The diagnosis of spontaneous bacterial peritonitis is an ascites-polymorphonuclear neutrophil count that is greater than or equal to 250 cells/mm3. However, empirical antibiotics therapy that is based on the patient’s clinical symptoms and polymorphonuclear neutrophil counts can lead to the excessive use of antibiotics and the occurrence of multi-drug resistant organisms. The current traditional culture for peritoneal diagnosis have insufficient sensitivity. Therefore, it is urgent to introduce more accurate and rapid etiological diagnosis methods. Droplet digital polymerase chain reaction has the advantage of high sensitivity, simplicity and fastness. In addition, digital PCR method has an optimal applicational prospect in future clinical practice due to its detection time of 4 hours and an economical cost (e.g.$5.20 per sample). Therefore, we aimed to analyze the diagnostic value and applicational prospect of the bacterial DNA load in spontaneous bacterial peritonitis.

Methods: Study design was composed of laboratory and clinical studies. First, we developed and validated the digital PCR assay, that is the depletion of cell free DNA from ascites using Benzonase and the identification of pathogens by gram-positive and gram-negative primers and probes. And the performance metrics were analyzed. Subsequently, 250 patients with decompensated cirrhosis were collected on admission from October 2019 to April 2021. Cancerous ascites, secondary peritonitis and incomplete clinical data were excluded. The ascitic samples from 191 patients were obtained and ascitic bacterial DNA was detected by digital PCR. Receiver operating characteristic curve was plotted to establish diagnostic thresholds for peritoneal infection and analyze the patients' diagnostic accuracy based on composite standard (polymorphonuclear neutrophil and clinical manifestation). Next, we performed bacterial DNA quantification analysis on 13 patients with a PMN less than 250 cells/mm3 but with clinical symptoms. The available

consecutive samples were collected for 3 or more times to observe dynamic changes of bacterial DNA loads, one cohort was that 14 patients without infection on admission developed SBP during hospitalization and the other cohort was to analyzed the change of bacterial DNA load from 8 infectious patients (before, during, and after antibiotics).

Results: The improvement of accuracy of bacterial DNA detection in spontaneous bacterial peritonitis diagnosis compared to culture

After the depletion of cell free DNA, the bacterial DNA detected by digital PCR were generally decreased(1.75 vs. 1.5 log copies/μl, P<0.001), thus all detected DNA came from live bacteria. The bacterial DNA loads showed that the digital PCR sensitivity, specificity, positive predictive value, and negative predictive value relative to clinical composite criteria were 80.5%, 95.3%, 82.5%, and 94.7% compared to 53.7%, 88.0%, 55.0%, and 87.4% of culture, respectively (Fig 1A,B).

The bacterial DNA load facilitates the diagnosis of spontaneous bacterial peritonitis relative to polymorphonuclear neutrophil

Our study showed that bacterial DNA loads in ascites of peritoneal patients (2.8 log copies/μl) were significantly higher than that of the patients with bacterascites (1.7 log copies/μl) and the ascites without infection (2.0 log copies/μl), and the bacterial load of Gram-positive was generally lower than Gram-negative bacteria (Fig 1C). In patients with a polymorphonuclear neutrophil count below 250 cells/mm3, the bacterial DNA load of 13 patients with symptoms was significantly higher than those without symptoms (2.7 vs 1.7 log copies/μl, P<0.001, Fig 1D). We also found that the false negative rate of spontaneous bacterial peritonitis diagnosis was 31.7% when the polymorphonuclear neutrophil threshold was 250/mm3, comparing to 26.8% atthe threshold of 192/mm3, whereas the false-positive rate dropped to 19.5% at the bacterial DNA load threshold of 103 copies/μl, which indicated that the bacterial DNA load improved the diagnostic value for patients with polymorphonuclear neutrophil count below 250/mm3, and we considered that the threshold of 250/mm3 was too high for the peritonitis diagnosis, especially for Gram-positive infections.

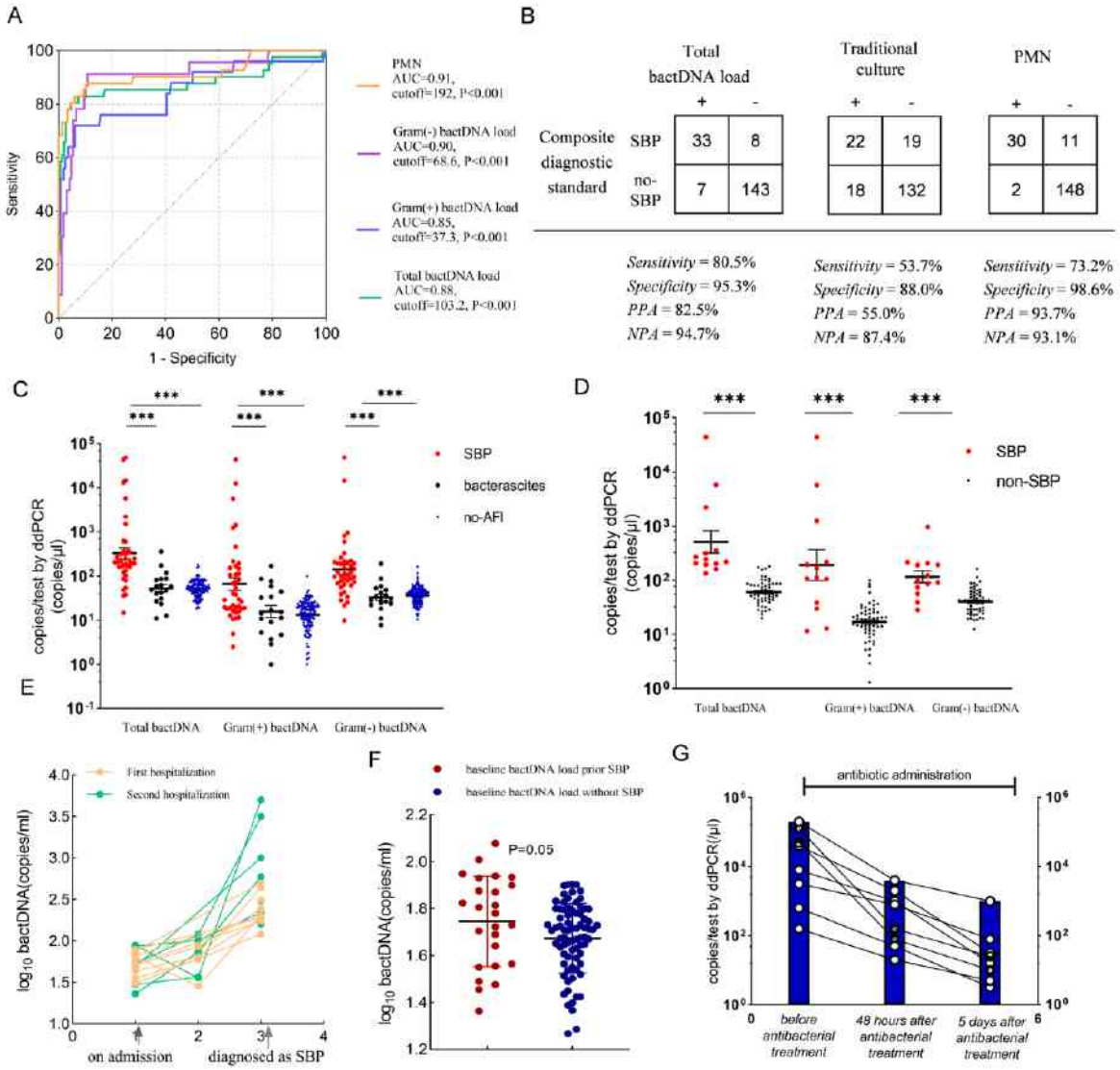
Bacterial DNA loads assist to predict the development of spontaneous bacterial peritonitis and monitor antibiotic therapy in clinical practice.

We compared the baseline bacterial DNA load with and without peritonitis development in 14 patients and found that baseline bacterial DNA load was higher in patients with peritonitis development (55.7 copies/μl vs 46.7 copies/μl, P=0.05, Fig 1E,F), indicating that

the higher bacterial DNA load, the higher incidence of peritonitis. The bacterial DNA in eight patients had peritonitis that decreased by 1.6 log copies/μl after 48 hours of antibiotic treatment and by 1.0 log copies/μl after 3 days of continued treatment (Fig 1G).

Conclusion: Bacterial DNA detection can be used to further enhance the diagnostic efficiency of spontaneous bacterial peritonitis. especially for patients who are symptomatic and have a PMN less than 250 cells/mm3. Therefore, the application of digital PCR assay can not only be used to discriminate and quantify bacteria but also be used in the clinical assessment for antibiotics treatment and prediction for peritonitis development.

Figure:



❖OP-019

Research on Factors Linked with Occurrence of Minimal/Covert Hepatic Encephalopathy and Development of Overt Hepatic Encephalopathy

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Object/Background: The clinical factors associated with the occurrence of minimal hepatic encephalopathy (MHE) and overt hepatic encephalopathy (OHE) progression are still unclear. To analyse the relationship between common laboratory indicators and neuropsychological tests with MHE incidence and OHE development.

Methods: We prospectively incorporated 123 healthy controls and 266 patients with potential hepatic encephalopathy (HE) developing diseases (mainly cirrhosis) in our study. Data from healthy group was used to get the reference standard for diagnosis of MHE/ CHE . All 266 patients were identified by the PHES, ANT, VNT, AVNT and 57 patients were assessed by Encephalstroop App. All patients were followed for the occurrence of OHE in 30 days.

Results: The prevalence rate of MHE diagnosed by the gold standard PHES was 27.1%. The risk of developing into OHE in patients with MHE (16/72, 22.2%) was over triple higher than those without MHE (13/194, 6.7%). Those who progressed for OHE suffered from decompensated liver cirrhosis (23, 79.3%), EASL-ACLF (9, 31.0%) and APASL-ACLF (26, 89.7%). Statistical analysis showed IBIL, LDH, eGFR were correlated with MHE ($p<0.05$). Multivariate regression analysis indicated that age, ammonia and K (potassium) level at baseline were independent risk factors for MHE. Age, gender, comorbidity, primary OHE, GGT, TT at the time of study inclusion were correlated with OHE development ($p<0.05$). Univariate regression analysis showed age, prior OHE, MHE, AVNT<18, complications, HE treatments (including lactulose, LOLA, probiotics, etc.), MELD, IL6, L%, TBIL, DBIL, IBIL, TBA, eGFR, PT, PTA, APTT, Offtime, OffTime+OnTime were significant in OHE progression in 28 days ($p<0.1$).However, after Multivariate regression analysis, there remained no significant independent risk factors for OHE development.

Conclusion: Age, ammonia and K (potassium) level at baseline were independent risk factors for MHE. Age, prior OHE, MHE incidence, abnormal neuropsychological test results(AVNT<18, Offtime, OffTime+OnTime), complications, HE treatments, MELD, IL6, L%, bilirubin,TBA, eGFR, coagulation function (PT, PTA, APTT) were significant in OHE progression in 28 days.

❖OP-020

Safety, Efficacy, Pharmacokinetics, and Pharmacodynamics of Recombinant Human Albumin in Cirrhotic Patients with Ascites

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Object/Background: Recombinant human albumin is an alternative to human serum albumin for the management of ascites in cirrhotic patients. This phase Ib study was designed to evaluate the safety, tolerability, and pharmacokinetics/pharmacodynamics of rHA in cirrhotic patients with ascites.

Methods: This open-label, multicenter, multi-dose, positive controlled, dose-escalation study enrolled 36 Chinese subjects divided into 3 dose cohorts. Each cohort included 12 subjects who were randomized 9:3 to receive recombinant human albumin or human serum albumin, respectively, at a dose of 10 g/day, 20 g/day, or 30 g/day, either for 14 days or until the serum albumin level reached 35g/L. All subjects were followed-up for 28 days after the treatment was concluded. The primary objective was to assess the safety, tolerability, and pharmacokinetics/pharmacodynamics of recombinant human albumin. Safety and tolerability were determined by the incidence, intensity, and seriousness of adverse events. pharmacokinetics/pharmacodynamics were assessed by monitoring serum albumin concentration and plasma colloid osmotic pressure before and after each dose of recombinant human albumin or human serum albumin. The time required for the serum albumin concentration to reach 35 g/L was also monitored.

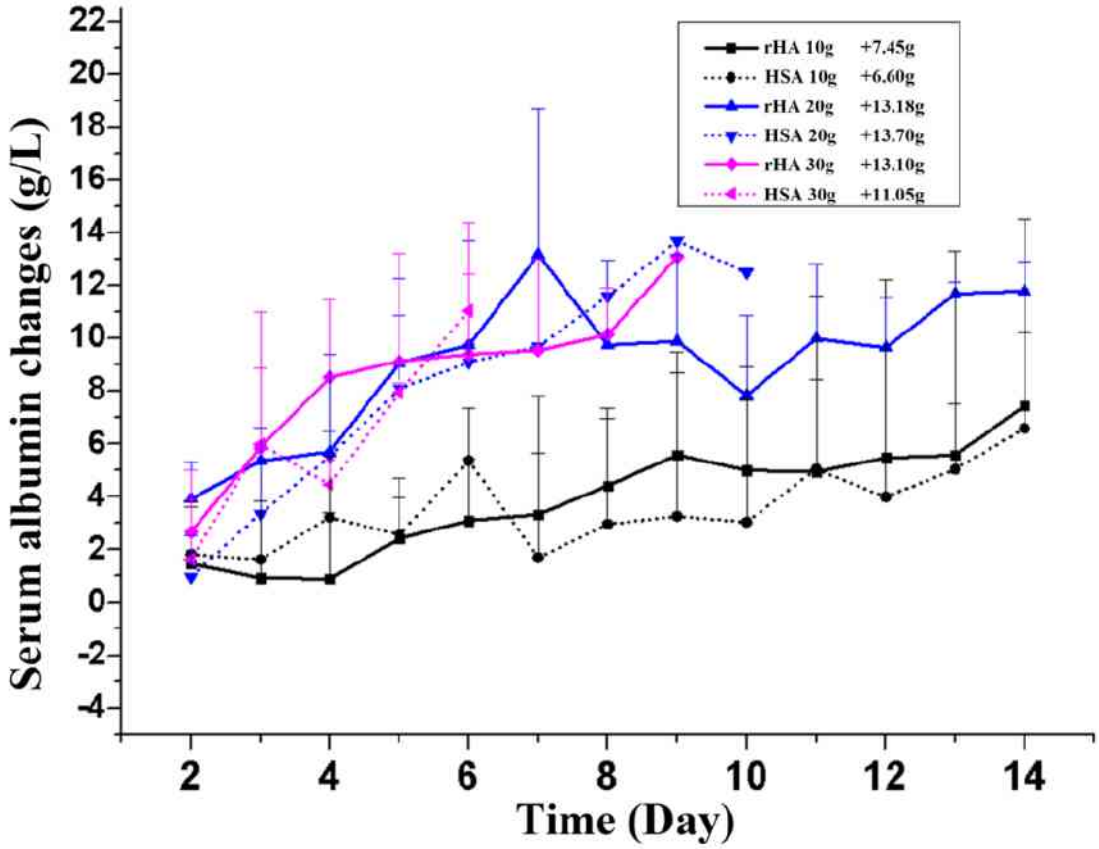
Results: Recombinant human albumin treatment was well tolerated in Chinese patients with ascites due to cirrhosis. The criteria specified in the protocol for termination of dose escalation were not met, so all 3 planned doses were tested in their respective cohorts. The incidence of adverse events was similar between the recombinant human albumin and human serum albumin cohort, and there were no dose response relationships were observed between the incidence of adverse events and the doses of the product given. Regarding the pharmacokinetics/pharmacodynamics, it was observed that the

improvement of the levels of albumin in the recombinant human albumin cohort was similar to the human serum albumin cohort during the study. The trends of changes in serum albumin concentration were similar between the recombinant human albumin cohort and the human serum albumin cohort throughout the entire study. During the treatment period, the average concentrations of serum albumin increased with increasing the doses of recombinant human albumin or human serum albumin. During the follow-up period, the serum albumin concentrations declined but remained higher than the baseline value at 29 days after the last infusion. The recombinant human albumin and human serum albumin treatments resulted in similar improvements in plasma colloid osmotic pressure. Mean plasma colloid osmotic pressure was greatly increased compared with the baseline on the second day of treatment with 20 g/day or 30 g/day of either recombinant human albumin or human serum albumin, and it was further increased by the time of the last administration. The increases of the mean plasma colloid osmotic pressure were maintained until the 29th day after the last administration, although no statistically significant differences were observed. No anti-drug antibody was found in immunogenicity study.

Conclusion: Recombinant human albumin was safe and well-tolerated in cirrhotic patients with ascites and had similar pharmacokinetics/pharmacodynamics and efficacy profiles compared to human serum albumin. These results support further evaluation of the safety and efficacy of recombinant human albumin in a phase II clinical study.

❖OP-020

Figure:



❖OP-021

Difference between type 2 gastroesophageal varices and isolated fundic varices in clinical profiles and portosystemic collaterals

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Object/Background: There is significant heterogeneity between gastroesophageal varices (GOV2) andisolated gastric varices (IGV1). The data on the difference between GOV2 andIGV1 are limited.The aim of our study was to determine the etiology, clinical profiles, endoscopic findings, imaging signs,portosystemic collaterals in patients with GOV2 and IGV1.

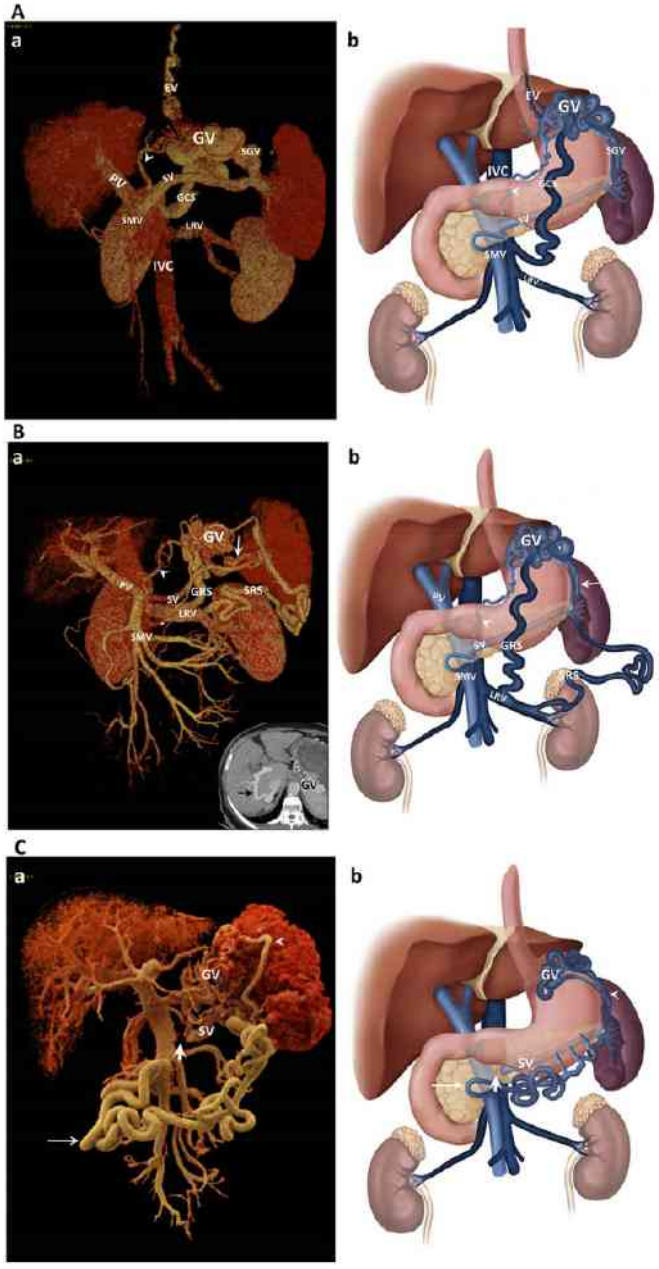
Methods: Medical records of 252 patients with gastric fundal varices were retrospectivelycollected, and computed tomography images were analyzed.

Results: Significant differences in routine blood examination, Child–Pugh classificationand MELD scores were found between GOV2 and IGV1. The incidence of pepticulcers in patients with IGV1 (26.55%) was higher than that of GOV2 (11.01%),while portal hypertensive gastropathy was more commonly found in patientswith GOV2 (22.02%) than in those with IGV1 (3.54%). Typical radiological signs ofcirrhotic liver were more commonly observed in patients with GOV2 than in thosewith IGV1. In patients with GOV2, the main afferent vessels were via the leftgastric vein (LGV) (97.94%) and short gastric vein (SGV) (39.18%). In patients with IGV1, the main afferent vessels were via the LGV (75.61%), SGV (63.41%) and posterior gastric vein(PGV) (43.90%). In IGV1 patients with pancreatic diseases, spleno-gastromental-superiormesenteric shunt (48.15%) was a major collateral vessel. In patients with fundic varices, the sizes ofgastric/esophageal varices were positively correlated with afferent vessels (LGVs and PGVs) andefferent vessels (gastrorenal shunts). The size of the esophageal varices was negatively correlatedwith gastrorenal shunts in GOV2 patients.

Conclusion: Significant heterogeneity in the etiology and vascular changes between GOV2 and IGV1 is usefulin making therapeutic decisions.

❖OP-021

Figure:



❖OP-022

Mechanism of MIF promoting inflammatory polarization of liver macrophages mediating acute liver failure

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Object/Background: Liver failure (LF), also known as Severe hepatitis, is a syndrome caused by large or sublarge necrosis of Liver tissue in a short period of time under the action of multiple pathogenic factors, resulting in Liver failure. The etiology of severe hepatitis is diverse. In the United States, Europe and Japan, drugs are the main cause of acute liver failure (ALF) (accounting for more than 50% in the United States, of which 36% are non-steroidal anti-inflammatory drugs, especially acetaminophen), while in China, it is mainly caused by hepatitis virus infection. Especially hepatitis B virus (HBV). However, the latest study estimates that the annual incidence of drug-induced liver injury (DILI) in China is about 23.80/100,000. A clinical analysis of 25,927 CASES of DILI in China suggested that 1.08% of the patients developed liver failure. The mortality rate among these patients was 43% (102/280); In addition, 44.40% of patients with hepatocellular DILI met Hy's Law criteria (ALT≥3xULN and TBL>2xULN, the prognosis is usually poor, and the fatality rate may reach 10%-50% even if relevant drugs are discontinued in the absence of biliary obstruction). Due to the diversity of clinical manifestations and pathological types, and the lack of specific diagnostic biomarkers, the diagnosis is difficult. The unavoidable use of a large number of hepatotoxic drugs in clinical practice, as well as the use of a large number of hepatotoxic herbs and dietary supplements; In addition, once liver failure occurs in DILI patients, the disease is severe, the disease develops rapidly, and the complications are numerous and difficult to treat. There is a lack of sensitive early warning indicators and effective specific intervention methods in clinical practice, resulting in high mortality. Therefore, it is necessary to further study the pathogenesis of DILI, especially drug-induced ALF, to provide scientific basis and intervention targets for clinical treatment.
At present, it is considered that in addition to the direct cellular stress caused by drugs and their metabolites, the abnormal immune response of the host under certain conditions is the main mechanism of DILI. Of the dominance of the liver is a natural immune immune organs, including macrophages Kupffer cells (Kupffer cells, KCs) occupies the highest percentage, it laid the macrophages in the pillar position in the study of liver diseases, therefore further study of innate immune stage and its regulation of macrophage function, is of great significance to understand the mechanism of liver injury. Existing studies have

shown that liver KCs, like other macrophages in the body, can be divided into two continuous polarization types according to their cell phenotypes and secreted cytokines and chemokines. That is, Classically activated M1 type and Alternatively activated M2 type macrophages. M1-type macrophages mainly mediate pro-inflammatory effects, characterized by the release of large amounts of pro-inflammatory cytokines (such as IL-12, IL-1β, IL-23 and tumor necrosis factor (TNF)), active nitrogen intermediates and reactive oxygen intermediates, and high expression of major histocompatibility complex II (MHC II) and costimulatory molecules. Highly efficient antigen presentation, as well as microbiological or antitumor activity. M2 macrophages can inhibit inflammation, regulate immunity and repair tissues by expressing anti-inflammatory factors. It exhibits more phagocytic activity, high expression of scaven receptors, glycine and galactose receptors, ornithine and polyamine production through arginase pathway, and low phenotype of IL-12, high expression of IL-10, IL-1 decoy receptors and IL-1RA. In general, these cells are involved in the TH2 response, helping with parasite clearance, suppressing inflammation, promoting tissue remodeling and tumor progression, and having immunomodulatory functions. In different histopathological environments, macrophages can affect the progression and prognosis of disease through M1/M2 polar balance shift. Macrophage migration inhibitory factor (MIF) was discovered in the late 1960s and named after its ability to inhibit Macrophage migration. Subsequent studies have found that MIF is actually a multipotent protein, which has the characteristics of cytokines, endocrine molecules, chaperone proteins and enzymes. At the same time, MIF can also act as an unassociated ligand of chemokine receptors, such as CXCR2, CXCR4 and CXCR7. These also determine the complexity of MIF's role. Binding of MIF to its cell membrane high affinity receptor CD74 (also known as HLA Class II histocompatibility antigen γ chain) results in the recruitment of the cell surface glycoprotein CD44 which then activates a variety of signaling pathways by mediating intracellular signaling, including: Mitogen-activated protein kinases/extracellular signal-associated kinases (MAPK/ERK), Src, phosphoinositol 3 kinase (PI3K)/Akt, and nuclear factor (NF) -KB pathways. We previously found that MIF expression was positively correlated with the severity of liver inflammation, and macrophages polarized towards M1 during the onset of acute liver failure animal model. Based on the previous work, this project intends to focus on macrophages and MIF molecules that play a core role in the innate immune response of liver, and combine animal model and cell model in vitro experiments to carry out in-depth research on the immunological mechanism of liver injury. Relevant research results are helpful for further revealing the pathogenesis of acute liver failure. Especially, the mechanism of immune-

mediated inflammatory injury in liver region is of great significance and will provide new ideas and methods for immunotherapy of severe hepatitis B.

Methods: 1. The mouse ALF model was established by intraperitoneal injection of excessive Acetaminophen (APAP). At 0h, 6h, 12h, 24h, 48h, and 96h after APAP injection, HE staining of liver sections and detection of ALT levels in plasma were performed to evaluate the degree of liver inflammation. Further, the expression of MIF in serum of mice was dynamically detected by Elisa, and the expression of MIF in liver at 0h, 6h and 12h was detected by Western Blot.
2. Hepatic parenchymal cells were extracted by portal vein perfusion digestion and density gradient centrifugation after injection of excess APAP at 0h and 12h. Flow cytometry was used to detect the change of proportion of M1 macrophages M1 (F4/80+CD86+) and M2 (F4/80+CD206+) in liver. The expression of MIF ligand CD74 in M1-type and M2-type macrophages was further detected.
3. Mice were randomly divided into treatment group and control group. MIF specific antibody (treatment group) and IgG antibody (control group) were injected into tail vein with high pressure within 1 hour after APAP injection, respectively. The survival time of all mice in the control group and treatment group was recorded, and the survival rate of the two groups was compared. The liver tissues were fixed, embedded and sectioned for HE staining, and the pathological changes of the two groups were observed and compared under a microscope. The levels of ALT in serum were detected to compare the degree of liver injury between the control group and the treatment group, The proportion of M1-type and M2-type macrophages in the liver of the treatment group and the control group was further detected at the peak of inflammation at 12H.
4. Bone marrow cells from femur and tibia of normal mice were cultured with macrophage colony stimulation factor. Bone Marrow Derived Macrophage (BMDM) was cultured in M-CSF medium for 7 days and induced differentiation into Bone Marrow Derived Macrophage (BMDM), which were divided into four in vitro treatment groups: Blank group, LPS group, LPS+MIF protein group and LPS+MIF antibody group. Cytometric Bead Array (CBA) was used to analyze the factors related to M1 polarization of supernatant, and PCR was used to detect the genes related to M1 polarization of macrophages in each group, Repeat the experiment three times.

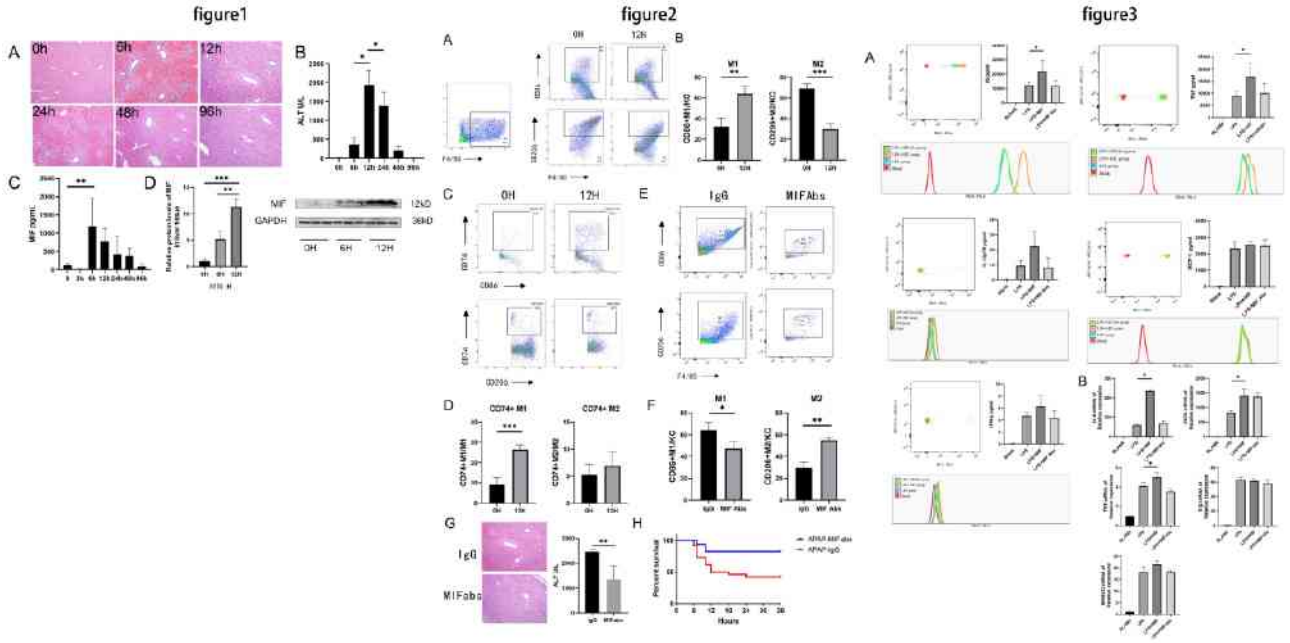
Results: 1. In ALF mice model was established successfully, 6 hours started dying mice began to appear in succession, 12 hours peak, mouse liver flake and massive necrosis,

serum ALT levels rise sharply, mice liver inflammation peak, a large number of mice in the time of death, death rate is as high as 60%, no death in mice after 24 hours, Return to normal gradually over time;
2. MIF in serum and liver of ALF model mice increased with the aggravation of hepatitis, and the recovery period of inflammation decreased gradually as time went by.
3. In healthy mouse liver macrophages, mouse liver macrophages were in a relatively anti-inflammatory state, dominated by M2 type (CD206+) macrophages, while in ALF model mice (12h after excessive APAP injection), mouse liver macrophages were in a pro-inflammatory state, dominated by M1 type (CD86+) macrophages. The expression of MIF receptor CD74 was increased in M1-type macrophages of liver in ALF model mice.
4. Compared with IgG antibody injection, HE staining necrosis and biochemical ALT were significantly reduced in MIF antibody injection group. The survival rate of mice injected with MIF antibody was nearly 80%, while that of mice injected with IgG antibody was 60%, In the MIF antibody injection group, compared with the IgG antibody injection group, the M1 polarization of liver macrophages of ALF mice decreased at the peak of inflammation 12H, and the M2 polarization increased relatively.
5. After BMDM was stimulated by LPS and MIF protein in vitro, the cytokines released by BMDM (IL-6, TNF) were significantly increased compared with the blank group, and the mRNA levels of M1-related genes (IL-6, INOS, TNF) were significantly increased in LPS+MIF protein group compared with the blank group. However, there was no significant difference between LPS+MIFabs group and LPS group.

Conclusion: 1. During the development of ALF in mice, the expression level of MIF in mice was significantly increased, and the intervention of MIF antibody could significantly reduce liver injury of ALF mice and improve the survival rate of mice. Macrophages polarized to pro-inflammatory M1 type during ALF, and MIF ligand CD74 was highly expressed in M1 type macrophages. After the use of MIF antibody, the polarization of macrophages towards pro-inflammatory M1 in ALF process was weakened. These results suggest that MIF can promote the progression of ALF, and may play a pro-inflammatory role through CD74 mediated M1-type polarization of macrophages.
2. MIF can enhance the pro-inflammatory polarization effect of LPS on macrophages in vitro. However, MIF specific antibody intervention could not reduce the pro-inflammatory polarization effect of LPS on macrophages. It is suggested that MIF can promote the polarization of macrophages, but macrophages do not produce MIF in IPS-mediated pro-

inflammatory polarization of macrophages, so the antibody is ineffective. It is speculated that MIF proteins in mice are secreted by other cells, such as liver cells.

Figure:



❖OP-023

Defection of GLT25D1 Exacerbated Con A Induced Liver Injury by Promoting Polarization of M1-type Macrophage via MAPK Signaling Pathway

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Beijing Ditan Hospital, Capital Medical University

Object/Background: Liver macrophages account for 20-25% of the hepatic interstitial cells, which contribute to the innate immunity and adaptive immunity in the liver. Glycosyltransferase GLT25D1 modified collagens or proteins with collagen-like structure, which was verified to be involved in liver fibrosis and metabolic associated fatty liver disease. But its role in autoimmune hepatitis (AIH) has not been investigated. Here we investigated the role of GLT25D1 in AIH and mainly focused on the effect of macrophages.

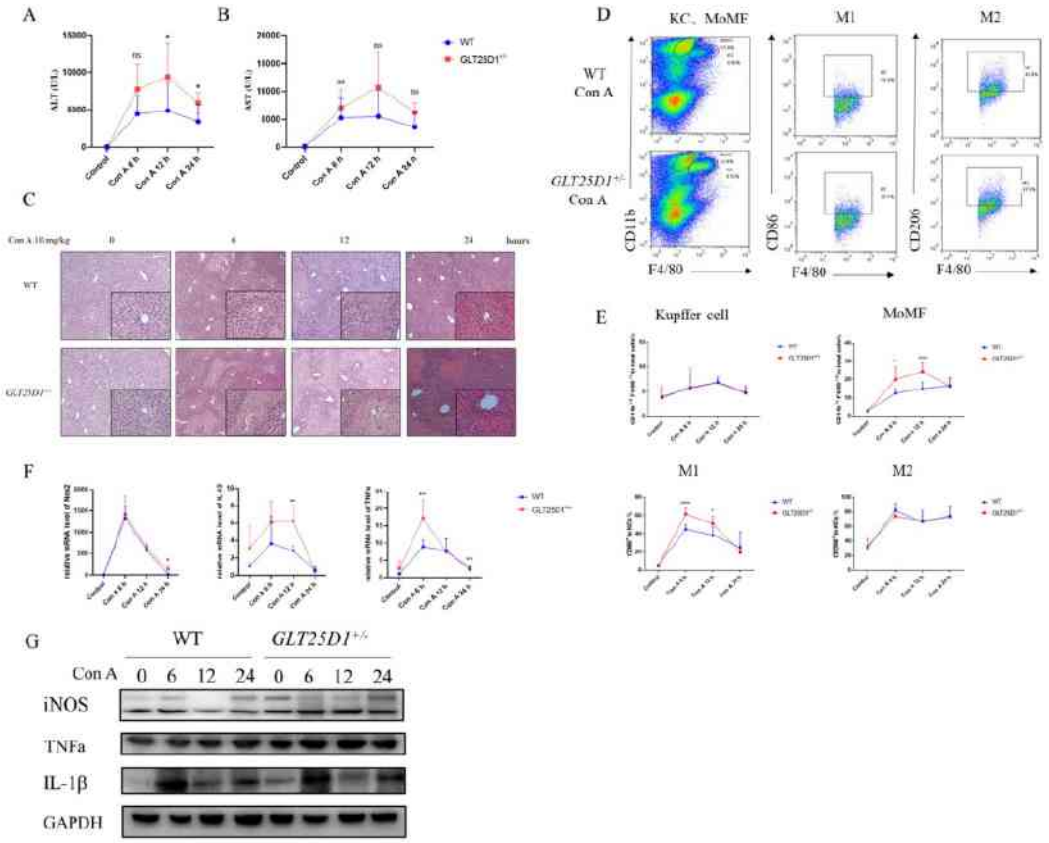
Methods: Con A (10 mg/kg body weight) was intravenously injected to establish an immune-mediated hepatitis mouse model. WT and GLT25D1+/- mice were randomly allocated into control and Con A challenged group. Liver injury was evaluated by serum aminotransferase and histological H&E staining. The percentages of M1 and M2 macrophages in liver tissues were marked by CD86 and CD206 respectively and detected by flow cytometry. Some markers of M1 (such as IL-1β, TNFα, inducible nitric oxide synthase (iNOS)) and M2 macrophages (such as CD206, Arginase-1, Ym-1) were detected by qRT-PCR and western blot. In vitro, we isolated bone marrow cells from WT and GLT25D1+/- mice, and used granulocyte-macrophage colony-stimulating factor (GM-CSF) for 7 days to induced bone marrow derived macrophage (BMDM). Then lipopolysaccharide (LPS) and IFNγ was used to derive M1 cells and IL-4 was used for M2 cells in vitro. Similarly, we detected the markers of M1 and M2 macrophages by qRT-PCR and western blot. Furthermore, MAPK signaling was detected via western blot.

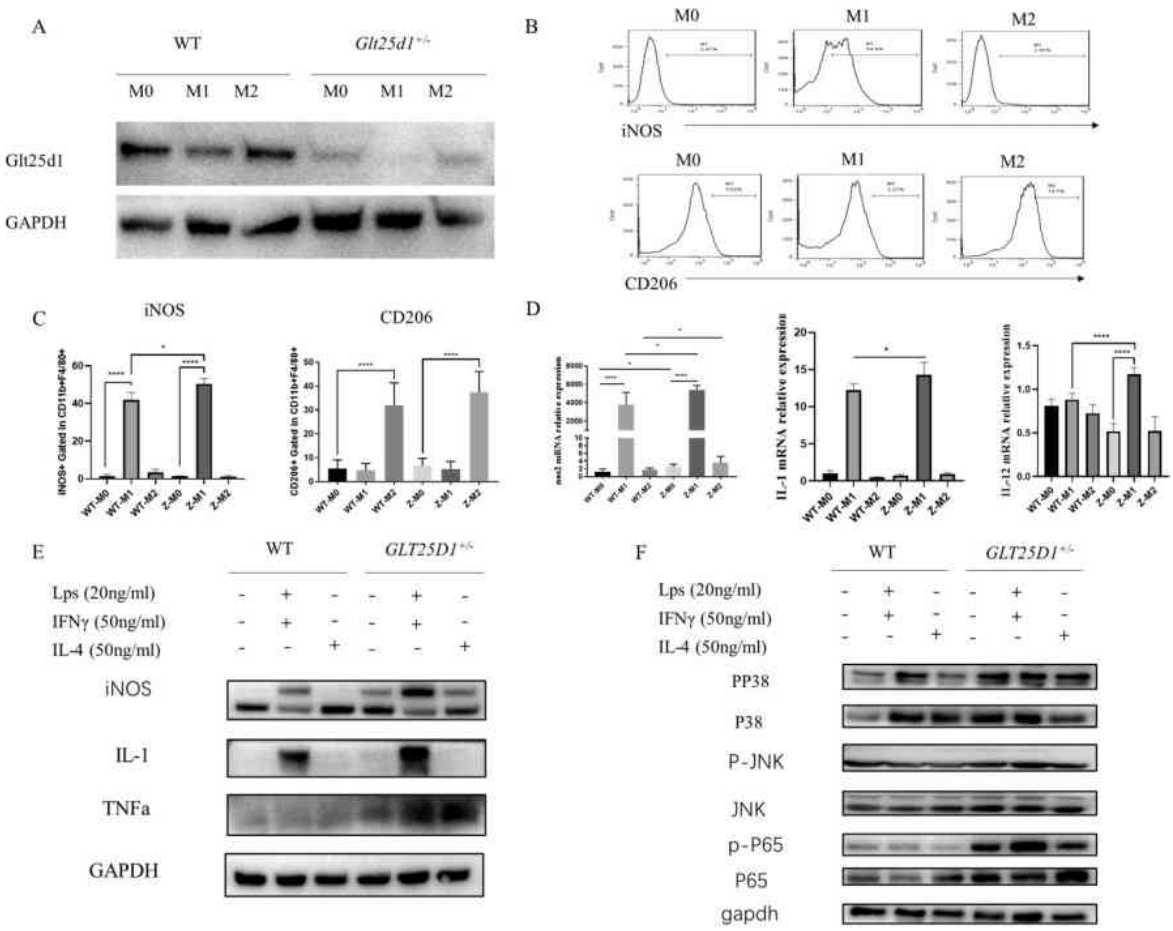
Results: We found that defect of GLT25D1 significantly elevated the levels of serum ALT and AST, and aggravated liver histopathological damage after Con A administration for 12 h and 24 h. Liver kupffer cells (CD11bintF4/80hi) and CD206+ M2 cells had no differences between WT and GLT25D1+/- mice, while monocyte-derived macrophages (MoMF, CD11bhiF4/80int) and CD86+ M1 macrophages were increased in GLT25D1+/- mice to compared with that of WT mice after Con A administration for 6 h and 12 h. Furthermore, the mRNA levels of NOS2, IL-12, and Tnfα in the liver were significantly elevated in GLT25D1 defection mice after Con A administration. Consistently, the expression levels of

iNOS, IL-1β and TNFα in liver tissues were increased in GLT25D1+/- mice compared to that of WT mice, especially in Con A challenged for 6 and 12 h. The results in vivo showed that deficiency of GLT25D1 induced the polarization of M1 macrophage. Similarly, we found that GLT25D1 was reduced in M1 cells derived from BMDM compared with that of the M0 cells. And frequency of iNOS+ M1 cells was increased in GLT25D1+/- BMDM compared with that of WT BMDM. And mRNA levels of iNOS, IL-1β and IL-12 were up-regulated in GLT25D1+/- M1 cells than that of the WT M1 macrophages. Besides, the results of western blotting showed that deficiency of GLT25D1 induced increasement of expression of iNOS, IL-1β and TNFα in M1 cells. Furthermore, we found that the levels of pp38, p-JNK and pp65, which was known as a pro-inflammatory signaling pathway, were significantly elevated in GLT25D1+/- M1 cells then that of WT M1 cells in vitro.

Conclusion: We found that defect of GLT25D1 exacerbated Con A-induced liver injury and promoted M1 macrophage polarization in vivo and in vitro, which might be related to the activation of PP38, P-JNK and NFκB signaling pathway.

Figure:




A
WT
Glt25d1+/-
M0 M1 M2 M0 M1 M2
Glt25d1
GAPDH
B
M0 M1 M2
iNOS
M0 M1 M2
CD206
C
iNOS
CD206
D
E
WT
GLT25D1+/-
Lps (20ng/ml)
IFNγ (50ng/ml)
IL-4 (50ng/ml)
iNOS
IL-1
TNFa
GAPDH
F
WT
GLT25D1+/-
Lps (20ng/ml)
IFNγ (50ng/ml)
IL-4 (50ng/ml)
PP38
P38
P-JNK
JNK
p-P65
P65
gapdh

❖OP-024

Observation on short-term effect of sequentially combined multi-modal artificial liver treatment on HBV-related acute-on-chronic liver failure

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wuxi NO.5 people's hospital

Object/Background: To observe the short-term effect of sequentially combined multi-modal artificial liver treatment (SCMALT) on HBV-related acute-on-chronic liver failure (HBV-ACLF).

Methods: A total of 86 HBV-ACLF patients undergoing artificial liver treatment in Wuxi Fifth People's Hospital from January 2018 to June 2021 were retrospectively analyzed, and they were divided into the SCMALT group and the conventional-modal artificial liver treatment (CALT) group. The clinical data of all patients were recorded and the serum levels of interleukin-6 (IL-6), interleukin-8 (IL-8) and chemokine interferon-inducible protein-10 (IP-10) were detected. The changes in 30-day survival rate, cytokine level, model for end-stage liver disease (MELD) score and complications of artificial liver treatment were analyzed. Cox regression was used to analyze the risk factors of death, and Kaplan Meier method was used to calculate the survival rate of HBV-ACLF patients. $P < 0.05$ indicated that the difference was statistically significant.

Results: After being followed up for 30 days, 63 patients survived and 23 died. At the end of the whole-course treatment, the decreases in IL-6, IL-8 and IP-10 levels and MELD score in the SCMALT group were greater than those in the CALT group. Cox regression analysis suggested that Cr level（OR=1.045，95% CI 1.009-1.083，P=0.014）,INR（OR=2.241，95% CI 0.941-5.334，P=0.048）and AT-III activity（OR=0.959，95% CI 0.901-1.020，P=0.017）at baseline, artificial liver treatment mode（OR=0.529，95% CI 0.129-2.173，P=0.003), number of artificial liver treatments（OR=0.146，95% CI 0.059-0.363，P=0.000）,post-treatment levels of IL-6(OR=0.836,95% CI 0.740-0.945，P=0.004）and IP-10（OR=0.944,95% CI 0.888-1.005, P=0.032), spontaneous peritonitis (OR=0.161, 95% CI 0.029-0.881,P=0.035）, and hepatic encephalopathy（OR=0.018，95% CI 0.001-0.219，P=0.002）were independent influencing factors of 30-day survival rate. SCMALT can

significantly prolong the survival period of the patient. There were no significant differences in the proportions of bleeding, deep vein thrombosis and circulation instability between the two groups（P >0.05）, and the decrease in platelet count in the SCMALT group was smaller than that in the CALT group.

Conclusion: Compared with the CALT, SCMALT can more effectively remove inflammatory mediators and reduce the MELD score in HBV-ACLF patients, which can significantly improve the prognosis of patients, with less effect on the platelet count.

Figure:

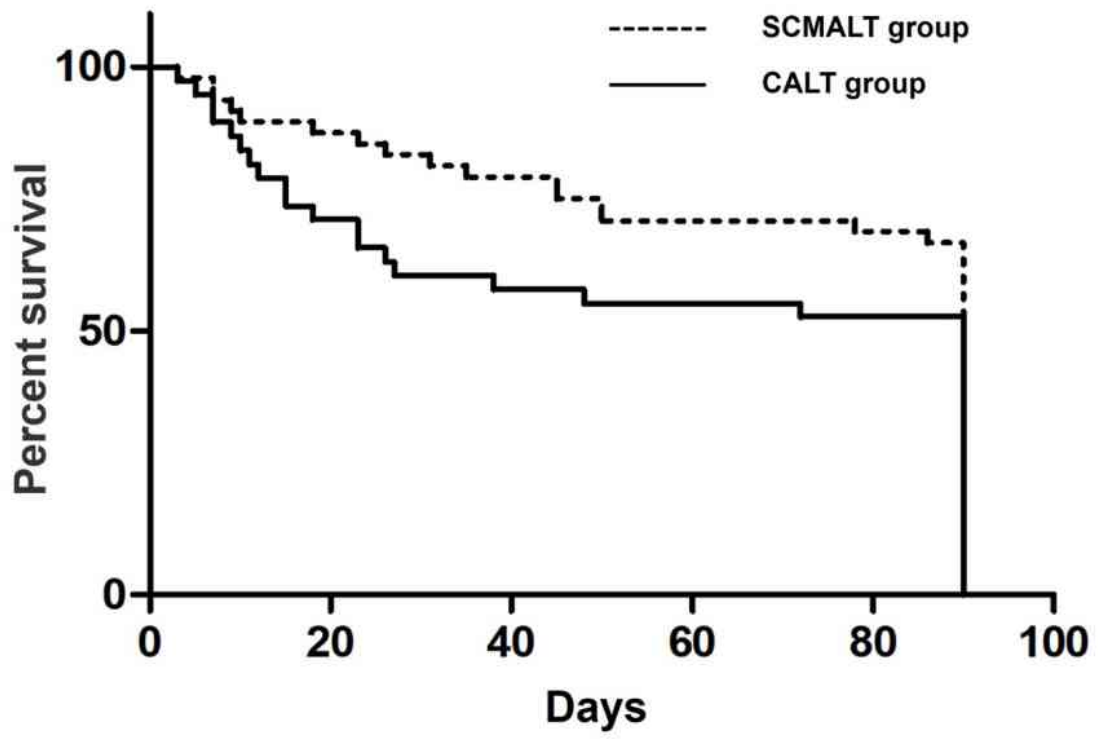

Figure 1 Comparison of survival curve of HBV-ACLF patients between the SCMALT group and CALT group

❖OP-025

G protein-coupled receptor-35 alleviates non-alcoholic steatohepatitis by reprogramming cholesterol homeostasis in hepatocytes

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Object/Background: Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. Fat accumulation “sensitizes” the liver to the insult and leads to non-alcoholic steatohepatitis (NASH), which manifests as injury, inflammation, and fibrosis of the liver. Hepatic lipotoxicity implies that exposure to, or accumulation of, certain lipid species in hepatocytes may cause cytotoxicity directly or act in a proinflammatory or pro-fibrotic manner. Potentially lipotoxic molecules include free cholesterol (FC) and free fatty acids (FFAs) and their derivatives. G protein-coupled receptor 35 (GPR35) is involved in metabolic stresses, but its role in NAFLD is not known. In this study, we investigated the effect of gain or loss of GPR35 function on the development and progression of NAFLD in mice.

Methods: To clarify the effect of GPR35 expression in hepatocytes on steatohepatitis, we generated hepatocyte GPR35 knockout (Gpr35hep−/−) mice via injection (i.v.) with AAV8-TBG-SaCas9-2A-EGFP-sgGpr35 in WT mice. Subsequently, Gpr35hep−/−, control (Gpr35hep-null1), and WT mice were fed the HFCF diet or NCD separately for 16 weeks. In addition to the loss-of-function approach, mice with GPR35 overexpression in hepatocytes (Gpr35hep-oe mice) were established by injection of AAV8-TBGp-MCS-EGFP-3Flag-SV40 PolyA-Gpr35 into WT mice to determine if GPR35 overexpression in hepatocytes could attenuate HFCF diet-induced NAFLD. Gpr35hep-oe, control (Gpr35hep-null2), and WT mice were fed the HFCF diet or NCD for 16 weeks. We undertook in-depth quantitative proteomic analysis of liver tissues in Gpr35 KO and WT mice fed with an HFCF diet or NCD through tandem mass tag (TMT) labeling that led to identification. To test the hypothesis stated above, we injected (i.v.) AAV8-TBGp-MCS-Stard4 into Gpr35hep-null1 or Gpr35hep−/− mice to restore StARD4 expression in hepatocytes, who were then fed an HFCF diet for 16 weeks. Furthermore, we injected (i.v.) Gpr35hep-null2 or Gpr35hep-oe mice with AAV8-TBG-SaCas9-sgStard4 to downregulate StARD4 expression. C57BL/6J WT and Gpr35hep−/− mice were administrated by the GPR35 agonist kynurenic acid (KYNA) and then were fed an HFCF diet for 16 weeks. A multiple sample test controlled by a false-detection rate (FDR) threshold of 0.05 was applied to identify significant differences in protein abundance (≥1.5-fold change and $p<0.05$).

Results: GPR35 overexpression in hepatocytes protected against high fat/cholesterol/fructose (HFCF) diet-induced steatohepatitis, whereas loss of GPR35 had the opposite effect. An endogenous agonist of GPR35, KYNA, could promote cholesterol esterification and bile-acid production, and reduce liver FC content, thereby resulting in significantly ameliorated experimental steatohepatitis. GPR35 overexpression prevented hepatic steatosis and inflammation by inducing StAR-related lipid transfer protein 4 (StARD4) expression, a steroidogenic acute regulatory-related lipid-transfer protein, ultimately resulting in hepatic cholesterol esterification and bile-acid synthesis (BAS). StARD4 led to a marked increase in the classic pathway of BAS initiated by cytochrome P450 family 7 subfamily A member 1 (CYP7A1) and CYP8B1. The protective effect induced by GPR35 overexpression in hepatocytes disappeared in hepatocyte StARD4-knockout mice. StARD4-overexpression in hepatocytes could rescue aggravation of HFCF diet-induced steatohepatitis caused by the loss of GPR35 expression in hepatocytes in mice.

Conclusion: GPR35 expression in hepatocytes mitigates NASH by regulating hepatic cholesterol homeostasis. We provide, for the first time, compelling evidence demonstrating that the GPR35-StARD4 axis is a promising therapeutic target for NAFLD.

❖OP-026

Etiology and prognostic criteria of liver failure in southeast China: A multicenter retrospective cohort study between 2018 and 2020

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Object/Background: The prognosis of patients with liver failure (LF) depends significantly on the etiologies and clinical indicators. This study aimed to determine the etiological and prognostic criteria for two cohorts of patients with hepatitis B virus-related LF (HBV-LF) and non-HBV-related LF (non-HBV-LF).

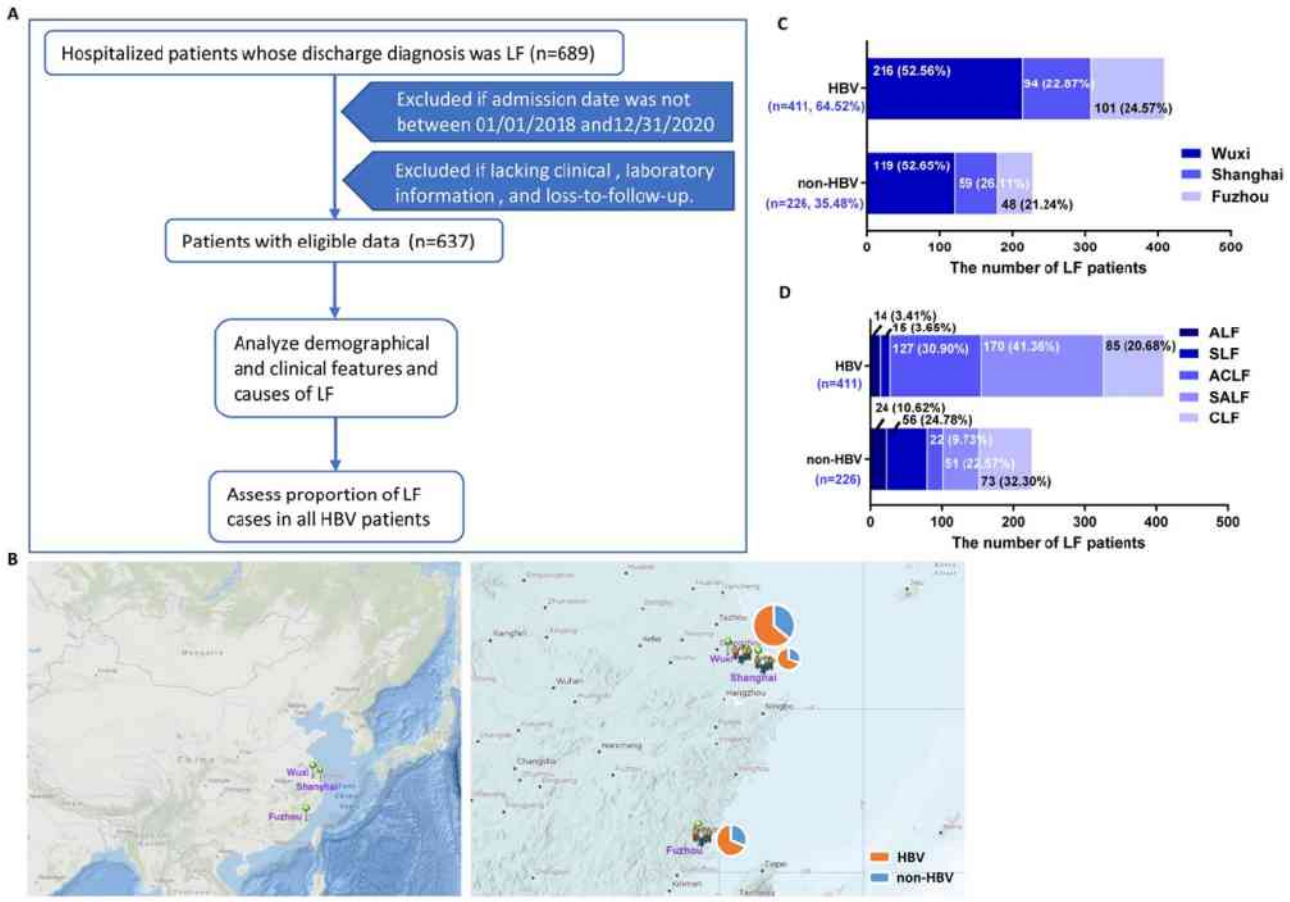
Methods: The retrospective cohort study included 637 LF patients between 2018 and 2020, including the subclasses of acute liver failure (ALF), subacute liver failure (SLF), acute-on-chronic liver failure (ACLF), subacute-on-chronic liver failure (SALF), and chronic liver failure (CLF). Multivariate logistic regression analysis was used to screen clinical indicators of death patients. We analyzed the receiver operating characteristic curves (ROCs) and cut-off values to assess prognosis criteria.

Results: HBV infection was present in 64.52% of LF patients. SALF (41.36%) is the main subclass of the HBV-LF group, while CLF (32.30%) is the main subclass of the non-HBV-LF group. Between 2018 and 2020, the incidence of HBV-LF decreased significantly, ranging from 72.36% to 59.74%, and the spontaneous survival (SS) rates of HBV-LF patients (36.43~44.93%) were substantially lower than those of the non-HBV-LF group (58.97~63.64%). The number of ACLF and SALF patients in the HBV-LF group was 2.50~8.50-fold higher than those of the non-HBV-LF group over three years. Infection and cirrhosis were the primary causes of both groups. In the HBV-LF group, the fatality rate (82.98%) after the onset of hepatic encephalopathy, artificial liver support mortality rates (67.62%), cirrhosis mortality rate (58.23%), and liver cancer mortality rate (94.87%) were higher than those of the non-HBV-LF group. Furthermore, the age and total bilirubin (TBil) value of the HBV-LF dead patients were significantly higher, and the number of days of hospitalization was significantly shorter than those of the survivors. The age and prothrombin time-international normalized ratio (PT-INR) value of the dead patients of the non-HBV-LF group were significantly higher, and the antithrombin III (AT III) value was significantly lower than those of the survivors. The PT-INR of 2.05, 1.92, or 2.11, and AT III

of 24.50%, which were proposed as prognostic criteria for the HBV-SALF, non-HBV-SLF, non-HBV-ACLF, and HBV-ALF subclasses, respectively.

Conclusion: The incidence of HBV-LF is decreasing yearly, and the resulting hepatic encephalopathy, cirrhosis, and liver cancer are the main factors of death. AT III, as a new prognostic criterion, has an excellent discriminative ability on the outcomes of the HBV-ALF subclass.

Figure:



❖OP-027

Enoxaparin attenuates monocrotaline-induced hepatic sinusoidal obstruction syndrome by inhibiting hepatocellular oncostatin M expression

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Object/Background: Definitive treatment for pyrrolizidine alkaloids (PAs)-induced hepatic sinusoidal obstruction syndrome (HSOS) is not available. The effectiveness of anticoagulation therapy remains controversial. The efficacy of low molecular weight heparin (LMWH) should be investigated in patients and animal models, and the underlying mechanism should be explored.

Methods: The prognosis of patients with PAs-HSOS who received anticoagulation therapy was retrospectively analyzed. The effect of enoxaparin on liver injury was determined in animal models of monocrotaline (MCT)-induced HSOS was determined, and the underlying mechanism was investigated using murine model.

Results: The cumulative survival rate of patients with PAs-induced HSOS was 90.90% and 60.00% in non-anticoagulation group and anticoagulation group. Enoxaparin attenuated liver injury effectively in rat model of MCT-induced HSOS. Additionally, the improvement of severe liver injury was observed in MCT-treated mice after the administration of enoxaparin (40 mg/kg). The alleviation of liver injury was observed in mice with hepatocyte-specific deletion of oncostatin M (Osm△Hep). In MCT-treated mice administrated with enoxaparin, no significant differences in liver injury was observed between Osm△Hep mice and Osm flox/flox mice. Additionally, adenovirus-mediated overexpression of Osm resulted in severe liver injury in MCT-induced mice after the administration of enoxaparin.

Conclusion: LMWH attenuated severe liver injury in patients with PAs-Induced HSOS and animal models of MCT-induced HSOS, which provides a rationale for the application of anticoagulation therapy.

❖OP-028

FGL2-MCOLN3-autophagy axis-triggered neutrophil extracellular traps exacerbate liver injury in fulminant viral hepatitis

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Object/Background: Fulminant viral hepatitis (FVH) is a life-threatening disease, but its pathogenesis is not fully understood. Neutrophil extracellular traps (NETs) were an unrecognized link between inflammation and coagulation, which are two main features of FVH. Here, we aim to investigate the role and mechanism of NETs in the pathogenesis of FVH.

Methods: A mice model of FVH was established by murine hepatitis virus strain-3 (MHV-3) infection. Liver leukocytes of infected or uninfected mice were used for single cell RNA sequencing and whole transcriptome sequencing. NETs depletion was achieved using DNase1. Acetaminophen was used to establish a mice model of non-virus caused acute liver failure. Clinically, NETs-related markers in liver, plasma and peripheral neutrophils were assessed in patients with HBV-related acute liver injury (HBV-ALI).

Results: Increased hepatic NETs formation was observed in MHV-3-infected mice but not in acetaminophen-treated mice. NETs depletion improved the liver damage and survival rate in FVH by inhibiting hepatic fibrin deposition and inflammation. Adoptive transfer experiment showed that neutrophil-specific FGL2 promoted NETs formation. FGL2 was found to directly interact with mucolipin3 (MCOLN3), which regulated calcium influx and initiated autophagy, leading to NETs formation. Clinically, elevated plasma NETs level was associated with coagulation dysfunction in patients with HBV-ALI. Colocalization of FGL2, NETs and fibrin in liver was observed in these patients.

Conclusion: NETs aggravated liver injury in FVH by promoting fibrin deposition and inflammation. NETs formation was regulated by the FGL2-MCOLN3-autophagy axis. Targeting NETs may provide a new strategy for the treatment of FVH.

❖OP-029

Soluble ST2 Serum Concentration Predicts Efficacy of Artificial liver support system in Acute-on-chronic liver failure.

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Object/Background: It has been proven that artificial liver support system (ALSS) treatment can reduce the short-term mortality rate of acute-on-chronic liver failure (ACLF) by 30% and increase the long-term survival rate of ACLF patients by 50%. However, at present, there are poor specificity and sensitivity of a single index for predicting the efficacy of ALSS treatment. Therefore, it is found that the biological detection indexes with higher sensitivity and specificity will further improve and simplify the evaluation system of ALSS treatment. Studies related to sST2 and ACLF showed that sST2 is inversely proportional to the survival time of patients, and sST2 can be used as an index to judge the prognosis of ACLF. However, the existing studies have not explored the relationship among sST2, ACLF and ALSS, and whether sST2 can be an independent biological index for predicting the efficacy of ALSS treatment remains to be further verified.

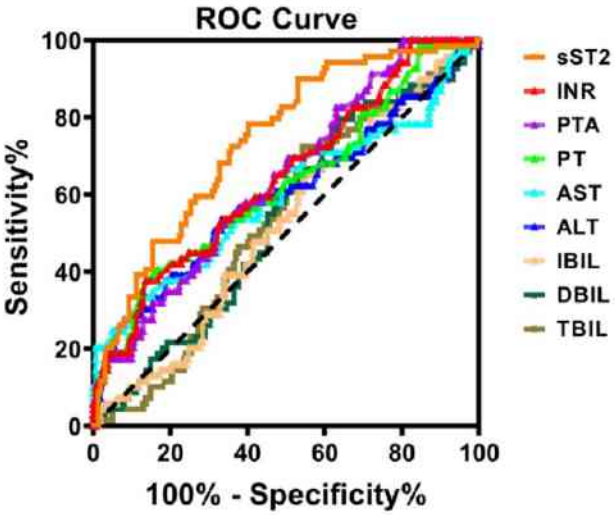
Methods: In this study, ACLF patients from October 2017 to July 2021 in the Affiliated Infectious Disease Hospital of Soochow University were selected. The residual sera of ACLF patients were collected within 30 days after ALSS treatment, and the content of sST2 in serum was determined by ELISA method, Survival curve, ROC curve and other statistical methods were used to analyze the relationship between sST2 and the efficacy of ALSS treatment.

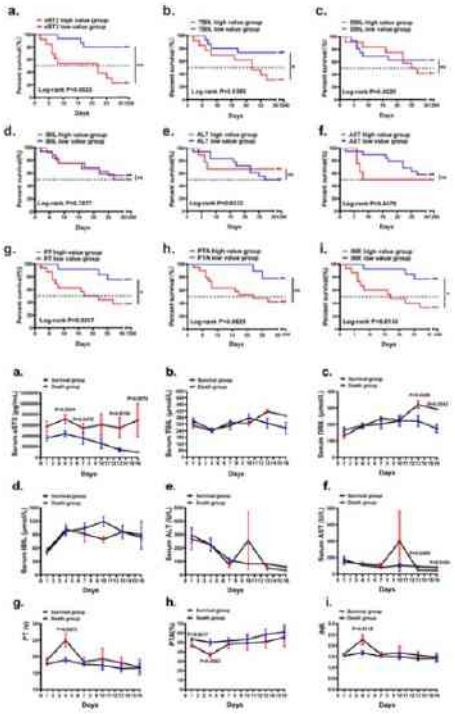
Results: This study found that similar to other independent influencing factors (TBIL, ALT, AST, PT, PTA and INR), the expression of sST2 in peripheral blood of ACLF patients after ALSS treatment decreased significantly, which was positively correlated with the levels of inflammatory injury index ALT ($P < 0.0001$) and coagulation indexes PT ($P < 0.0001$) and INR ($P < 0.0001$), and negatively correlated with PTA ($P < 0.0001$). Further survival curve analysis showed that after ALSS treatment, the short-term mortality (30 days) of patients with high sST2 value was higher than that of patients with low sST2 value, and the survival time was lower. After 20 days, the survival rate was lower than 50%, with statistically significant difference (Log-rank $P=0.0023$) and the statistical difference was greater than

that of known biological indexes such as TBIL (Log-rank P=0.0385), PT (Log-rank P=0.0357) and INR (Log-rank P=0.0138). ROC curve analysis also showed that the AUC value of sST2 was significantly higher than other indexes, and its cut-off value was 312,500.00 pg/ml, and its sensitivity and specificity were 78.3% and 59.9%, respectively. Based on the time axis curve, it was that the level of sST2 in the survival group was significantly lower than that in the death group (P=0.0244) from the 4th day, and the difference between the survival group and the death group widened with the passage of time, suggesting that sST2 is less affected by time.

Conclusion: In this study, the relationship between sST2 and the efficacy of ALSS treatment on ACLF is analyzed for the first time, and it is found that sST2 is an independent factor for predicting the efficacy of ALSS treatment. Compared with traditional indicators TBIL, ALT, AST, PT, PTA and INR, sST2 has better predictive value, higher sensitivity and specificity, which is less affected by time factors. The results of this study provide more accurate, sensitive and earlier biological indicators for predicting the efficacy of ALSS treatment.

Figure:





❖OP-030

Upregulation of microRNA-125b-5p alleviates acute liver failure through regulating Keap1/Nrf2/HO-1 pathway

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Object/Background: Liver failure is a life-threatening clinical problem with a high short-time mortality. Although liver transplantation is an effective therapeutic option, its application is limited due to the shortage of donor organ. MicroRNAs (miRNAs) are a group of small endogenous noncoding interfering RNA molecules involved in the biological processes and in the development of various diseases. Regulation of miRNAs and their target genes may serve as promising interventions for the treatment of liver failure. This study aimed to explore the role of miR-125b-5p in the development of liver failure.

Methods: Collected human liver tissue samples were categorized into 2 groups of mild-moderate liver injury and liver failure. The differentially expressed-miRNAs (DE-miRNAs) were screened and identified with the progression of liver injury through high-throughput sequencing. Among these DE-miRNAs, miR-125b-5p was selected to further investigate its role in lipopolysaccharide (LPS)/D-galactosamine (D-GalN) -induced acute liver failure (ALF) in vivo and in LPS/D-GalN-challenged huh7 cells in vitro.

Results: Total of 75 DE-miRNAs were obtained, including 28 upregulated and 47 downregulated miRNAs in liver failure group as compared to the mild-moderate liver injury group. Among these DE-miRNAs, miR-125b-5p was selected for further study. It revealed that miR-125b-5p not only reduced huh7 cell apoptosis in vitro, but also relieved ALF in vivo with evidence of improved liver histology, decreased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, and reduced tumor necrosis factor -α (TNF-α) and IL-1β levels. Based on the result of a biological prediction website, microRNA.org, Kelch-like ECH-associated protein 1 (Keap1) was predicted as one of the potential target genes of miR-125b-5p, which was verified by the dual-luciferase reporter gene assay. Subsequent experiments in vivo and in vitro both revealed that miR-125b-5p could decrease the expression of Keap1 and cleaved caspase-3, while upregulate the expression of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and heme oxygenase-1(HO-1).

Conclusion: Upregulation of miR-125b-5p can alleviate acute liver failure through regulating Keap1/Nrf2/HO-1 pathway, and regulation of miR-125b-5p may serve as an alternative therapeutic intervention for liver failure.

❖OP-031

Decreased vitamin D-binding protein level portends poor outcome in acute-on-chronic liver failure caused by hepatitis B virus

hepatitis B virus
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3. Jinan Microecological Biomedicine Shandong Laboratory

Object/Background: Aims: Acute-on-chronic liver failure (ACLF) is a catastrophic illness with high short-term mortality. A limited number of studies have investigated the prognostic value of vitamin D-binding protein (VDBP) for hepatitis B virus (HBV)-related ACLF (HBV-ACLF) and have resulted in conflicting results.

Methods: Two prospective HBV-ACLF cohorts (n=287 and n=119, respectively) were enrolled to assess and validate the prognostic performance of VDBP for HBV-ACLF.

Results: VDBP levels in the non-survivors were significantly lower than in the survivors (P<0.001). Multivariate Cox regression demonstrated that VDBP was an independent prognostic factor for HBV-ACLF. The VDBP level at admission gradually decreased as the number of failed organs increased (P<0.001), and it was closely related to coagulation failure. The areas under the receiver operating characteristic curve (AUCs) of the Child-Pugh-VDBP and chronic liver failure-sequential organ failure assessment (CLIF–SOFA)-VDBP scores were significantly higher than those of Child-Pugh (P<0.001) and CLIF-SOFA (P=0.0013). The AUCs of model for end-stage liver disease (MELD)-VDBP were significantly higher than those of MELD (P= 0.0384) only in the case of cirrhotic HBV-ACLF patients. Similar results were validated using an external multicenter HBV-ACLF cohort. By longitudinal observation, the VDBP levels gradually increased in survivors (P=0.026) and gradually decreased in non-survivors (P<0.001). Additionally, the VDBP levels were found to be significantly decreased in the deterioration group (P=0.012) and tended to decrease in the fluctuation group (P=0.055). In contrast, they showed a significant increase in the improvement group (P=0.036).

Conclusions: The VDBP was a promising prognostic biomarker for HBV-ACLF. Sequential measurement of circulating VDBP shows value for the monitoring of ACLF progression.

❖OP-032

Galectin-3 critically mediates the hepatoprotection conferred by M2-like macrophages in acute-on-chronic liver failure by inhibiting pyroptosis but not necroptosis signalling

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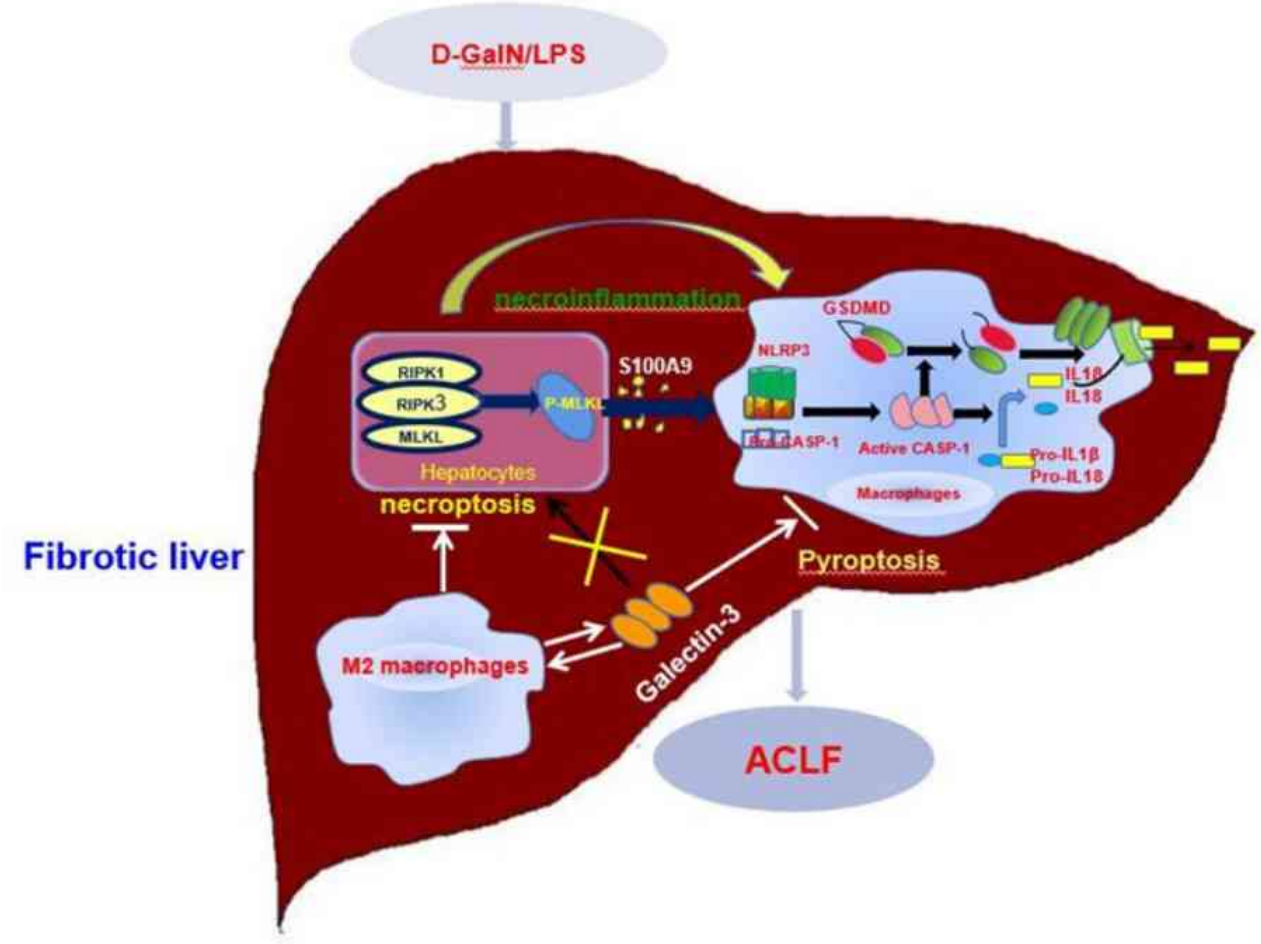
Object/Background: We previously documented that M2-like macrophages exert a hepatoprotective effect in acute-on-chronic liver failure (ACLF) by inhibiting necroptosis signalling. Nevertheless, the molecular mechanism behind this hepatoprotection still needs to be further dissected. Galectin-3 (GAL3) has been reported to be critically involved in the pathogenesis of multiple liver diseases, whereas the potential role of GAL3 in ACLF remains to be explored. Herein, we hypothesised that GAL3 plays a pivotal role in the hepatoprotection conferred by M2-like macrophages in ACLF by inhibiting necroptosis.

Methods: To test this hypothesis, we first assessed the expression of GAL3 in control and fibrotic mice with or without acute insult. Second, loss- and gain-of-function experiments of GAL3 were performed. Third, the correlation between GAL3 and M2-like macrophage activation was analysed, and the potential role of GAL3 in M2-like macrophage-conferred hepatoprotection was confirmed. Finally, the molecular mechanism underlying GAL3-mediated hepatoprotection was dissected.

Results: GAL3 was found to be obviously upregulated in fibrotic mice with or without acute insult but not in acutely injured mice. Depletion of GAL3 aggravated hepatic damage in fibrotic mice upon insult. Conversely, adoptive transfer of GAL3 provided normal mice enhanced resistance against acute insult. The expression of GAL3 is closely correlated with M2-like macrophage activation. Through adoptive transfer and depletion experiments, M2-like macrophages were verified to act as a major source of GAL3. Importantly, GAL3 was confirmed to hold a pivotal place in the hepatoprotection conferred by M2-like macrophages through loss- and gain-of-function experiments. Unexpectedly, the depletion and adoptive transfer of GAL3 resulted in significant differences in the expression levels of pyroptosis but not necroptosis signalling molecules.

Conclusion: Taken together, GAL3 plays a pivotal role in the hepatoprotection conferred by M2-like macrophages in ACLF by inhibiting pyroptosis but not necroptosis signalling. Our findings provide novel insights into the pathogenesis and therapy of ACLF.

Figure:



❖OP-033

Contrast-free ultrasensitive ultrasound imaging for in-vivo quantitative evaluation of hepatic microcirculation in cirrhotic rats

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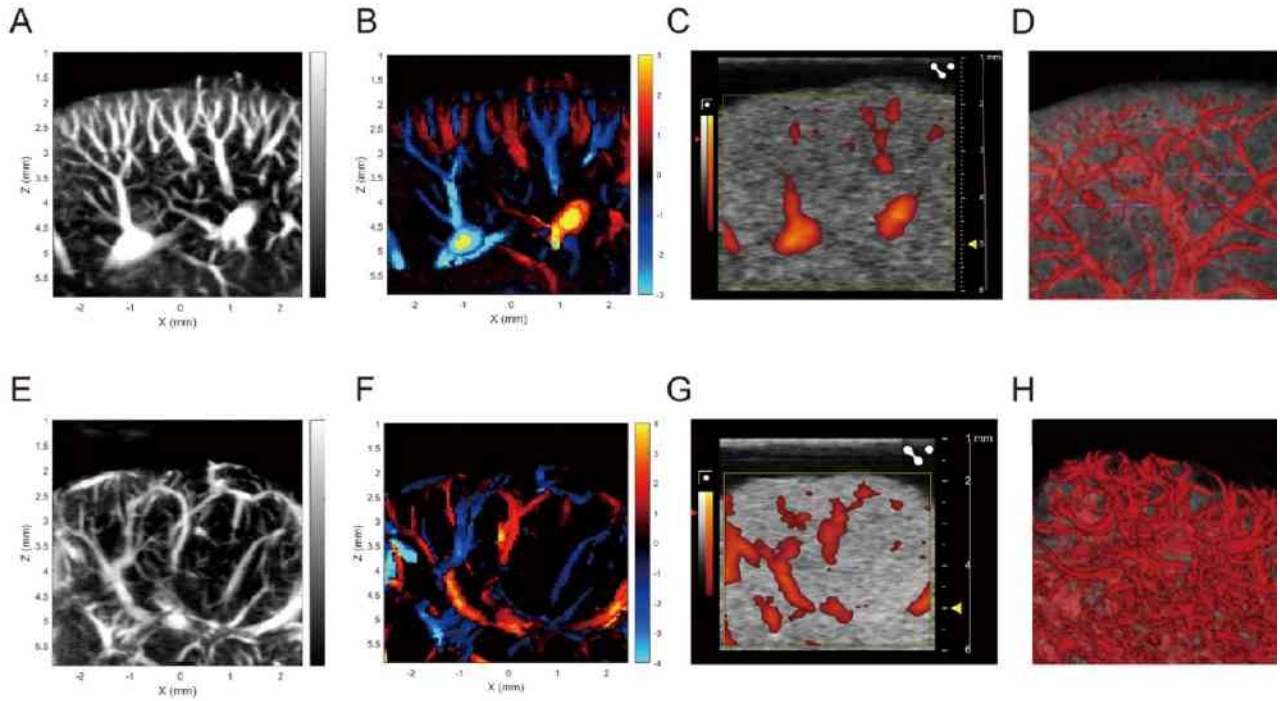
Object/Background: Liver microcirculation dysfunction plays a vital role in the occurrence and development of liver diseases, and thus there is a clinical need for in vivo and noninvasive evaluation of liver microcirculation.The objective was to evaluate the feasibility of ultrasensitive ultrasound microvessel imaging (UMI) in visualization and quantification of hepatic microvessels in rats.

Methods: In vivo studies were carried out to image hepatic microvasculature in Sprague Dawley rats (five control and five cirrhotic rats). Contrast-free ultrasensitive UMI was achieved by removing tissue clutters and extracting blood flow signals using a spatial-temporal singular value decomposition-based clutter filter. In vivo conventional power Doppler (PD) and in vitro micro-CT were performed as benchmarks. UMI-based quantifications for describing perfusion status, tortuosity, and integrity of microvessels were compared between control and cirrhotic groups by using Wilcoxon test. Spearman correlations between quantification parameters and pathological fibrosis, perfusion function, and hepatic hypoxia were evaluated.

Results: UMI showed good performance in detecting minute vessels below the liver capsule, as compared with the conventional power Doppler and micro-CT. Lower perfusion indicated by vessel density (22.11 (19.5, 27.46) % vs. 41.43 (37.16, 45.54) %, P=.008) and fractional moving blood volume (FMBV) (6.35 (4.78, 8.61) % vs. 13.18 (11.54, 14.26) %, P=.008), and higher tortuosity indicated by sum of angles metric (SOAM) (2.99 (2.93, 3.03) vs. 2.74 (2.60, 2.88), P=.008) were demonstrated in the cirrhotic rat group compared with the control group using UMI. Vessel density (r=0.85, P=.003), FMBV (r=0.86, P=.002) and SOAM (r=-0.83, P=.003) showed strong correlations with pathologically-derived microvessel density labeled with Dextran. Vessel density (r=-0.81, P=.005) and SOAM (r=0.87, P=.001) also showed strong correlations with hepatic tissue hypoxia.

Conclusion: Contrast-free ultrasensitive ultrasound microvessel imaging provided noninvasive in vivo imaging and quantification of hepatic microvessels in cirrhotic rat liver. These results in an animal model demonstrate potential feasibility of transition to a human application in the noninvasive evaluation of hepatic microcirculation dysfunction in liver diseases.

Figure:



❖OP-034

Monocyte subsets predicts outcome of hepatitis B virus related Acute-on-Chronic Liver Failure patients

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Object/Background: Hepatitis B virus related Acute-on-Chronic Liver Failure (HBV-ACLF) is a sever clinical syndrome with the intensity of systemic inflammation and the high short-term mortality. Monocytes are known for their very important role in inflammation, immune defense, phagocytosis or tissue repair. Monocytes are classically categorized into three functional subgroups: CD14++CD16− (classical monocytes), CD14++CD16+ (intermediate monocytes), and CD14+CD16++ (non-classical monocytes). Three monocyte subsets have different function in homeostasis and diseases. In this study, we try to identify the distribution of monocyte subsets and its prognostic value in patients with HBV-ACLF.

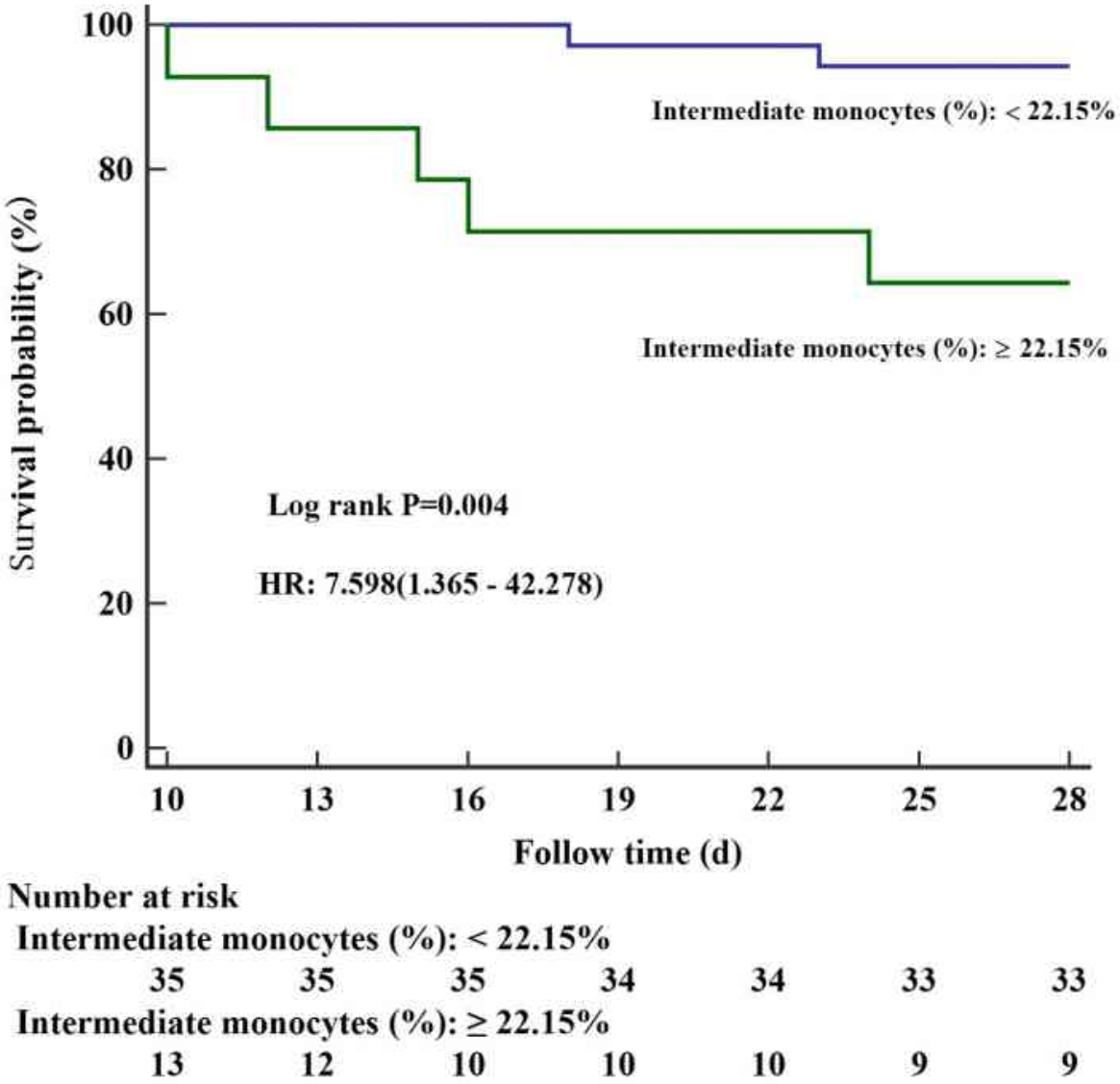
Methods: 49 HBV-ACLF patients and 24 HBV related chronic liver disease (CLD) patients were enrolled. Monocyte subsets were quantified by flow cytometry based on CD14 and CD16 expression. Patients with HBV-ACLF have been followed-up for at least 28 days.

Results: Proportion of intermediate monocytes in ACLF patients was higher than CLD patients (14.60 [6.75-26.50] vs 9.14 [4.02-14.60], P=0.045), and proportion of non-classical monocytes was lower than CLD patients (1.21 [0.56-3.95], P=0.008). But there was no significant different in proportion of classical monocytes between two groups (ACLF vs CLD: 77.90 [66.60-85.40] vs 70.35 [62.05-83.90], P=0.161). The follow-up results of ACLF patients showed that 42/49 (85.71%) survived and 7/49 (14.29%) died at 28 days. By comparing the distribution of monocyte subsets, we found that the proportion of intermediate monocytes in dead patients was higher than survival patients (12.75 [6.51-21.40] vs 27.00 [17.20-29.95], P=0.04); however, there was no significant difference in the proportion of classical and non-classical monocytes. The AUROC of proportion of intermediate monocytes was 0.745 (95% CI: 0.575-0.914) to prognosis 28 days outcome of HBV-ACLF patients. The cutoff value of the proportion of intermediate monocytes was identified as 22.15% based on the ROC curve. Furthermore, the Kaplan-Meier curves showed that higher short-term mortality was observed in high proportion of intermediate

monocytes (≥22.15%) patients compared to low proportion of intermediate monocytes (<22.15%) (HR: 7.598 [1.365-42.278], P=0.004).

Conclusion: The distribution of monocyte subsets related to short-term outcome of HBV-ACLF patients, and it may be used as a potential biomarker for prognosis and even a potential target for treatment of HBV-ACLF patients.

Figure:



❖OP-035

Prediction of prognosis of hepatitis B virus-related acute-on-chronic liver failure by LEAP-HBV model based on whole liver volume

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Object/Background: Hepatitis B virus-related acute-on-chronic liver failure (HBV-ACLF) is a clinical syndrome with rapid progression and high mortality. Thus, early and accurate evaluation of clinical progression and prediction of clinical outcome might be the key to optimizing the timing of liver transplantation, adjusting clinical strategies in a timely manner and raising the survival rates. The main indicators in the commonly used prediction models of liver failure included biochemical testing, demographic data, complications and other indicators, without liver morphology. During the process of liver failure, massive/submassive necrosis, shrinkage and structural collapse of liver tissue occur in liver tissue, leading to volume decrease. Although liver volume could impart significant prognostic information for liver failure, it has not been included in current prognostic models of ACLF. Zabron's study demonstrated that changes in LV were heavily dependent on etiology. ACLF is the most common type of liver failure and HBV infection is the most dominant pathogen of ACLF in China. However, the predictive value of liver volume in the progression of HBV-ACLF is still unclear, and there are few studies on prognostic models based on liver volume.
Objective: To explore the correlation between liver volume and the short-term mortality of HBV-ACLF, to construct a new prognostic model based on liver volume, to evaluate the accuracy and efficacy of the newly prediction model, hoping to help guide clinical treatment strategies to improve survival.

Methods: 323 patients from 2 liver centers were recruited to the deriving cohort, and 163patients from 5 external centers were enrolled to the validation cohort. The primary end-point was death or liver transplant within 28 days since diagnosis. Estimated liver volume (ELV) was calculated by the formula: ELV=203.3 -(3.61 ×age)+(58.7×thoracic width (cm))-463.7, wherein the thoracic width refers to the distance between the left and right rib corners. We acquired actually measured liver volume (LV) from computed tomography images using the automated software Volume Viewer. Ratio of LV to ELV(LV/ELV%)was used to eliminate various individual differences for liver atrophy. Univariate and multivariate logistic models were used to select independent predictors and establish a

new prediction model for predicting short-term mortality of HBV-ACLF patients by combining patients' demographic characteristics, biochemical tests, HBV DNA, liver volume ratio (LV/ELV%) and complications. The accuracy of the prognostic model was assessed using the area under the curve(AUC) of the receiver operating characteristic curve, sensitivity and specificity. Model performance was assessed in terms of calibration, evaluated by calibration plot. Bootstrap method was used to perform the internal validation. To validate the generalizability of the model, we used data from 5 external liver centers. We also used the ACs to compare the accuracy of the new prognostic model with previous models, and the differences between scoring systems were analyzed by Z-test. The Kaplan-Meier curve was used to compare the prognosis of patients with different LV/ELV% or LEAP-HBV. The data were analyzed using SPSS version 19.0, and graphs were plotted using Graphpad Prism version 7.0 and MedCalc version 18.11.3.All statistical tests were two-tailed, and a probability level of $P<0.05$ was considered statistically significant.

Results: The median age of non-survivors was higher than that of survivors ($P =0.005$) in the deriving cohort, and most of them were male. The incidences of hepatic encephalopathy and ascites were significantly higher in non-survivors than survivors ($P<0.05$). The level of total bilirubin (lnTBIL) of non-survivors was higher than that of survivors ($P=0.058$). The levels of blood urea nitrogen, international normalized ratio, prothrombin time, HBV DNA, HBsAg and HBeAg were much higher in non-survivors ($P<0.05$). However, the levels of hemoglobin, platelet, cholesterol, albumin, potassium and alpha-fetoprotein were much lower in non-survivors ($P<0.05$).There were no significance between two groups in white blood count, C-reactive protein, procalcitonin, alanine aminotransferase, aspartate aminotransferase. creatinine and sodium($P＞0.05$). There was no significant difference in ELVs between non-survivors and survivors in deriving cohort ($P = 0.583$), which indicates that there was no difference in baseline liver volume between two groups. However, measured LV and LV/ELV% were significantly lower in non- survivors than survivors in deriving cohort ($P <0.05$), indicating that atrophy of liver volume was much more severe in non-survivors. The AUC of LV for 28-day mortality of HBV-ACLF patients was 0.782, while the AUC of LV/ELV% for 28-day mortality of HBV-ACLF patients was 0.835 with a cut-off value, 82%. Kaplan-Meier survival curve analysis showed that the cumulative survival time of HBV-ACLF patients with LV/ELV%<82% was much shorter than that of HBV-ACLF patients with LV/ELV%＞ 82% (P

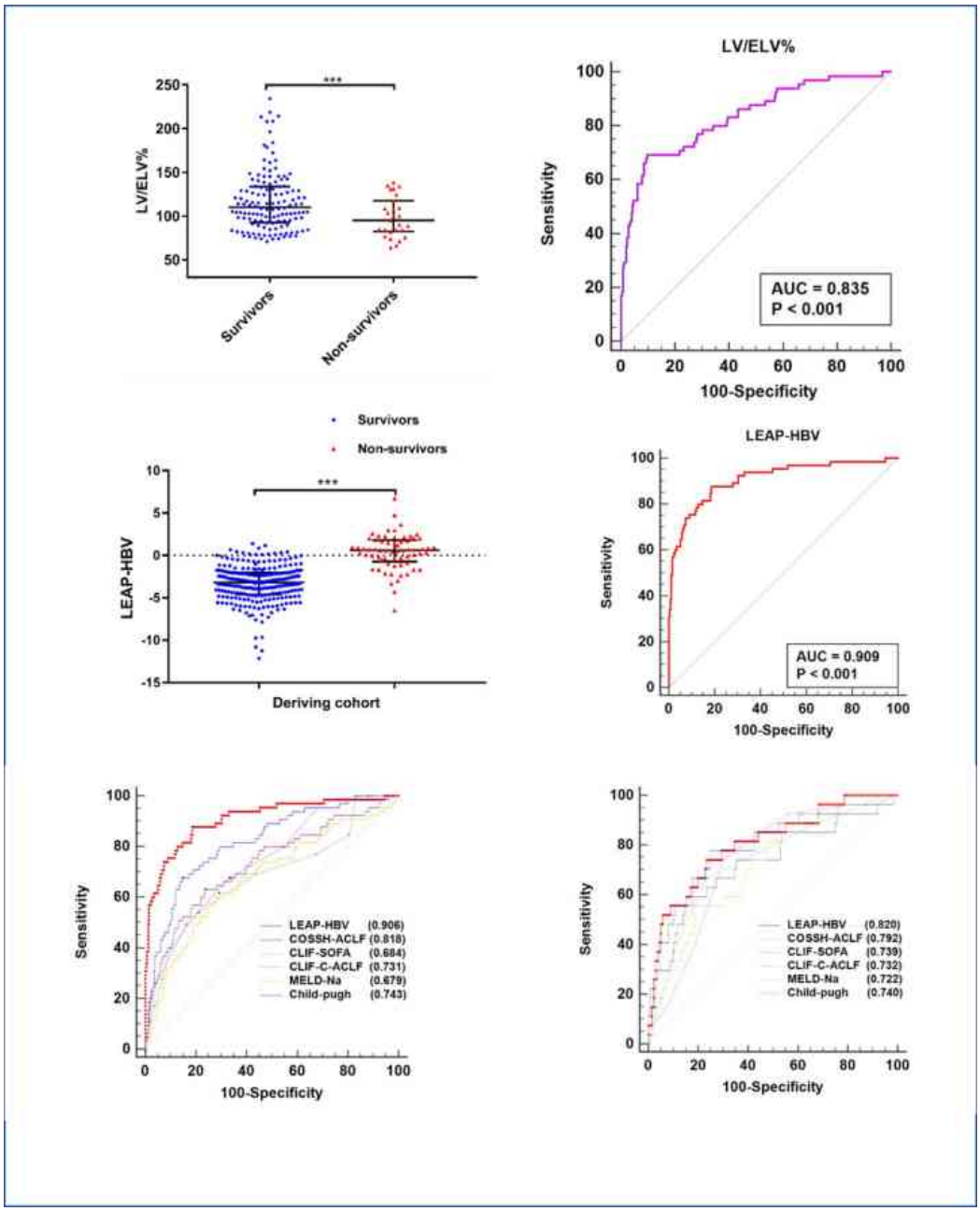
<0.001), suggesting the higher predictive value of LV/ELV% for the short-term mortality of HBV-ACLF.

The independent risk factors for predicting 28-day mortality of HBV-ACLF patients were selected by logistic regression analysis as followings: LV/ELV%, age, PT, HE, lnTBIL, HBV DNA, and a prognostic model based on liver volume was constructed as: LEAP-HBV=-0.0679×LV/ELV% +0.0345×Age +0.0381 ×PT+ HE +0.6390×ln[TBIL (umol/L)]-0.2583 ×HBY DNA (lg IU/mL)-0.8680, where HE =0 for patients without HE; HE =1.1527for mild HE, and HE =2.8108 for advanced HE. By internal bootstrap validation, the mean AUC of LEAP-HBV for the short-term mortality of HBV-ACLF in the deriving cohort was 0.906, with a sensitivity of 87.69% and specificity of 81.40% at a cut-off value of -1.71, which indicated that LEAP-HBV was a satisfactory prognostic model with high prediction efficiency. Kaplan-Meier survival curve analysis showed that the cumulative survival time of HBV- ACLF patients with LEAP-HBV＞1.71 was much shorter (P <0.001). The calibration curve analysis and bootstrap internal verification showed that the LEAP-HBV model was robust. The accuracy of the LEAP-HBV model in the validation cohort was similar to that of the deriving cohort with an AUC of 0.820, indicating good external generalization. Compared with current prediction models, LEAP-HBV (AUC 0.906) showed significantly higher predictive value for the short-term mortality of HBV-ACLF patients than Child-Pugh (AUC0.743)，MELD-Na(AUC0.679)，CLIF-SOFA(AUC0.684)，CLIF-CACLF(AUC0.731)，and COSSH-ACLF scores (AUC 0.818)(P<0.05).

Conclusion: Liver volume is an independent risk factor for the short-term mortality in HBV- ACLF patients, and patients with a low LV/ELV% indicates poor prognosis. The newly prediction model, LEAP-HBV, is constructed based on liver volume ratio (LV/ELV%), age, PT, lnTBIL and HBV DNA. LEAP-HBV model shows higher predictive value for the short- term mortality in HBV-ACLF patients than previous prediction models, such as Child-Pugh, MELD-Na, CLIF-C ACLF,CLIF-SOFA, and COSSH-ACLF scores.

Figure:

❖OP-035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LV/ELV%
Survivors
Non-survivors
LV/ELV%
Sensitivity
100-Specificity
AUC = 0.835
P < 0.001
Survivors
Non-survivors
***
LEAP-HBV
Deriving cohort
LEAP-HBV
Sensitivity
100-Specificity
AUC = 0.909
P < 0.001
Sensitivity
100-Specificity
LEAP-HBV (0.906)
COSSH-ACLF (0.818)
CLIF-SOFA (0.684)
CLIF-C-ACLF (0.731)
MELD-Na (0.679)
Child-pugh (0.743)
Sensitivity
100-Specificity
LEAP-HBV (0.820)
COSSH-ACLF (0.792)
CLIF-SOFA (0.739)
CLIF-C-ACLF (0.732)
MELD-Na (0.722)
Child-pugh (0.740)

❖OP-036

A novel prognostic nomogram for older patients with acute-on-chronic liver diseases (AoCLD): a nationwide, multicenter, prospective cohort study

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Object/Background: Acute-on-chronic liver diseases (AoCLD), defined as acute exacerbation of chronic liver disease (CLD), is currently an increasing global disease burden. Little is known about the clinical features and risk factors in elderly patients with AoCLD. We aim to investigate the clinical features and risk factors and to construct an effective prognostic nomogram model for elderly patients with AoCLD aged over 60 years old.

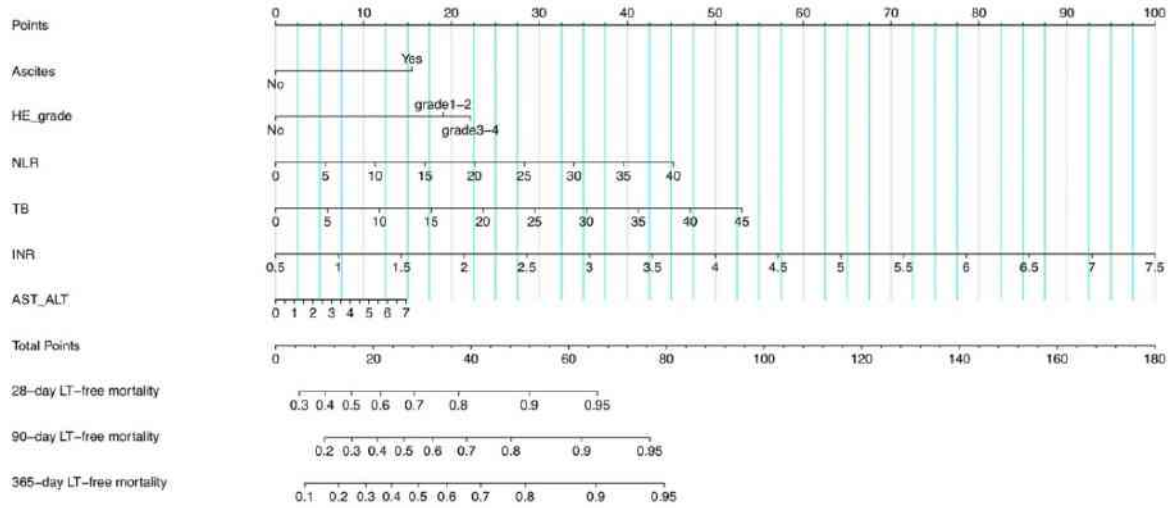
Methods: Two nationalwide, multicentre and prospective cohorts of 3970 AoCLD patients were established from January 2015 through December 2016 and July 2018 through January 2019. The multivariate Cox regression analyses was performed to identify predictive risk factors of elderly individuals, and an easy-to-use nomogram was established. The performance was assessed using the area under the curve (AUC), calibration plots, and decision curve analysis (DCA).

Results: A total of 3,949 AoCLD patients were included in the CATCH-LIFE study, of whom 3,140 were aged <60 years (older) and 809 were aged ≥60 years (youger). The proportion of men in the older group was lower than that in the younger group (58.3% vs. 77.6%, P<0.001). The top three of etiology in both groups were HBV infection, alcohol abuse, and autoimmune-related causes. HBV infection was common in the younger group (50.4% vs. 76.1%, P<0.001), while autoimmune-related causes (16.2% vs. 8.28%, P<0.001), HCV infection (6.43% vs. 2.90%, P<0.001), and schistosomiasis (3.96% vs. 0.73%, P<0.001) were significantly higher in the older group than in the younger group.The older group tended to develop liver cirrhosis (85.9% vs. 63.9%, P<0.001). Additionally, younger patients were more likely to develop ACLF than older patients (17.2% vs. 14.0%, P=0.03). Regarding AD events, there was a significantly high incidence of HE (11.24% vs. 7.90%), infection (25.1% vs. 20.3%), ascites (57.2% vs. 44.2%), and gastrointestinal bleeding (21.5% vs. 12.9%) and a significantly low incidence of jaundice (36.8% vs. 49.6%) in the older group (P<0.001). Several laboratory indices that the level of WBC, PLT, HGB, ALB, NLR , AST/ALT, CR, BUN were higher than the younger group, and the level of TB, ALT, AST and INR were lower than the younger group. The final nomogram was developed based on the results of the multivariate Cox analysis using the six risk factors of ascites, HE grades, NLR, TB, INR and AST/ALT. Based on the ordinary nomogram, we developed a dynamic nomogram web-based application (https://catch-life.shinyapps.io/DynNomapp/) to precisely calculate the survival probability of older patients with AoCLD at any time point within 1 year. The areas under the curve (AUC) of predicting 28-day ,90-day and 365-day LT-free mortality in the training set were 0.892, 0.839 and 0.853, respectively. Likewise, for validation set, it was 0.843, 0.841 and 0.85, respectively. The calibration plots showed well calibration (p > 0.05), indicating suitable performance of the nomogram model. Decision curve analysis showed that the nomogram added more net benefit compared with the treat-all strategy or treat-none strategy with a wider range of threshold probabilities.

Conclusion: Our study described the baseline characteristics, illustrated the risk factors, and constructed a nomogram with high accuracy and validity for the first time, which is promising for prognosis prediction and clinical course management in older patients with AoCLD.

❖OP-036

Figure:



❖OP-037

Establishment and validation of a quick bedside test with number connection test A and animal and vegetable naming test for minimal hepatic encephalopathy

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Object/Background: Minimal hepatic encephalopathy (MHE) is one of the most overlooked complications in decompensated liver diseases due to its inconspicuous symptoms, complex and time-consuming diagnosis. To establish a quick diagnostic tool of MHE for inpatients.

Methods: A total of 123 healthy volunteers and 252 inpatients with decompensated liver diseases were prospectively included and underwent the psychometric hepatic encephalopathy score (PHES), animal naming test (ANT), vegetable naming test (VNT), animal and vegetable naming test (AVNT), and simplified EncephalApp Stroop test. Serum ammonia was measured among patients. A diagnotic tool was established in a development cohort of 152 patients and tested in a validation cohort of 100 patients. Meanwhile, the newly occurred overt hepatic encephalopathy (OHE) during hospitalization was observed.

Results: A diagnostic tool named AVNA with AVNT and number connection test A (NCT-A) was established, with an AUC of 0.98 (97.1% sensitivity, 93.2% specificity and 94.1% accuracy) and 0.91 (87.9% sensitivity, 79.1% specificity and 82.0% accuracy) for the development and the validation cohort, respectively. The predicted probability of MHE was consistent with the actual probability by calibration curve analysis. Decision curve analysis (DCA) demonstrated that serum ammonia did not add additional positive net benefits than AVNA for almost all of the threshold probabilities. Patients with AVNA diagnosed MHE exhibited higher risk of 30-day OHE development (P <0.001). Moreover, the average time of AVNA test was significantly shorter than the PHES test (151±77 sec to 398±192 sec, P <0.001).

Conclusion: AVNA with AVNT and NCT-A was established for inpatients with decompensated liver disease, which showed quick and competent capacities for diagnosis of MHE and provided a potential auxiliary solution for early prognosis in clinical practice.

❖OP-038

Targeting GPR65 alleviates hepatic inflammation and fibrosis by suppressing the JNK and NFκB pathways

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2. Tianjin Union Medical Center、Tianjin Medical University

Object/Background: Liver fibrosis has emerged as a major chronic liver pathological process, which results from a perpetuated liver damage and wound healing reaction induced by various etiologies. If unresolved, the fibrotic process would result in organ failure and cirrhosis, which have caused more than 1.32 million deaths globally. Unfortunately, up to now, no effective pharmacological strategies for liver fibrosis have been established and approved. On the other hand, G-protein coupled receptors (GPCRs) are recognized as attractive targets for drug therapy. However, it remains poorly understood how GPCRs, except chemokine receptors, regulate the progression of liver fibrosis. Here, we aim to reveal the role of GPR65, a proton-sensing receptor, in liver fibrosis and to elucidate its underlying mechanism.

Methods: The expression level of GPR65 was evaluated in both human and mouse fibrotic livers. Furthermore, GPR65-deficient mice were treated with bile duct ligation (BDL) for 21 days or carbon tetrachloride (CCl4) for eight weeks and RNA-seq was performed to investigate the role of GPR65 in liver fibrosis. And silenced or over expressed GPR65 in hepatic macrophages, hepatocytes and hepatic stellate cells to analyze the function of GPR65 in vitro. Mechanistically, the siRNAs of Gα and GPR65 specific antagonist or endogenous and exogenous agonists were used to investigate the major signaling molecules acting downstream of GPR65. In addition, the specific antagonist of GPR65 was used in CCl4-induced liver fibrosis mice to study the further application of GPR65.

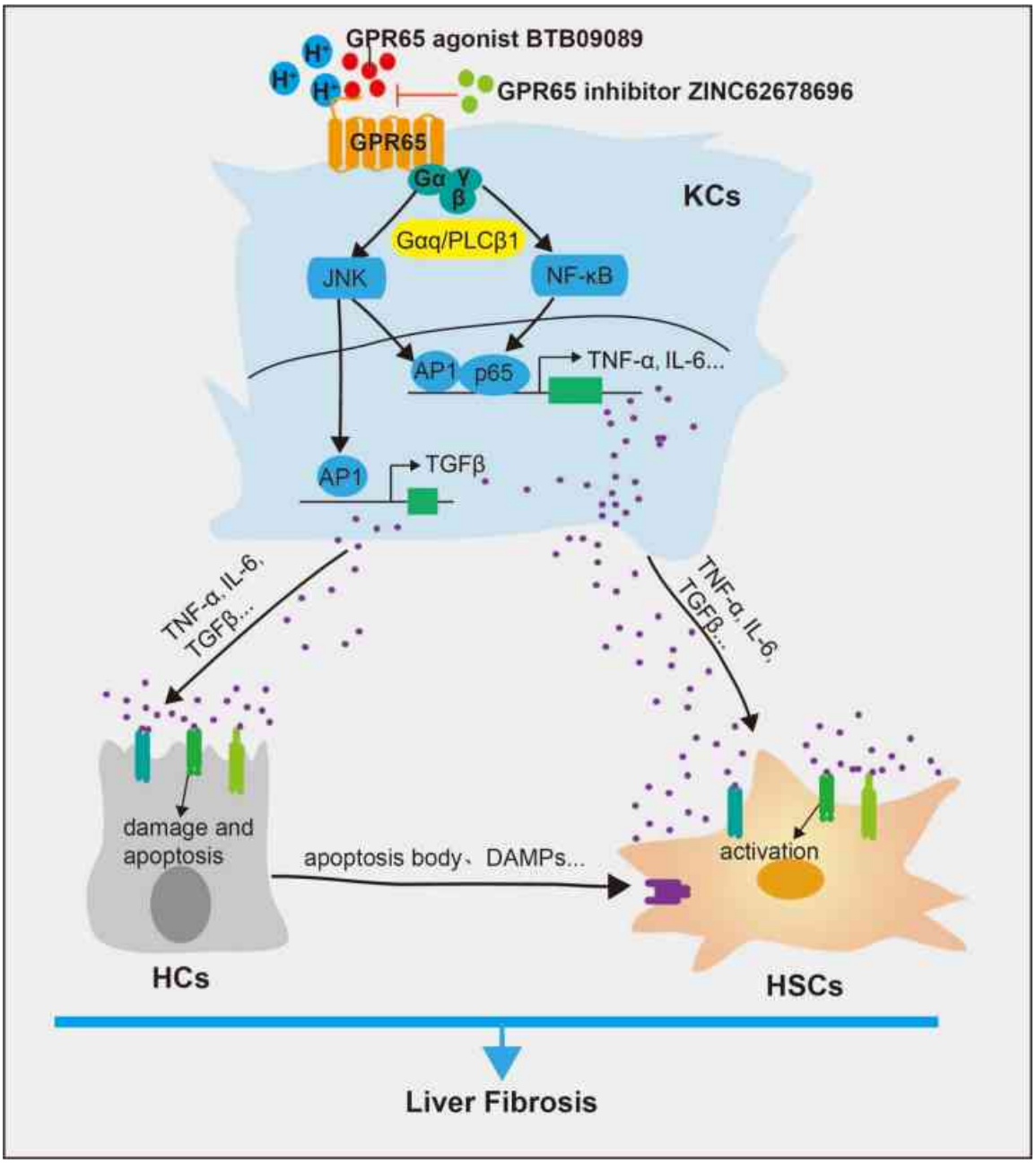
Results: We found that the proton-sensing receptor GPR65, enriched in hepatic macrophages, was upregulated in both human and mouse fibrotic livers. Moreover, knockout of GPR65 significantly alleviated BDL- and CCl4- induced liver inflammation, injury and fibrosis in vivo. In addition, the GO and KEGG pathway analysis revealed that loss of Gpr65 affected a list of genes associated with retinol metabolism, arachidonic acid metabolism, cytokine-cytokine receptor interaction, ECM-receptor interaction,

drugmetabolism, PI3K-AKT signaling pathway, NFκB signaling pathway and MAPK signaling pathway, suggesting that Gpr65 deficiency regulated liver metabolic dysfunction, inflammation and fibrosis. In addition, in vitro data demonstrated that GPR65 silencing and the application of GPR65 antagonist inhibited, while overexpression of GPR65 and the application of GPR65 endogenous and exogenous agonists enhanced the expression and release of IL-6, TNF-α and TGF-β, which subsequently promoted the activation of hepatic stellate cells and the damage of hepatocytes. Mechanistically, GPR65 over-expression, the acidic pH 6.6 and GPR65 exogenous agonist induced up-regulation of TNF-α and IL-6 via the Gαq-JNK/NFκB pathways, while promoted the expression of TGF-β through the Gαq-JNK pathway. Notably, pharmacological GPR65 inhibition reversed the development of inflammation, hepatocyte injury and fibrosis in vivo.

Conclusion: In summary, our results provide convincing evidence that the hepatic macrophages-enriched proton-sensing GPR65 is involved in liver fibrosis in both human and mice. Given the role of GPR65 as a physiologic integrator of the microenvironment (especially inflammation condition), these data provide insight into the molecular mechanisms by which the acidosis-activated GPR65 may contribute to the progression of fibrosis. It also brings some understanding of the anti-inflammatory effects of the GPR65 inhibitor, which may open novel avenues of research for drug development in the treatment of liver fibrosis and inflammation-related diseases.

❖OP-038

Figure:



❖OP-039

Single-Cell Profiling Reveals the Metastasis-Associated Immune Signature of Hepatocellular Carcinoma

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Object/Background: Tumor-infiltrating immune cells (TILs) determine the immune microenvironment and affect the development, growth and metastasis of hepatocellular carcinoma (HCC). Metastasis is the main cause of poor prognosis in HCC patients, while the metastasis-associated immune signature is poorly characterized. The aim of our study was to reveal the crosstalk between landscape of TILs and HCC metastasis.

Methods: We performed bioinformatic analysis baed on the single-cell transcriptome data derived from GSE149614 datasets in Gene Expression Omnibus (GEO). CD45+ immune cells were identified in 4 tumor samples from metastasis HCC patients (TNM stage IIIB/IV) and 6 tumor samples from non-metastasis HCC patients (TNM stage I/II/IIIA). Clusters of immune cells were identified through mutual nearest neighbors (MNN) and t-distributed stochastic neighbor embedding (t-SNE). Heterogeneity of immune cells between metastasis and non-metastasis group were characterized. Pseudotime state transition and cell-cell interaction were further analyzed in specific cell subtypes.

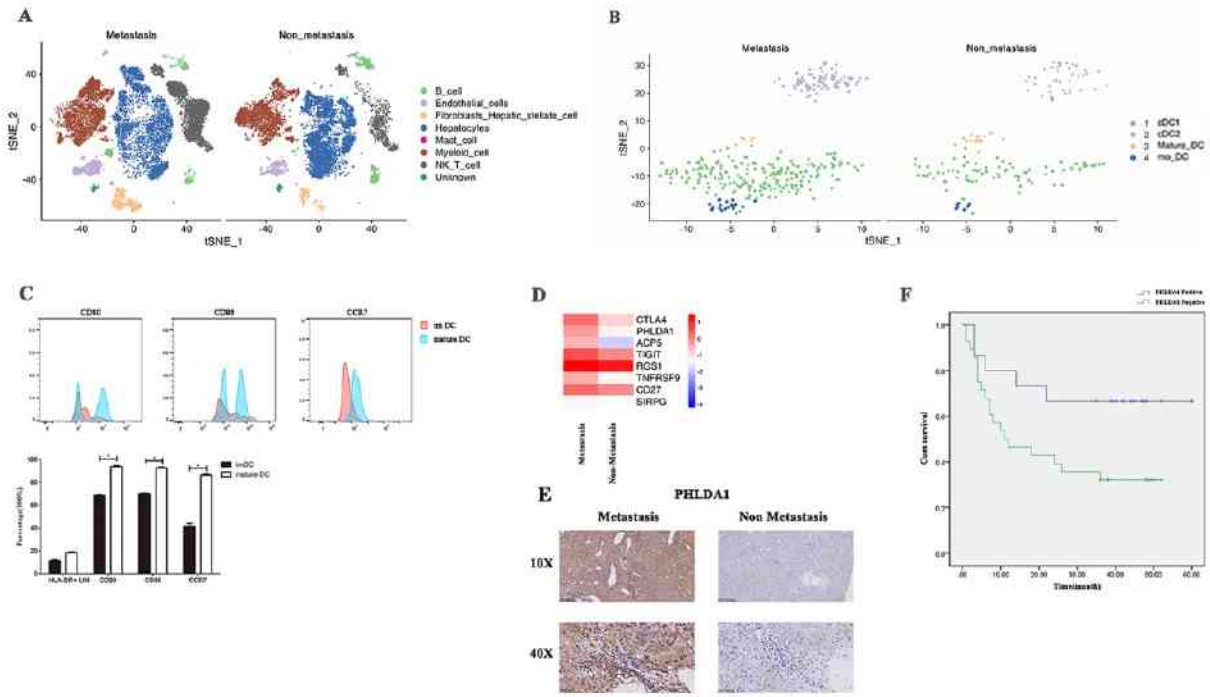
Results: A total of 20 Clusters including 6 hepatocyte clusters, 4 myeloid cell clusters, 3 natural killer (NK) cell clusters, 3 B cell clusters, 2 endothelial cell clusters, 1 mast cell cluster, 1 fibroblast cluster were identified. Myeloid cells were further divided into 12 clusters, 4 of which were dendritic cells including cDC1, cDC2, CCR7+ mature DC, mo-DC. CCR7+ mature DC cells were enriched in non-metastasis group and deficient in metastasis group ($P<0.05$). Cells in CCR7+ mature DC expressed the maturation markers CD80, CD86, DC maturation stimulator gene CCR7 and its ligand CCL19. CCR7 and CCL19 were also significant differential genes between metastasis group and non-metastasis group. Pseudotime state transition analyze showed that the branch containing CCR7+ mature DC cells and cDC2 cells flows toward the hub during HCC progression. Mo-DC and cDC1 cells were terminally DC cells in metastasis state. Further cell-cell analysis also showed that CCR7/CCL19 receptor-ligand interaction play an important role in CD 8+ T cell/DC cell communication. We conducted flow cytometry to verify the expression of CCR7+ mature DC cells. Results showed that during the maturation of DC

cells in vitro culture, expression of CCR7, CD80, CD86 were significantly upregulated. As for NK/T clusters, NK/T cells were further divided into 9 clusters, Treg cells were significantly enriched in metastasis group. 3 clusters of Treg cells were identified including eTreg, non-Treg and nTreg cells. Pseudotime state transition analyze showed that FOXP3- non-Treg cells flow toward to hub and eTreg cell cluster was the terminally Treg cells. Exhausted CD8+ T cells and Treg cells common genes CTLA4, PHLDA1, TIGIT were significant differential genes in Treg cells between metastasis group and non-metastasis group. Immunohistochemistry was conducted in 46 tumor tissues from HCC patients to verify the expression of PHLDA1 in tumor tissues. Results showed that expression of PHLDA1 was significantly higher in HCC metastasis patients (2.24 vs 0.81, $P<0.05$). Patients with positive PHLDA1 had significantly worse prognosis than patients with negative PHLDA1 (1 year survival rate 46.4% vs 80%, $P<0.05$).

Conclusion: The present study revealed that down regulation of CCR7+ mature DC cells and enrichment of effector Treg cells were main metastasis-associated immune features. Which indicated that function of antigen presentation and T cell activation was impaired and immunosuppression was enhanced. Our findings provide new insights into immune therapy in the future.

❖OP-039

Figure:



❖OP-040

microRNA-223 attenuates hepatocarcinogenesis by blocking hypoxia-driven angiogenesis and immunosuppression

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Object/Background: and Aims: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide. Although current treatment to block angiogenesis and immunosuppression provides some benefits only for a subsets of HCC patients, optimized therapeutic regimens are unmet needs, which require a thorough understanding of the underlying mechanisms by which tumor cells orchestrate inflamed tumor microenvironment that is associated with significant myeloid cell infiltration. microRNA-223 (miR-223), a neutrophil-specific miRNA, is well known to act as an important anti-inflammatory regulator, thereby inhibiting liver disease progression by controlling neutrophil infiltration and activation; however, whether and how miR-223 affects HCC development remain unclear.

Methods: miR-223 knockout (miR-223KO) mice and wild-type (WT) littermates were generated and subjected to two mouse HCC models induced by injection of diethylnitrosamine (DEN) with chronic carbon tetrachloride (CCl4) or orthotopic HCC cell implantation with chronic CCl4 injection. In detail, the mice were administered with DEN (single i.p. injection of 25 mg/kg at 15 days of age), and CCl4 (starting at 8 weeks of age; 25% dissolved in olive oil; 2 ul/g; once per week via i.p injection) was challenged for continuous 21 weeks, where 100% male mice developed HCC. For HCC cell line-derived xenograft HCC model, the Hepa1-6 cell mixture was orthotopically implanted at the margin of major lobes in mouse livers, and the implanted mice were continuously administrated with CCl4 treatment (2 ml/kg, 10% dissolved in olive oil, once per week via i.p. injection) for 2 more weeks. In addition, adenovirus-mediated hepatic miR-223 overexpression was also used to treat HCC.

Results: In the current study, we demonstrated that genetic deletion of miR-223 markedly exacerbated tumorigenesis in two mouse models of inflammation-associated HCC induced by injection of DEN with chronic CCl4 or orthotopic HCC cell implantation with chronic CCl4 injection as demonstrated that miR-223KO mice had greater tumor masses and

number of DEN+CCl4 HCC compared with WT mice. Correspondingly, miR-223KO mice showed more progressively proliferative capacity than WT mice as demonstrated by higher mRNA levels of HCC biomarkers (Afp, Gpc3, Golm1 and Tff3) and Ki67 index (a marker for tumor proliferation). Interestingly, compared to WT mice, miR-223KO mice had more infiltrated programmed cell death 1 (PD-1+) T cells and programmed cell death ligand 1 (PD-L1+) macrophages after DEN+CCl4 administration. Furthermore, as a hallmark of tumor growth, angiogenesis of HCC tumor region in miR-223KO mice was significantly greater than that in WT mice, which was evidenced by higher CD31 (a marker for endothelial cell) expression. Bioinformatic analyses of RNA-Seq data revealed a strong correlation between miR-223 levels and tumor hypoxia, a condition that is well-documented to regulate PD-1/PD-L1. In vivo and in vitro mechanistic studies demonstrated that miR-223 did not directly inhibit PD-1 and PD-L1 in immune cells rather than indirectly downregulated them by modulating tumor microenvironment in HCC. Our in vivo and in vitro data further suggested that miR-223 targeted hypoxia inducible factor 1α (HIF-1α) in HCC tumor cells and subsequently suppressed immune checkpoint PD1/PD-L1 expression, thereby inhibiting HCC. Moreover, gene delivery of miR-223 via adenovirus inhibited angiogenesis and hypoxia-mediated PD-1/PD-L1 axis activation in both HCC models, thereby hindering HCC progression.

Conclusion: We identify miR-223 as a key orchestrator for tumor hypoxia and inflammatory tumor microenvironment in controlling HCC progression. Mechanistically, HIF-1α is a direct target of miR-223 in HCC, and miR-223 ameliorates HCC growth, angiogenesis and PD-1/PD-L1 activation in HCC surrounding regions by limiting HIF-1α, suggesting that miR-223 plays a critical role in modulating hypoxia-induced tumor immunosuppression and angiogenesis, which may serve as a novel therapeutic strategy for HCC.

A Chinese system of staging and treatment strategy for post-hepatectomy recurrent hepatocellular carcinoma

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Object/Background: Treatment strategy for recurrent hepatocellular carcinoma remains scantily defined. Recommendations based on the Barcelona Clinic Liver Cancer staging and treatment system in treating primary hepatocellular carcinoma may not be simply copied to treat recurrent hepatocellular carcinoma. Not only the recurrent tumor data, the patient's present hepatic functional reserve and basic characteristics, but also the surgical and pathologic profiles of the previous resected tumor need to be considered in the decision making process on treatment of hepatocellular carcinoma recurrence. This study was aimed to establish a system of staging and treatment strategy to manage post-hepatectomy recurrent hepatocellular carcinoma, and report the clinical outcomes.

Methods: From January 2006 to December 2016, 556 consecutive patients who developed recurrence after curative liver resection of hepatocellular carcinoma were retrospectively enrolled, clinical outcomes and prognostic factors were investigated. A draft system based on the findings was established by the multi-disciplinary team. From January 2018 to December 2019, a prospective cohort of patients with post-hepatectomy recurrent hepatocellular carcinoma was enrolled to confirm and improve the system. Clinical data and survival times were prospectively collected and analyzed.

Results: In the retrospective cohort of 556 patients with recurrent hepatocellular carcinoma, 341 (61.3%) patients received transarterial chemoembolization, 90 (16.2%) received repeat resection, 74 (13.3%) received local ablation, 17 (3.1%) received radiotherapy and 34 (6.1%) received palliative treatment. At a median follow-up of 31 months (range 10-176 months), the 1-, 3-, and 5-year overall survival rates after recurrence were 72.2%, 34.3%, and 25.0%, respectively. Patients who received curative treatments in the form of repeat resection and local ablation achieved best survival outcomes. On multivariate analysis using the Cox regression model, four independent risk factors for the survival of recurrent hepatocellular carcinoma including extrahepatic recurrence (HR: 2.704, 95%CI: 1.569-5.871, P=0.001), multiple tumors (HR: 1.561, 95%CI: 1.023-2.743, P<0.001), macrovascular invasion (HR: 3.495, 95%CI: 1.379-7.647,

P<0.001), microvascular invasion positive of the resected tumor in initial hepatectomy (HR: 1.558, 95%CI: 1.056-2.434, P<0.001) and disease-free interval ≤1 year (HR: 1.795, 95%CI: 1.404-2.294, P<0.001) were addressed. Accordingly, a draft system including staging and treatment strategy was established, and then we made some improvements due to some new treatment options involved recently (Figure 1). In the prospective cohort of 236 patients, 96(40.7%), 93(39.4%), 35(14.8%) and 12(5.1%) patients were assigned to Early, Intermediate, Advanced and Terminate stages, respectively. The treatment options were recommended according to the system. After a median follow up of 36 months (range 3-77 months), the 1-, 3-, and 5-year overall survival rates after recurrence were 82.7%, 48.8% and 39.3%, respectively, for the Early stage patients, while 73.6%, 33.8% and 19.9%, respectively, for the Intermediate stage patients. The median survival times were 30 months (range 5-80 months) for the Advance stage patients and 6.5 months (range 1-11 months) for Terminal stage patients. The long-term survival outcomes of the four stages as plotted by the Kaplan-Meier curves separated nicely (Figure 2).

Conclusion: A system of staging and treatment strategy has been successfully established to manage post-hepatectomy recurrent hepatocellular carcinoma. This system derived from the Barcelona Clinic Liver Cancer staging system, but with some important modifications which made it more suitable for the recurrent hepatocellular carcinoma.

Figure:

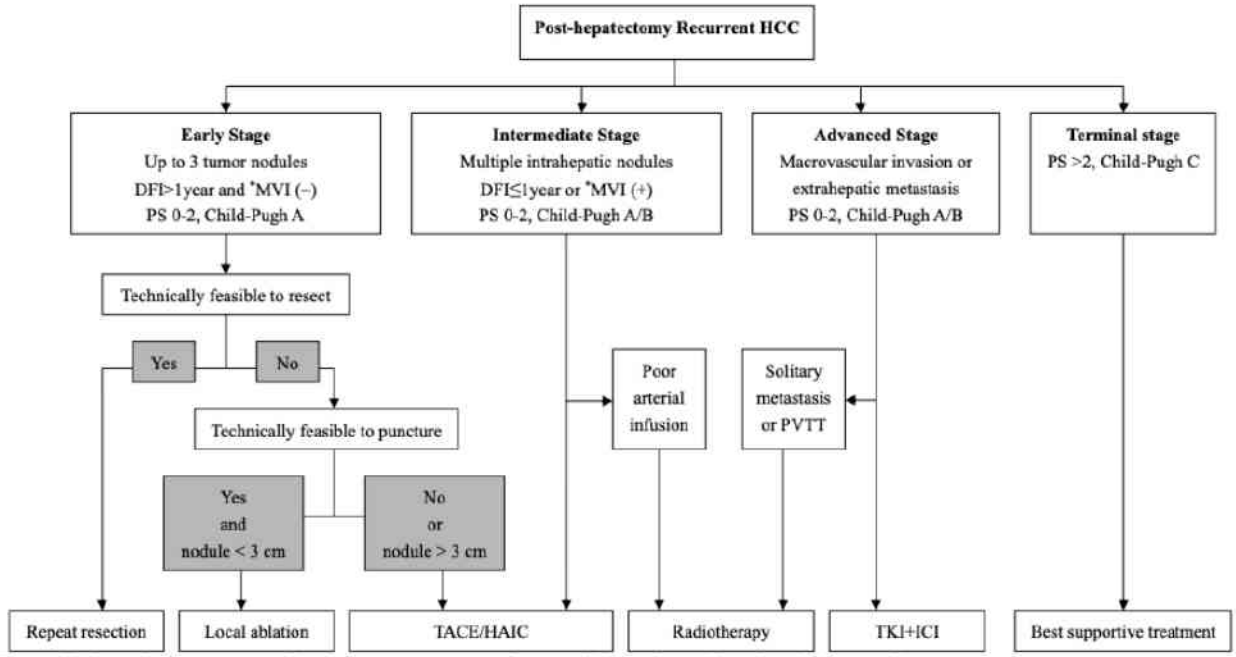

Figure 1. Staging and treatment strategy for post-hepatectomy recurrent hepatocellular carcinoma. *Data from initial resected tumor
HCC, hepatocellular carcinoma; DFI, disease-free interval; MVI, microvascular invasion; PS, performance status; PVTT, portal vein tumor thrombus; TACE, transarterial chemoembolization; HAIC, hepatic artery infusion chemotherapy; TKI, tyrosine kinase inhibitor; ICI, immune checkpoint inhibitors

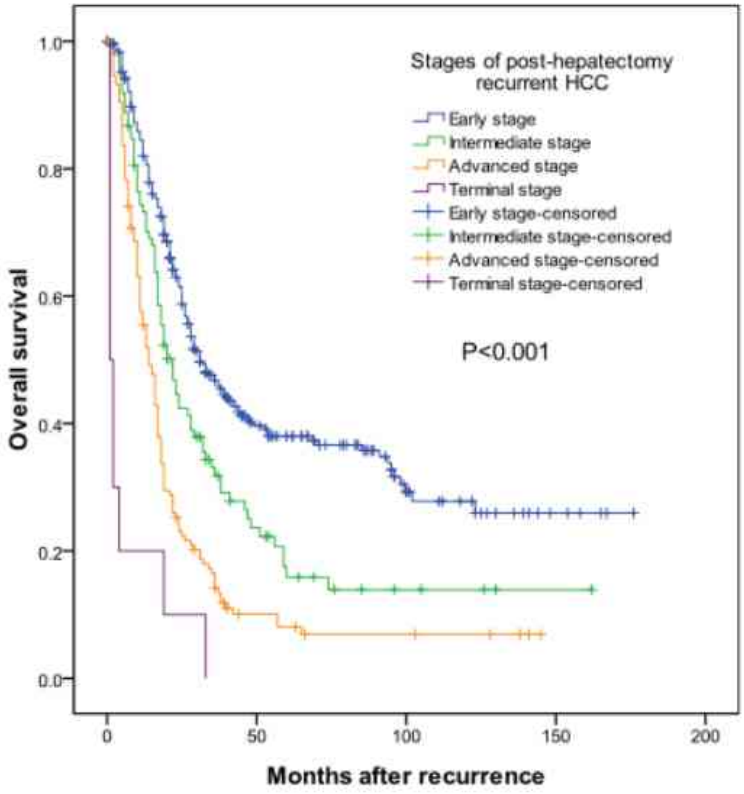

Figure 2. Survival curves in the prospective cohort of patients with four stages of recurrent hepatocellular carcinoma.

HCC, hepatocellular carcinoma

❖OP-042

Presence of liver inflammation and fibrosis in Asian patients with chronic hepatitis B in the grey zone

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Object/Background: Over a quarter of chronic hepatitis B (CHB) patients did not meet criteria of the traditional natural phases and hence classified as grey zone (GZ). However, few studies reported the liver inflammation and fibrosis of Asian CHB patients in the GZ.

Methods: This multicenter, retrospective study included 1,064 CHB patients underwent liver biopsy (LB). Phases of natural history were determined according the AASLD 2018 Guidance. GZ patients were divided into four categories: HBeAg-positive, normal ALT and HBV-DNA≤106 IU/ml (GZ-A); HBeAg-positive, elevated ALT and HBV-DNA<2×104 IU/ml (GZ-B); HBeAg-negative, normal ALT and HBV-DNA≥2×103 IU/ml (GZ-C); HBeAg-negative, elevated ALT and HBV-DNA<2×103 IU/ml (GZ-D).

Results: 263 (24.7%) patients were in the GZ with the median age of 41.5 years and male of 67.2%. Among the GZ patients, GZ-D (44.1%) was the dominate category, followed by GZ-C (37.6%), GZ-A (9.9%) and GZ-B (8.4%). Surprisingly, as high as 60.4% of GZ patients had significant inflammation (≥G2) and 89.7% GZ patients had significant fibrosis (≥S2), which were higher than that of patients with immune-tolerant and inactive phases. Over half of patients had significant inflammation or fibrosis in each GZ category. GZ-B patients had the highest proportions of significant inflammation (95.5%) and fibrosis (81.9%) compared to other GZ categories (GZ-A: 76.9% and 69.3%; GZ-C: 53.5% and 52.5%; GZ-D: 56.1% and 60.4%, respectively).

Conclusion: A substantial GZ patients had significant liver inflammation or fibrosis, especially for CHB patients with GZ-B. Using liver biopsy to assess the liver histological activity should be encouraged in GZ patients.

❖PP-001

Short term mortality risk value of multiple models in predicting IPA in patients with HBV-ACLF

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Object/Background: Acute-on-chronic liver failure (ACLF) means on the basis of chronic liver disease, patients suffer from acute liver function decompensation under the action of a variety of pathogenic factors, resulting in a series of clinical symptoms. The disease is serious and complex. At present, there is no ideal treatment, and the short-term mortality is high. The main causes of ACLF are reactivation of hepatitis virus, bacterial infection and alcoholism, the main cause of ACLF in China is HBV infection. Patients with ACLF have severe impairment of immune functions and are prone to various infections. Due to the use of broad-spectrum antibiotics, the increase of invasive procedures and the use of glucocorticoids, the incidence of fungal infection has increased. The mortality of ACLF patients with fungal infection is high. Early prediction of short-term mortality risk in patients with ACLF is of great significance to the prognosis of patients. There are few studies on the short-term death risk prediction model of ACLF complicated with pulmonary aspergillosis. This study used MELD score, MELD-Na score, CLIF-C OF score, and CLIF-C ACLF score to predict the patients' short-term mortality risk in Acute-on-chronic liver failure (ACLF) with invasive pulmonary aspergillosis (IPA), to explore and compare the predictive value of various scoring systems for the short-term case fatality rate in patients with ACLF incorporate IPA.

Methods: Collected the clinical data of ACLF patients, who admitted to the First Affiliated Hospital of Nanchang University from January 2019 to December 2021. A total of 110 patients were collect, which divided into IPA group (n=10) and control group (n=100) according to whether IPA occurred. The MELD score, MELD-Na score, CLIF-C OF score and CLIF-C ACLF score were calculated respectively, and the subject operating characteristic curve (ROC curve) was applied to compare the diagnostic value of the above scoring system. Comparisons between two groups who fit the normal distribution were performed by the t-test,Comparisons between two groups who did not fit the normal distribution were performed using the Mann-Whitney U test.

Results: Among the 110 patients,there were 89 males and 21 females, aged 39 (31 ~ 49) years. There were 4 patients with type 2 diabetes. 45 patients were treated with voriconazole, 106 with broad-spectrum antibiotics. There were significant differences in the occurrence of WBC and ascites between the two groups (P < 0.05); The incidence of WBC and ascites in blood was significantly higher than that in control group (p<0.05). 36 patients (32.7%) died after 28 days of treatment.The area under the ROC curve (AUC) of the MELD score, MELD-Na score, CLIF-C OF score and CLIF-C ACLF score was 0.77,0.74,0.93 and 0.89, respectively. The cut-off value of the MELD score, MELD-Na score, CLIF-C OF score and CLIF-C ACLF score was 37.4,37.4,10.5,41.7,respectively. The sensitivity of the MELD score, MELD-Na score, CLIF-C OF score and CLIF-C ACLF score was 69.2%,71.8%,84.6%,87.2%,respectively. The specificity of the MELD score, MELD-Na score, CLIF-C OF score and CLIF-C ACLF score was 76.1%,70.4%,88.7%,84.5%,respectively.The Youden index of the MELD score, MELD-Na score, CLIF-C OF score and CLIF-C ACLF score was 0.453,0.422,0.733,0.717,respectively. The largest AUC was CLIF-C OF score, and there was no significant difference with CLIF-C ACLF score (P> 0.05).The comparative difference between CLIF-C OF score with MELD score and MELD-Na score AUC was significant (P <0.05).

Conclusion: The CLIF-C OF score and CLIF-C ACLF score can effectively predict the 28-day risk of death in patients with ACLF incorporate the IPA.

❖PP-002

The risk factors and establishment of predictive model of developing invasive pulmonary aspergillosis in HBV related acute-on-chronic liver failure.

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Object/Background: Liver failure is serious and complex, has a high short-term mortality. Acute on chronic liver failure (ACLF) refers to the acute decompensation of patients with chronic liver disease under the action of pathogenic factors. The main causes of ACLF are reactivation of hepatitis virus, bacterial infection and alcoholism, and the main cause of ACLF in China is HBV infection .The patients with ACLF have low immune function due to massive injury or death of Kuffer cells, and are prone to severe complications such as hepatorenal syndrome and hepatic encephalopathy, resulting in multiple organ failure and high mortality. At the same time, ACLF patients are prone to infection. Due to the extensive use of antibiotics, long-term hospitalization and invasive operation, the rate of invasive fungal infection increases. The early symptoms of ACLF complicated with fungal infection are atypical. A large number of studies have reported the risk factors and prognosis of ACLF, but the research on ACLF complicated with fungal infection is still insufficient.The study aimed to explore the risk factors of invasive pulmonary aspergillosis (IPA) and death of short-term (90 days) in patients with hepatitis B virus-related acute-on-chronic liver failure (HBV-ACLF), establish the prediction model.

Methods: HBV-ACLF patients from the First Affiliated Hospital of Nanchang University were retrospectively screened from January 2017 to December 2021, a total of 128 patients were collect, which divided into IPA group (n=17) and control group (n=111) according to whether IPA occurred. Gender, age, diabetes, complications of liver failure and relevant laboratory examinations were collected. The prognosis of all patients were observed and followed up for 90 days. The independent risk factors of IPA and of 90-day death in HBV-ACLF patients were analyzed by multivariate logistic regression, the prediction model of IPA was established. Kaplan-meier method was used to draw survival curves.

Results: Among the 128 patients with HBV-ACLF, 17 patients developed IPA, the incidence rate was 13.28%. peritoneal effusion, diabetes, hepatorenal syndrome, respiratory dysfunction or failure, and the use of glucocorticoid were independent risk

factors for IPA in HBV-ACLF patients, while the use of voriconazole was a protective factor for IPA in ACLF patients. The six factors were combined to establish the prediction model, the area under the curve of subject operating characteristic curve(AUC)(95%CI) was 0.856 (0.782~0.912), the cut-off value was 1.097, the sensitivity was 83.13%, the specificity was 90.70% ,the Youden index was 0.74. IPA, peritoneal effusion and hepatic encephalopathy were independent risk factors for 90-day death in HBV-ACLF patients, while the use of voriconazole was a protective factor. The survival rate of patients in IPA group was lower than in control group(17.6% vs 45.95%, P = 0.007), the survival rate of patients with use of voriconazole was higher than non users(54.5% vs 32.9%, P = 0.004), the survival rate of patients with hepatic encephalopathy was lower than that without hepatic encephalopathy (5.88% vs 66.23%, P < 0.001). and the survival rate of patients with peritoneal effusion was lower than that without peritoneal effusion (25.64% vs 49.44%, P = 0.007).

Conclusion: peritoneal effusion, diabetes, hepatorenal syndrome, respiratory dysfunction or failure, the use of glucocorticoid are independent risk factors for IPA in HBV-ACLF patients. The prediction model has good predictive value for the risk of IPA in HBV-ACLF patients. IPA, peritoneal effusion and hepatic encephalopathy are independent risk factors for 90-day death in patients with HBV-ACLF.

❖PP-003

A patient of Visceral Leishmaniasis combined with bone marrow infection, pancytopenia and splenomegaly in a non-endemic area in China

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Object/Background: Kala-azar is a zoonotic chronic endemic infectious disease caused by Leishmania donovani infection, also known as visceral leishmaniasis. It is an important vector-borne disease. At present, it is endemic worldwide, mainly transmitted by the bite of female sandflies, inducing chronic infection. Its clinical manifestations are diverse and similar to those of many diseases, such as malaria and pulmonary tuberculosis, which complicates its diagnosis and leads to the lack of timely and effectively treatment, seriously threatening the life and health of patients.Many of these diseases can also co-infect with visceral leishmaniasis, resulting in accumulation of Leishmania donovani in the spleen, bone marrow and lymph nodes, which further complicates the problem.

Methods: This study reports a female patient with visceral leishmaniasis accompanied by bone marrow infection, pancytopenia and splenomegaly. Leishmania donovani was observed in the bone marrow smear of this patient. After diagnosis, the patient received short-term treatment with amphotericin B liposome in time.
And then switched to sodium stibogluconate on June 27th, 2021 for continued treatment due to poor therapeutic effect of amphotericin B liposome.reexamination of abdominal ultrasound showed that the spleen of the patient was smaller than before, and the effect of antimonial was significant. The patient continued to use antimonial, and the fatigue symptoms were significantly relieved.

Results: The patient was discharged on July 8th, 2021, and the spleen was palpable 2 cm below the costal arch at discharge. The spleen size was significantly reduced, and the skin pigmentation basically subsided. The proliferation of parasites in macrophages in the liver, spleen and bone marrow of patients with visceral leishmaniasis may leads to progressive hepatosplenomegaly and bone marrow suppression, and then develop allogeneic cytopenia and immunosuppression, resulting in increased host susceptibility to bacterial

infections and eventually death if not given timely and effective treatment. Physical examination of the patient showed rash and diffuse skin pigmentation during hospitalization. ELkhair EB et al. found that skin hyperpigmentation may be the result of cytokine-induced increased adrenocorticotropic hormone production , so the analysis of a large number of cellular inflammatory factors can provide a more comprehensive understanding of the immunopathogenesis of visceral leishmaniasis. We could find from the examination of 12 cytokines at admission that the levels of IL-1β, IL-5, IL-6 and IL-8 were >10 times higher than the normal values. However, what needs most attention is the microbiome on the IL-17 immune axis, which plays an indispensable role in the host immune response to pathogens and symbionts. This provides new ideas for the establishment of immune mechanisms and immunological detection methods for visceral leishmaniasis.

Conclusion: This paper mainly summarizes the epidemiology of visceral leishmaniasis in the world and China, and analyzes the clinical features and treatment protocols in conjunction with various indicators of this patient, in order to better explore visceral leishmaniasis in non-endemic areas and better provide some reference significance for the detection, diagnosis, treatment and later prevention and treatment of black fever in non-endemic areas.

At present, although there are a few reports of visceral leishmaniasis in non-endemic areas, it hasn’t been paid much attention to in recent years. This study suggests that visceral leishmaniasis should still attract the attention of the world by reporting cases in non-endemic areas. Whether from the pathogenesis, rapid diagnostic methods and effective treatment, it is necessary to carry out continuous detection in endemic areas and case reports nationwide or even worldwide, striving to make further breakthroughs in the infection caused by Leishmania donovani and providing the basis for the elimination of leishmaniasis all over the world.

❖PP-004

Chronic Q fever endocarditis complicated by Epstein-Barr virus infection was diagnosed by mNGS: A case report and literature review

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Object/Background: Q fever is a zoonotic disease, and humans are primarily infected by inhalation of infected aerosols. The clinical presentation is commonly non-specific, and Chronic Q fever is usually characterized by endocarditis and persistent fever, which seriously threatens the lives of patients. Timely and effective pathogen diagnosis becomes the key to effective control of the disease. Here we reported a case of chronic Q-fever endocarditis complicated with Epstein-Barr virus infection in a patient with symptoms such as intermittent fever, pancytopenia and hepatosplenomegaly. The patient had a heart valve replacement 8 years ago and a past history of living in Xinjiang. Multiple blood cultures and various test parameters failed to confirm the diagnosis, and Coxiella burnetii and Epstein-Barr virus were finally detected in the blood by mNGS. The treatment was timely adjusted to minocycline combined with linezolid for anti-infective treatment, and the patient's condition was significantly improved. To our knowledge, this is the first reported case employing mNGS to diagnose C. Burnetii and EBV co-infection.

Methods: PET-CT and liver puncture pathological biopsy did not reveal the causes, while enhanced liver MRI scan showed diffuse liver damage. Bone marrow cytomorphology examinations revealed mononuclear phagocytic histiocytes and a few abnormal lymphocytes. Serologies (IgG) for cytomegalovirus, rubella virus, Toxoplasma, and herpes simplex virus were all positive and showed past infections. Additional examinations performed consisted of CMV virus test, T cell spot test for tuberculosis infection, Epstein-Barr virus test, Brucella agglutination test, and novel bunyavirus nucleic acid detection, which were all negative. The bacterial culture and fungal culture of blood samples were taken while no pathogen was identified.
In order to definite cause and pathogenesis, approximately 5 mL of blood sample was collected and sealed using a sterile technique, transported at 4℃ to Hugobiotech Co., Ltd. (Beijing, China) to perform mNGS .

Results: In this study, we reported the first known case of Q fever endocarditis complicated with EB virus infection diagnosed by mNGS in a patient with a heart valve replacement. The patient had pancytopenia, hepatosplenomegaly and intermittent fever for more than six months, and had a past history of living in Xinjiang. He was not definitely diagnosed by a series of laboratory tests, and finally C. burnetii and Epstein-Barr virus were detected in the blood by mNGS. This case suggests that patients with persistent intermittent fever and a history of heart-related disease or possible livestock exposure should be considered seriously as Q fever.

Conclusion: This paper emphasizes that as a new generation of detection methods in clinical application in recent years, the feasibility and superiority of mNGS can assist in rapid clinical diagnosis and avoid delayed treatment in future pathogenic microorganism detection.mNGS can be the most promising and sensitive diagnostic tool for Q fever, especially when the clinical symptoms are non-specific. The diagnosis and treatment process described in this case provided evidence for the clinical characterization and diagnosis of chronic Q fever, which could help reduce misdiagnosis and unnecessary over-diagnosis and treatment, and provide a certain degree of guidance for the rapid diagnosis of Q fever in epidemic and non-epidemic areas.

❖PP-005

Research on the effect of multi-department interaction in preventing multi-drug resistant bacteria infection in XX Hospital

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Object/Background: To explore the infection status of multidrug resistant Organism (MDROs) in XX Hospital and the role of multi department linkage in implementing prevention and control measures.

Methods: The patients admitted to Macheng people's Hospital (June 1, 2017 - June 31, 2019) were taken as the control group (n = 201), the routine prevention and control measures were adopted, and the patients admitted to Macheng people's Hospital (July 1, 2019 - June 31, 2021) were taken as the experimental group (n = 150), and the multi department linkage was adopted to implement the prevention and control measures. Analyze the current situation of MDROs infection in the two groups, and compare the use intensity of antibiotics, the implementation compliance of prevention and control measures, the hospital infection rate of MDROs on hospitalization day, the community colonization rate of MDROs and the hospital colonization rate of MDROs between the two groups.

Results: The number of detection (5.25 ± 1.12), the number of cases (2.01 ± 0.23), the type of infection (CA 25.33%, ha 32.67%, uninfected 42.00%) in the experimental group were compared with those in the control group (5.32 ± 1.20), (2.00 ± 0.21), and the type of infection (CA 28.86%, ha 36.82%, uninfected 34.33%) (P > 0.05).The number of defined daily dose of antibiotics consumed per 100 person days (DDD) (5124.25 ± 101.01), the total length of hospital stay (4387.24 ± 124.36) in the experimental group were lower than those in the control group (6598.63 ± 123.52), (6485.52 ± 122.30), and the use intensity of antibiotics (114.25 ± 1.20) The implementation compliance of prevention and control measures (70.20 ± 15.52) and the average length of hospital stay (28.98 ± 3.54) were higher than those in the control group (98.52 ± 2.30), (45.62 ± 10.20), (27.20 ± 4.51), P < 0.05.The nosocomial infection rate of MDROs in the experimental group (2.00%) was lower than that in the control group (10.95%), P < 0.05. The community colonization rate of MDROs (1.33%) and hospital colonization rate of MDROs (0.67%) in the experimental group were lower than 9.95% and 8.96% in the control group (P < 0.05).

Conclusion: multi department linkage implementation of prevention and control measures can improve the implementation compliance of MDROs prevention and control measures of clinical medical staff, promote the rational application of antibiotics and reduce the incidence of MDROs nosocomial infection.

Figure:

表 2 对比两组抗菌药物使用强度、防控措施落实依从性

组别	例数	抗菌药物累计DDD数	抗菌药物使用强度	依从率（%）	平均住院天数（d）	总住院天数（d）
对照组	201	6598.63±123.52	98.52±2.30	45.62±10.20	27.20±4.51	6485.52±122.30
实验组	150	5124.25±101.01	114.25±1.20	70.20±15.52	28.98±3.54	4387.24±124.36
t 值		119.392	76.347	17.873	4.000	157.870
P 值		<0.050	<0.050	<0.050	<0.050	<0.050

❖PP-006

CRISPR/Cas13a-assisted Hepatitis B Virus Covalently Closed Circular DNA Detection

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Object/Background: The formation of an intranuclear pool of covalently closed circular DNA (cccDNA) in the liver is the main cause of persistent hepatitis B virus (HBV) infection. HBV cccDNA is a template for the replication of HBV RNA and virus-derived progeny because it can be continuously and stably stored in the hepatocyte nucleus and cannot be eliminated by any antiviral drugs. Therefore, it is necessary to detect the persistence of HBV cccDNA, which contributes to guiding further clinical therapeutics. The clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein (Cas) system is an acquired immune system for the cleavage of foreign genetic elements from invading viruses and phages and was first identified in bacteria and archaea, which also is used to detect a wide variety of pathogens. Advantages of the CRISPR-Cas detection system can compensate for the shortcomings of the current HBV cccDNA testing methods.

Methods: We used plasmid-safe ATP-dependent DNase (PSAD) enzymes and HindIII to digest loose circle rcDNA and double-stranded linear DNA, amplify specific HBV cccDNA fragments by rolling circle amplification (RCA) and PCR, designed and screened crRNA for CRISPR detection, and detect the target gene by using CRISPR-Cas13a technology. HBV cccDNA plasmid and plasmid-transfected cellular DNA were detected by ddPCR, qPCR and PCR-CRISPR, respectively. 40 HBV-related liver tissues samples and 24 HBV-related plasma, whole blood and peripheral blood mononuclear cells (PBMCs) samples were detected and compared by ddPCR, qPCR, RCA-qPCR, PCR-CRISPR and CRISPR-Cas13a-based assay.

Results: Based on the sample pretreatment step, the amplification step and the detection step, we established a new CRISPR-Cas13a-based assay for the detection of cccDNA. For HBV cccDNA plasmid, the sensitivity of the PCR-CRISPR method for HBV cccDNA was 1 copy/μL, which was higher than that of ddPCR detection approaches; For plasmid-transfected cellular DNA, the sensitivity of the ddPCR, qPCR and PCR-CRISPR method for HBV cccDNA were 102 copies/μL, 103 copies/μL and 10 copies/μL, respectively; For HBV cccDNA-positive samples, the sensitivity of the CRISPR-cccDNA assay was as low

as 1 copy/μL, which was consistent with ddPCR and qPCR methods, while higher PCR-CRISPR and RCA-qPCR methods detected 10 copies/μL concentration of HBV cccDNA. We used ddPCR, qPCR, RCA-qPCR, PCR-CRISPR and RCA-PCR-CRISPR methods to detect 20, 4, 18, 14 and 29 positive samples in liver tissue samples from 40 HBV-related patients, respectively. The results suggested that the positive coincidence rate of the CRISPR-cccDNA assay was higher than that of other methods. HBV cccDNA was almost completely undetected in the 20 blood samples of HBV patients (including plasma, whole blood and PBMCs) by the above five methods.

Conclusion: In summary, Here, we established a high-sensitivity and high-specificity HBV cccDNA detection method using the CRISPR-Cas system combined with RCA and PCR methods. It provides a powerful tool for clinical treatment and useful guidance for patients undergoing long-term anti-HBV therapy to improve their administration. Moreover, But too few samples were detected, and there was no correlation and statistical significance between these results, so we concluded that HBV cccDNA was not present in plasma, whole blood or PBMCs. Further study will be required to optimize the amplification detection steps of the method and verify the connection between liver tissue and extrahepatic HBV cccDNA.

❖PP-007

Clinical, biochemical, and molecular characteristics of infantal-onset Dubin-Johnson syndrome: A case-series study

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2. Xi'an Children's Hospital

Object/Background: To analyze the clinical and biochemical characteristics of patients with infantal-onset Dubin Johnson syndrome (DJS), so as to provide some evidence for the etiological diagnosis and differential diagnosis of infantile cholestasis. To Summarize the molecular characteristics of DJS and further enrich the mutation spectrum of ABCC2 gene.

Methods: 8 infants with DJS diagnosed in Xi'an Children's Hospital from January 2015 to June 2022 were enrolled in the study, and their clinical and biochemical characteristics, ABCC2 gene mutations and prognosis were retrospectively analyzed. The gene detection of all patients was performed by next generation sequencing with liver disease related gene panel, and sanger sequencing was performed in the family. The mutations of the pathogenic gene were compared with Human Gene Mutation Database, and the related software was used to predict the harmfulness and protein structure of the novel mutation, and the pathogenicity was evaluated.

Results: Among the 8 patients with DJS, 6 were male and 2 were female. The age of onset was (3.4 ± 1.2) months, and the age of diagnosis was (5.6 ± 3.0) months. The main manifestation of all patients was the delayed regression of jaundice. 7 cases (70.0%) were accompanied by dark urine, and there were no stool discoloration, hepatomegaly and splenomegaly. Cholestasis was present in all 8 children. Abnormal biochemical indicators included total bilirubin [(115.0 ± 54.5) umol/L], direct bilirubin [(73.9 ± 36.6) umol/L], g-glutamyl transpeptidase [(111.4 ± 45.1) umol/L], total bile acid [(100.9 ± 51.9) umol/L]. Alanine aminotransferase <40U/L, 40~80 U/L and >80 U/L were 3 cases (37.5%), 4 cases (50.0%) and 1 case (12.5%), respectively. Aspartate aminotransferase <40 U/L and 40~80 U/L were 4 cases (50.0%). Among the 8 children, 2 were homozygous and 6 were compound heterozygous. A total of 11 gene mutations were detected, including 6 missense mutations (c.4024T>C, c.1177C>T, c.2148G>T, c.4103T>A, c.2366C>T and

c.2063T>C), 2 deletion mutations (c.1063delA and c.4210_4212delGAG), 1 nonsense mutation (c.2443C>T), 1 insertion mutation (c.4237_4238insCT) and 1 splicing mutation

(c.2439+5G>A). Among the 16 mutations, 6 (37.5%) were c.1177C>T, and 1 (6.3%) for each of the other 10 mutations. 7 novel mutations were found, which were c.2148G>T(p.K716N), c.4103T>A(p.I1368N), c.2063T>C (p.M688T), c.1063delA (p.T355Hfs*36), c.2439+5G>A, c.4237_4238insCT (p.H1414Lfs*18) and c.4210_4212delGAG (p.1404delE), where c.1063delA and c.4237_4238insCT were probably pathogenic, and the other five mutations were of unknown significance. All patients were treated with ursodeoxycholic acid, and 2 cases had taken phenobarbital orally. After 12.8 (12.0, 14.4) months of follow-up, total bilirubin fluctuated between 15.9 and 55.6umol/l and direct bilirubin fluctuated between 10.0 and 34.9 umol/l. Alanine aminotransferase, aspartate aminotransferase, g- glutamyl transpeptidase and total bile acid were normal.

Conclusion: The clinical manifestations of infantal-onset DJS are nonspecific, and the transaminase is mostly lower than 80U/L. Next generation sequencing is helpful for the early diagnosis of the disease, and c.1177C>T may be a high-frequency mutation. A total of 7 novel mutations were found to further enrich the ABCC gene mutation spectrum.

❖PP-008

Stimulator of interferon gene aggravates Concanavalin A-induced acute immune hepatitis by triggering hepatocyte ferroptosis

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Object/Background: & Aims: Ferroptosis is a new type of cell death discovered in recent years, which is characterized by iron-dependent accumulation of lipid peroxidation. However, the specific mechanism of ferroptosis in acute immune hepatitis (AIH) remains to be clarified. The aim of this study is to investigate how stimulator of interferon gene (STING) modulates hepatocyte ferrptosis during Concanavalin A (ConA)-induced AIH.

Methods: WT and STING-/- mice aged 6-8 weeks were utilized to construct ConA-induced liver injury models. Ferrostatin-1, an inhibitor of ferroptosis, and iron chelator Defetoxamine were used in the experiments.

Results: Our study found that ferroptosis was associated with ConA-induced AIH, and pretreatment with the iron chelator Defetoxamine and the ferroptosis inhibitor Fer-1 could alleviate hepatocyte ferroptosis by reducing ferric iron content and inhibiting lipid peroxidation respectively. In addition, hepatic STING up-regulated in acute immune hepatitis and STING knockout mice showed decreased liver injury. Histopathology, immunofluorescence and immunohistochemical staining demonstrated that knockout of STING effectively reduced ferroptosis products malondialdehyde and 4-hydroxynonenal. Meanwhile, deficiency of STING reversed ConA-induced up-regulation of transferrin and transferrin receptor (Tf/TfR), as well as down-regulation of solute carrier family 7 members 11 and glutathione peroxidase 4 (xCT/GPX4) pathways, which inhibited cellular iron overload and lipid peroxidation, thereby ameliorating liver damage. These results suggested a regulatory role for STING in ferroptosis.

Conclusions: Ferroptosis is involved in ConA-induced AIH, and the inhibition of STING alleviates ConA-induced liver injury by inhibiting ferroptosis through suppressing the iron overload pathway Tf/TfR and promoting xCT/GPX4 expression against lipid peroxidation. Therefore, STING is a novel and pivotal therapeutic target in AIH.

❖PP-009

Magnesium isoglycyrrhizinate ameliorates concanavalin A-induced liver injury by regulating autophagy

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Object/Background: and Aims: Acute liver failure (ALF) is a type of liver injury that is caused by multiple factors and leads to severe liver dysfunction. Although the use of liver transplantation has drastically improved the survival rate, there are still many deficiencies in the treatment of ALF. Magnesium isoglycyrrhizinate (MgIG), a novel glycyrrhizin extracted from the traditional Chinese medicine licorice, have many pharmacological activities, such as antiviral, antimicrobial, anti-inflammatory, antitumor and other activities and has a significant protective effect against concanavalin A (ConA)-induced liver injury. However, its underlying curative mechanism is unclear. Hence, this study aims to explore the potential curative mechanism of MgIG against ConA-induced immune liver injury.

Methods: ConA (20 mg/kg, i. v.) was administered for 12 h to construct an immune liver injury model, and the treatment group was given MgIG (30 mg/kg, i. p.) injection 1 h in advance. Autophagosomes in liver tissue were observed under electron microscopy, and the expression of autophagy-related genes and proteins was detected by RT-PCR and Western blot. The intervention group was given rapamycin (2mg/kg) 1 h in advance to regulate autophagy to further observe the effect of MgIG on liver injury. The lethality, liver injury, inflammatory cytokines, autophagy, and apoptosis of each group of animals were evaluated. The hepatocyte death was assessed by flow cytometry to further explore whether MgIG can reduce hepatocyte damage by regulating autophagy in vitro.

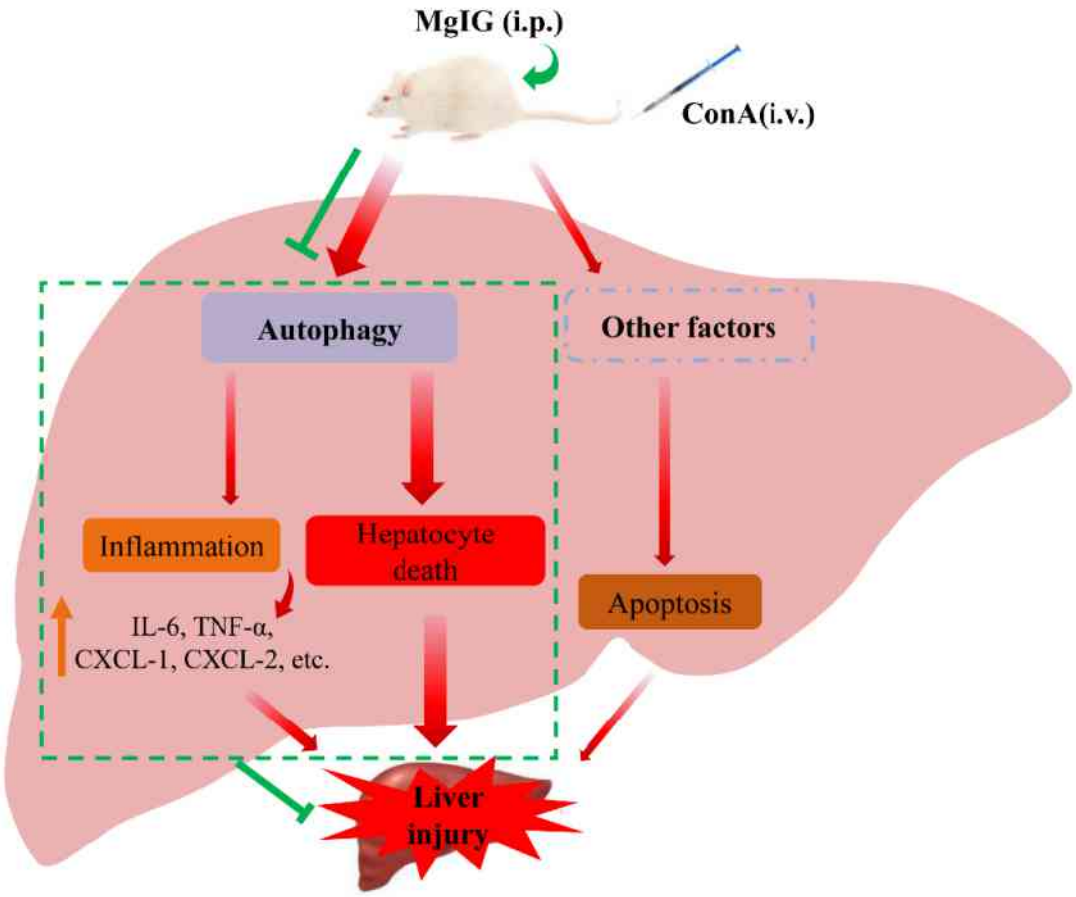
Results: MgIG significantly increased the survival rate of mice and ameliorated severe liver injury mediated by ConA. The decrease in the number of autophagosomes, downregulation of LC3b expression and upregulation of p62 expression indicated that MgIG significantly inhibited ConA-induced autophagy in the liver. Reactivation of autophagy by rapamycin (RAPA) reversed the protective effect of MgIG against ConA-induced liver injury. Compared with model group, MgIG treatment decreased the

expression of liver inflammation markers (IL-1β, IL-6, TNF-α, CXCL-1, CXCL-2, CXCL-10, etc.) and hepatocyte death. Notablely, compared with MgIG treatment, activation of autophagy by RAPA also promoted the expression of liver inflammation markers (IL-1β, IL-6, TNF-α, CXCL-1, CXCL-2, CXCL-10, etc.) and hepatocyte death. Furthermore, in vitro experiments, MgIG concentration gradually increased, autophagy was gradually inhibited, as manifested by a gradual decrease in the level of LC3 and a gradual increase in the level of p62. However, the difference in caspase-3, cleaved caspase-3, Bax and Bcl-2 levels was not obvious after MgIG treatment. Importantly, the flow detection results also showed that MgIG reduced ConA-induced hepatocyte death but did not decrease hepatocyte apoptosis by inhibiting autophagy.

Conclusions: Our study clarified that MgIG played a significant role in ameliorating ConA-induced liver injury in mice and then proved that MgIG can ameliorate liver injury by inhibiting autophagy. MgIG inhibits the expression of some inflammatory factors by inhibiting autophagy to attenuate the inflammatory response, thereby indirectly ameliorating liver damage. In addition, studies have shown that MgIG alleviates the direct damage caused by abnormal autophagy flux by inhibiting autophagy, mainly by reducing the death of hepatocytes rather than inhibiting apoptosis. This study provides proof that strategies that target autophagy can be used to treat diseases and a solid theoretical basis for the treatment of liver disease with MgIG.

❖PP-009

Figure:



❖PP-010

Hepatocyte-specific Mas activation enhances lipophagy and fatty acid oxidation to protect against acetaminophen-induced hepatotoxicity

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Object/Background: Acetaminophen (APAP) is the most common cause of drug-induced acute liver failure (ALF), however the treatment options are quite limited. Mas is a G protein-coupled receptor, whose role in APAP-induced hepatotoxicity has not been examined.

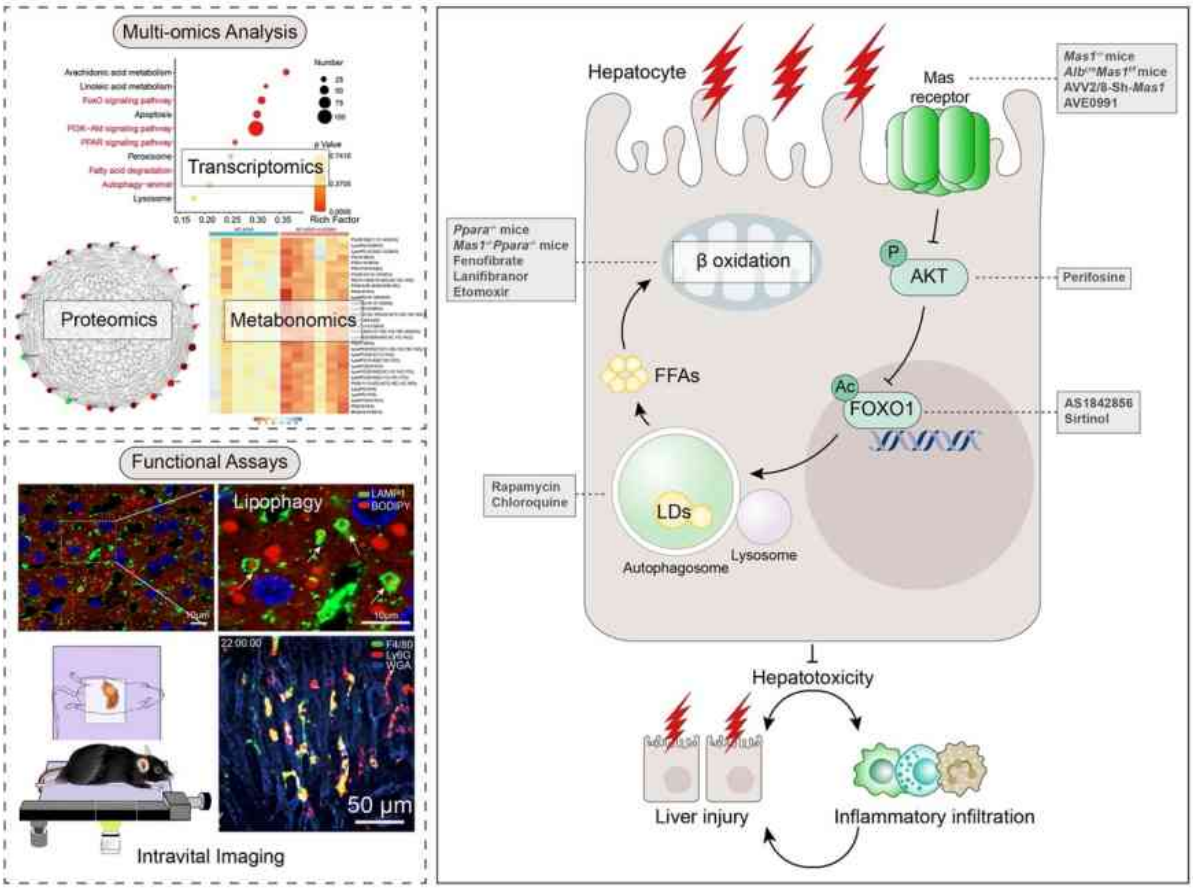
Methods: Gene expression was analyzed in APAP-induced ALF in patients and mice.Mas1-/-, AlbcreMas1f/f, Ppara-/-, Mas1-/-Ppara-/- and wild-type (WT) mice were challenged with APAP for the in vivo analysis of the Mas-AKT-FOXO1 axis dependent lipophagy and fatty acid oxidation (FAO), in the assistance of small-molecule inhibitors and agonists. Liver samples were collected for RNA-seq, proteomics and metabonomics analyses. Living mouse liver imaging, histological, biochemical, and molecular studies were carried out to evaluate APAP-induced hepatotoxicity in mice. Human and mouse hepatocyte cell lines were also exposed to confirm the existence of Mas-dependent lipophagy and FAO in vitro.

Results: The hepatic expression of Mas1 was increased in APAP-induced ALF in patients and mice.Surprisingly,mice with the systemic, liver-specific or hepatocyte-specific deficiency of Mas1 were all vulnerable to APAP-induced hepatotoxicity. Meanwhile, they exhibited the substantially impaired lipophagy and downstream FAO, which was accompanied with activation of AKT and suppression of FOXO1. Besides, the systemic activation of Mas by AVE0991 showed unbelievably ideal effects to protect mice from APAP challenge, along with the remarkably enhanced lipophagy and subsequent FAO which were dependent on suppression of AKT and activation of FOXO1. Moreover, the protective effects of AVE0991 could be substantially diminished by blocking either lipophagy or FAO.

Conclusion: The activation of Mas on hepatocytes enhances the AKT-FOXO1 dependent lipophagy and downstream FAO to protect mice against APAP-induced hepatotoxicity, thus suggesting hepatocyte-specific Mas as a novel therapeutic target of drug-induced ALF.

❖PP-010

Figure:



❖PP-011

Single-cell and spatial analysis reveal Mas-dependent Immunometabolic remodeling in Acute liver failure

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Object/Background: Acute liver failure (ALF) is a fulminant complication of multiple etiologies, characterized by rapid hepatic destruction, multi-organ failure and mortality. ALF treatment is mainly limited to supportive care and liver transplantation.Mas is a G protein-coupled receptor, whose role in acute liver failure has not been examined.

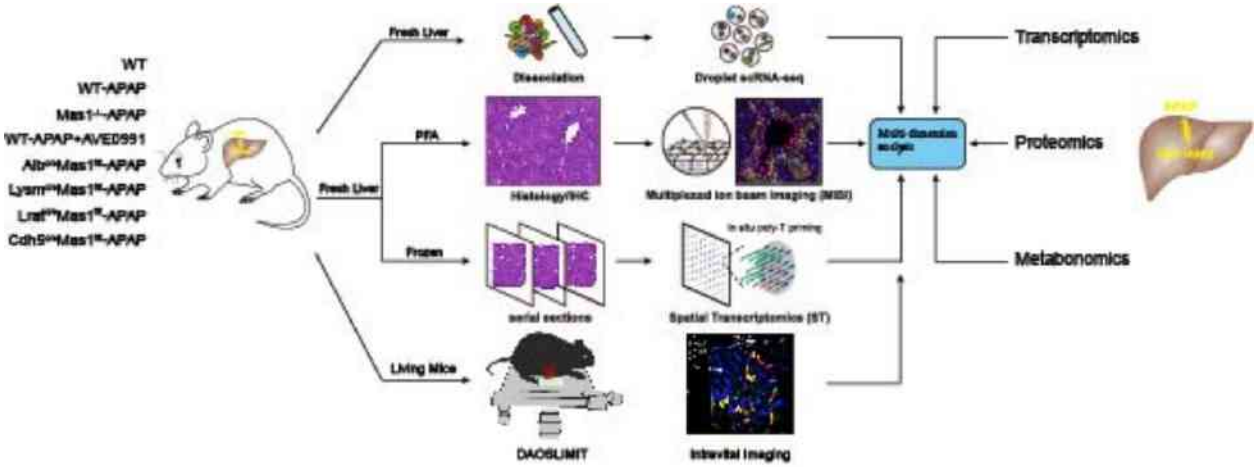
Methods: Multiplexed immunofluorescence (IF) was performed to analyze the gene expression in APAP-induced ALF in patients and mice. Mas1-/-, AlbcreMas1f/f, LysmcreMas1f/f, LratcreMas1f/f, Cdh5creMas1f/f, Nlrp3-/-, Mas1-/-Nlrp3-/- and wild-type (WT) mice were created and subjected to APAP-induced ALF. Single-cell RNA-seq and Spatial Transcriptomics were carried out to evaluate the immunometabolic microenvironment. A variety of bulk RNA-sequencing, immunohistochemical staining, immunofluorescence staining and functional experiments were employed to study the Mas-denpendent Immunometabolic remodeling.

Results: The hepatic expression of Mas1 was increased in APAP-induced ALF in patients and mice. Surprisingly, mice with the systemic, myeloid cell-specific or hepatocyte-specific deficiency of Mas1 were all vulnerable to APAP-induced hepatotoxicity. Loss of Mas signal in myeloid cells can promote the liver infiltration of pro-inflammatory monocytes, which can further achieve metabolic remodeling of liver sinusoidal endothelial cells through glycolysis. Further, we demonstrate that the MYC+ liver sinusoidal endothelial cells and MMP12+ Kupffer cells were positively correlated with APAP-induced liver injury by multiplexed immunofluorescence, single-cell RNA-seq and spatial transcriptomics. This interaction might be regulated by glycolysis. Pharmacological inhibition of MYC and glycolysis attenuate ALF. In humans, we also demonstrate upregulated MYC+ liver sinusoidal endothelial cells and MMP12+ Kupffer cells in ALF transplant recipients compared to healthy donors.

Conclusion: Mas can regulate the dynamic interaction between liver sinusoidal endothelial cells, Kupffer cells and myeloid cells , and form a unique and highly

inflammatory immunometabolic microenvironment, thereby promoting the progression of liver injury, suggesting that myeloid cells Mas receptors may be a new therapeutic target for acute liver failure.

Figure:



❖PP-012

A novel AIHI-nomogram to predict advanced liver inflammation in patients with autoimmune hepatitis

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6. The Affiliated Infectious Diseases Hospital of Soochow University

Object/Background: The assessment of liver inflammation plays a vital role in the management of patients with autoimmune hepatitis (AIH). We aimed to establish and validate a nomogram to predict advanced liver inflammation in AIH patients.

Methods: AIH patients underwent liver biopsy were included and randomly divided into a training set (n=142) and a validation set (n=71). Independent predictors of advanced liver inflammation were selected by the least absolute shrinkage and selection operator regression from the training set and used to conduct a nomogram. Receiver characteristic curves (ROC), calibration curves and decision curve analysis (DCA) were adopted to evaluate the performance of nomogram.

Results: Of the 213 patients, female patients accounted for 83.1% and the median age was 53.0 years. The albumin, gamma-glutamyl transpeptidase, total bilirubin, red cell distribution width, prothrombin time and platelets were independent predictors of advanced inflammation, and an AIHI-nomogram was established. The calibration curve revealed that the AIHI-nomogram had a good agreement with actual observation in the training and validation sets. The area under the ROCs of AIHI-nomogram for predicting advanced inflammation were 0.795 in the training set and 0.759 in the validation set, showing significantly better performance than alanine aminotransferase and immunoglobulin G in the training and validation sets, as well in the subgroup of patients with normal ALT in the training set. DCA indicated that the AIHI-nomogram was clinically useful.

Conclusion: This novel AIHI-nomogram provided an excellent prediction of advanced liver inflammation in AIH patients, which could be used for the better management of AIH.

❖PP-013

Indoleamine 2, 3-dioxygenase 1 aggravates acetaminophen-induced liver injury by triggering hepatocyte ferroptosis

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Southern Medical University

Object/Background: & Aims: Ferroptosis is a new type of cell death discovered in recent years, which is characterized by iron-dependent accumulation of lipid peroxidation. However, the specific mechanism of ferroptosis in drug-induced liver injury (DILI) remains to be clarified. The aim of this study is to investigate how indoleamine 2,3-dioxygenase 1 (IDO1) modulates hepatocyte ferrptosis during drug-induced liver injury.

Methods: Acetaminophen (APAP)-induced liver injury model was constructed. Zebrafish with fluorescent labeling of hepatocytes and inflammatory cells, and human hepatic LO2 cell were used. Key protein IDO1 was modified by lentiviral in vitro. Ferroptosis inducer Erastin and IDO1 inhibitor 1-Methyl-DL-tryptophan (1-MT) were utilized in the experiment.

Results: Our study found that ferroptosis is involved in APAP-induced liver failure, and pretreatment with the ferroptosis inhibitor Fer-1 effectively ameliorated liver injury. At the same time, APAP induced the up-regulation of IDO1, and the liver function of IDO1-knockout mice was reduced. Histopathology, immunofluorescence and immunohistochemical staining suggested that IDO1-knockout could reduce F4/80 macrophage infiltration and inhibit lipid peroxidation. The levels of the products malondialdehyde and 4-hydroxynonenal ameliorated liver injury. The results of zebrafish experiments suggested that the ferroptosis inducer Erastin effectively aggravates APAP-induced liver injury and inflammatory infiltration, and the intervention of IDO1 inhibitor 1-MT could improve liver cell death and inflammatory damage in zebrafish. In addition, we modified the IDO1 gene in human hepatocyte LO2 in vitro and found that IDO1 knockdown effectively reduced Erastin-induced up-regulated expressions of transferrin and transferrin receptor (Tf/TfR) and promoted cellular iron overload, suggesting that IDO1 plays an essential regulatory role in ferroptosis.

Conclusions: Ferroptosis is involved in APAP-induced drug-induced liver injury, and inhibition of IDO1 can ameliorate APAP-induced liver injury by suppressing ferroptosis

through the inhibition of lipid peroxidation and iron overload pathway Tf/TfR. Thus, IDO1 is a novel and pivotal therapeutic target in DILI.

❖PP-014

Construction and validation of prediction model of extrahepatic metastasis of primary hepatic cancer base on commonly available clinical data

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Object/Background: This study aimed to investigate the clinical characteristics and risk factors of patients with primary hepatic carcinoma combined with extrahepatic metastases and establish an effective diagnostic nomogram for extrahepatic metastasis.

Methods: Clinical and pathological data of 456 primary hepatic cancer patients admitted to the Affiliated Hospital of Qinghai University between January 2015 and May 2018 were documented. General information, laboratory testsing, tumor imaging characteristics to clarify Clinical risk factors for extrahepatic metastasis of primary hepatic carcinoma. Those screened risks were selected into construct a clinical prediction model using the R. The predictive accuracy and discriminative ability of the model were determined by concordance index(C-index) and calibration curve. The results were validated using bootstrap resampling and 151 patients from June 2018 to December 2019 at the same institution.

Results: On multivariate analysis, independent factors for extrahepatic metastasis were neutrophils, prothrombin time, tumor number, tumor size, which were selected into the model. The C-index of the model for predicting extrahepatic metastasis was 0.672. In the validation cohort, the C-index of the model for predicting extrahepatic metastasis was 0.694. In the train cohort and validation cohort, the calibration curve for probability of extrahepatic metastasis showed good agreement between prediction by nomogram and actual observation.

Conclusion: The prediction model for extrahepatic metastasis of primary hepatic carcinoma constructed in this study has good evaluation ability.Using the model, the missed diagnosis rate of extrahepatic metastasis can be reduced.

❖PP-014

Figure:

Figure1:The nomogram predicting inital patient EHM.

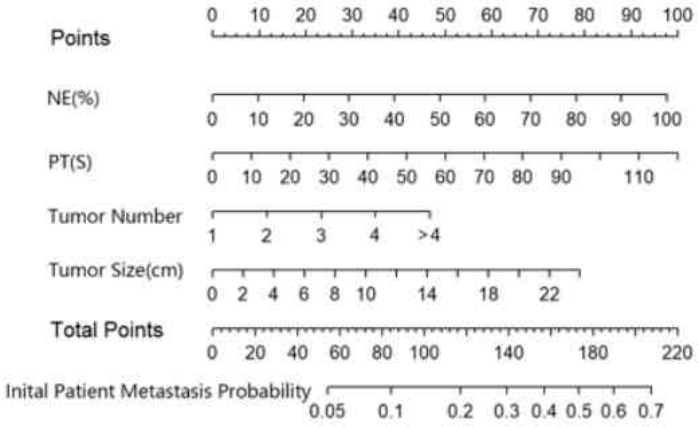
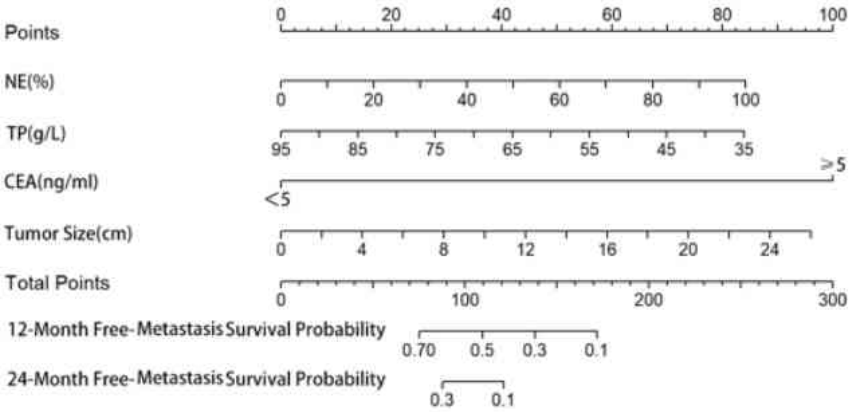


Figure2: The nomogram predicting 24 month-free-Metastasis survival probability



❖PP-015

Prognostic value of the ratio of IL4-to-IL10 in patients with hepatocellular carcinoma treated with anti-PD-1 therapy

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Object/Background: Immune cells play an important role in the tumor microenvironment and are closely related to cancer progression. However, there is currently a lack of recognized biomarkers to identify hepatocellular carcinoma (HCC) patients who may benefit from anti-PD-1 therapy. This study aimed to investigate the prognostic value of immune cytokines in HCC patients after anti-PD-1 therapy.

Methods: A total of 30 HCC patients consecutively treated with anti-PD-1 therapy in Sichuan Provincial People's Hospital between January 2021 and January 2022 were included in the retrospective study. The patients were divided into tumor progression and tumor progression free groups according to solid tumor clinical efficacy evaluation index (RECIST1.1). The determination of serum immune cytokines levels relied on the Cytometric Bead Array (CBA). According to the follow-up survival and prognosis information of the patients, the serological and immunological indexes of the patients with tumor progression and tumor progression free groups were compared. Clinical characteristics of patients were compared using Wilcoxon rank-sum test for categorical variables and independent samples t-test for continuous variables.The model were assessed for prediction of prognostic value using univariate and multivariate logistic regression analysis and receiver operating characteristic (ROC) curve and area under the ROC curve.

Results: Analysis of the data by independent samples t-test found that patients with tumor progression free had higherratio of IL4-to-IL10 than those with tumor progression. (3.96±2.37 vs 2.11±1.72, p =0.020) The multivariate analysis identified IL4-to-IL10 ratio (HR: 1.595, 95% CI: 1.038–2.453, p=0.033) as independent prognostic factors, thus to construct the prognostic prediction model. According to ROC analysis, the ratio of IL4-to-IL10 (AUC=0.739, 95% CI: 0.556–0.922, p=0.026) presented better prognostic performance than other traditional prognostic indicators, including the platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR). The area of the ratio

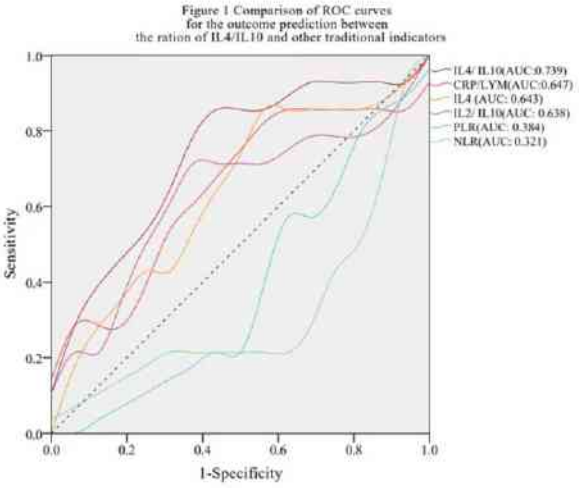
of IL4-to-IL10 under the ROC curve (AUC) was higher than that of the PLR and NLR for the prediction of tumor progression.

Conclusion: The ratio of IL4-to-IL10 ratio showed an discriminatory prognostic indicator for tumor progression in HCC patients treated with anti-PD-1 therapy. High ratio of indicated that patients should be considered for maintenance therapy and close follow-up. Immune cytokines directly reflect the autoimmune status of HCC patients, so the ratio of IL4-to-IL10 is worthy of clinical application, and should be further validated in large-scale clinical studies.

Figure:

Table 1 Comparison of the prognostic performance between the indices in tumor progression of HCC patients treated with PD- 1 inhibitors

Variables	Calculations	Cut off value	AUC (95% CI)	sensitivity	specificity	p value
IL4/IL10	IL4: IL10	0.482	0.739 (0.556-0.922)	0.857	0.375	0.026
hsCRP/LYM	hsCRP: LYM	0.294	0.647 (0.441-0.854)	0.857	0.563	0.170
IL4		0.357	0.643 (0.439-0.847)	0.857	0.5	0.183
IL2/IL10	IL2: IL10	0.401	0.638 (0.429-0.848)	0.714	0.313	0.198
PLR	Platelet count: lymphocyte count		0.384 (0.179-0.589)			0.28
NLR	Neutrophil count: lymphocyte count		0.321 (0.115-0.528)			0.096



❖PP-016

Research progress in portopulmonary hypertension

Jing Kuang、*Tongtong Shen*、*Wei Wang**、*Chuan Shen*、*Yadong Wang*、*Caiyan Zhao*
The Third Affiliated Hospital of Hebei Medical University

Object/Background: Portopulmonary hypertension is a serious complication of portal hypertension occurs most frequently in patients with liver cirrhosis, and it is often ignored and misdiagnosed in clinical practice due to the low incidence, nonspecific clinical manifestations and the diagnosis depending on invasive examination. Portopulmonary hypertension has a great impact on the prognosis of patients with liver disease, which makes people pay more and more attention to it. Advances in the research progress of its epidemiology, pathophysiology, pathogenesis, risk factors, clinical manifestations, diagnosis and treatment will help clinicians to improve their understanding of the disease.

Methods: The first author performed a computerized literature search on CNKI, Wanfang Medical database and PubMed with search items were “Portopulmonary hypertension” OR “Portal pulmonary hypertension” OR “POPH” OR “liver cirrhosis” OR “Portal hypertension” AND “pulmonary hypertension” until June 2022, and summarized the relevant literatures.

Results: Reviewing the relevant literature, portopulmonary hypertension refers to pulmonary hypertension formed on the basis of portal hypertension with or without liver disease, accounting for about 5.3%-8.5% of patients waiting for liver transplantation, the related risk factors are not clear, and the prognosis which related to underlying liver disease and cardiac function is poor. Hyperdynamic circulation, portosystemic shunt, endotoxemia, heredity and variation are thought to contribute to portopulmonary hypertension pathogenesis in multiple ways. The clinical manifestations are not specific and difficult to identify, transthoracic echocardiography is the best screening test, further right heart catheterization as a gold standard should be performed in all patients with a pulmonary artery systolic pressure >50mmHg on screening echocardiogram. In recent years, bone morphogenetic protein 9 and macrophage migration inhibitory factor have been found to be new biomarkers for diagnosis. The treatment of portopulmonary hypertension is difficult, liver transplantation is the main treatment method, but the perioperative mortality of liver transplantation for moderate and severe portopulmonary hypertension increases significantly, pulmonary hypertension specific therapies can

improve hemodynamics and functional outcomes to help become candidates for liver transplantation and live longer potentially, but further research is needed.

Conclusion: Portopulmonary hypertension is a complex disease occurs in the hepatic and pulmonary circulation, a serious complication of chronic liver disease. At present, we have limited understanding of it and further research is needed.

❖PP-017

Predictive value of kinetics changes of virological index on low-level viremia in nucleos(t)ide analogs treated chronic hepatitis B patients

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The First Affiliated Hospital of Nanchang University

Object/Background: To investigate the influencing factors for patient low-level viremia（LLV） in chronic hepatitis B (CHB) treated with nucleos(t)ide analogs (NAs), and to establish a prediction model， evaluate the predictive value of it and further analyze the kinetics changes of virological indexes during treatment， so as to provide some guidance for antiviral treatment and follow-up of CHB patients.

Methods: CHB patients who received NAs antiviral therapy for at least 12 mouths in the outpatient department of infection, the First Affiliated Hospital of Nanchang University from November 2020 to March 2022 were selected. According to the HBV DNA level during the treatment, the patients were divided into sustained virological response (SVR) group and LLV group. The baseline data were analyzed by univariate analysis, and the independent influencing factors of LLV were analyzed by multivariate logistic regression, Thirdly, the binary logistic regression model was used to establish the prediction model of LLV occurrence, and the subject working curve was used to evaluate the prediction efficiency of the model. At the same time, repeated measurement ANOVA was used to analyze the mean level and decline range of HBV DNA and HBsAg between the two groups at different time points.

Results: Among the 78 patients with CHB, there were 20 patients with LLV, and the incidence was 25.6%. Univariate analysis showed that there were significant differences in age, ALT, AST, LSM, HBeAg positive rate, HBV DNA and HBsAg between SVR group and LLV group ($P < 0.05$). Multivariate analysis showed that HBV DNA and HBsAg were independent risk factors for LLV in CHB patients, while ALT was an independent factor for LLV in CHB patients; The prediction model Logit (MLLV) = - 8.668 + 1.441 × lgHBsAg+0.598 × lgHBV DNA-0.016 × ALT, the area under the ROC curve is 0.931, which is better than the predictive value of HBV DNA, HBsAg and ALT alone. The negative conversion rate of HBV DNA was analyzed by Kaplan Meier method and log rank test. The

results showed that the negative conversion rate of HBV DNA in the group with HBV DNA < 7.61 log10 IU/ml was always higher than that in the group with HBV DNA > 7.29 log10 IU/ml; The negative conversion rate of HBV DNA in patients with HBsAg ≤ 4.38 log10 IU/ml was always higher than that in patients with HBsAg > 4.38 log10 IU/ml.During the treatment period, the levels of HBV DNA and HBsAg in the included patients showed a decreasing trend from fast to slow, but the levels of HBV DNA and HBsAg in the LLV group were higher than those in the SVR group at different time points, while the decreasing range of DNA and HBsAg in the LLV group was greater than that in the SVR group, but the difference was not statistically significant.

Conclusion: HBV DNA, HBsAg and ALT are independent influencing factors of LLV in CHB patients treated with NAS. The prediction model constructed based on this has good predictive value; The changes of HBV DNA and anti-virus kinetics can guide the treatment of CHB.

❖PP-018

Quantitative anti-HBc but not HBcrAg can predict HBsAg loss in sequential combination therapy with PEG-IFN-α in NAs-suppressed chronic hepatitis B patients

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2. The Fifth Medical Center of Chinese PLA General Hospital

Object/Background: Precise predictors are lacking for hepatitis B surface antigen (HBsAg) clearance under the combination therapy of nucleos(t)ide analogs (NAs) and pegylated interferon-alpha (PEG-IFN-α) in patients with chronic hepatitis B (CHB). This study aimed to determine the quantitative anti-hepatitis B core antibody (qAnti-HBc) and quantitative hepatitis B core-related antigen (qHBcrAg) as predictors for HBsAg clearance in NAs-suppressed patients with CHB receiving PEG-IFN-α add-on therapy.

Methods: Seventy-four CHB patients who achieved HBV DNA suppression (HBV DNA < 20 IU/ml) and quantitative HBsAg (qHBsAg) < 1500 IU/ml after ≥ 1 year of NAs treatment were enrolled. Fifteen patients continued on NAs monotherapy, while 59 patients received PEG-IFN-α add-on therapy. Serum qAnti-HBc and qHBcrAg levels were detected every 12 or 24 weeks for add-on and NAs alone groups, respectively.

Results: Serum qAnti-HBc but not qHBcrAg levels at baseline were negatively correlated with duration of prior NAs therapy. After 48-week treatment, both qAnti-HBc and qHBcrAg levels declined further, and 17/59 (28.81%) and 0/15 (0%) achieved HBsAg clearance in add-on and NAs groups, respectively. In add-on group, the rate of HBsAg clearance was significantly higher in patients with baseline qAnti-HBc < 0.1 IU/ml (52.63%). Logistic regression analysis identified baseline qAnti-HBc but not qHBcrAg was an independent predictor for HBsAg loss. Receiver operating characteristic curve analysis showed that the combination of qAnti-HBc and qHBsAg had a better predictive value for HBsAg clearance.

Conclusion: Baseline qAnti-HBc levels may represent a new and early predictor of HBsAg clearance in NAs-suppressed CHB patients receiving PEG-IFN-α add-on therapy.

❖PP-019

An online web-based calculator accurately diagnoses immune tolerant phase in chronic HBV-infected patients

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1. Peking University First Hospital
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Object/Background: There were different antiviral treatment strategies and prognoses between immune tolerant (IT) and non-IT in chronic hepatitis B (CHB) patients. However, existing non-invasive diagnostics were not precision. We aimed to explore new noninvasive models for diagnosing IT and assessed the risk of hepatocellular carcinoma (HCC) in IT patients.

Methods: We included treatment-naive CHB patients with liver biopsy who serological met the diagnostic criteria of IT (HBeAg-positive, HBV DNA>5lgIU/mL, normal ALT). This study included four parts: in step 1, we described the clinical characteristics of IT patients and calculated ORs for noninvasive markers between patients who were IT and those who were not. In step 2, we evaluated the value of noninvasive markers combinations recommended by the guidelines for the diagnosis of IT. In step 3, due to the limitations of the current noninvasive models, a new model for the diagnosis of IT with non-invasive markers were developed and validated. In step 4, to assess the risk of developing HCC using 15 HCC prediction models for IT and non-IT.

Results: According to the criteria for the diagnosis of IT by WHO 2015 guidelines, 196 patients were finally included in this study, of which 83 were liver biopsy-proven IT.
In the IT group age, qAnti-HBc, ALT, AST and LSM were lower. While, HBV DNA and HBsAg were higher. The risk of non-IT increased 1.2-fold and 3.92-fold in patients aged 30-40 (95%CI 1.00-4.81, P=0.049) and >40 years (95%CI 2.28-10.60, P<0.001), respectively, compared to those aged<30 years. Compared to HBV DNA >8 lg IU/mL, the risk of non-IT was increased 4.33-fold and 9.96-fold in patients 7-8 lg IU/mL (P=0.001) and <8 lg IU/mL (P<0.001), respectively.
The accuracy of noninvasive marker combinations for the diagnosis of IT did not exceed 0.800, whether in accordance with EASL2017/APASL2015, AASLD2018 or CHINA2019 criteria (0.709, 0.658 and 0.765, respectively). The lowest misdiagnosis rate of IT was

CHINA2019 criteria (EASL2017/APASL2015 criteria plus HBsAg>4 lg IU/mL) which was 21.2%. Nevertheless, misdiagnosis rate of EASL2017/APASL2015 or AASLD2018 criteria was 34.5%.
Using univariate analysis, LASSO regression and multivariate analysis, we created a CALA model (qAnti-HBc, LSM, AST, ALP) to diagnosing IT. The AUROC of CALA model reached 0.890 and 0.892 in training and validation sets, respectively, which were significantly higher than APRI, FIB-4 and LSM. For clinical convenience, we have made CALA model in an online web-based calculator and QR code.
We included 15 hepatitis B related HCC prediction models (REACH-B, mREACH-BI, mREACH-BII, GAG-HCC, CU-HCC, LSM-HCC, PAGE-B, mPAGE-B, NGM1-HCC, NGM2-HCC, CAMD, RWS-HCC, AASL-HCC, REAL-B, aMAP) by searching the PubMed database. These models were used to estimate the difference in the occurrence of HCC between biopsy-proven IT and biopsy-proven non-IT, respectively. The risk of HCC was significantly lower in biopsy-proven IT than in biopsy-proven non-IT population (all $P<0.01$). Subsequently, the CALA model was used to differentiate IT and the same results were obtained as those confirmed by liver biopsy (all $P<0.01$, except CU-HCC model $P=0.066$).

Conclusions: Online web-based calculator of CALA model can accurately and conveniently diagnose IT. Patients with liver biopsy or CALA model-proven IT were at a lower risk of developing HCC.

❖PP-020

Combination therapy in HBeAg-negative chronic hepatitis B patients with low-level viremia to nucleos(tide) analogues

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Object/Background: Considering that the ultimate goals of treatment are to decrease the morbidity and mortality related to chronic hepatitis B (CHB), the recently updated guidelines of liver disease recommend that people with low-level viremia(LLV) to nucleos(tide) analogues(NAs) should switch or add another drug for people with a suboptimal virological response.To compare and evaluate the efficacy and safety of pegylated interferon (peg-IFN)α in combination with NAs and NAs in combination with NAs in HBeAg-negative CHB patients with LLV to NAs, so as to investigate optimal treatment regimen with end point of therapy.

Methods: Totally 240 HBeAg-negative CHB patients with LLV to NAs were enrolled in the study, and divided into 2 groups receiving Peg-IFN+NAs (IFN group) or NAs+NAs (NA group) according to administration of NAs. Complete virological response (HBV DNA concentration of less than 20 IU/ml or10IU/ml) rate, HBsAg loss/seroconversion rate at 48 weeks of treatment were compared between the 2 groups

Results: There were 178 patients in IFN group and 89 patients in NA group, and 162 patients in the IFN group and 78 patients in NA group received 48-week treatment duration.At 12 weeks of treatment, the complete virological response rate was 51.1%(40/78) in the NA group, which was seemed higher than that in the PegIFN group (45.1%,73/162), but the difference was not statistically significant (X2=0.52, P=0.47). At 48 weeks, the complete virological response rates of PegIFNα group and NA group were 97.50% (158/162) and 85.90%(67/78), respectively, with statistically significant differences (X2=10.56, P=0.001). There was no significant difference in HBsAg levels at baseline between the PegIFN group and NA group (823.97 vs. 988.50,t=0.80, P=0.43). In the PegIFNα group, HBsAg titer decreased significantly from baseline to week 12 (823.97 vs. 527.7,t=2.31, P=0.02), and then it decreased slowly from week 12 to week24 (399→324.9IU/ mL).While in the NA group, HBsAg titer did not change significantly from baseline to week 48,there was no statistically significant difference (988.5 vs. 847.1,t=0.84, P=0.41).

The HBsAg loss rates at 12,24,36 and 48 weeks in PegIFNα group were 1.2% (2/162), 4.94% (8/162), 16% (26/162) and 30.9% (50/162), respectively. In NA group, only 1.3% (1/78), 1.3% (1/78), 2.6% (2/78), and 5.2% (4/78) were observed. There was no significant difference in HBsAg clearance rate between the two groups at 12 and 24 weeks (X2=0.01, P=0.97; X2=1.95, P=0.16), but the proportion of HBsAg≧500IU/ ml at baseline in PegIFNα group was 50% (81/162), and the proportion decreased sharply to 30.8% (50/162) and 24.1% (39/162) at 12 and 24 weeks. In particular, the proportion of HBsAg titer between 0~20IU/ml increased from 4.3% at baseline to 26.5% at 24 weeks , the difference was statistically significant (X2=18.27, P<0.001). At 36 weeks and 48 weeks, the HBsAg loss rates in PegIFNα group was significantly higher than that in NA group, and the difference was statistically significant (X2=9.29, P=0.02; X2=19.89, P<0.001). There was no significant difference in HBsAg level between the two groups at baseline (823.97IU/ mL vs. 988.50IU/ mL, t =0.80, P=0.43), but HBsAg significantly decreased in PegIFNα group at 12 weeks (823.97 → 527.7IU/ mL, t =2.31, P=0.02). After 24 weeks, HBsAg decreased slowly (399→324.9IU/ mL), while the titer of HBsAg in NA group from baseline to 48 weeks showed no significant difference (988.5 vs. 847.1,t=0.84, P=0.41).

Conclusion: The treatment regimens of Peg-IFN+NAs and NAs+NAs are effective in suppression of HBV replication in HBeAg-negative CHB patients with LLV to NAs, but Peg-IFN+NAs is superior to NAs+NAs in . Combination therapy with IFN as the basis will achieve reliable end point of therapy in HBeAg-negative CHB patients with LLV to NAs.

A novel nomogram for predicting significant liver inflammation in patients with chronic hepatitis B in the indeterminate phase

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Object/Background: The presence of significant liver inflammation is an important indication for antiviral treatment in patients with chronic hepatitis B (CHB) in the indeterminate phase. We aimed to establish a non-invasive nomogram to predict significant liver inflammation in CHB patients in the indeterminate phase.

Methods: One hundred ninety-five CHB patients in the indeterminate phase were retrospectively included and randomly divided into a training set and a validation set. The least absolute shrinkage and selection operator and logistic regression were applied to identify risk factors and establish a predictive model. A calibration curve, decision curve analysis (DCA) and receiver operating characteristic (ROC) curve were used to evaluate the performance of the nomogram.

Results: The median age of 195 CHB patients was 42.0 years and 116 (59.5%) patients were male. Alkaline phosphatase, γ-glutamyl transpeptidase and prothrombin time were determined as independent predictive factors for significant liver inflammation and selected to establish the AGP-nomogram. The calibration plot demonstrated that the predicted results matched the actual values. The DCA showed a high net benefit when the threshold probability was 25-83% in the training set and 31-100% in the validation set. The areas under ROC curves of the AGP-nomogram in predicting significant inflammation were significantly higher than ALT in the training set (0.744 vs. 0.642, P = 0.049) and validation set (0.766 vs. 0.660, P = 0.047). The ability of APG-nomogram in predicting advanced inflammation was superior to ALT in both two sets.

Conclusion: The AGP-nomogram using conventional laboratory parameters can accurately identify significant inflammation in CHB patients with indeterminate phase, and its application may reduce the necessity of liver biopsy and help identify candidates who require antiviral treatment.

❖PP-022

Discontinuation of Oral Antivirals in Hepatitis B-Related Liver Cirrhosis: A Systematic Review

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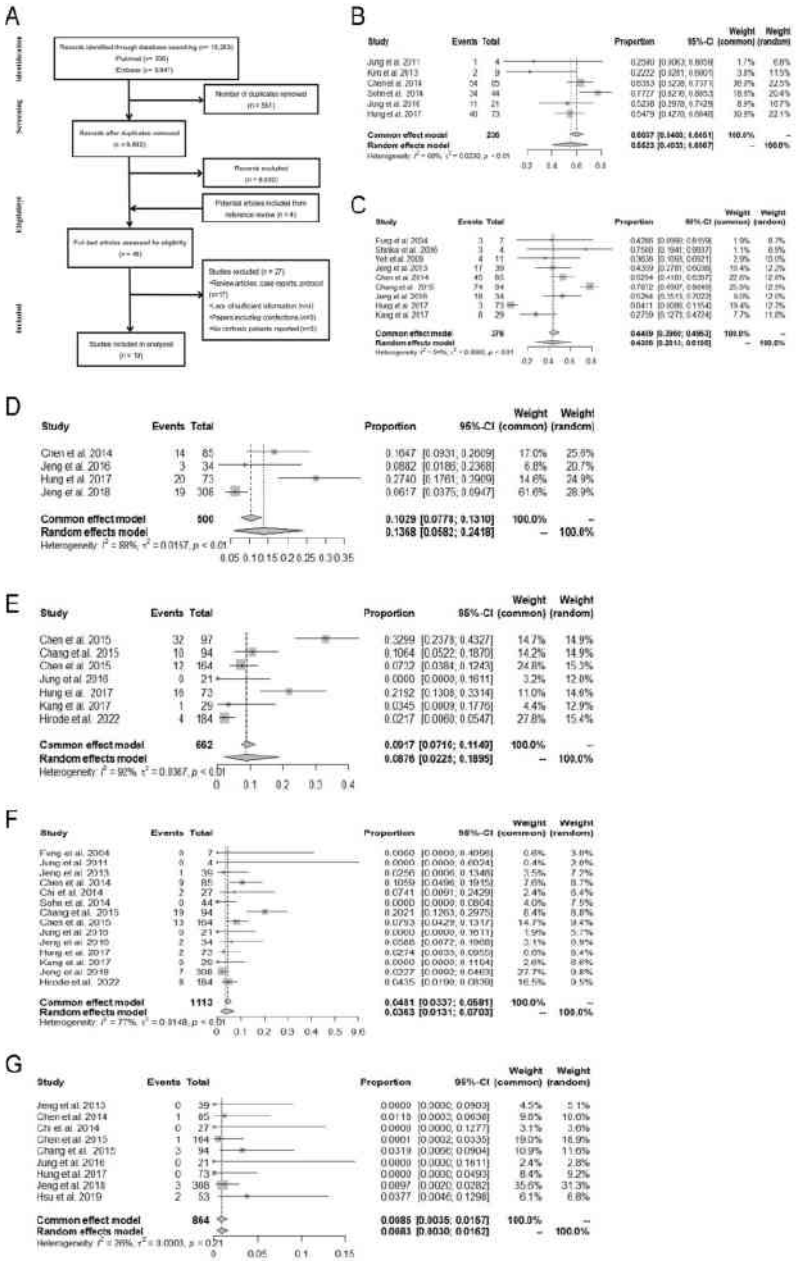
Object/Background and Aim: The feasibility and safety of Nucleos(t)ide analogues discontinuation remains one of the most controversial topics in the management of hepatitis B-related liver cirrhosis. Current guidelines have not fully harmonized the criteria for discontinuing Nucleos(t)ide analogues in patients with chronic hepatitis B cirrhosis. Therefore, the aim of this review is to systematically evaluate existing data on Nucleos(t)ide analogues discontinuation in chronic hepatitis B patients with cirrhosis and to potentially identify the feasibility, safety, and potential benefits of Nucleos(t)ide analogues withdrawal.

Methods: The Medline and EMBASE databases were searched (until May 2022) with the following terms: "Antiviral Agents or Lamivudine or entecavir or adefovir or telbivudine or tenofovir or nucleoside or nucleotide" AND "Hepatitis B or hepatitis B or HBV" AND "liver cirrhosis or Hepatic Cirrhosis or Cirrhosis, Hepatic or Cirrhosis, Liver" AND "discontinuation or withdrawal or end". Estimation of pooled proportions were calculated using transformed proportions using Freedman-Tukey double arcsine transformation.

Results: Nineteen studies with 1,287 chronic hepatitis B cirrhosis patients were included (Figure A). Most cirrhotic patients achieved complete virological suppression when they stopped the drug. The pooled proportions of virological relapse and clinical relapse after Nucleos(t)ide analogues discontinuation were 55.23% (95% CI 40.33-69.67) (Figure B) and 43.56% (95% CI 26.13-61.85) (Figure C), respectively. HBsAg loss was observed in 56 of 500 (pooled proportion = 13.68%, 95% CI 5.82-24.18) (Figure D) cirrhotic patients. And the pooled proportions of hepatocellular carcinoma, hepatic decompensation and death were 8.76% (95% CI 2.25-18.95) (Figure E), 3.63% (95% CI 1.31-7.03) (Figure F) and 0.85% (95% CI 0.35-1.57) (Figure G), respectively, after discontinuation Nucleos(t)ide analogues in patients with chronic hepatitis B cirrhosis.

Conclusion: In patients with chronic hepatitis B cirrhosis, discontinuation of oral antivirals still carries a high relapse rate, but the incidence of adverse events is generally low and controlled, and contributes to the fact that discontinuation Nucleos(t)ide analogues achieves a high rate of HBsAg clearance. In summary, we suggests that discontinuing Nucleos(t)ide analogues in virological remission may be a feasible and worthwhile alternative to indefinite treatment in chronic hepatitis B compensated cirrhotic patients.

Figure:



❖PP-023

Meta-analysis and trial sequential analysis of Anluo Huaxian Wan in the treatment of hepatic cirrhosis in hepatitis B

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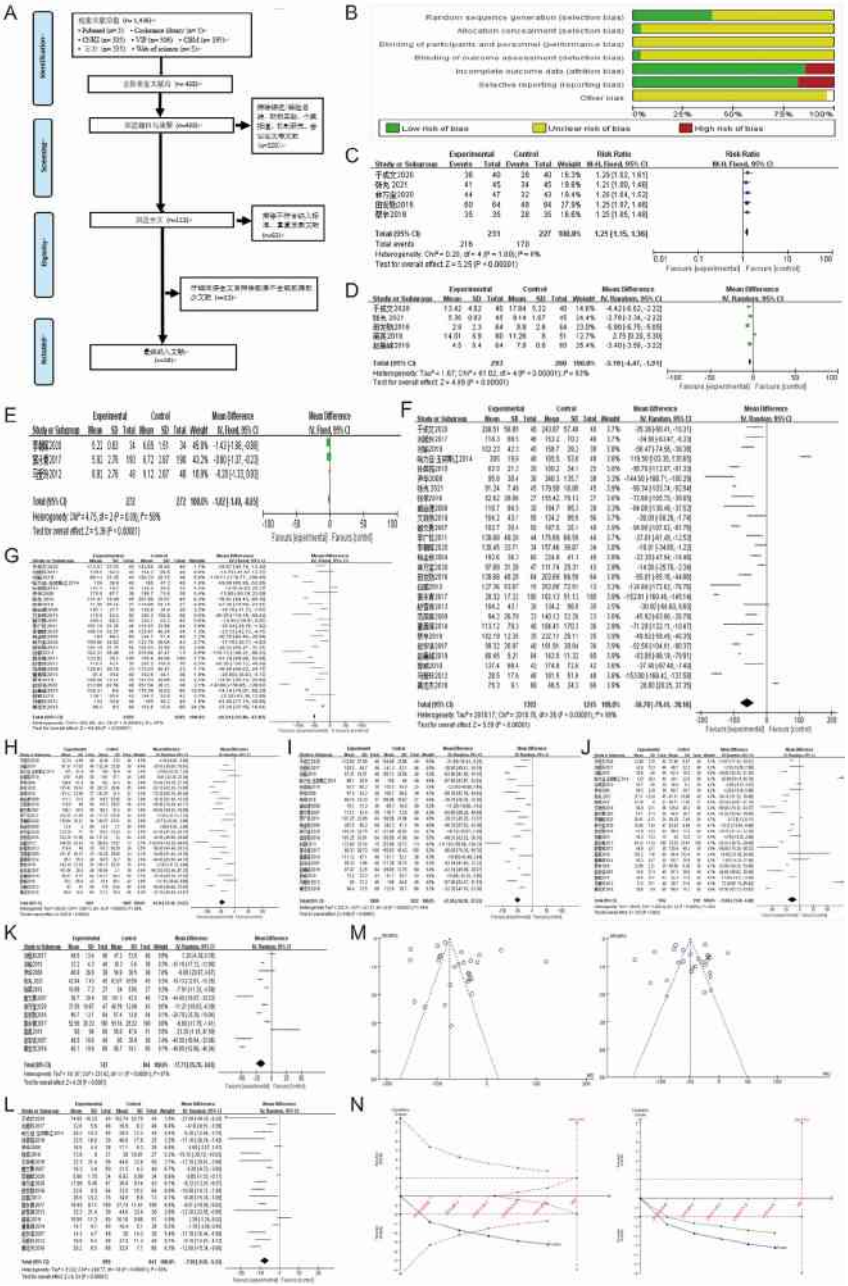
Object/Background: To evaluate the effect of Anluo Huaxian Wan on hepatitis B-related cirrhosis.

Methods: Computer searches were conducted on PubMed, Cochrane Library, Web of Science, CNKI, Wanfang Data Resource System, CBM, and VIP. The search was conducted from the time of the establishment of each database until 01 March 2022. All randomized controlled trials of Anluo Huaxian Wan for hepatitis B cirrhosis were retrieved, and two researchers each independently and rigorously performed the quality evaluation and data extraction for the included studies, Meta-analysis using Review Manager 5.4 software, and trial sequential analysis (TSA) using TSA 0.9.5 software.

Results: A total of 28 articles and 2,493 patients were included. Meta-analysis results showed that the overall effective rate of the experimental group (RR = 1.25; 95 % CI 1.15, 1.36; P < 0.00001) was better than that of the control group. In improving patients' liver stiffness (MD-3.19,95% CI -4.47, -1.91, P < 0.00001) and reducing Child-Pugh score (MD-1.02,95% CI -1.40, -0.65, P < 0.00001), hyaluronic acid (HA) levels (MD-62.81,95% CI -80.94, - 44.68, P < 0.00001), laminin (LN) levels (MD-48.03, 95% CI -60.36, -35.70, P < 0.00001), procollagen type III (PC-III) levels (MD-45.42, 95% CI -55.33, -35.51, P < 0.00001), collagen type IV (IV-C) levels (MD-45.49, 95% CI -54.96, -36.03, P < 0.00001), alanine aminotransferase (ALT) levels (MD-16.18, 95% CI -23.81, -8.56, P < 0.00001), aspartate aminotransferase (AST) levels (MD-19.90, 95% CI -27.30, -12.50, P < 0.00001), and serum total bilirubin (TBIL) levels (MD-7.36, 95% CI -9.56, -5.16, P < 0.00001) were also better than those in the control group, and the differences were statistically significant (P < 0. 05), and no significant adverse reactions occurred in both groups. The results of the TSA showed that the cumulative clinical effectiveness of the included studies crossed both the traditional and TSA cut-offs, further confirming the clinical efficacy of the trial group.

Conclusion: The efficacy of Anluo Huaxian Wan in combination with conventional western antiviral treatment for hepatitis B liver fibrosis/cirrhosis was superior to conventional antiviral western treatment with fewer adverse effects, but due to the low quality of the included studies and small sample size, the above results still need to be further validated in well-designed, large sample randomized controlled trials.

Figure:



❖PP-024

Twelve-month systemic consequences of COVID-19 in patients with or without corticosteroid therapy in Wuhan: a prospective study

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Object/Background: The coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has officially been declared a global pandemic. Long-term systemic consequences of COVID-19 in patients with or without corticosteroid therapy remains unclear.

Methods: We prospectively evaluate the long-time prognosis and the possible sequelae of COVID-19 survivors with or without corticosteroid therapy by using propensity score matching (PSM) analysis.

Results: The prevalence of fever, and dyspnea were higher in patients with corticosteroid therapy compared with those without corticosteroid therapy at admission, which gradually decreased to similar level during the follow-up period. There was no difference between two groups in most of the laboratory abnormalities and the proportion of patients with these laboratory abnormalities except total bilirubin, lymphocyte count, and lactate dehydrogenase. Frequencies of symptoms related to respiratory and cardiovascular systems and frequencies of abnormal biomarkers showed no difference in all subgroups of patients with different corticosteroid dose. When comparing the radiological findings, pulmonary function and electrocardiogram between two groups, only the frequencies of patchy shadows and fibrous stripes showed a difference.

Conclusion: Corticosteroid treatment did not demonstrate serious complication for overall patients with COVID-19 over SMT in the one-year follow-up period.

❖PP-025

Coronavirus murine hepatitis virus strain 3 induces intestinal injury via caspase 3 dependent apoptosis

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Object/Background: Diarrhea was typical symptoms of the coronavirus disease 2019 (COVID-19). However, the underlying mechanism had not been fully understood.
Aim: The study aimed to explore the mechanism of intestinal injury during COVID-19 in a coronavirus murine hepatitis virus strain 3 (MHV-3) induced acute mouse model.

Methods: MHV-3 induced acute infection Balb/cJ mice model was established. Intestine samples were collected at indicated time points as 0h, 24h, 48h and 60h post infection. The mRNA and protein expression of IL1β, TNFα, IL6, caspase 3 and cleaved caspase 3 were examined by real-time quantitative PCR (qPCR) and western blot respectively. The intestine injury and apoptosis were measured by HE staining and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). Moreover, Z-DEVD-FMK (caspase 3 inhibitor) pre-treated MHV-3 infection mice model were established, in which the apoptosis of intestine was evaluated as well. Meanwhile, the murine intestinal cell MODE-K was infected by MHV-3 in vitro for evaluation of virus induced apoptosis.

Results: Post MHV-3 infection, the histopathology of intestine tissue showed extraordinary injury with time dependence, as well as high level of TUNEL positivity. The mRNA levels of inflammatory cytokine IL1β, TNFα and IL6 were significantly increased. The protein expressions of caspase 3 and cleaved caspase 3 in the intestine was found significantly elevated from 24h to 48h post MHV-3 infection. Z-DEVD-FMK pretreatment inhibited caspase 3 and cleaved caspase 3 expression and decreased TUNEL positivity. Meanwhile, alleviated gut injury and inhibited TNFα expression were observed. In vitro treated by MHV-3, intestinal cell line MODE-K showed nine-fold increase of apoptosis by comparison with saline treated ones. The expressions of apoptosis crucial protein caspase3 and cleaved caspase3 significantly elevated, as well as TNFα.

Conclusion: Coronavirus murine hepatitis virus strain 3 induces intestinal injury via caspase 3 dependent apoptosis, which might shed light on the treatment of intestinal complications in COVID-19.

❖PP-026

A prognostic nomogram for progression-free survival of patients with primary liver cancer after transarterial chemoembolization

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Object/Background: For patients with liver cancer treated with tansarterial chemoembolization (TACE), the correct and accurate assessment of prognosis is the key to guide the subsequent treatment. Various new biomarkers and scoring systems have emerged internationally, but they have not been well applied to the prognositic assessment of survival of patients with liver cancer due to various limitations. Our aim was to identify relevant risk factors and construct a predictive model of nomogram for hepatocellular carcinoma (HCC) patients receiving TACE.

Methods: A total of 346 patients with primary liver cancer who underwent TACE as initial treatment were retrospectively included, of which 208 patients were allocated to derivation cohort randomly. 12-month Progression free survival (PFS) was used as follow-up time endpoint according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST). Univariate analysis by Kaplan-Meier and multivariate analysis by COX regression model screened out indicators associated with short-term prognosis, and R language was further used to construct a nomogram. Then the remaining 138 patients were allocated to validation cohort. And the nomogram was compared with the classical BCLC staging system.

Results: After univariate and multivariate analyses of the derivation cohort, some independent predictors affecting the PFS in patients with liver cancer undergoing TACE included: 1. baseline indicators: age (P=0.013), ALBI grade (grade 2 vs grade 1, P=0.029, grade 3 vs grade 1, P=0.000), and portal vein tumor thrombus (P=0.000); 2. indicators of 1 month follow-up after initial TACE treatment: NLR (P=0.032), the change of AFP (P<0.05), and the change of DCP (P=0.000). 3. the numbers of TACE in 6 months after initial TACE (P=0.007). These predictors were used to construct a prognostic nomogram for PFS. In the derivation cohort, calibration curve of the nomogram showed high consistency between the predicted and the actual PFS probability with C-index=0.712, which outperformed BCLC staging system (C-index=0.688, P=0.004). This result was confirmed

in the validation cohort, whose C-index was 0.734 and better than BCLC staging system (C-index=0.663, P=0.012).

Conclusion: Our study showed the prognostic nomogram had good predictive efficacy and could be used as a complementary assessment to predict the survival and prognosis of patients with liver cancer treated with TACE.

❖PP-027

A case of giant hydrocele accompanying cirrhosis and causing massive scrotal enlargement: forward or backward?

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Object/Background: A 56-year-old man was admitted with abdominal distention and progressive scrotal enlargement. The patient had an 8-year history of HBV-related cirrhosis and had undergone endoscopic selective varices devascularization several times for upper gastrointestinal bleeding.

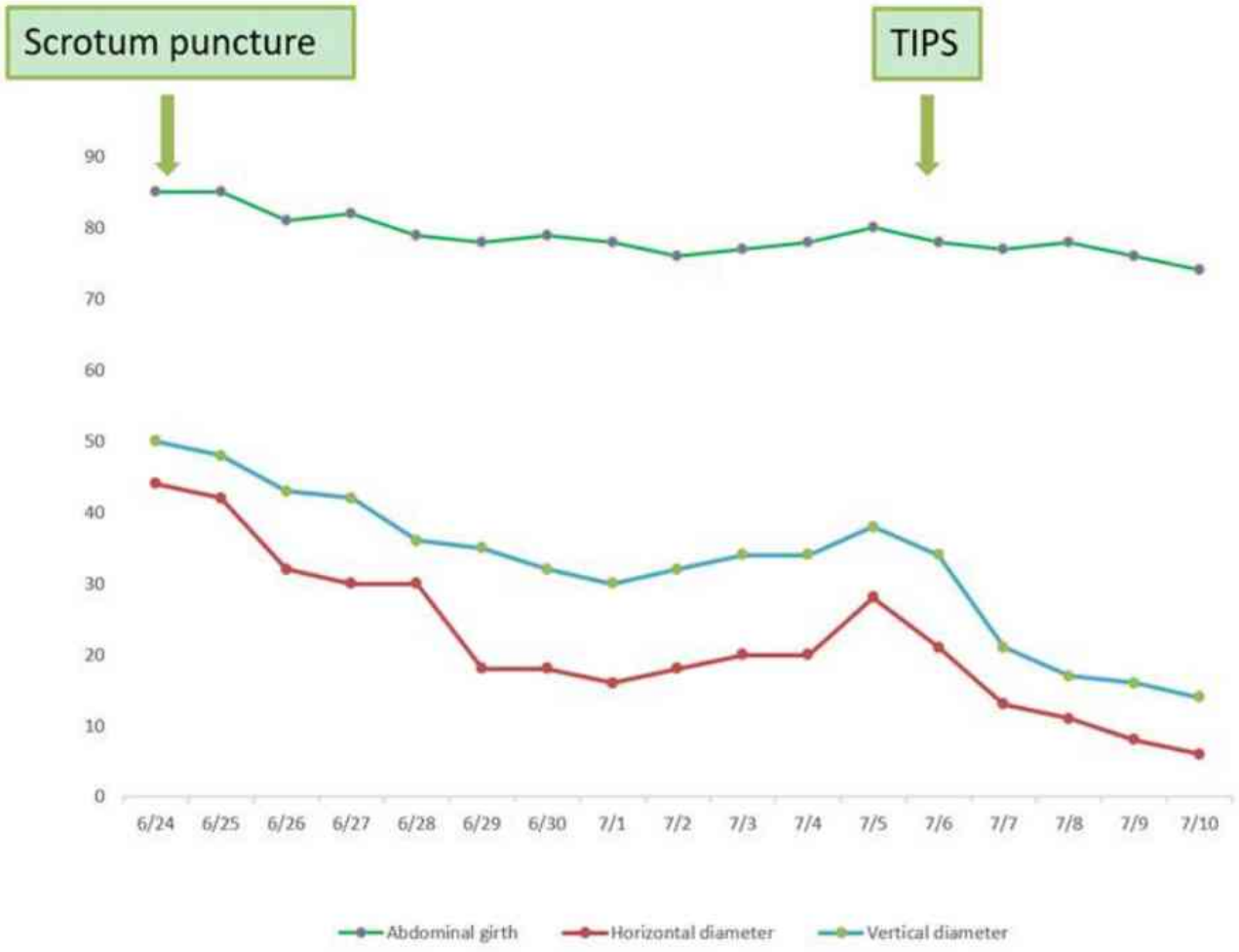
Methods: Physical examination and ultrasound examination showed that there was a large amount of fluid (30*20cm) in the scrotum and a positive transillumination test, but ultrasound examination showed minimal ascites. Enhanced CT showed portal hypertension and venous thrombosis.

Results: Scrotal distension was rapidly relieved after scrotal puncture (Figure 1), and the property of Testicular puncture fluid was similar to that of previous ascites, which indirectly proved the source. Although a large amount of fluid was discharged every day, there was no significant retraction of scrotum before transjugular intrahepatic portosystemic shunt (TIPS) (Figure 2). With the postoperative decrease of portal vein pressure, hydrocele disappeared rapidly(Figure 3) and the urinary system disorder were solved(Figure 4).

Conclusion: Adult hydrocele is usually secondary. With the increasing use of peritoneal cavity in peritoneal dialysis, ventriculoperitoneal shunt and kidney transplantation, the incidence rate of adult hydrocele is rising[1, 2]. Ascites in patients with decompensated cirrhosis may leak into the pleural cavity and cause hydrops[2, 3]. However, in some rare cases, ascites in the abdominal cavity may communicate with the tunica vaginalis and cause hydrocele[4, 5]. Diuretic therapy is ineffective for these patients, although some surgery or transplantation may be the treatment option. Liver cirrhosis with giant hydrocele is rare, and its pathogenesis may be related to hypoproteinemia, because the synthetic function of the liver is decreased and a patent processus vaginalis[6]. It is demonstrated that TIPS is feasible and effective in the treatment of refractory ascites or variceal bleeding secondary to hepatic cirrhosis [7]. In this case, ultrasound-guided scrotal puncture and

TIPS can rapidly relieve local tension and clinical symptoms, but its long-term efficacy remains to be proved.

Figure:



❖PP-028

Influencing factors for the partial virological response of chronic hepatitis B patients treated with nucleos(t)ide analogs

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Object/Background: To investigate the influencing factors of partial virological response in patients with chronic hepatitis B treated with nucleos(t)ide analogs, and to analyze the dynamic changes of virology and liver function during the occurrence of partial virological response.

Methods: 84 chronic hepatitis B patients who received nucleos(t)ide analogs treatment for at least 48 weeks from September 2019 to April 2021 in The First Affiliated Hospital of Nanchang University were retrospectively analyzed. According to HBV DNA levels during treatment, patients were divided into complete virological response group and partial virological response group. Demographic characteristics and laboratory test indexes of patients were observed. Univariate analysis of patient baseline data. Multivariate logistic regression was used to analyze the risk factors for the occurrence of partial virological response, and receiver operating characteristic curve was used to evaluate the effectiveness of each indicator in predicting the occurrence of partial virological response.

Results: A total of 84 patients were included, including 60 cases of complete virological response and 24 cases of partial virological response. Univariate analysis was conducted between the two groups. There were statistically significant differences in age, baseline HBV DNA, 12-week HBV DNA, baseline HBsAg, 12-week HBsAg, baseline alanine aminotransferase, 12-week alanine aminotransferase, gamma-glutamyltransferase, albumin, neutrophils, and non-invasive fibrosis aspartate transaminase and platelet ratio index scores (all $P<0.05$). Multivariate logistic regression analysis showed that HBsAg at baseline, alanine aminotransferase and HBV DNA at 12 weeks of treatment were independent risk factors for partial virological response in chronic hepatitis B patients treated with nucleos(t)ide analogs ($P<0.05$). Baseline HBsAg, 12-week HBV DNA, and baseline alanine aminotransferase all predicted partial virological response ($P<0.05$ or <0.01). Baseline alanine aminotransferase was less predictive of partial virological response than HBV DNA at 12 weeks ($Z= 3.436$, $P<0.001$) and baseline HBsAg ($Z= 2.436$, $P=0.04$).There was no significant difference between the predictive value of HBV DNA at

12 weeks and HBsAg at baseline (Z= 0.815, P=0.39). HBsAg, HBV DNA and alanine aminotransferase were significantly different at different time points after treatment (F=12.692, 121.466 and 8.346, P<0.05). HBV DNA in complete virological response group and partial virological response group (F=11.772 and 3.092, P<0.05) and HBsAg (F=93.004 and 52.639, P<0.05) gradually decreased, and at each time point, all indicators in both groups showed statistically significant differences from baseline at 12 weeks (P<0.05). HBsAg, HBV DNA and alanine aminotransferase were significantly different at different time points group (F=11.772 and 3.092, P<0.05) and HBsAg (F=93.004 and 52.639, P<0.05) gradually decreased, and at each time point, all indicators in both groups showed statistically significant differences from baseline at 12 weeks (P< 0.05).HBsAg and HBV DNA in partial virological response group were higher than those in complete virological response group (F=26.468 and 101.433, P<0.05), HBsAg and HBV DNA in partial virological response group were higher than those in complete virological response group at baseline, 12, 24, 36 and 48 weeks (P<0.05). There was no significant difference in alanine aminotransferase level between the partial virological response group and the complete virological response group (F=3.389, P=0.069). At each time point, alanine aminotransferase level in complete virological response group was significantly higher than that in partial virological response group (P<0.05), alanine aminotransferase level in complete virological response group was lower than that in partial virological response group at 12, 24, 36 and 48 weeks (P<0.05).HBV DNA (F=6.898, P<0.05) and alanine aminotransferase (F=2.798, P<0.05) there was significant interaction effect between time point and group, but HBsAg (F=1.499, P=0.226) had no interaction effect between time point and group.

Conclusion: The chronic hepatitis B patients with high HBsAg level before treatment, high HBV DNA load after 12 weeks of treatment and low alanine aminotransferase level before treatment are more likely to develop partial virological response. Therefore, dynamic monitoring of HBsAg, HBV DNA and alanine aminotransferase levels in this population is of great significance.

❖PP-029

Research progress on short-term prognosis of acute-on-chronic liver failure

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Object/Background: Acute-on-chronic liver failure (ACLF) is a clinical syndrome that can rapidly progress to multiple organ failure and is characterized as a severe condition with rapid progression, a poor therapeutic response and poor prognosis. Early and timely evaluation of the prognosis of ACLF is helpful for providing appropriate clinical intervention, making correct clinical decisions, and prolonging patient survival. Thus, this article reviews the progress in evaluating the short-term prognosis of ACLF to provide future directions for more dynamic prospective large-scale multicenter studies and a basis for individualized and precise treatment for ACLF patients.

Methods No.

Results No.

Conclusion: ACLF is a clinical syndrome that progresses to multiple organ failure in a short time and is characterized as a severe condition with rapid progression, a poor therapeutic response and poor prognosis. Early and timely assessment of the prognosis of ACLF patients is helpful not only for doctors to make timely clinical decisions and provide appropriate clinical intervention but also for rational allocation of LT resources and to prolong the survival of patients. There are individual differences in the clinical features, evoked events and incidence of organ failure among patients with ACLF; therefore, there is no prognostic evaluation system suitable for all ACLF patients at present. It is necessary to explore and identify new indices and prognostic models with high sensitivity.
New prognostic markers are developing not only in the fields of genetics and histology but also towards diversification combined with imaging. ACLF is a dynamic process, and the best prognostic marker is the clinical evolution of organ failure over time, rather than an assessment at a single time point. More large-scale, multicenter, prospective studies are needed in the future to dynamically assess the relationship between changes in certain indicators and outcomes. Multimodal therapy for ACLF, a potentially reversible disease, may be beneficial to the survival of patients, but not all treatments have a positive effect on short-term prognosis. In the future, more studies are needed to assess the significance of

monotherapy or combination therapy in prolonging survival in patients with ACLF. Determining which patients will benefit from continued advanced life support and which patients will be unresponsive to treatment is a formidable challenge, and accurate short-term prognostic assessments of ACLF are a good approach to addressing this issue. Accurate evaluations of the prognosis of ACLF patients are conducive to the development of individualized, accurate treatments and can improve the survival rate of ACLF patients.

❖PP-030

Systematic review and network Meta-Analysis: Corticosteroid combined with SAMe has great promising to improve short-term survival of SAH patients

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Object/Background: The short-term survival of severe alcoholic hepatitis (SAH) patients was not optimistic, but the optimal treatment has been still controversial. Recently, some new therapy had been reported, such as corticosteroid combined with S-adenoidal-L-methionine (SAMe) or granulocyte colony-stimulating factor (G-CSF) related therapy, but few studies compared the survival effects of these therapies. We aimed to compare the effect of different treatment regimens on the survival and complications of SAH patients with network meta-analysis.

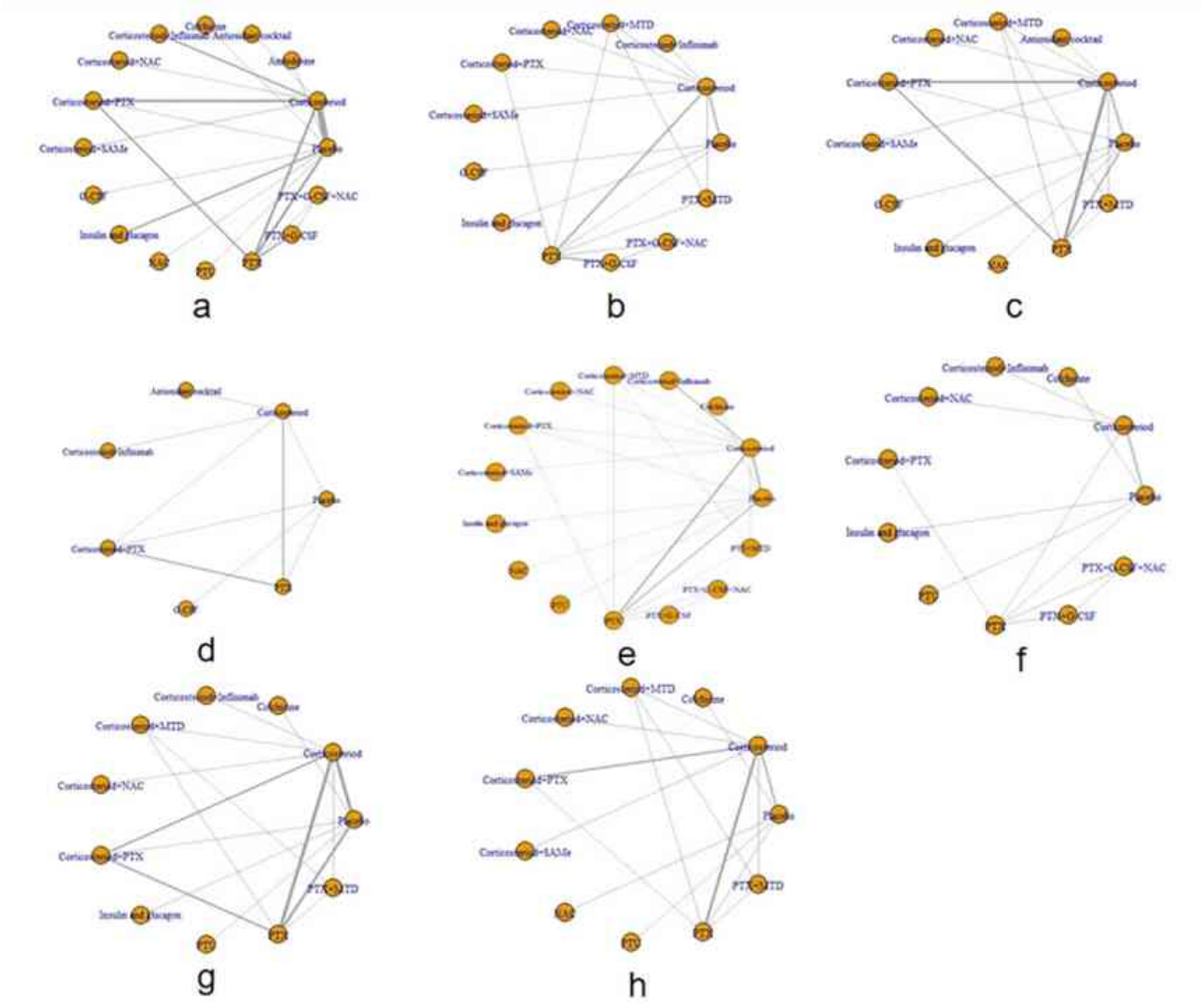
Methods: Four databases (Pubmed, Embase, MELDLINE, Cochrane) and one website (https://www.clinicaltrials.gov/) were searched from establishment to January 2021. SAH patients treated with pharmacotherapy were included in our study and the primary outcome was short-term survival and secondary outcomes consists of medium or long-term survival and complications after the treatments. R software was used to established network meta-analysis models. The result of network meta-analysis was expressed by the odd ratio (OR) value and 95% confidence interval ($p<0.05$).

Results: Ultimately, 31 randomized controlled trial studies including 18 different treatment regimens were enrolled in our study. Total of 8 consistency models were established. As a primary outcome, Corticosteroid+SAMe showed significant superiority compared to Placebo (OR=19.11, 95%CI:1.87-56.43), Corticosteroid (OR=18.55, 95% CI:1.31-55.87), G-CSF (OR=18.65, 95%CI:1.15-56.25) in the 1-month survival of SAH patients. As for the second outcomes, it was found that Corticosteroid+SAMe could significantly reduce the occurrence of hepatorenal syndrome, while pentoxifylline (PTX)+G-CSF was effective in reducing the incidence of infection. In terms of medium-term and long-term survival and occurrence of gastrointestinal bleeding, Corticosteriod+SAMe or G-CSF related therapy did not show significant superiority.

Conclusion: In conclusion, it was found that corticosteroid combined with SAMe had a significant superiority in improving the 1-month survival and reducing the occurrence of

hepatorenal syndrome of SAH patients. In addition, G-CSF combined with PTX was proven to be effective to reduce the risk of infection of these patients.

Figure:



RNA sequencing profiling of human hepatic stellate cells and the potential biomarkers for liver cirrhosis.

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Object/Background: Liver cirrhosis, which presents with the distortion of hepatic architecture, is a significant global health burden. Hepatic stellate cells (HSCs) participate in the core regulation of fibrotic occurrence and reversion; however, the exact mechanisms remain incompletely established. Here, we used RNA sequencing (RNA-seq) to analyze the changes in the transcriptional patterns of patient-derived HSCs, revealing the diagnostic and therapeutic targets for cirrhosis.

Methods: Primary HSCs were isolated from human liver tissues by recirculating perfusion. RNA-seq was performed to profile the mRNA expressions of HSCs by two comparative schemes [scheme A: Group_Cir (n=9) versus Group_Noncir (n=6); scheme B: Group_Act (n=7) versus Group_Fre (n=7)]. The dysregulated mRNAs of HSCs induced by hepatic cirrhosis were identified through the corresponding screening conditions, and bioinformatics analyses were depicted. Protein-protein interaction (PPI) networks were constructed to search for candidates, which were verified by quantitative real time PCR.

Results: In the sequencing data of scheme A, we observed 3828 differentially expressed genes (DEGs) from the HSCs of cirrhotic tissues, of which 2251 and 1577 genes were significantly upregulated and downregulated, respectively. A total of 2262 genes were differentially expressed upon HSC activation in vitro (scheme B), including 1032 and 1230 significantly upregulated and downregulated genes, respectively. According to the functional annotations of these two schemes, the DEGs were significantly enriched in focal adhesion; retinol metabolism; and the formation, assembly, or degradation of collagen and the extracellular matrix. Thereafter, through PPI analysis and PCR verification, CAV1, ESR1, APP, SHC1, BCR, and LPL were screened as hub genes.

Conclusion: Our study offers the sequencing data of human HSCs from the perspective of horizontal and vertical comparison; reveals the changing characteristics of HSCs; and investigates potential targets associated with liver cirrhosis. It therefore sheds light on the

molecular mechanisms underlying liver cirrhosis and provides information for its detection and treatment.

Figure:

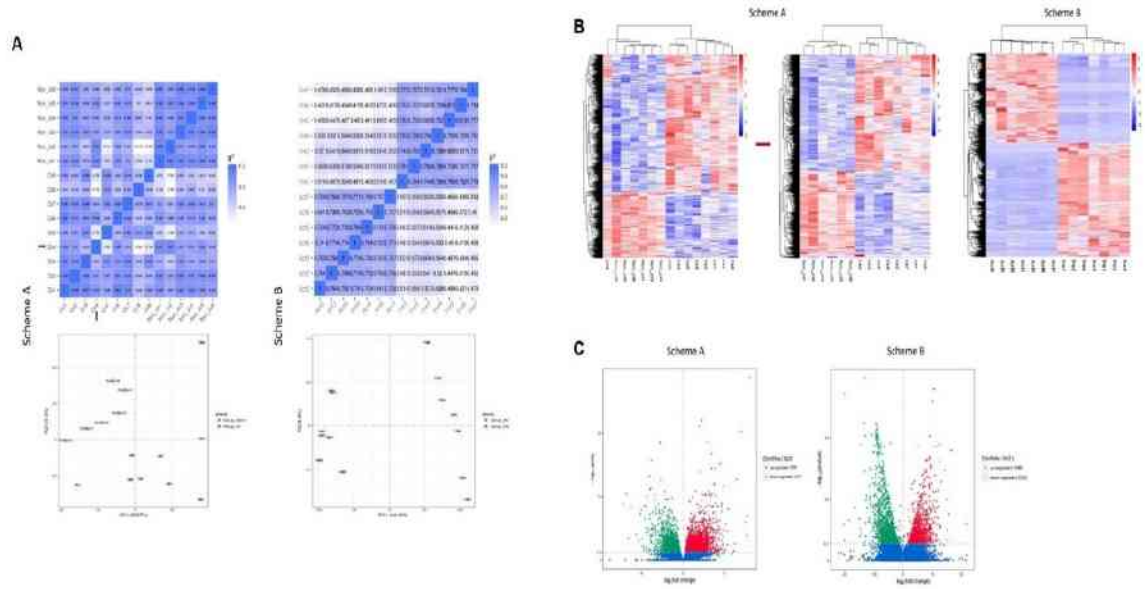

Figure 1. Distinct transcriptional patterns in HSCs during activation. A) Pearson's correlation coefficient (upper panel) and PCA analysis (lower panel) were used to evaluate the homogeneity among samples; B) Hierarchical clustering heatmaps of DEGs. Left: 15 HSC samples in scheme A; Middle: The sample named "Cir 4" was deleted from scheme A; Right: seven paired HSC samples in scheme B; C) The volcanic plot of DEGs.

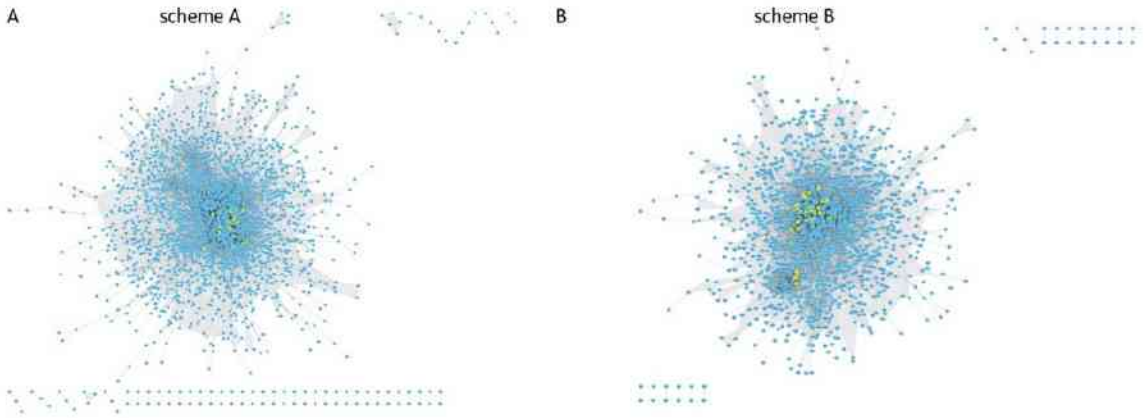

Figure 3. PPI networks of DEGs. Interaction networks of DEGs in scheme A (A) and scheme B (B) were constructed by the PPI analysis.

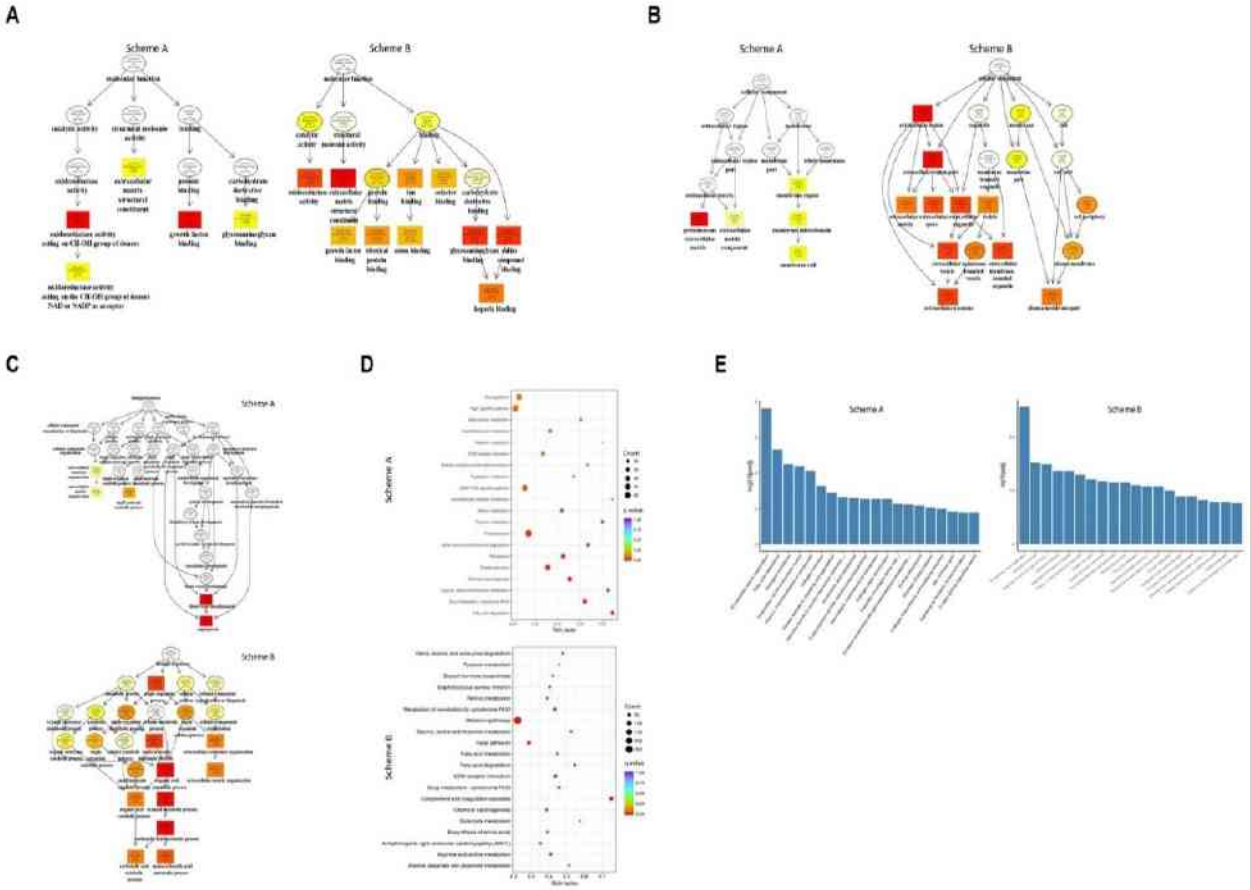

Figure 2. Enrichment analyses of DEGs. The GO analysis was performed to identify the A) molecular functions, B) cellular components, and C) biological processes of the DEGs; D) KEGG analysis of the DEGs. The top 20 significantly enriched pathways are shown. E) Reactome analysis of DEGs. The top 20 significantly enriched pathways are shown.

❖PP-032

DECELERATION OF LIVER REGENERATION BY KNOCKDOWN OF HEPATIC STIMULATOR SUBSTANCE GENE EXPRESSION IN MICE

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Object/Background: Hepatic stimulator substance (HSS) acting as a hepatotrophic growth factor, specifically stimulated proliferation of cultured hepatocytes as well as hepatoma cells in vitro, promoted liver regeneration and recovery of damaged hepatocytes in vivo. Hepatic stimulator substance (HSS) is expressed in the liver cytosol of weanling or partially hepatectomized adult rats, and was first described by LaBrecque and Pesch . As a unique growth factor that can specifically activate hepatic origin cells to grow regardless of animal species, HSS is able to stimulate the proliferation of hepatocytes and hepatoma cells in vitro and in vivo, promote liver regeneration after PHx.To demonstrate if HSS plays a role in the regulation of liver regeneration and its possible mechanisms, we detect liver regeneration related index changes by knockdown of HSS after partial hepatectomy (PH).

Methods: In this study, 50 mice were randomly assigned to two groups. In the control group, 100 μg of scramble-control plasmid (pGPU6/GFP/Neo-negative control) was given through the portal vein injection immediately after PHx in mice. In the group of HSS knockdown, 100 μg pGPU6/GFP/Neo-shRNA HSS RNA plasmid was given similar as the portal vein injection. We established a hepatic gene transfer system via portal vein immediately after PH to deliver HSS shRNA plasmid and analyzed the relationship between HSS and liver regeneration.HSS shRNA plasmid injection through portal vein was found to be an effective method. The data reports HSS mRNA and protein expression were reduced by 56% and 60%, respectively at day 4 after PH.Mouse serum samples were collected at time of killing. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) were assayed.

Results: We showed that knockdown of HSS lead to decreased liver regeneration, inhibit hepatocyte proliferation and impair mitochondrial. HSS is apparently required for normal completion of liver mass restore after PH in mice. Whereas in control mice liver weight returns to the original weight prior to hepatectomy, in HSS RNAi mice liver weight is inferior to (at day 5) that of the prehepatectomy weight. It show that, whereas in control

mice liver weight returns to the original size prior to hepatectomy, in HSS RNAi mice liver regeneration inhibited (at day 7). Serum HSS content were reduced after PH. In control mice, compared to ‘ time 0 ’ value, an increase of ALT and AST level was soon registered after PH; its maximal expression was reached by the first day, subsequently declining to the ‘ time 0 ’ level for the rest of the period of observation. Knockdown of HSS aggravate liver dysfunction. In both types of mice there is a typical initial increase (2 and 3 days post-PHx) as assessed by PCNA immunohistochemistry. Compared to control mice, the HSS RNAi mice exhibited a slightly slower increase in in cell proliferation at 2, 3 days post-PHx. HSS RNAi elicited morphological abnormalities in the mitochondrial ultrastructure, including decrease in number and deficient or swollen cristae.

Conclusion: Inhibition of HSS expression by shRNA during liver regeneration obviously delayed the process .our results present here for the first time that HSS is apparently required for normal completion of liver mass restore after PHx in mice. Inhibition of HSS expression by shRNA during liver regeneration obviously delayed the process as result of comparatively weakened DNA synthesis.Knockdown of HSS aggravate liver dysfunction.

Keywords: Hepatic stimulator substance, partial hepatectomy,liver regeneration, hepatocyte proliferation

❖PP-033

Weighted hypothalamic-pituitary-thyroid axis dysfunction in prognosis of hepatitis b virus related acute-on-chronic liver failure

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Object/Background: To weighted the prognostic value of hypothalamic-pituitary-thyroid axis (HPT) dysfunction in catastrophic HBV-ACLF.

Methods: A retrospective cohort (n=635) and a multicenter prospective cohort (n=353) were enrolled in this study.

Results: TSH was identified as potential prognostic predictor for HBV-ACLF among hormones in HPT. The novel score (mCLIF-OFs) was developed with weighted TSH and other failed organs in CLIF-OFs using the retrospective cohort (n=635). The C-index of mCLIF-OFs (0.885 [0.883-0.887]) for 30-day mortality was significantly higher than that of the CLIF-OFs, CLIF-SOFAs, CLIF-C ACLFs, MELD, and Child-Pugh (all $p<0.001$). The absolute improvements of prediction error rates of the mCLIF-OFs comparing to the above five scores were from 19.0% to 61.1%. After the analysis of probability density function (PDF), the mCLIF-OFs showed the least overlapping coefficients (27.9%) among above prognostics scores. The predicted risk and observed probabilities of death were comparable across the deciles of mCLIF-OFs (Hosmer-Lemeshow $\chi 2 = 4.28$, $p=0.83$; Brier scaled=11.9). The mCLIF-OFs showed better goodness-of-fit than that of CLIF-SOFAs and MELD, but had the minimum Brier scaled among above five prognostic scores. Additionally, the mCLIF-OFs show greater net benefit than other five scores over a wide range of risk threshold of death. Similar results were validated in a multicenter prospective cohort (n=353).

Conclusions: Decreased TSH was a representative hormone presaging HPT dysfunction, which can be treated as an “organ failure” in HBV-ACLF prognosis. The novel mCLIF-OFs is high-performance prognostic score with better discrimination power, calibration capacity, and clinical net benefit.

❖PP-034

Single-Cell Sequencing Reveals the Hepatic Immune Microenvironment in Patients with Acute-on-chronic Liver Failure

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Object/Background: Acute-on-chronic liver failure (ACLF) is a critically-ill disease caused by an acute exacerbation of underlying chronic liver disease and characterized by multiple organ failure and high short-term mortality. The liver is one of the frequently involved organs in the pathogenesis of acute-on-chronic liver failure (ACLF). Mounting evidence suggests that the intricacy of the hepatic immune microenvironment of ACLF patients. It is now increasingly recognized that traditional approaches including flow cytometry, immunofluorescence and analysis on bulk transcriptomic profiles yielded limited information on cell-specific markers and therefore resulted into our inability to fully elucidate the actual complexity of immune cell subsets. The single-cell RNA sequencing (scRNA-seq) technology has emerged in recent years and has proved to be a powerful tool to uncover the molecular heterogeneity of cell populations in a comprehensive and unbiased manner. Herein, we applied scRNA-seq to precisely define leukocytes within the hepatic compartment in ACLF. The distribution, differentiation, functionality, and interaction of specific cell types were also explored. This study aimed to delineate the components and characteristics of the interactive network of the hepatic immune microenvironment in ACLF via high-throughput technologies.

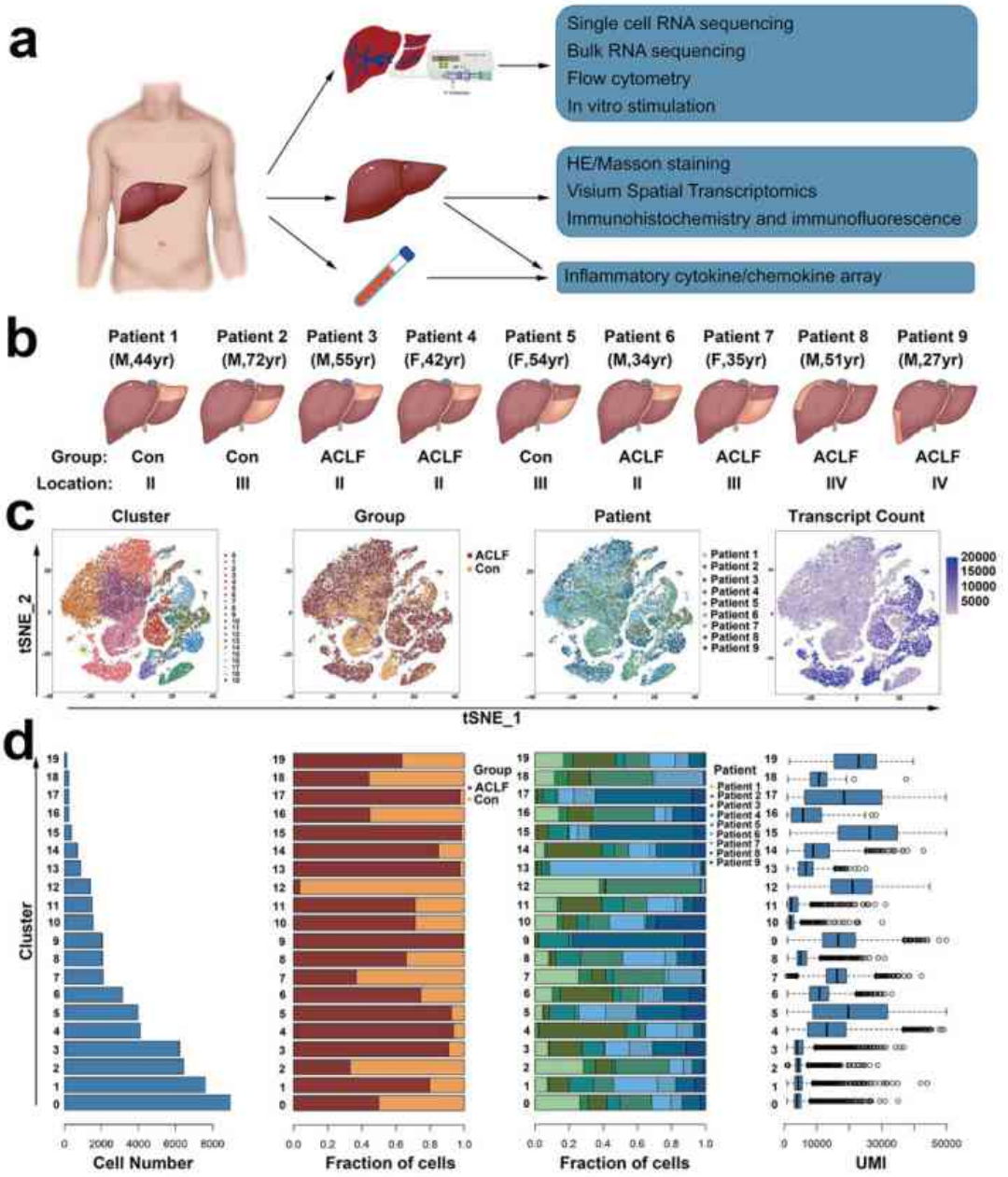
Methods: We sequentially enrolled patients with ACLF who underwent liver transplantation or partial hepatectomy at the First Affiliated Hospital of Zhejiang University from August 2018 to January 2021. Single-cell RNA sequencing of CD45+ cells isolated from liver of 6 ACLF patients and 3 disease controls was performed. The phenotypes of the macrophage clusters was confirmed by flow cytometry. A visium spatial gene expression was performed to analyze the distribution of macrophage clusters, which was confirmed by immunofluorescence assays. In vitro experiments was performed to explore the cytokines in the inflamed liver resulting in the differentiation of monocytes. A measurement of cytokines and chemokines within the circulating and hepatic compartment of ACLF was conducted using the MSD high-output approach.

Results: Single-cell RNA sequencing of CD45+ cells isolated from liver of 6 ACLF patients and 3 disease controls was performed to identify 19 types of immune cells. CD9+ and TFRC+ macrophages, CD8+ T cells and plasma cells were enriched in patients with ACLF. Spatial gene expression and immunofluorescence assays showed that CD9+ and TFRC+ macrophages were significantly increased in the peri-lobular area of the inflammatory livers. By the in silico trajectory analysis, the two macrophage subets exhibited different differentiation trajectories despite their monocyte origin. Furthermore, monocytes could undergo differentiation to express CD9 and TFRC in response to the ACLF liver homogenates and anti-inflammatory cytokines IL-10. CD74-MIF was predicted to mediate the interaction between macrophages and adaptive immune cells. A manifold of cytokines was altered either in the hepatic or circulating compartment or both in ACLF patients.

Conclusion: We collectively used scRNA-seq to characterize the immune cell heterogeneity within the liver of ACLF patients and provided the basis for uncovering the pathophysiological mechanisms. The identification of repair-associated macrophages subsets is a key step for developing macrophage-based cell therapy for ACLF. Our findings also emphasized the importance of the liver as the origin of systemic inflammation in the pathogenesis of ACLF precipitated by acute hepatic insults.

❖PP-034

Figure:



❖PP-035

Enhanced ASPP2 Promotes Liver Injury by Way of an Inflammatory Immune Regulatory Mechanism in Acute Liver Failure

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Object/Background: Acute liver failure (ALF), an inflammation-mediated hepatocellular injury process, is a clinical syndrome that results from hepatocellular apoptosis and hemorrhagic necrosis. The apoptosis-stimulating protein of p53 (ASPP) family is a newly identified family protein including ASPP1, ASPP2 and inhibitor of ASPP (iASPP), by which the tumor protein 53 (TP53)-mediated apoptotic process is selectively regulated. Downregulation of ASPP2 was revealed to be associated with a poor prognosis and metastasis in several types of cancer. The ASPP2 could enhance apoptosis at least in part through a p53-mediated pathway. Previous research had shown that ASPP2 is an independent haploinsufficient tumor suppressor in vivo. However, the role of ASPP2 in the pathogenesis of ALF and its regulatory mechanisms remain unclear.

Methods: The expression of ASPP2 were analysed using the liver biopsy samples from HBV-related hepatocarcinoma (HCC) patients and ALF patients. The different groups of mice were sacrificed after D-GalN/LPS injection 2, 4, 6 hours. Liver injury blood markers, including ALT and AST, were detected to evaluate the acute liver failure modle of mice whether success. Liver tissues were fixed in formalin, washed with phosphate-buffered saline (PBS), embedded in paraffin and stained with hematoxylin and eosin (H&E) in 5-μm sections with a standard protocol, and then the samples were observed with light microscopy. The gene expression was detected by real time polymerase chain reaction(PCR), and the protein expression was observed by western blot and Immunofluorescence. ASPP2+/- and ASPP2+/+ Balb/c mice were used to examine the effects of ASPP2 on liver injury induced by D-galactosamine(D-GalN)/ lipopolysaccharide(LPS) in vivo. The inflammatory immune mechanism of ASPP2 were also explored in bone marrow-derived macrophages (BMDM) in vitro.

Results: Both the gene and protein expression of ASPP2 was significantly upregulated in liver tissue of ALF patients with HBV infection and ALF mice induced by D-GalN/LPS, and significantly down-regulated in HCC patients. In ALF mice, the expression of ASPP2 increased with prolonged administration. Compared with wildtype mice, the ablation of

ASPP2 (ASPP2+/-) significantly ameliorated the hepatocellular damage, evidenced by reduced serum alanine aminotransferase (sALT and sAST) levels, well-preserved liver architecture compared with controls. The liver protective effect of ASPP2+/- was dependent on an inflammatory immune regulatory mechanism, because ASPP2 is required to regulate liver inflammation by selectively depressing tumor necrosis factor-α (TNF-α) and promoting interleukin-6 (IL-6) in vivo and in vitro, moreover, the luciferase assay results showed that overexpression of ASPP2 could bind to the promoter sequence of TNF-α and IL-6. Aspp2 knockdown protects mice from D-GalN/LPS -induced liver injury by promoting autophagy and inhibiting hepatocyte apoptosis. The molecular mechanistic investigations elucidated that the enhanced ASPP2 promoted liver injury by regulating PPARα-autophagy pathway, because inhibition of PPARα by siRNA abrogated liver protection and decreased autophagy again induced by ASPP2+/-.

Conclusion: Our results, which define a mechanism whereby ASPP2 knockdown protects mice from D-GalN/LPS -induced liver injury by promoting autophagyoverexpression, open a new avenue for promoting autophagy in treatments to cure hepatocellular carcinoma. Our novel findings document the key inflammatory immune regulatory function of ASPP2 in the pathophysiology of ALF, and provide a rationale to target ASPP2 as a refined therapeutic strategy to ameliorate acute liver injury.

Figure:

Figure 1 Increased expression of Aspp2 in the liver of ALF

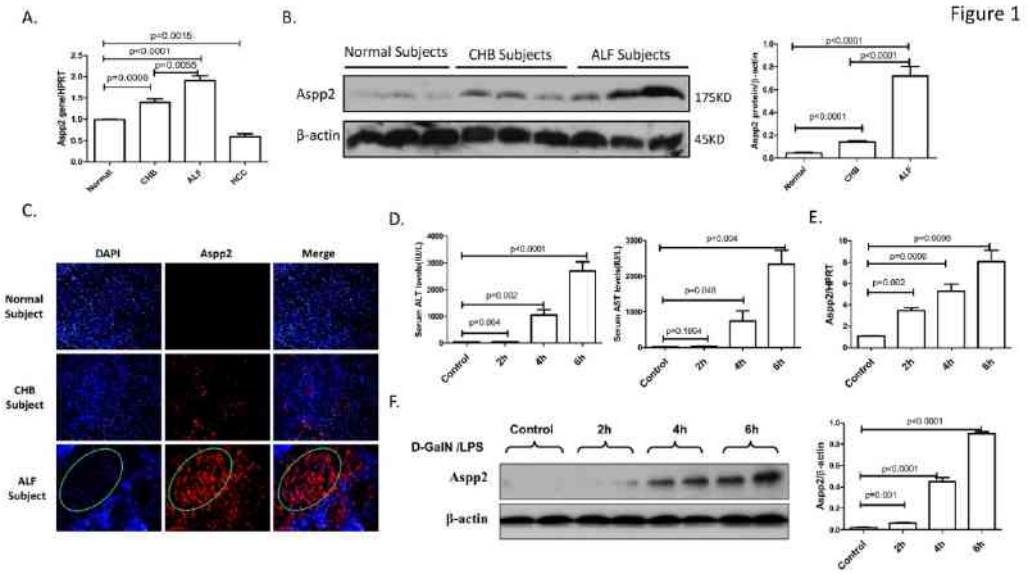


Figure 2 Aspp2 knockdown protects mice from D-GalN/LPS -induced liver injury

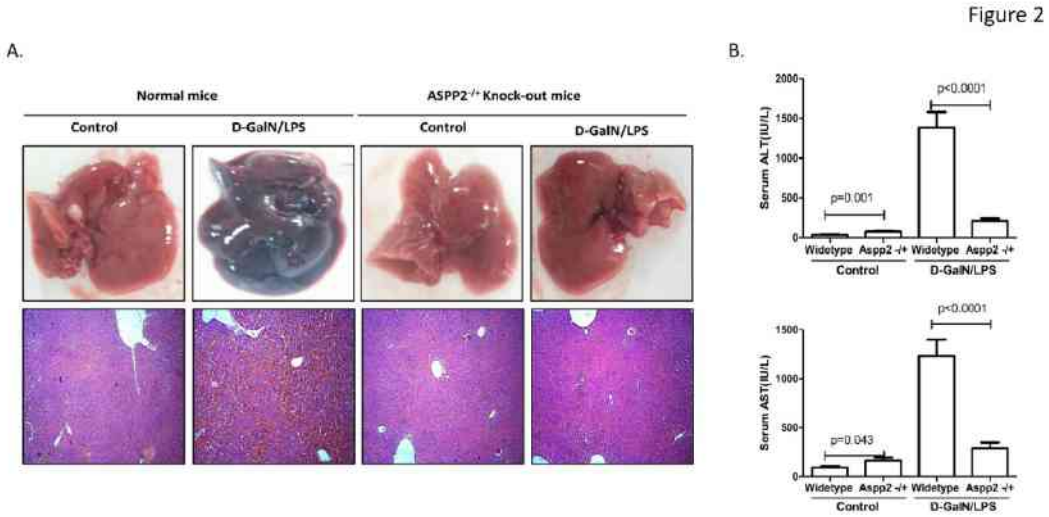


Figure 3 Aspp2 knockdown protects mice from D-GalN/LPS -induced liver injury by suppressing liver inflammation

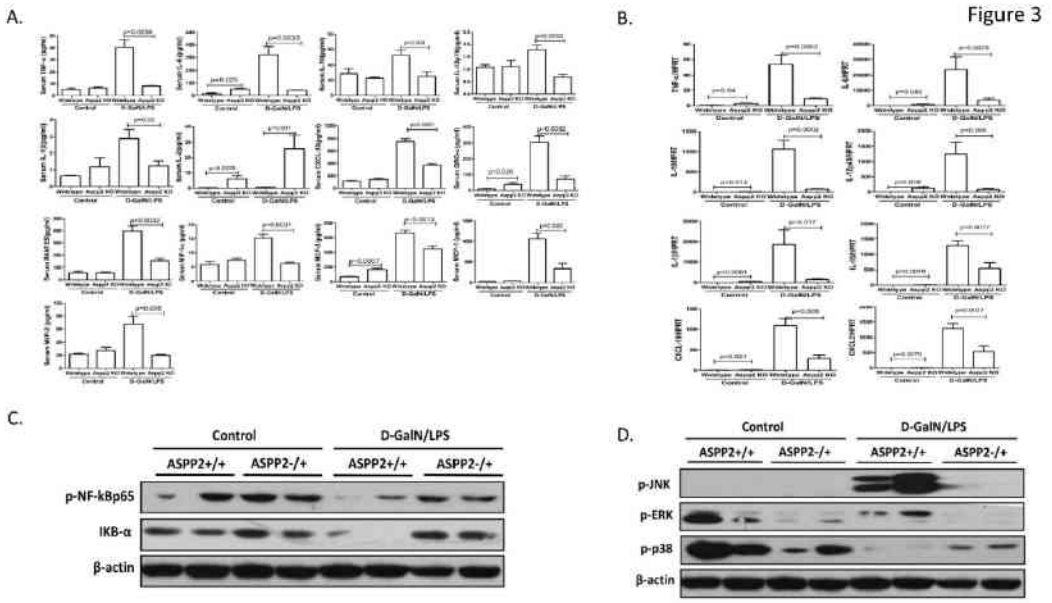


Figure 4 Aspp2 knockdown inhibits hepatocyte apoptosis to protects mice from D-GalN/ LPS -induced liver injury

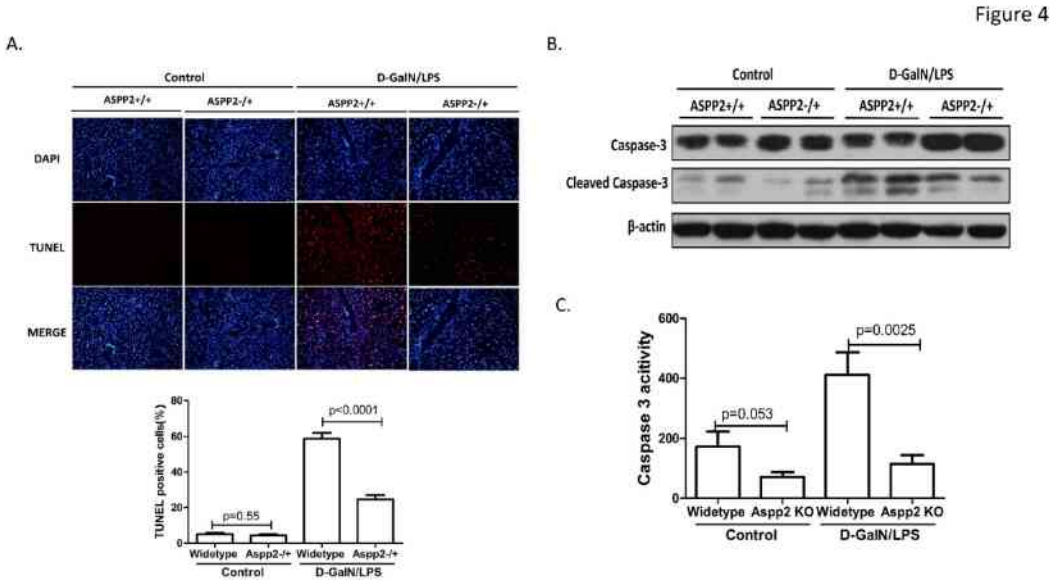


Figure 5 Aspp2 selectively regulates TNF-α and IL-6 expression in macrophage induced by LPS

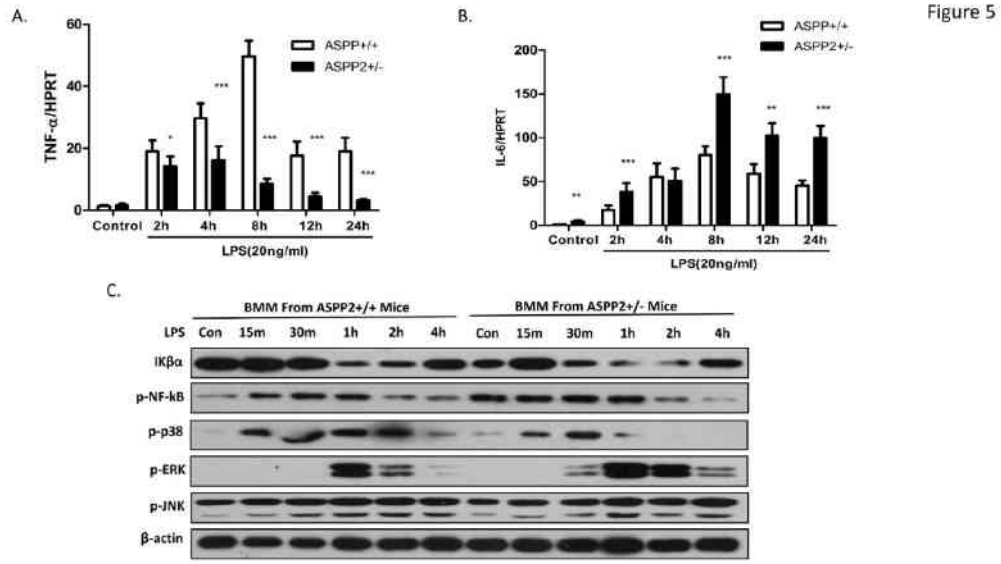


Figure 6 Aspp2 knockdown protects mice from D-GalN/LPS -induced liver injury by promoting autophagy

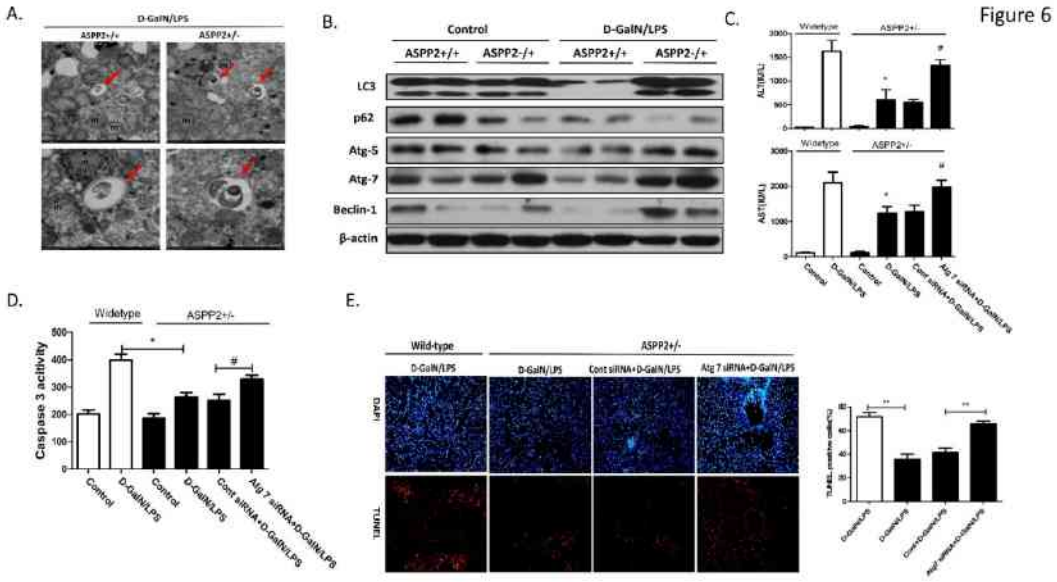
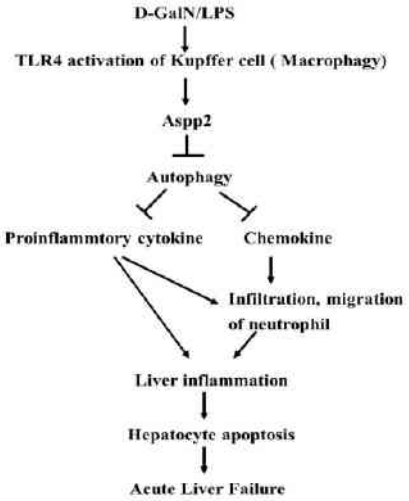


Figure7 A proposed model for the Aspp2-autophagy pathway in acute liver failure



❖PP-036

Single-cell RNA Sequencing Shows T Cell Exhaustion Landscape in Peripheral Blood of patients with hepatitis B virus associated acute-on-chronic liver failure

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Object/Background: Acute-on-chronic liver failure (ACLF) is a complex clinical syndrome that is featured by the acute deterioration of liver function in a patient with chronic liver disease and is associated with organ failure and a high short-term mortality rate. Secondary infection is the most common complication during the development of hepatitis B virus-associated ACLF (HBV-ACLF) and also one of the leading causes of acute liver failure.Immune dysfunction is the main factor for secondary infection in patients with HBV-ACLF, but the exact mechanism remains unclear.T cell exhaustion is an important component of immune dysfunction. T cell exhaustion occurs in many chronic infections or cancers and is characterized by a progressive loss of T effector functions , increased expressions of multiple inhibitory receptors. Here we explored the heterogeneity of peripheral blood T cell subsets and the characteristics of exhausted T lymphocytes in patients with HBV-ACLF, in an attempt to identify potential therapeutic target molecules for immune dysfunction in ACLF patients.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from six patients with HBV-ACLF and three healthy controls (HC), and single-cell RNA sequencing (scRNA-seq) was performed on a 10× genomics platform. Step 1: unsupervised clustering of the cells was performed based on the gene expression profiles using the Seurat package and transmitted to the Uniform Manifold Approximation and Projection (UMAP) to analyze the clustering of immune cells and T cells. Cell subsets were identified based on marker genes. Step 2: the differences in the expressions of exhausted T-cell subsets were compared between HBV-ACLF patients and healthy controls. Step 3: the developmental trajectory of exhausted T cells was inferred by pseudotime analysis. Step 4: the expression of exhausted T cells was validated by flow cytometry in 30 HBV-ACLF patients with co-

existing infection and 30 healthy controls, along with the validation of the differences in the secretion functions of cytokines (IL-2, TNF-a, and IFNγ) between exhausted T cells and non-exhausted T cells.

Results: T lymphocytes in the peripheral blood of HBV-ACLF patients had distinct differentiation trajectories and could be differentiated into eight clusters with different gene characteristics, among which CD4+ TIGIT+ subset and CD8+ LAG-3+ subset, with high expression of exhaust genes, were significantly higher in HBV-ACLF patients than in normal controls. As shown by pseudotime analysis, the CD4+ TIGIT+ T cells and CD8+ LAG-3+ T cells experienced a transition from naïve T cells to effector T cells and then exhausted T cells in the later stage of T cell differentiation. Flow cytometry confirmed that the CD4+TIGIT+ subset and CD8+LAG-3+ subset in peripheral blood of ACLF patients were significantly higher than those in healthy controls. Moreover, in-vitro cultured CD8+LAG-3+ T cells were significantly less capable of secreting cytokines (IL-2, TNF-a, and IFNγ) than CD8+LAG-3- subset.

Conclusion: Peripheral blood T cells are heterogeneous in HBV-ACLF. The exhausted T cells, featured by CD4+TIGIT+ T lymphocyte subset and CD8+LAG-3+ T lymphocyte subset, markedly increase during the pathogenesis of ACLF, suggesting that T cell exhaustion is involved in the immune dysfunction of HBV-ACLF patients. These results may help to provide potentially effective target molecules for the treatment of immune dysfunction in ACLF patients.

❖PP-037

Ercao Qinggan Decoction regulates apoptosis of hepatocytes through TLR4-mediated PI3K/Akt/GSK3β signal pathway in mice with acute liver failure

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Object/Background: To investigate the inhibitory effect of Ercao Qinggan Decoction (EQD) on apoptosis of hepatocytes in mice with acute liver failure (ALF) and the related mechanisms.

Methods: RAW264.7 cells were stimulated with lipopolysaccharide (LPS) to induce cell apoptosis. Cells were pretreated with TLR4 inhibitor CLI-095, GSK3β inhibitor LiCl and different doses of EQD. Cell apoptosis rate was detected by flow cytometry. GSK3β nuclear translocation was examined by immunofluorescence. The mRNA expression levels of TNF-α and IL-6 were detected by qPCR. ALF mouse model was established by intraperitoneal injection of D-Gal/LPS. The mice were gavaged with different doses of EQD. Liver tissues were stained with hematoxylin and eosin, and the apoptosis of hepatocytes was detected by TUNEL assay. Expression of Bax and Bcl-2 mRNA in liver tissues was detected by qPCR. Serum AST and ALT levels were examined by commercial kits. TNF-α and IL-1β levels in the serum were detected by ELISA. Western blot was used to detect Akt, p-Akt, GSK3β and p-GSK3β in liver tissues.

Results: CLI-095, LiCl and EQD significantly inhibited cell apoptosis, TNF- α and IL-6 mRNA expression, and GSK3β nuclear translocation of LPS-stimulated RAW264.7 cells. EQD significantly inhibited hepatocyte apoptosis, downregulated serum TNF-α and IL-β levels, and suppressed the ratios of p-GSK3β/GSK3β and p-Akt/Akt in the liver tissues of ALF mice.

Conclusion: EQD can inhibit the apoptosis of hepatocytes in ALF mice through regulating TLR4/PI3K/Akt/GSK3β signaling pathway.

❖PP-038

G-CSF Promotes the Angiogenesis of Injured Liver via Direct Effects on Liver Cells

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Object/Background: Presently, liver transplantation is the only treatment strategy for liver failure (LF). Although granulocyte colony-stimulating factor (G-CSF) exhibits protective functions in LF, its direct affects on liver cells is still unknown. In this study, we explored the direct roles of G-CSF in VEGF-A expression of injured liver cells, and the indirect stimulation on human umbilical vein endothelial cells (HUVECs). In addition, we investigated the role of AKT and ERK signaling pathways.

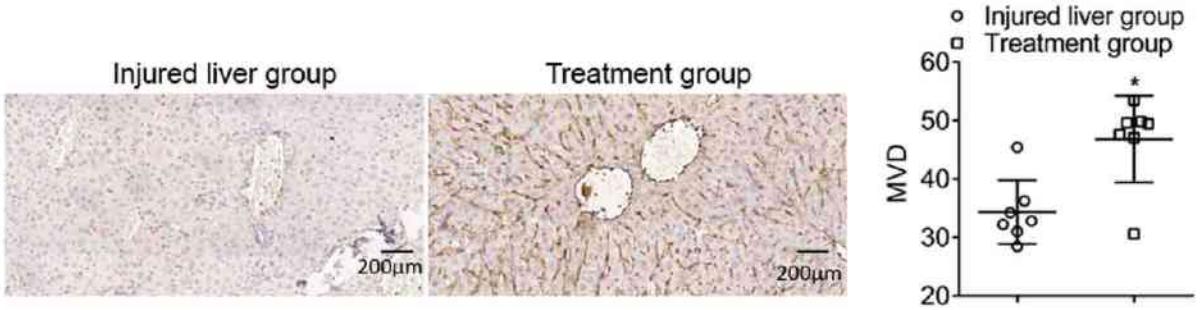
Methods: We established an injured liver cell model and examined the effect of G-CSF treatment on VEGF-A expression. Thereafter, HUVECs were cultured in a conditioned medium (CM) collected from the G-CSF-treated injured liver cells. Ki67 expression in HUVECs' were detected by western blot, HUVECs' proliferation was analysed using CCK8 assay, and tubule formation tests were performed to detect the angiogenic ability of endothelial cells. Furthermore, we established injured liver mouse model, and evaluated changes of serum alanine aminotransferase activity and micro vessel density (MVD) after G-CSF treatment. Additionally, AKT and ERK signal targets were explored.

Results: G-CSF treatment directly enhanced VEGF-A expression in injured liver cells. Compared with injured untreated liver cells, cells pre-treated with G-CSF at different concentrations showed higher expression of VEGF-A both at the RNA and protein levels. Further, CM from injured liver cells treated with G−CSF stimulated HUVECs. Compared with HUVECs cultured in CM from injured untreated liver cells, those cultured in CM from injured liver cells treated with G-CSF exhibited a higher expression of Ki67 at the protein level, a significant increase in cell viability and an increase in the number of tubular structures. In vivo, HE staining indicated less severe liver injury in G-CSF (250 μg/kg)-treated mice compared with injured and untreated mice. G-CSF-treated mice also showed significantly lower serum ALT levels (166.73±62.12 IU/L, $p = 0.003$) than untreated mice with injured liver (293.24±22.55). MVD was significantly higher in the treatment group

(47.78±5.86, p < 0.001, Fig.1) than in the injured untreated liver group (35.06±5.46). Additionally, it was demonstrated that the effects of G-CSF on injured liver cells were mediated through the AKT and ERK signalling pathways.

Conclusion: G-CSF promotes injured liver angiogenesis by directly affecting injured liver cells via the AKT and ERK signalling pathways. These findings enrich our understanding of the role of G-CSF in recovery from LF.

Figure:



❖PP-039

Coagulation function in patients with liver failure

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none

Object/Background: In patients with liver failure, the production of coagulation factors is reduced due to the decline in the synthesis function of the liver, and clinically, it will be manifested as abnormality of various indicators in the coagulation routine. There is an urgent need for an index that can be used to more accurately evaluate coagulation function in clinical practice.

Methods: The primary hemostasis under physiological conditions is mainly the interaction of platelets and endothelial cells, thereby forming platelet thrombus to play the role of hemostasis. In patients with liver cirrhosis, his platelet count is decreased, but the increase of vWF level and the decrease of ADAMTS13 can increase the adhesion of platelets to promote platelet adhesion to vascular endothelial cells, making up for the bleeding tendency caused by the decrease in platelet number. . It is mainly a cascade reaction of activation of coagulation factors of intrinsic and extrinsic pathways, and finally forms fibrin clots and microthrombi. In patients with liver cirrhosis, due to the reduced synthesis function of the liver, procoagulant factors and anticoagulant factors will decline synchronously, and coagulation activation also maintains a dynamic balance. During the fibrinolytic phase, the synthesis of antiplasmin and fibrinolytic inhibitors by the liver decreases, while the levels of plasminogen activator and plasminogen activator inhibitor produced by endothelial cells increase, which is also achieved during the fibrinolytic phase. a new balance.
A single coagulation evaluation index is defective. Compared with conventional coagulation tests, thromboelastography can reflect the whole process of blood from coagulation to dissolution. It is a detection method that can evaluate the dynamic process of coagulation and fibrinolysis as a whole. A comprehensive evaluation of factors such as factor activity, fibrinogen, and platelet count and function can more comprehensively reflect the coagulation status of patients with liver failure.

Results: The coagulation function of patients with liver failure is actually in a fragile state of rebalancing at a low level, and this balance is easily affected by various factors and changes to a hypocoagulable or hypercoagulable state.

Conclusion: Although the coagulation routine indexes and platelet counts of these patients are obviously abnormal, the abnormality of these indexes cannot fully reflect the real coagulation function status of patients with liver cirrhosis.

❖PP-040

Characterization of acute-on-chronic liver diseases: a multicenter prospective cohort study

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Object/Background: Patients with chronic liver diseases (CLD) will develop acute liver injury (ALI) under the attack of various precipitants, leading to significantly elevated alanine aminotransferase and/or total bilirubin levels, liver failure, or acute decompensation (AD) of liver cirrhosis, which is called acute on chronic liver diseases (AoCLD). This study intends to explore the clinical type, etiology, inducement, prognosis and prognostic factors of AoCLD, so as to provide theoretical guidance for the accurate diagnosis and prognosis of AoCLD.

Methods: Patients with AoCLD from the Chinese Acute-on- Chronic Liver Failure (CATCH-LIFE) study cohort were included in this study, the demographic and clinical data, including age, gender, etiology, and predisposing factors, clinical examination and test data, complications, and outcomes (survival, liver transplantation, death) were analyzed. The 28-day and 90-day prognosis of each clinical type of AoCLD was analyzed using Kaplan-Meier, and the survival rate was compared using the log-rank test method.

Results: A total of 3375 patients with AoCLD were included in this study, including 1679 (49.7%) patients with acute decompensation of liver cirrhosis (LC-AD), 850(25.2%)

patients with acute-on-chronic liver failure (ACLF) (type-C ACLF in 535 patients, type-A ACLF in 215 cases, type-B ACLF in 100 cases), 269 (8.0%) cases with LC-A patients, and 577 (17.1%) cases with chronic hepatitis acute exacerbation (CHAE). The most common cause of CLD was HBV infection (71.4%), followed by alcoholic liver disease (19.2%). Among the inducements of AoCLD, HBV reactivation was the most common (61.4%), followed by bacterial infection (22.8%). At the end of 90 days of follow-up, the mortality rates of each clinical subtype of AoCLD were: 43.4% (232/535) in type-C ACLF, 36.0% (36/100) in type-B ACLF, 27.0% (58/215) in type-A ACLF, 9.0% (151/1679) in LC-AD, 3.0% (8/269) in LC-A, and 1.2% (7/577) in CHAE, respectively.

Conclusion: In China, HBV infection is the main cause of AoCLD, and HBV reactivation is the main inducement of AoCLD. The most common clinical type of AoCLD is LC-AD, followed by ACLF, CHAE, and LC-A. Patients with LC-AD or ACLF have high short-term mortality, and early diagnosis and timely intervention should be applied to reduce the mortality of patients.

❖PP-041

Effect of risk allele of PNPLA3 rs738409 on liver fibrosis

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Object/Background: Long-term hepatic steatosis can cause chronic liver damage, leading to varying degrees of liver fibrosis. Liver cirrhosis is the end stage of liver fibrosis progression, and late stage is prone to complications of liver cirrhosis, such as esophageal and gastric varices rupture bleeding, ascites, hepatic encephalopathy, etc. The presence of cirrhosis complications is an important factor in predicting the prognosis of patients with chronic liver disease and making treatment plans. The molecular mechanism of fatty liver disease is not well understood. Many researchers believe that the accumulation of triglycerides can cause lipotoxicity, thereby promoting the release of DAMage-endogenously related molecules (DAMPS) after excessive oxidative stress induces hepatocyte injury, leading to hepatic stellate cell activation, and then hepatocyte fibrosis. Potato glycoprotein-like phospholipase protein 3 (PNPLA3) has triacylglycerol hydrolase and acyltransferase activities in liver. The rs738409(CG) polymorphism of PNPLA3 gene results in the conversion of amino acid from isoleucine to methionine at position 148, which limits the activity of hydrolase and causes the increase of liver fat. Limited data are available on the role of PNPLA3 rs738409 gene polymorphisms in the progression of liver fibrosis. Therefore, we aimed to evaluate the effect of RS738409 gene polymorphism on patients with cirrhosis.

Methods: A prospective study was conducted in the Second Hospital of Jilin University from November 2020 to May 2022. The patients were divided into control group and cirrhosis group, and the patients in cirrhosis group were further divided into compensated cirrhosis group and decompensated cirrhosis group according to whether there were complications of cirrhosis. The complications of liver cirrhosis include varicose vein rupture bleeding, hepatic ascites, hepatic encephalopathy and so on. To assess the role of PNPLA3 rs738409 gene polymorphism in the progression of liver fibrosis, odds ratios (OR) and relative risks of rs738409 variants in cirrhotic and non-cirrhotic groups, as well as compensated and decompensated cirrhosis groups in the entire cohort were calculated.

Results: Finally, 200 cases in cirrhosis group and 70 cases in control group were enrolled. The frequency of G allele was significantly different between the two groups (OR=1.751;

P< 0.001). The OR value of liver cirrhosis group was significantly higher than that of control group (OR = 2.348; P< 0.001) OR two other alleles (OR= 1.671; P = 0.039) significantly increased. There was no significant difference in the frequency of G allele between compensated cirrhosis group and decompensated cirrhosis group (OR=1.36, P=0.150). However, when two mutant alleles were present, the OR value of decompensated cirrhosis group was significantly higher (OR = 1.858; P = 0.005).

Conclusion: This is the first study to evaluate the effect of RS738409 gene polymorphism on the progression of liver fibrosis in patients with cirrhosis. In patients with liver disease, the G allele of RS738409 increases the risk of fibrosis progression. In particular, the occurrence of two mutant alleles is a risk factor for complications in cirrhosis group. GG genotype may be a potential predictor of severe liver fibrosis.

❖PP-042

Natural killer and hepatitis B virus-specific T cells are associated with virologic relapse and liver damage after nucleos(t)ide analogue cessation

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Object/Background: Nucleos(t)ide analogue (NA) therapy has no direct inhibitory effect on covalently closed circular DNA (cccDNA), and it is extremely difficult to eradicate HBV. Treatment cessation of traditional nucleos(t)ide analogues is often accompanied by virologic relapse (VR) and liver damage. Natural killer (NK) and virus-specific T cell responses are compromised, and their antiviral ability are profoundly weakened in chronic HBV infection. We aimed at investigating the roles of natural killer (NK) and hepatitis B virus (HBV)-specific T cells in mediating viral suppression and liver damage after NA cessation.

Methods: The phenotype and function of circulating NK cells, as well as HBV-specific T cell responses ex vivo and in vitro, were longitudinally analyzed in 24 HBeAg-negative patients with NA cessation. In addition, 19 inactive carrier (IC) patients and 28 treatment-naive HBeAg-negative hepatitis (ENEG) patients with HBsAg >200 IU/mL were also enrolled as the control group. The presence of HBV-DNA >1×104 copies/mL within one year was defined as VR.

Results: Fourteen patients experienced VR during 1-year follow-up after NA cessation. However, only two patients with inactive carrier had serum HBV-DNA level >1×104 copies/mL within 1-year follow-up. Serum HBsAg levels at EoT were significantly lower in VR and non-VR group than those in ENEG group, but were similar to those in IC group. The expressions of inhibitory receptor— CD96 in total NK cells were obviously higher in VR and ENEG group than those in NVR and IC group. Likewise, the expressions of CD96 at the 1th and 6th month, and NKG2A at the 3th month were obviously higher in VR group than those in non-VR group. The ability of NK cells-producing IFN-γ at EoT was partially restored in VR group when compared with that in ENEG group, which was still obviously weaker than that in non-VR and IC group, independent of stimulators. NK cell TNF-α production at EoT was relatively attenuated in VR group in comparison to that in IC group. NK cell IFN-γ production at the 1th, 3th, 6th and 12th month was still significantly lower in VR group than that in non-VR group (P <0.05). In comparison to that at the end of treatment, the increased frequency of NK cells-expressing perforin and CD107a was

positively correlated with serum ALT levels during VR, respectively. HBV core-specific T cells were evaluated via intracellular cytokine staining to IFN-γ and IL-2 after 10 days in vitro expansion. IFN-γ and IL-2 production by CD4 T cells at EoT was partially restored only in non-VR group compared with that in ENEG group ($P < 0.05$). Noteworthily, IFN-γ production by CD4 T cells at EoT was significantly lower in VR group than that in non-VR group ($P < 0.05$). Compared with that in VR and ENEG group, IFN-γ and IL-2 production by CD4 and CD8 T cells was markedly increased in IC group ($P < 0.05$). HBV core-specific T cell responses ex vivo were determined by the IFN-γ ELISPOT . Spot-forming cells (SFC) were significantly increased in non-VR group when compared with that in ENEG group. IC group had the significantly higher SFC number than ENEG and VR group. In addition, SFC number at the 3th and 6th month differed markedly between VR and non-VR group ($P < 0.05$)

Conclusion: Weak NK cytokine production and core-specific T cell responses are associated with VR after NA cessation, and the augmented NK cell cytotoxicity correlates with liver damage during VR. Our findings are helpful in better understanding the immunological mechanisms under clinical manifestations after NA cessation, and providing the guidance for safe withdrawal.