factors predictive of death were a higher serum AST ($p < 0.04$), ALP ($p < 0.02$), sodium ($p < 0.02$), and INR ($p < 0.01$) and the development of HE ($p < 0.000$). Of the 51 patients known to be alive, 27 were available for review. Of these, 21 (78%) had some degree of HE; less than 30% were on anti-encephalopathy treatment. The factors predictive of HE were older age ($p < 0.01$), with the risk of developing HE increasing by 6.5% for every year of age, and non-British white ethnicity ($p < 0.03$).
Conclusion: HE is one of the most important predictors of mortality post-TIPS. Patients are often unaware of the risk of HE development and are rarely monitored beyond the immediate post-TIPS period. Better assessment of the risk of developing HE pre-TIPS as well as closer and longer term follow-up and treatment may help preventing the development of HE and hence improve survival.

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ACTIVATION OF THE AIM2 INFLAMMASOME IS ASSOCIATED WITH THE SEVERITY OF LIVER DISEASE AND THE INFLAMMATORY RESPONSE IN CIRRHOTIC PATIENTS WITH STERILE ASCITES

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Background: The inflammasome is a cytosolic multiprotein complex that triggers the activation of Caspase-1 and the maturation of IL-1b and IL-18. Different PRRs (e.g. NLRPs) are able to form inflammasomes upon recognition of different PAMPs and/or DAMPs. *Absent in melanoma 2* (AIM2) triggers inflammasome formation in response to cytosolic dsDNA irrespectively of its origin. Here, we investigate the role of different inflammasomes in the inflammatory response and the severity of liver disease in cirrhotic patients with sterile ascites.
Methods: Ten healthy controls and 60 patients with cirrhosis and non-neutrocytic ascites were included in the study. Ascitic fluid (AF)- and PBMC-derived macrophages were isolated and transfected with polydA:dT to activate the AIM2 inflammasome. Inflammasome activation was evaluated by immunoblot (caspase-1-p20) and ELISA (IL-1b/IL-18) in culture supernatants and AF.
Results: AF-macrophages showed an exacerbated inflammasome activation when compared to PBMC-macrophages isolated from the same patients or healthy controls. qPCR and Immunoblot analysis showed a marked increase in the basal expression of AIM2 (but not NLRPs or NLRC4) in both PBMC- and AF-macrophages from the ascitic patients. Moreover, stimulation with bacterial DNA or LPS further enhanced the expression of AIM2 as well as the mRNA levels of Caspase-1 and IL-1b in PBMC-macrophages from both patients and controls. Functional activation of the AIM2 inflammasome in PBMC-macrophages required previous stimulation with TLR ligands, with TLR9 having a much higher effect than the other TLRs tested. Unlike PBMC-macrophages, AF-macrophages did not require TLR pre-stimulation to mount a robust AIM2-inflammasome response, demonstrating the highly pre-activated state of these cells. Accordingly, positive detection of bacterial DNA in the AF of a subgroup of patients (31.6%) was associated with higher inflammasome activation. Noteworthy, Child–Pugh class C patients showed significantly higher inflammasome activation and IL-1b levels in blood and AF than those with a Child–Pugh class B.
Conclusions: The activation of the AIM2-inflammasome in response to dsDNA triggers an exacerbated ***inflammatory response in sterile AF***. Notably, inflammasome activation also correlates with a ***higher degree of liver disease***. These results lay the foundation to investigate novel therapeutic options aimed at blocking the activity of this inflammatory pathway in ascitic patients.

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RELAXIN IS A RENAL VASODILATOR IN EXPERIMENTAL MODELS OF CIRRHOSIS AND A POTENTIAL NOVEL THERAPY FOR HEPATORENAL SYNDROME (HRS) IN HUMANS

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Background: HRS is a feared complication of cirrhosis with a high mortality rate and limited treatment options. The hallmark of HRS is profound renal vasoconstriction, resulting in functional renal failure but normal kidney histology. The peptide hormone relaxin (RLN) mediates maternal haemodynamic adaptations to pregnancy, including increased renal blood flow (RBF) and glomerular filtration rate (GFR). We hypothesised that RLN could modulate RBF in cirrhosis.
Methods: Cirrhosis was induced in rats by 16 weeks i.p. carbon tetrachloride (CCl_4) and decompensated biliary cirrhosis by 3 weeks bile duct ligation (BDL). We measured the effect of acute i.v. and extended (72 hr) s.c. RLN on systemic haemodynamics, RBF, GFR and organ histology. Subgroups of rats were co-treated with the nitric oxide (NO) synthase inhibitor L-NAME. Blood oxygen dependent-magnetic resonance imaging (BOLD-MRI) was used to quantify changes in renal oxygenation. Tissue expression and distribution of RLN receptor (RXFP1) was determined by qPCR and immunohistochemistry. Expression of vasoconstrictor genes was quantified by qPCR array.
Results: RXFP1 was detected in glomerular podocytes, renal pericytes, renal, segmental and interlobar arteries of cirrhotic rats. In CCl_4 cirrhosis, acute i.v. RLN (4 μg) induced a 50% increase in RBF after 60 minutes ($p < 0.01$ vs. placebo, $n = 6$). BOLD-MRI showed increased tissue oxygenation at the same timepoint in renal cortex and medulla. Extended s.c. RLN increased RBF by 54% in CCl_4 ($p < 0.01$ vs. placebo, $n = 8$) and 87% in BDL ($p < 0.05$ vs. placebo, $n = 3$) and increased GFR by 138% in CCl_4 ($p < 0.01$ vs. placebo, $n = 8$) and 70% in BDL ($p < 0.05$ vs. placebo, $n = 3$). Mean arterial pressure was unaffected by RLN. L-NAME (250 mg/L) p.o. abrogated the effect of RLN on RBF and GFR. Relative expression of vasoconstrictor genes in kidney was markedly reduced by RLN treatment.
Conclusion: RLN increased RBF in experimental cirrhosis. Critically, RLN also improved renal function and oxygenation but did not induce systemic hypotension even in decompensated disease. The effects of RLN were mediated via augmentation of NO and downregulation of vasoconstrictor genes known to be important in the pathogenesis of HRS. RLN has potential as a treatment for HRS and further translational studies are warranted.

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MARKERS OF ENDOTHELIAL DYSFUNCTION AS PREDICTORS OF ASCITIC DECOMPENSATION IN PATIENTS WITH LIVER CIRRHOSIS

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Background and Aims: Natutal history of liver cirrhosis (LC) is accompanied by an intra- and extrahepatic endothelial dysfunction (ED), which is considered to have a pivotal role in the development of portal hypertension (PH). Serum levels of markers of ED (MED) are increased in LC patients, and they correlate with the stage of liver disease. Aims of the present study were to assess, (1) differences between MED serum levels in patients with compensated and decompensated LC; (2) possible prognostic

role of MED in the development of ascites in patients with compensated LC.
Methods: 90 consecutive LC patients (mean age 65±9 years, 24 female) underwent a complete clinical, radiological and biochemical evaluation in order to assess clinical characteristics and the stage of disease; all subjects were assessed for MED [P-selectin, von Willebrand factor (vWF), endothelin-1 (ET-1), thrombomodulin (TM) and nitric oxide (NO)] serum levels. The 70 patients (mean age 65±9 years, 19 female) with compensated LC (no ascites, cLC) underwent a 2 years-follow-up for ascites development; their data were also compared with those of 20 (mean age 63±10 years, 5 female) LC patients with decompensated LC (presence of ascites, dLC) and those of 11 healthy controls (mean age 26±6.6 female).
Results: ET-1, P-selectin and TM serum levels were significant higher in LC and in dLC patients with respect to controls. NO e vWF serum levels were higher in dLC patients, whereas no difference was observed in cLC with respect to controls. 33/70 (47.1%) of cLC patients developed ascites during follow-up. At univariate analysis, predictors of ascites development in cLC patients were serum concentrations of ET-1 (OR = 3.56, p = 0.000), TM (OR = 1.95, p = 0.000) and P-selectin (OR = 1.033, p = 0.004) and Child–Pugh score (OR = 1.05, p = 0.041). At multivariate analysis (Cox regression), serum ET-1 and diabetes were independent predictors of early development of ascites during the two-years follow-up period (HR = 2.631, p = 0.004) in cLC patients. Efficiency (measured by ROC method) of high levels (cut-off value=6 pg/ml) of ET-1 in predicting the ascites development was good (AUC=0.803).
Conclusions: Among serum MED, vWF, ET-1, NO, P-selectin and TM resulted significantly higher in dLC patients as compared to cLC patients. Plasma ET-1 resulted as an independent predictor of ascites development in cLC patients. Considering also the possible pathophysiological role of ET-1 in PH development, this result may have important implications in early detection and prevention of ascites in cLC patients.

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CULTURE-INDEPENDENT CHARACTERISATION OF VIABLE BACTERIA IN ASCITES REVEALS A BROAD RANGE OF SPECIES INCLUDING THOSE OF NON GUT ORIGIN

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Introduction: Identification of pathogenic bacteria in ascites correlates with poor clinical outcomes. Ascites samples are commonly reported culture-negative by traditional microbiology, even where frank infection is evident. Culture-independent methods have previously reported bacterial DNA in ascites, however, whether this represents viable bacterial populations has not been determined. We report the first application of 16S rRNA gene pyrosequencing in conjunction with propidium monoazide sample treatment to characterise the viable bacterial composition of ascites.
Methods: Twenty five cirrhotic patients undergoing paracentesis provided ascites. Samples were treated with propidium monoazide to exclude non-viable bacterial DNA. Total bacterial load was quantified by 16S rRNA Q-PCR with species identity and relative abundance determined by 16S rRNA gene pyrosequencing. Clinical measures and diagnostic microbiology were recorded as routine. The composition of the microbiota revealed was correlated with clinical parameters.
Results: Viable bacterial signal was detected in 84% of ascites samples, both by Q-PCR and pyrosequencing (mean bacterial densities of 1.5 x 10^5 cfu/ml equiv., std dev 1.8 x 10^5, n = 21). Bacteria were also detected in patients with normal ascetic WCC and no clinical evidence of SBP. Approximately 190,000 ribosomal pyrosequences were obtained, representing 236 species across diverse array of primarily opportunistic pathogens including both gut and non gut-associated species. There was high species variation in the ascites microbiota between patients with high relative abundance species commonly unique to one patient. Statistically significant relationships were identified between the composition of the bacterial communities detected and clinical measures, including ascitic white cell count (clinical evidence of SBP) and Child–Pugh score.
Conclusions: Viable bacteria are present in the ascites of a majority of patients with cirrhosis including those with no clinical signs of infection. Entry of bacteria into ascites is not limited to translocation from the gut, raising fundamental questions about the processes that underlie the development of spontaneous bacterial peritonitis. The ascitic microbiota composition correlates with clinical status.

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COMPARTMENTAL REGULATION OF suPAR IN PATIENTS WITH DECOMPENSATED CIRRHOSIS: EVALUATION OF ORIGIN, REGULATION AND PROGNOSTIC RELEVANCE

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Background: Patients with decompensated cirrhosis are susceptible to bacterial infections that are associated with organ failure and a high mortality. Reliable biomarkers that predict unfavorable outcomes are essential to identify high-risk patients who require intensified treatment. In non-cirrhotic patients with infections and sepsis, elevated serum levels of soluble urokinase plasminogen activator receptor (suPAR) predict mortality but little is known about the regulation and short-term prognostic relevance of suPAR in patients with advanced cirrhosis.
Patients and Methods: To study suPAR in serum and ascitic fluid (AF) samples from 162 consecutive patients with decompensated cirrhosis undergoing paracentesis for suspected bacterial infection were enrolled in this study. suPAR levels were assessed by ELISA and prognostic value was calculated by cox-regression and Kaplan–Meier curve analysis. Ex vivo, immune cell subsets were stimulated with varying concentrations of TNF-alpha and various Toll-like-Receptor Agonists and suAPR release was measured. Surface-bound uPAR was determined by flow cytometry. Monocytic uPAR expression was quantified by RT-PCR.
Results: Circulating suPAR levels were increased in decompensated compared with compensated cirrhosis, correlated with the severity of liver dysfunction and surrogate markers of systemic inflammation and liver-related mortality but were not indicative of bacterial infection. Circulating suPAR equaled MELD in 28-days mortality prediction (AUC 0.711 for suPAR and 0.705 for MELD) and circulating suPAR levels above 14.4 ng/ml predicted 28-day mortality even after adjustment for MELD score and confounders (hazard ratio 3.0), whereas cut-off levels derived from non-liver cohorts were not applicable due to low specificity. AF suPAR levels were elevated during SBP but not during bacterascites or bacterial translocation. They correlated poorly with systemic suPAR but were associated with a more severe course of SBP and worse outcome. *In vitro* experiments revealed that monocytes and to a lesser extent