



ABSTRACTS

Abstract

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Plenary Session

Plenary Session 1

[L-OP-1287]

Multiplexed digital spatial protein profiling reveals distinct phenotypes of portal mononuclear phagocytes in livers with advanced fibrosis

Pil Soo Sung¹, Chang Min Kim², Jaejun Lee¹, Jung Hoon Cha¹, Jin Young Park², Yun Suk Yu², Eun Sun Jung¹, Si Hyun Bae¹

¹Gastroenterology, The Catholic University of Korea Seoul St. Mary's Hospital, Seoul, Republic of Korea, ²Cbsbioscience, Cbsbioscience, Daejeon, Republic of Korea

Corresponding author: Si Hyun Bae, Gastroenterology, The Catholic University of Korea Seoul St. Mary's Hospital, Seoul, Republic of Korea

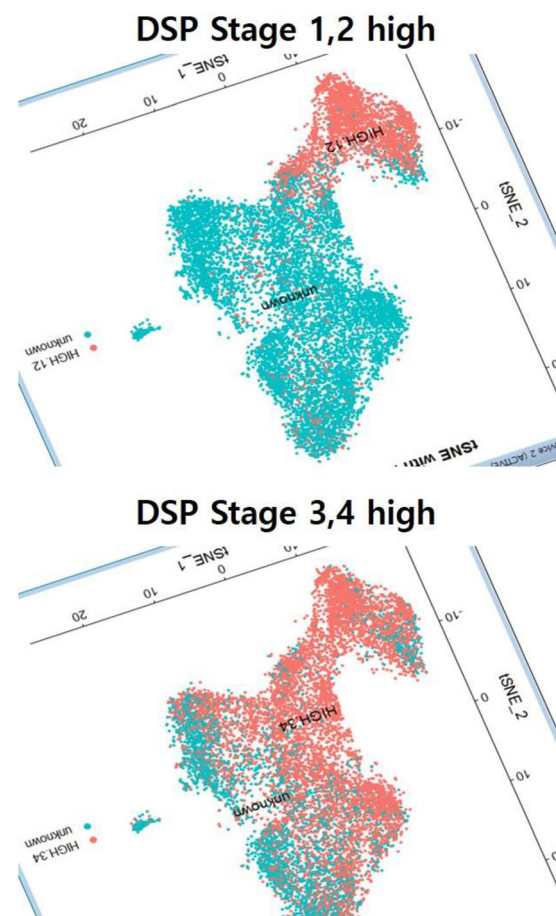
Objectives: In this study, using multiplexed digital spatial profiling, we aimed to identify distinct phenotypes of portal mononuclear phagocytes in livers with advanced fibrosis.

Materials and Methods: Snap-frozen liver tissues with various chronic liver diseases at different fibrosis stages were subjected to spatially-defined protein-based multiplexed profiling (Nanostring GeoMX™). CD3, CD68, and α -SMA markers were used to identify specific cell types. Single-cell RNA-Seq analysis was performed using GEO datasets from normal livers and cirrhotic livers.

Results: Eighty-eight portal ROIs were selected for the spatial profiling, with 41 ROIs classified into “inflammatory” (high T cell number) and 47 ROIs classified into “non-inflammatory” (low T cell number). In “non-inflammatory” ROIs, which were used for the further analyses, there were liver tissues with various fibrosis grade as F0 (n = 7), F1 (n = 12), F2 (n = 3), F3 (n = 12), and F4 (n = 13). In CD68⁺ cells, protein markers for activation of mononuclear phagocytes (CD66b, STING, OX40L, VISTA, CD80) were detected significantly stronger in early stage fibrosis (F1 and F2) than advanced fibrosis (F3 and F4). Conversely, CD68 and HLA-DR, which are known to be upregulated in scar-associated macrophages (SAMacs) rather than Kupffer cells, were detected stronger in advanced fibrosis. Combined analysis of single cell RNA-Seq data from GEO datasets (GSE136103) and spatially-defined protein-based multiplexed profiling revealed that most proteins upregulated in F1 and F2 livers in portal CD68⁺ cells were specifically marked in Kupffer cell clusters, whereas proteins upregulated in F3 and F4 livers were marked in

SAMacs, Kupffer cells, and tissue monocytes, highlighting the different phenotypes of portal CD68⁺ cells according to the fibrosis stages.

Conclusion: This is the first study that used spatially-defined protein-based multiplexed profiling demonstrating the critical difference in the phenotypes of mononuclear phagocytes between livers of early stage fibrosis and those of late stage fibrosis.



Results: Of the outpatients, 17% (n = 153) aged 54 ± 12 had LC and 97% patients had compensated LC without clinical manifestations. Five (5) patients had regular alcohol consumption (RAC). Most cases (93%)—chronic hepatitis C (CHC). All patients with chronic hepatitis B (CHB) had replication. Of the hospitalized patients, 633 (75%) age of 50 ± 14 (29–74) had LC and 90%—subcompensated/decompensated. RAC was seen in 60%. CHC was accounted for 30%, CHB – for 34%, and out of these, 67% had occult hepatitis B (OHB), 22%—mixed, 14%—unknown etiology. All 455-deceased aged 48 ± 12 (27–76) had LC. RAC was seen in 65%. The share of CHB was 23.7%, out of these 71%—OHB, CHC—35.2%, mixed—31.5%, 19.6%—unknown etiology. In the study of liver micropreparations from 97-deceased age 45 ± 12 , only 77% of patients with subcompensated LC and 70%—with decompensated LC showed signs of completed cirrhotic transformation. In all cases of LC, the fact of multimorbidity was noted.

Conclusion: CHC dominates. There is insufficient screening OHB at the outpatient stage. Outpatient with LC are persons of working age, socially adapted and employed, without alcohol dependence, with compensated LC, with minimal clinical manifestations. Among inpatients and deceased socially maladapted individuals, and a high percentage of alcohol addicts are more common. Thirty percent (30%) of patients die from causes not related to decompensation of the underlying disease. This confirms the effect of comorbid pathology on CVH.

[OP-0569]

Frequency of sarcopenia in patients with cirrhosis and factors predicting sarcopenia

Ammara Abdul Majeed¹, Hafeezullah Shaikh¹, Abdur Rasheed¹, Raheela Khalid¹

¹National Institute of Gastrointestinal and Liver Disease, Dow University of Health Sciences, Karachi, Pakistan

Corresponding author: Ammara Abdul Majeed, National Institute of Gastrointestinal and Liver Disease, Dow University of Health Sciences, Karachi, Pakistan

Objectives: To determine frequency of sarcopenia in patients with cirrhosis and factors predicting sarcopenia.

Materials and Methods: A total of 386 patients with cirrhosis were enrolled. Sarcopenia was determined based on the Asian Working Group for Sarcopenia-2019. Patients' clinical and laboratory characteristics were recorded. Chi square test was applied to find out the association between sarcopenia and other demographic variables. Logistic regression analysis was performed to identify independent predictors for sarcopenia. P value of ≤ 0.05 was considered as significant.

Results: Out of 386 patients, sarcopenia was found in 314 patients (81.34%). Sarcopenia was more prevalent in females (86.8% vs 78.1%, $p = 0.036$). Most common etiology was chronic viral hepatitis (85.5%). 33% had hepatoma, while 32% were on statins. Mean MELD-Na score was 14.49 ± 5.543 . Mean age and BMI of study participants were 52.13 with SD of 8.46. and 23.8 with SD of 4.11 respectively. On multivariate analysis, female gender (odds ratio (OR): 1.813, 95%CI 1.033 – 3.182, $P = 0.038$), Age > 49.5 years (OR: 2.925, 95%CI: 1.727 – 4.955, $P < 0.001$), and albumin > 2.45 g/dl (OR: 0.28, 95%CI: 0.129 – 0.606) were associated with sarcopenia.

Conclusion: Sarcopenia is very common in patients with cirrhosis. Special measures should be taken for patients with risk factors of sarcopenia to improve their outcomes.

[OP-0576]

Bone mineral density loss in liver cirrhosis patients

I Dewa Nyoman Wibawa¹, Cokorde Istri Yuliandari Kk^{1,2}

¹Gastroentero-hepatology Div, Dept of Internal Medicine, Gastroenterology-hepatology Div, Dept of Internal Med, Udayana Univ, School of Medicine, Denpasar, Bali, Indonesia, ²Internal Medicine, Udayana Univ.hospital, Div.of Gastroentro-Hepatology, Denpasar, Bali, Indonesia, Indonesia

Corresponding author: I Dewa Nyoman Wibawa, Gastroentero-hepatology Div, Dept of Internal Medicine, Gastroenterology-hepatology Div, Dept of Internal Med, Udayana Univ, School of Medicine, Denpasar, Bali, Indonesia

Objectives: One of the liver cirrhosis complications is related to bone metabolism is known as hepatic osteodystrophy (HO). Pathologic fracture caused by HO can significantly affect the quality of life and life expectancy of patients. The mechanism is multifactorial. Our study was carried out to assess the prevalence of HO and to identify the factors that related to the loss of bone mineral density in liver cirrhosis patients.

Materials and Methods: Consecutive eligible patients with liver cirrhosis were recruited. Bone mineral densitometry (BMD) was evaluated by dual-energy X-ray absorptiometry (DEXA). BMD at both femoral neck and lumbar spine were collected, and the lowest T score was used. Hepatic osteodystrophy was defined as a T score of ≤ -1 . Continuous variables were presented by mean or median as appropriate, and categorical variables by percentage. The univariate and multivariate analyses were performed to identify factors related to the development of hepatic osteodystrophy.

Results: Among 80 participants, 75% (n = 60) were men, 25% (n = 20) were women, and the median duration of liver disease was 3 years. The mean age and body mass index (BMI) was 48.89 ± 7.32 years old; 24.27 ± 0.06 respectively. Viral hepatitis was the most common etiology of cirrhosis (97.5%). There were 52.5% (n = 42) participants with Child-Turcotte-Pugh (CTP) score grade A, 30% (n = 24) with CTP score grade B, and 17.5% (n = 14) with CTP score grade C. The mean of lowest T score was -1.398 ± 1.252 . HO were present in 61.3% (n = 49) participants (41.3% osteopenia and 20% osteoporosis). On both univariate and multivariate analysis, BMI and duration of liver disease showed a significant association with HO ($p = 0.003$; $p = 0.001$).

Conclusion: Our study found a high prevalence (61.3%) of hepatic osteodystrophy in cirrhosis patients. Low BMI and a longer duration of liver disease were related to the development of HO in cirrhosis patients.

[PP-0581]

Combination treatment with curcumin and silibinin prevents oxidative stress and attenuates experimentally induced liver injury and fibrosis in rats

Joseph George¹, Mikihiro Tsutsumi¹, Mutsumi Tsuchishima¹

¹Hepatology, Kanazawa Medical University, Uchinada, Ishikawa, Japan

Corresponding author: Joseph George, Hepatology, Kanazawa Medical University, Uchinada, Ishikawa, Japan

Objectives: Hepatic fibrosis is characterized by excessive synthesis and deposition of connective tissue components, especially collagens in the extracellular matrix of the liver. Curcumin is a food additive with established antioxidant and anti-inflammatory properties. Silibinin is the active constituent of silymarin and a potent antioxidant

with proven pharmacological effects against alcoholic liver disease and non-alcoholic steatohepatitis. We studied the protective effects of curcumin and silibinin to prevent oxidative stress and attenuate experimentally induced hepatic fibrosis in rats.?

Materials and Methods: Liver injury and hepatic fibrosis was induced with intraperitoneal injections of N-nitrosodimethylamine (NDMA) in a dose of 10 mg/kg body weight daily for 10 consecutive days. Groups of animals received curcumin 200 mg/kg body weight and/or silibinin 20 mg/kg body weight everyday orally 2 h prior to the administration of NDMA and also until the end of the study. All the animals were sacrificed on day 21 from the beginning of exposure. Levels of AST, ALT, malondialdehyde, glutathione, ascorbic acid, and hyaluronan were measured either in serum, liver, or both. Immunohistochemistry was carried out for alpha-SMA, collagen type I and type III, and 4-hydroxy-2-nonenal (4-HNE). RT-PCR was performed for alpha-SMA, type I and type III collagens.

Results: Serial administrations of NDMA produced well developed fibrosis and early cirrhosis in rat liver. Combination treatment with curcumin and silibinin significantly reduced serum/hepatic levels of AST, ALT, malondialdehyde, glutathione, ascorbic acid and hyaluronan and completely prevented deposition of collagen fibers in the liver. Immunohistochemical staining and RT-PCR depicted marked decrease in the expression of alpha-SMA, 4-HNE, and collagens type I, and type III.

Conclusion: Combination treatment with curcumin and silibinin efficiently decreased oxidative stress, markedly reduced expression of collagens, and prevented hepatic fibrosis. The data demonstrated that curcumin and silibinin could be used as potent therapeutic agents to prevent liver injury and hepatic fibrosis.

[PP-0623]

Salvianolic acid A is the active compound of *Salvia miltiorrhiza* against the activation of NLRP3 inflammasomes on macrophages

Yuan Peng¹, Jingshu Qi¹, Dabing Ping¹, Zhao Yang¹, Yanyan Tao¹, Chenghai Liu¹

¹Institute of Liver Diseases, Shuguang Hospital affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China

Corresponding author: Chenghai Liu, Institute of Liver Diseases, Shuguang Hospital affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China

Objectives: *Salvia miltiorrhiza* (SM) is a Chinese herbal medicine that is widely used to treat liver diseases in clinic. We previously found SM was effective in treating liver injury via inhibiting expression of NLRP3 in vivo, but the active compound of SM and the underlying mechanism remained unclearly. Here, we aimed to screen its active substances against NLRP3 inflammasomes and explore the mechanism involved.

Materials and Methods: A cell culture model was investigated to detect NLRP3-dependent IL-1 β release using bone marrow-derived macrophages (BMDMs). 16 ingredients of SM, including danshensu, magnesium lithospermate B, tanshinone I, tanshinone IIA, miltirone, manool, salvianolic acid A, salvianolic acid B, salvianolic acid C, dhydrotanshinone I, dihydroisotanshinone I, caffeic acid, neocryptotanshinone, cryptotanshinone, lithospermic acid, were screened out to confirm the maximum non-toxic concentration for BMDMs by LDH and CCK8 kits, respectively. For NLRP3 inflammasome activation, BMDMs were primed with lipopolysaccharide (LPS), and subsequently were stimulated with nigericin or adenosine triphosphate (ATP). The most active substances against NLRP3 inflammasomes in SM was screened out and the underlying mechanism were explored.

Results: Maximum non-toxic concentration and IC₅₀ of 16 ingredients for BMDMs were assayed. Among the ingredients,

Salvianolic acid A was the most anti-inflammatory substances in SM that played a dose dependent (25, 50 and 100 μ M) in alleviating the release of IL-1 β on activated NLRP3 inflammasomes. Levels of mitochondrial ROS production and expression of LC3 and LC3-II in LPS (50 ng/ml)/ATP (2.5 mM)- and LPS (50 ng/ml)/nigericin (4 μ M)-stimulated BMDMs were significantly reduced respectively, which revealed that Salvianolic acid A might confer mitochondrial protection via promoting autophagy pathway.

Conclusion: Salvianolic acid A might be the efficacious compound in SM against activation of NLRP3 inflammasome on macrophages in vitro. The mechanism was associated with alleviating mitochondrial oxidative stress and promoting autophagy pathway in vitro.

[OP-0625]

Neutrophillymphocyte ratio and the risk of 30-day mortality in patients with overt hepatic encephalopathy

Ke Shi¹, Qun Zhang¹, Yi Zhang¹, Xianbo Wang¹

¹Center of integrative medicine, Beijing Ditan Hospital, Capital Medical University, Beijing, China

Corresponding author: Xianbo Wang, Center of integrative medicine, Beijing Ditan Hospital, Capital Medical University, Beijing, China

Objectives: Overt hepatic encephalopathy (OHE) is related to a risk of adverse outcomes and short survival. However, the association between different neutrophil to lymphocyte ratio (NLR) levels and the 30-day mortality risk in cirrhotic patients with OHE has not yet been well assessed.

Materials and Methods: We retrospectively included 1301 patients with OHE at Beijing Ditan Hospital between August 2008 and December 2018. By adjusting for important risk variables, we investigated the association between NLR and the 30-day mortality risk using Cox regression analysis and restricted cubic splines. The discrimination and clinical usefulness of NLR were assessed by receiver operating characteristic curves and decision curve analysis.

Results: All patients were divided into four groups according to quartiles of the baseline NLR (< 2.5, 2.5 – 4.3, 4.3 – 7.5, > 7.5). The 30-day mortality were 7.8%, 12.7%, 19.5%, and 34.1%, respectively ($p < 0.001$). Compared with the lowest quartile, increased NLR was correlated with increasing 30-day mortality after multivariable adjustment (NLR 2.5–4.3: adjusted hazard ratio [AHR], 1.17 (95% confidence interval [CI], 0.70–1.95); NLR 4.3 – 7.5: AHR, 1.58 (95% CI, 1.01 – 2.47); NLR > 7.5: AHR, 2.32 (95% CI, 1.50 – 3.57). A non-linear association between NLR and the adjusted probability of 30-day mortality was observed. There was a significant increasing 30-day mortality in the range of $4.3 < \text{NLR} < 12$. The performance of NLR + MELD (0.839) was higher than that of NLR + MELD-Na (0.829), MELD (0.814), and MELD-Na (0.812) in patients with OHE. The decision curve showed that NLR + MELD had superior standardized net benefit than MELD and NLR. Notably, elevated NLR was linked to increased incidence of 30-day mortality in the subgroup analysis of patients with OHE (HR > 1.0).

Conclusion: Elevated NLR is independently associated with 30-day mortality in cirrhotic patients with OHE.