GS002

Anti-fibrotic effect of rifaximin in early alcohol-related liver disease: a double-blind, randomised, placebo-controlled trial

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Background and aims: Alcohol is the leading cause of liver cirrhosis and liver-related mortality. Alcohol abstinence remains the only therapeutic option. The gut-liver axis with gut dysbiosis and "leaky gut" is considered a key driver of inflammation and fibrogenesis in alcohol-related liver disease (ALD). Rifaximin-α is known to reduce recurrent hepatic encephalopathy and has been linked to improved gut barrier function and reduced systemic inflammation in cirrhosis. We hypothesized that long term rifaximin-αwould reduce inflammation and fibrosis in patients with ALD.
Method: We conducted an 18-months randomised double-blind trial in patients with biopsy-verified ALD and no previous decompensation. We stratified patients according to fibrosis stage and alcohol abstinence within the previous six months. Patients were randomly allocated 1:1 to 550 mg rifaximin-α twice daily or placebo. The primary end point was an improvement of at least one fibrosis stage. Secondary outcomes included progression in fibrosis stage, lobular inflammation, ballooning, steatosis, and transient elastography (TE). We assessed liver biopsies and performed the statistical analyses blinded.
Results: We randomised 136 patients, 108 (79%) completed the trial with paired liver biopsies of adequate quality and 14 dropped out in each group. At baseline, the distribution of fibrosis stages (F0/F1/F2/F3/F4) were 5/31/49/17/6, 84% were male, median TE 8.7 kPa (IQR = 6.5–11.8) and mean age was 59 ± 6 years. The proportion of patients who improved according to fibrosis stage was 26% in the rifaximin-α group and 28% in the placebo group (OR = 1.10, p = 0.821). The proportion who remained stable or progressed in fibrosis stage was 48% and 26% in the rifaximin-α group, versus 30% and 43% in the placebo group and consequently, rifaximin-α reduced the risk of progression compared to placebo (OR = 0.37, p = 0.035) (Figure). Rifaximin-α reduced lobular inflammation compared to placebo (OR = 0.48, p = 0.067), but did not affect hepatic steatosis or ballooning. TE decreased from a median of 8.8 kPa to 8.3 kPa in the rifaximin-α group and 8.7 kPa to 8.3 kPa in the placebo group (p = 0.757). In total, 13 SAEs were reported, and the most frequent reported adverse event (n = 20) was diarrhoea with no difference between the groups.

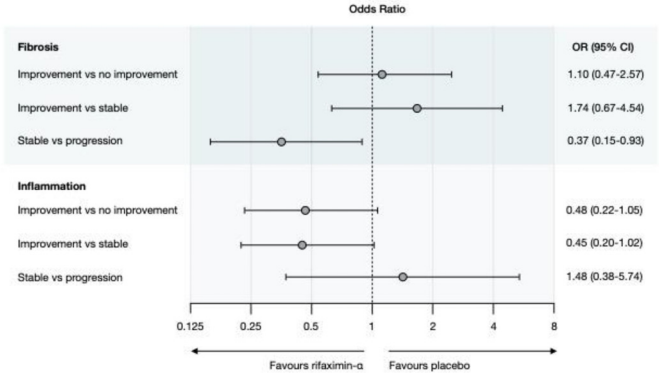

Figure: Rifaximin-α protects against fibrosis progression and reduces lobular inflammation compared to placebo.

Conclusion: Our results show that 18 months treatment with rifaximin-α does not improve fibrosis in early ALD. However, rifaximin-α seems to prevent histological progression of liver fibrosis and tends to decrease hepatic inflammation.

GS003

The spatial distribution and detailed composition of infiltrating immune cells define autoimmune- and checkpoint-therapy associated hepatitis

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Background and aims: Immune-checkpoint blockade (ICB) therapy has revolutionized clinical oncology, but frequently results in immune-related adverse events (irAE), such as ICB-Hepatitis. To date, there is limited information regarding key involved immune subsets and their spatial interaction in ICB-Hepatitis-moreover, differences to spontaneous autoimmune hepatitis (AIH) remain unclear. We therefore set out to perform a detailed analysis of the immune cell composition and their spatial interaction in the liver in in both disease entities.
Method: We performed high dimensional, spatially resolved analysis of immune cell populations in liver biopsies of patients with ICB-Hepatitis (n = 15), AIH (n = 22) or control tissue (n = 10), using a 40-marker Imaging Mass Cytometry (IMC) panel with single-cell resolution (1 μm^2). Single cells were segmented and normalized utilizing a machine learning pipeline and subsequently classified high dimensionally. In addition, PBMCs from ICB-Hepatitis- and AIH-patients were analyzed by mass cytometry.
Results: Analysis of the composition of the immune infiltrate revealed major differences between ICB-Hepatitis and AIH. An unbiased clustering identified disease-specific, phenotypically well defined, immune cell cluster, which show distinct spatial interaction patterns. Specifically, $CD8^+$ T cell clustering resulted in enrichment of several Ki-67^+ Granzyme B^+ T-bet^+ Cluster in ICB-Hepatitis. In contrast, AIH was defined by a higher expression of exhaustion markers (PD-1^+ TOX^+ $Eomes^+$ cluster) as well as a tissue resident memory (Trm) phenotype. Remarkably, one Granzyme B^+ $CD8^+$ T cell cluster and the Trm cluster correlated with ALT levels in ICB-Hepatitis and AIH, respectively.
Clustering of tissue phenotypes according to spatial liver zonation revealed a biased accumulation of $CD8^+$ T cells near the central vein in ICB-Hepatitis, while in AIH a closer interaction with the periportal triad was observed. Neighborhood analysis identified closely engaged immune cell clusters in both diseases. In ICB-Hepatitis, we observed significant interactions between myeloid clusters and $CD8^+$ T cells, especially between a Ki-67^+ CD8+ T cell cluster and metabolically distinct macrophage populations. In AIH, a stronger B-T cell interaction was noted.
Conclusion: The composition of the immune infiltrate, as well as a distinct spatial distribution and interaction between immune

subsets, points towards major differences in the immune mechanisms and pathogenesis between ICB-Hepatitis and AIH. Our results indicate T-cell-myeloid interactions as likely drivers of ICB-Hepatitis.

GS004

Prospective randomized controlled trial of biomarkers for early detection of hepatocellular carcinoma

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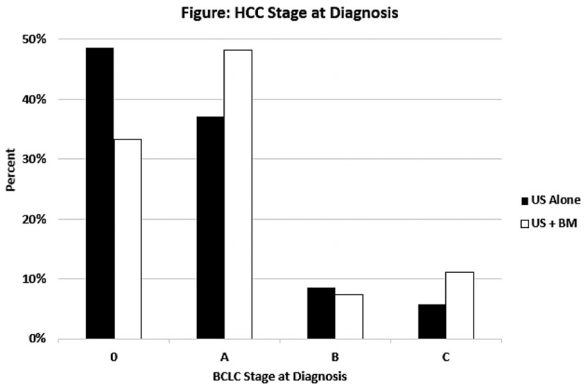
Background and aims: Surveillance for hepatocellular carcinoma (HCC) is key to early diagnosis and access to potentially curative therapy. Small studies have suggested that addition of serum biomarkers (BM) to 6-monthly ultrasound (US) surveillance may allow for earlier HCC diagnosis. Prospective validation of the utility of BMs is lacking.

Method: Adults with cirrhosis or high-risk HBV infection (REACH-B score >8) followed at the Toronto Centre for Liver Disease were randomized to HCC surveillance with US alone (Group A) or US + BM (Group B) with measurement of alpha-fetoprotein (AFP), lectin-reactive fraction of AFP (AFP-L3) and des-gamma-carboxy prothrombin (DCP). Elevated BM levels and/or findings on US triggered CT/MRI for confirmation of HCC diagnosis. The primary outcome was the proportion of HCCs diagnosed at a curable stage (BCLC 0/A) within Milan criteria. Cox regression was used to evaluate the association of the GALAD score (BM + age/sex) with HCC.

Results: 1, 208 patients were enrolled with median age 59 (18–88) years; 72% were male. HBV and HCV were the most common underlying liver diseases (64% and 21%, respectively) and 770 (64%) were cirrhotic. In Group A, after a median follow-up of 34 (1.2–70.4) months and 9.2 US per patient, 35 HCCs were diagnosed, 31 (87%) in cirrhotics, of which the BCLC was 0 for 17 (49%), A for 13 (37%), B for 3 (9%) and C for 2 (6%). In Group B, after a median follow-up of 19 (0.1–60.4) months and 7.9 US per patient, 27 HCCs were diagnosed, 24 (89%) in cirrhotics, of which BCLC was 0 for 9 (33%), A for 13 (48%), B for 2 (7%) and C for 3 (11%). In Group A, 30/35 (86%) HCCs were diagnosed at a curable stage (BCLC 0/A), compared to 22/27 (81%) in group B ($p = 0.63$) (Figure). In group A, 32 (91%) HCCs were identified first by the study US, while 3 (9%) were found incidentally by imaging done for other reasons with a negative prior study US giving an overall sensitivity for US of 27/35 (77%). In group B, 21 (78%) HCCs were evident on US and 9 (33%) were associated with elevated BM of which 6 (22%) were found by BM with a negative corresponding US. Of these 6, BCLC was 0 for 2, A for 3 and C for 1, confirming that curable HCCs may be identified by BM when US is negative. The GALAD score was associated with HCC risk (HR 1.86, $CI_{95\%}$ 1.48–2.32, $p < 0.001$). The previously identified threshold of >-0.63 was associated with increased HCC risk (HR 6.05, $CI_{95\%}$ 2.4–15.3, $p < 0.001$) and 3 of the 5 advanced (BCLC B/C) HCCs in the BM group would have had imaging triggered by GALAD >–0.63 at an earlier visit.



Conclusion: The probability of diagnosing HCC in a curable stage was similar with US and US + BM. However, some HCCs were diagnosed by elevated BM with a negative US and the GALAD score may have identified additional HCCs earlier. Further analyses will determine if certain patient populations may benefit from use of these BM in routine HCC surveillance.

GS005

Development and validation of the gender-equity model for liver allocation (GEMA) to prioritize liver transplant candidates

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Background and aims: The model for end stage liver disease (MELD) and its sodium-corrected variant (MELD-Na) have created gender disparities in accessing liver transplantation (LT). We derived and validated a new model that replaced creatinine with the Royal Free glomerular filtration rate (PMID: 27779785) within the MELD and MELD-Na formulas.

Method: The "Gender-Equity Model for liver Allocation" (GEMA) and its sodium-corrected variant (GEMA-Na) were trained and internally validated in adults listed for LT in the United Kingdom (2010–2020) using generalized additive multivariate Cox regression. The models were externally validated in an Australian cohort (1998–2020). The primary outcome was mortality or delisting due to clinical deterioration at 90 days. The Greenwood-Nam-D'Agostino test was used to test calibration.

Results: The study comprised 9, 320 patients: 5, 762 patients for model training, 1, 920 patients for internal validation, and 1, 638 patients for external validation. The prevalence of the primary outcome ranged from 5.3% to 6%. In the internal validation cohort, GEMA and GEMA-Na showed a Harrell's c-statistic = 0.752 and 0.766, respectively, for the primary outcome, which were significantly higher than those of the MELD score (0.712) and the MELD-Na score (0.742). Results were consistent in the external validation cohort. Among women, these differences were more pronounced (see Harrell's c-statistics in the table). GEMA and GEMA-Na were adequately calibrated and prioritized differently 43.9% and 41.8% of