Thieme

38. Jahrestagung der Deutschen Arbeitsgemeinschaft zum Studium der Leber

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Prof. Dr. Steven Dooley

Lecture Session I Basic Hepatology (Fibrogenesis, NPC, Transport) 28/01/2022, 12.40 pm – 13.25 pm, Lecture Hall

L 1.01 mRNA therapeutics for liver diseases: HNF4A mRNA delivery via lipid nanoparticles attenuates liver fibrosis in preclinical models.

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Messenger RNA (mRNA)-based drug technologies are expeditiously reaching the clinic and bear the capacity to benefit millions of individuals globally. However, therapeutic targeting of injuries that demand transient restoration of proteins by mRNA delivery requires more in-depth analyses. In this study, we investigated therapeutic applicability of mRNA delivery in liver fibrosis and cirrhosis, which causes millions of fatalities, annually. Here, we envisioned to demonstrate the therapeutic utility of the human transcription factor, hepatocyte nuclear factor alpha (HNF4A) encoding mRNA, in chronically injured mouse liver leading to fibrosis and cirrhosis. We demonstrated restoration of hepatocyte functions by HNF4A mRNA transfection in vitro, and examined the inhibition of liver fibrosis and cirrhosis in numerous murine models, by administering hepatocyte-targeted lipid nanoparticles (LNP) encapsulating HNF4A mRNA. The potential mechanisms of HNF4A mRNA in attenuation of liver fibrosis were discovered by performing microarray-based gene expression profiling, single cell RNA sequencing, and chromatin immunoprecipitation. Primary liver cells and human liver buds were used for functional validation.
Expression of HNF4A mRNA restored metabolic functions of fibrotic primary human hepatocytes in vitro. Repeated in vivo administration of HNF4A mRNA encapsulated-LNP triggered a strong suppression of fibrogenesis in four independent murine models of hepatotoxin- and cholestasis-induced liver fibrosis. We identified that HNF4A targets paraoxonase 1, which mediates HFF4A-directed inhibition of liver fibrosis by regulating liver macrophages and hepatic stellate cells.
Taken together, our results provide the first direct pre-clinical evidence that HNF4A mRNA therapeutics would be a promising strategy for the treatment of liver fibrosis.

L 1.02 Generation of human liver organoids – An in vitro model of liver diseases

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DOI 10.1055/s-0041-1740649

Introduction Research on liver diseases is hampered by the lack of hepatic models that can faithfully recapitulate complex disease phenotypes. Emerging effort is created to generate 3-dimensional tissue culture models, so called organoids, that sufficiently reflect the in vivo situation. The goal of the present study was therefore to generate organoids from cells isolated from resected human liver tissue (personalized model) as well as from upcyte® hepatocytes and hepatoma celllines to study the development of liver diseases and the effects of drugs.

Methods Human liver tissue was dissolved using different mixtures of enzymes. Also human upcyte® HC's as well as hepatoma celllines were embedded in Matrigel and cultured using diverse recipes of culture medium. We analyzed the cell type populations in organoids from liver tissue using real time PCR and characterized also the upcyte®HC´s and hepatoma cellline - organoids regarding functionality markers and we performed H&E – and immunofluorescent stainings.

Results Our data show that the generation of liver organoids derived from primary human tissue is possible but the general success depends on many different factors like enzymes used for tissue dissection, media composition and underlying disease (e. g. normal tissue surrounding HCC or a CRC liver metastasis). Use of upcyte® cells or hepatoma celllines for organoid generation can be a good alternative in order to overcome some of these obstacles and obtain a standardized setup that can be used for diverse research questions like e. g. liver toxicity tests or basic research questions.

L 1.03 Expression of Amyloid Beta42 and its metabolizing proteins is altered in steatosis and non-alcoholic steatohepatitis (NASH)

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DOI 10.1055/s-0041-1740650

Amyloid-Beta (Aß), a major player in the pathogenesis of Alzheimer disease (AD), is also generated and cleared in the liver. Hepatic Aß improves sinusoidal permeability and protects against fibrogenesis. Furthermore, cirrhosis impairs Aß clearance in humans and NASH diet worsened AD in mouse models. Therefore we aimed to analyze expression of Aß and its metabolizing proteins in liver tissue from patients with steatosis or NASH. Aß-42 liver tissue levels were determined by multiplex technology and showed a significant decrease in histopathological proven NASH (n = 12) compared to steatotic (n = 21) or normal livers (n = 23). Lower Aß-42 levels correlated with steatosis, but not with fibrosis grade. Fluidigm Taqman-PCR analysis of liver samples in patients with steatosis / NASH (n = 64) revealed lower mRNA levels of genes generating or degrading Aß regarding a more severe fibrosis score. Additionally in absence of fibrosis (n = 53) we found that steatosis induces Aß precursor protein (APP), Aß generating and degrading genes. The latter was confirmed in vivo by qRT-PCR analysis in liver tissues from mice fed a standard or a high fat diet (n = 5 each) for 14 weeks. Furthermore, in vitro using Huh-7 cells, we investigated the impact of saturated (palmitic) and/or mono-unsaturated (oleic) fatty acids (FA) on Aß metabolizing genes. We found in FA treated cells enhanced Aß generating genes and this differential gene expression was dependent on FA concentration as well as was attenuated by addition of oleic acid. Our results demonstrate that NASH, but not (early) steatosis alters Aß levels causing fibrogenesis and cirrhosis.

Lecture Session II Clinical Hepatology, Surgery, LTX
28/01/2022, 14.40 pm – 15.25 pm, Lecture Hall

L 2.01 Macrophage-specific PLA2g6 deficiency exacerbates liver injury during bacterial sepsis via myelopoiesis activation in male mice

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Background PLA2g6 mutations lead to neurodegeneration and Parkinson's disease and polymorphisms of PLA2g6 are associated with increase of serum C-reactive protein. Hepatic inflammation was exaggerated in PLA2g6-null mice during autoimmune hepatitis and NASH. The role of PLA2g6 in macrophages (MΦ) is still elusive. Here, we studied the response of MΦ-specific PLA2g6 deficient (KO) mice to E.Coli lipopolysaccharide (LPS) treatment.

Methods Male PLA2g6 flox and KO mice were subjected to treatment with a single (1 mg/kg, 24 h) or multiple (1 mg/kg daily, 4 times) doses of LPS as a model of acute and persistent inflammation, respectively. Complete blood counts, plasma cytokines, and liver histology were analysed.

Results Without LPS treatment, MΦ-specific PLA2g6 deficiency led to an increase in spleen weights, plasma IL-6, blood mean platelet volume, hepatic necrosis, and ~25 % of KOs showed hepatic lymphoplasmacellular infiltration. Acute LPS caused the elevation of plasma IL-6, IL-10, KC, GM-CSF, MCP-1, and MIP1-α, the deficiency further elevated MIP1-α levels. After persistent LPS, KOs at 6 months old showed an increase in blood granulocytes and eosinophils without severe hepatic injury. KOs at 12 months old showed an increase in plasma AST, hepatic necrosis, and interstitial infiltration of immune cells.

Conclusions MΦ-specific PLA2g6 deficiency caused basal inflammatory abnormalities seen in blood, spleen, and liver. The deficiency further increased plasma MIP1-α resulting in an increase in myelopoiesis after acute and persistent LPS, respectively. The observed bacterial innate immune response demonstrated the effects of MΦ-PLA2g6 deficiency on hepatic inflammation which can lead to liver failure during sepsis.

L 2.02 Ultraschall in der Erkennung und Beurteilung von Gallenblasenpolypen bei PSC: Cut-off von 8,5mm Größe eignet sich zur Prädiktion eines malignen Polypen

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Bei PSC-Patienten ist das Risiko für Gallenblasenkarzinome erhöht. Zur Früherkennung werden bildgebende Surveillance-Untersuchungen alle 6 bis 12 Monate eingesetzt. Ziel der Studie war es, die Zuverlässigkeit von Ultraschall und MRT in der Erkennung von Gallenblasenpolypen bei Patienten mit PSC zu prüfen.

Aus einer Kohorte von 596 PSC-Patienten des YAEL-Centrums des UKE wurden 77 Patienten aus primärer Indikation cholezystektomiert. 37 Patienten (21m, 16w) mit der Indikation „Polypen in der Bildgebung" wurden in die Studie eingeschlossen. Zur Evaluation der Bildgebung wurden auch die übrigen 40 Patienten mit primärer Indikation hinzugezogen. Die Datenerhebung erfolgte retrospektiv. Es wurden Ultraschall- und MRT-Befunde erhoben, die Bestimmung der Polypenart erfolgte über den histologischen Befund der Gallenblase.

Polypen mit hochgradiger Dysplasie wurden der Gruppe der Malignome zugeordnet.
Ultraschall (Sens. = 100 %) zeigte sich signifikant sensitiver als MRT in der Erkennung von Gallenblasenpolypen (Sens. = 35 %) ($p < 0{,}001$). 3 der 8 malignen Befunde wurden in der MRT übersehen. Im Ultraschall fanden sich maligne Polypen (n = 8, M = 13mm) signifikant größer als nicht-maligne Polypen (n = 26, M = 6,6mm) ($p < 0{,}001$). Der Ultraschall konnte zuverlässig einen malignen Polypen erkennen (AUC = 0,92, $p < 0{,}001$). Der Cut-off zur Erkennung von malignen Polypen mit 100 %-Sensitivität betrug 8,5mm.
Bei Patienten mit PSC eignet sich Ultraschall aufgrund der signifikant höheren Sensitivität besser zur surveillance auf Gallenblasenpolypen als die MRT. Anhand der Größe der Polypen im Ultraschall kann gut zwischen malignen und benignen Befunden unterschieden werden. Kein maligner Polyp war im Ultraschall kleiner als 8mm. Ab dieser Größe erscheint daher eine Cholezystektomie indiziert. Eine multizentrische Validierung der Daten ist in Planung.

L 2.03 In vitro and in silico characterization of a novel NR1H4/FXR mutation causing Progressive Familial Intrahepatic Cholestasis Type 5

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Background Mutations in NR1H4/FXR underlie Progressive Familial Intrahepatic Cholestasis Type 5 (PFIC5). FXR is a member of the nuclear receptor family that, heterodimerized with RXRα, transactivates the ABCB11/BSEP promoter. PFIC5-associated NR1H4 mutations result in reduced or absent BSEP expression. Consequently, PFIC5 is a rare, low-γGT intrahepatic cholestasis to be considered in absence of disease-causing ATP8B1/FIC1 and ABCB11/BSEP mutations. Here, we describe and characterize a novel NR1H4/FXR mutation causing PFIC5 in a patient.

Methods NR1H4 cDNA obtained from human liver was cloned into a mammalian expression plasmid and the mutation introduced via site-directed mutagenesis. Expression and localization of FXR was analyzed via immunofluorescence (IF) and western blot (WB). HEK293 cells were transfected with an ABCB11-promoter-driven luciferase reporter and expression constructs for RXRα and FXR variants, then stimulated with 9-cis-retinoic acid and obeticholic acid before used in luciferase assays. Impact of the mutation on FXR function was investigated by in silico structural analysis.

Results and Conclusion Since our patient had normal γGT, strongly elevated serum bile acids and absent BSEP expression, yet no ABCB11 or ATP8B1 mutation, NR1H4 was sequenced yielding the homozygous c.887C > T;p.(T296I) mutation. T296I was introduced into both liver-relevant FXR isoforms (α1/α2). By IF, both wildtype and mutant FXR were localized to the nucleus in stimulated HEK293 cells co-transfected with RXRα. WB demonstrated similar expression levels of WT and T296I. However, T296I failed to induce substantial ABCB11 promoter transactivation. Preliminary in silico analysis suggested altered interaction of the mutated side chain with the α-helix-forming part of the activation function.

Lecture Session III Metabolism (incl. NAFLD) 28/01/2022, 17.35 pm – 18.20 pm, Lecture Hall

L 3.01 Hepatocellular ballooning in liver cirrhosis is due to glycogenotic metabolic aberrations with facultative steatosis and ground glass formation

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Background and aim Hepatocellular ballooning frequently occurs in chronic liver diseases, particularly alcoholic and non-alcoholic steatohepatitis, often associated with Mallory-Denk body and ground glass formation. Cytoplasmic enlargement and "rarefication" due to ill-defined degenerative changes have been related to these alterations, but their striking resemblance to glycogenotic/steatotic changes characterizing preneoplastic clear cell populations discovered and detailed in various animal models of hepatocarcinogenesis and chronic human liver diseases prone to develop hepatocellular carcinomas remained unrecognized.

Materials and methods Using specimens from 96 cirrhotic human livers we quantified the appearance of ballooned hepatocytes (BH), and studied their glycogen/lipid stores, Mallory–Denk-bodies, and ground glass features by light and electron microscopy. Enzyme activities and/or expression of proteins involved in glycolysis, lipogenesis and proto-oncogenic signaling cascades were investigated by cytochemical approaches in serial paraffin and cryostat sections.

Results BH were found in 43.8 % of cirrhotic livers with a mean volume fraction of 14 %. Regardless of the underlying etiology, ballooning was mostly associated with excessive storage of glycogen and/or fat, decreased glucose-6-phosphatase activity, and increased activity or protein expression of enzymes involved in glycolysis, lipogenesis and AKT/mTOR pathway.

Conclusion BH often appear in cirrhotic livers, irrespective of the underlying etiology, mostly showing excessive storage of glycogen and/or lipids. Ballooning is due to metabolic aberrations corresponding to those of preneoplastic glycogenotic/steatotic hepatocellular lesions well known from experimental hepatocarcinogenesis and chronic human liver diseases prone to develop hepatocellular carcinomas.

L 3.02 Direct impact of fructose on hepatic lipid and glycogen metabolism

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DOI 10.1055/s-0041-1740655

Background Overconsumption of fructose has been identified as major risk factor for the development of non-alcoholic fatty liver disease (NAFLD). Although the impact of dietary fructose on NAFLD development is multifactorial, a fructose-dependent increase in hepatic de novo fatty acid synthesis with an ensuing intrahepatic triglyceride accumulation is frequently claimed to be a relevant mechanism. However, direct evidence for such a mechanism is largely lacking.

Methods Rat hepatocyte cultures were incubated with varying concentrations of glucose and fructose ± insulin and the incorporation of a [14C]-label from glucose and fructose into glycogen and lipids was quantified.

Results [14C]-label from glucose and fructose was incorporated into glycogen. This incorporation was stimulated five to ten-fold by insulin. At low (5 mM) glucose concentrations and in absence of insulin, fructose (2 mM) enhanced the incorporation of [14C]-glucose into glycogen. This effect was no longer observed at high (20 mM) glucose concentrations or in presence of insulin.

Both [14C]-glucose and [14C]-fructose were incorporated into hepatic lipids. The incorporation was stimulated three to four-fold by insulin. Neither monosaccharide affected the incorporation of the other. Notably, however, both in absence and presence of insulin, the [14C]-hexose incorporation into lipids from a mixture of 2 mM fructose and 5 mM glucose was higher than from 7 mM Glucose. This effect of fructose was no longer observed at higher glucose concentrations.

Conclusion Thus, only at low physiological glucose concentrations but not at high postprandial glucose concentrations, fructose might directly drive lipid incorporation into hepatocytes.

L 3.03 Free fatty acids induce impairment of mitochondrial function and cytokine expression in mucosal-associated invariant T (MAIT) cells in NAFLD

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Non-alcoholic fatty liver disease (NAFLD) is becoming the most common chronic liver disease worldwide. Recent data suggest alterations of the immune landscape in NAFLD, rendering the liver microenvironment permissive for the development of hepatocellular carcinoma. Here, we analysed the phenotype and function of mucosal-associated invariant T (MAIT) cells in patients with NAFLD and aimed to decipher the effect of the metabolism on MAIT cell function in NAFLD. MAIT cells were isolated from peripheral blood of NAFLD patients or controls and stimulated in vitro in complete or fatty acid rich medium. MAIT cell phenotype and function were analysed by multi-colour flow cytometry. MAIT cell metabolism was investigated by metabolic flux analysis. We show that MAIT cell frequency is significantly decreased in peripheral blood of patients with NAFLD. Compared to controls, NAFLD MAIT cells expressed significantly higher levels of activation markers and effector cytokines ex vivo, suggesting MAIT cell activation in NAFLD. However, upon in vitro stimulation with IL-12 and IL18, MAIT cells from NAFLD patients failed to upregulate expression of effector cytokines, i. e. IFNg, Granzyme B and IL-17A. MAIT cell effector function was dependent on glycolysis and oxidative phosphorylation in NAFLD patients. When cultured with free fatty acids, MAIT cells preferentially took up oleic and linoleic acid, which induced a significant decline in IFNg expression by MAIT cells. Interestingly, oleic and linoleic acid disrupted mitochondrial potential in MAIT cells and induced cell death, while mitochondrial mass remained unaffected. Our results show that MAIT cells are highly activated but dysfunctional in NAFLD.

Lecture Session IV Tumors 29/01/2022, 8.30 am – 9.15 am, Lecture Hall

L 4.01 CXCR3 is a key regulator during macrophage differentiation and has a significant impact on tumor-associated macrophages

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Background It has been appreciated that in the presence of tumors, hematopoetic stem cells have the potential to proliferate and differentiate towards the monocytic and granulocytic lineages resulting in an intratumoral accumulation of suppressive immune cells like tumor-associated macrophages. Furthermore, recent studies have shown that the CXCR3 network is important for the efficacy of immune checkpoint inhibitor therapy and has an influence on the tumor-associated angiogenesis.

Method In vivo, HCC was induced in Cxcr3-/- and wild-type (WT) mice. The HCC-related immune response was analyzed, with focus on tumor-associated macrophages. In vitro, phenotype of Cxcr3-/- and WT hematopoietic progenitor cells, myeloid precursor cells and monocyte-derived macrophages were determined. To define changes in the macrophage lineage an epigenetic sequencing of WT and Cxcr3-/- myeloid precursor cells and monocytes was executed. To elucidate regulatory pathways in monocyte-derived macrophages, we performed multi-kinase arrays.

Results Cxcr3-/- mice displayed a significantly increased tumor burden compared to WT mice. Deletion of Cxcr3 promotes the myeloid progenitor lineage and modulates chromatin accessibility for key transcription factors. Furthermore, deletion of Cxcr3 leads to an altered kinase activity in macrophages and a pro-tumorigenic differentiation. The enhanced secretion of anti-inflammatory, pro-angiogenic and pro-proliferative mitogens characterizes this cell population. In addition, the inactivation of Cxcr3 in monocyte-derived macrophages results in an anti-inflammatory profile compared to WT cells.

Conclusion We here decipher the complex regulatory network of CXCR3 impacting macrophage polarization and cell biology and identify new targets to improve anti-PD-L1 and anti-VEGF therapy in HCC.

L 4.02 N-cadherin distinguishes intrahepatic cholangiocarcinoma from ductal adenocarcinoma of the pancreas

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E- and N-cadherin are transmembrane glycoproteins of adherens junctions that are pivotal for embryogenesis, cell differentiation, and maintenance of tissue integrity. Whereas E-cadherin was thought to be specific for epithelial cells, N-cadherin has been described in mesenchymal and neuroectodermal cells. We could previously show that E:N-Cadherin-heterodimers are constitutively expressed in hepatocytes and cholangiocytes. Therefore, we aimed to analyze E- and N-cadherin in tumors of the pancreatobiliary system in a large collective of patients with intrahepatic cholangiocarcinoma (iCCA, n = 313), carcinoma of the extrahepatic bile ducts (EHBD, n = 499), gallbladder carcinoma (GBC, n = 219), ductal adenocarcinoma of the pancreas (PDAC; n = 129), as well as respective precursor lesions (n = 342) and normal tissue (n = 363).

E- and N-cadherin was analyzed using protein biochemical and immunohistochemical methods. Manually scored tissue-microarray cores were compared to automated image analysis with QuPath software, allowing qualitative and quantitative assessment.

E-cadherin was positive in 99 % of iCCA, N-cadherin in 67 % of the small duct, and 45 % of large duct iCCA. In GBC, EHBD, and PDAC, E-cadherin was positive, but N-cadherin was only faintly expressed. N-cadherin expression in tumors was comparable to its expression in respective normal tissues and precursor lesions.

The expression of E- and N-cadherin in tumors of the pancreatobiliary tract recapitulates their expression in their normal counterparts. N-cadherin is a suitable marker for the differential diagnosis between iCCA and PDAC with a specificity of 96 % and a sensitivity of 67 % for iCCA of small duct type and a sensitivity of 50 % for iCCA of large duct type.

Z Gastroenterol 2021; 60: e1–e55

L 4.03 ACSL4-dependent ferroptosis is not a major tumor-suppressive mechanism during HCC initiation and progression

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Question Non-alcoholic fatty liver disease is a condition characterized by excess of fat in liver ranging from simple steatosis to steatohepatitis, cirrhosis, and hepatocellular carcinoma (HCC). In our study, we aimed to investigate the relevance of ferroptosis for disease progression using hepatocyte-specific ACSL4 deletion in an experimental HCC model.

Methods Primary hepatocytes from either wild-type mice or mice with hepatocyte-specific deletion of ACSL4 (ACSL4Δhepa) were treated with specific inducers (e.g., RSL3) and inhibitors (e.g., Liproxstatin-1) of ferroptosis. We used STZ (Streptozocin) with high-fat diet as HCC model, where different time points of disease progression were studied to investigate the role of ferroptosis.

Results Treatment of primary hepatocytes with RSL3 triggered increased ferroptotic cell death, which could be rescued by Liproxstatin-1 or by ACSL4 deletion. At the stage of tumor initiation in our HCC model, inhibition of ferroptosis in hepatocytes increases the severity of chronic liver disease as evidenced by elevated serum transaminase levels in ACSL4Δhepa mice. Importantly, the overall tumor burden was increased, too. Interestingly, ACSL4 deletion does not significantly affect inflammation. However, enhanced oxidative stress and remarkably decreased ferroptotic and apoptotic cell death was observed in ACSL4Δhepa mice. Moreover, evaluation of HCC progression at later stage showed no effect on overall tumor burden but significantly reduced proliferation in ACSL4Δhepa mice.

Conclusion Our results demonstrate that primary mouse hepatocytes are susceptible to ferroptosis induction and that it depends on functional ACSL4. In vivo in our HCC model, ACSL4 deletion increases tumor initiation, but has no significant impact on tumor progression.

Lecture Session V Viral Hepatitis and Immunology
29/01/2022, 11.00 am – 11.45 am, Lecture Hall

L 5.01 Enforced cytotoxic signature of HBV pol455-specific CD8 T cells in chronic HBV infection

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DOI 10.1055/s-0041-1740660

T-cell exhaustion represents a distinct T-cell differentiation program associated with chronic viral infections. Several studies have shown that exhausted CD8 + T cells are heterogeneous. In chronic HBV infection, we and others observed major differences in phenotype and function as well as the degree of dysfunction of HBV-specific CD8 + T cells targeting different antigens. The aim of this study was to investigate the molecular heterogeneity of these differences in antigen-specific HBV-specific CD8 + T-cell immunity.

We conducted single-cell RNA sequencing of HBV-specific CD8 + T cells targeting different antigens, HBVcore18 and HBVpol455, obtained from chronically HBV-infected patients. Cluster analysis of single-cell transcriptomes revealed a different subset diversification of HBVcore18- versus HBVpol455-specific CD8 + T cells. In particular, HBVcore18-specific CD8 + T cells were mostly comprised of precursor-like/memory-like exhausted T-cell subsets. Within HBVpol455-specific CD8 + T cells, we could identify a cluster of cells that highly expressed cytotoxic genes including GNLY, GRMB, NKG7, PRF1. The differential transcriptional profile of HBVpol455-specific CD8 + T cells was further confirmed ex vivo after pMHCI tetramer-based enrichment. Indeed, at the protein level, we also detected a higher cytotoxic potential of HBVpol455-specific CD8 T cells obtained from patients who endogenously control the viral infection in comparison to patients requiring antiviral therapy.

In sum, our data show different molecular and functional characteristics of virus-specific CD8 + T-cell responses targeting different HBV antigens linked to control of infection. This may have potential implications for the design of immunotherapeutic approaches in HBV cure.

L 5.02 Impaired neutrophil migration is association with adverse outcomes in patients with liver cirrhosis

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Neutrophil granulocytes are important determinants of the first line defense against bacterial and fungal infections. In the present study, we aimed to determine associations between neutrophil migration and outcome of liver cirrhosis and acute-on-chronic liver failure.

Patients with compensated (n = 11) or acutely decompensated liver cirrhosis (n = 84) or ACLF (n = 29) were recruited from a prospective cohort study. Neutrophils were isolated of whole blood by magnetic beads. Neutrophil migration was studied during steady-state-, fMLP-, CXCL1-, or CXCL8-stimulated conditions using time-lapse microscopy. Automated cell tracking enabled quantification of the proportion of migrating cells and of average migration speed. Regression analyses were used to determine associations between neutrophil migration and clinical endpoints.

The proportion of fMLP-stimulated migrating neutrophils was lower in decompensated patients (81.32 ± 1.6 %,P = 0.01) or ACLF (76.63 ± 3.4 %,P = 0.003) compared to healthy individuals (89.04 ± 0.8 %). Furthermore, CXCL8-stimulation was less effective in neutrophils of ACLF patients (77.56 ± 3.2 %) compared to healthy individuals (87.14 ± 2.0 %,P = 0.04). Average speed was reduced after fMLP- and CXCL8-stimulation in decompensated patients (fMLP 15.23 ± 3.3µm/min,P = 0.001; CXCL8 14.42 ± 0.3µm/min,P = 0.048) or ACLF (fMLP 14.91 ± 0.7µm/min,P = 0.005; CXCL8 13.82 ± 0.4µm/min,P = 0.008) compared to healthy individuals (fMLP 17.78 ± 0.5µm/min, CXCL8 15.78 ± 0.5µm/min). A low proportion of migrating neutrophils under steady-state or stimulated conditions was associated with the development of ACLF ($P < 0.05$), sepsis ($P < 0.05$), or the composite of ACLF, sepsis and death ($P < 0.01$) within 7 days.

Impaired neutrophil migration under steady-state conditions or after stimulation is associated with adverse outcomes in liver cirrhosis. Hence, neutrophil migration analysis might provide a novel early warning sign for the development of severe complications in liver cirrhosis patients.

L 5.03 Comprehensive characterization of Mucosal-Associated invariant T (MAIT) cells in patients with hepatitis E virus infection

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Hepatitis E virus (HEV) gt3 infection causes a self-limiting, usually asymptomatic disease in immunocompetent patients. However, in immunocompro-

mised patients, chronic HEV infection can develop, leading to liver cirrhosis. Since HEV is mainly transmitted orally, the first contact with the virus is through the mucosal barrier, including mucosal-associated invariant T cells (MAIT). Modulation of MAIT cells has been demonstrated in chronic viral hepatitis. Since little information is available on MAIT cells during HEV infection, we investigated the phenotype and function of MAIT cells in HEV-infected immunocompetent and immunosuppressed patients.

PBMCs from 48 HEV patients (17 immunocompetent (no-TX, acute and resolved); 31 immunocompromised (TX, acute, chronic, resolved)) were investigated. MAIT cells were phenotypically characterized and their function was measured after in vitro stimulation with IL12/IL18 or HEV protein.

The frequency of MAIT cells was comparable in the groups studied. A higher frequency of the activation marker CD69 on MAIT cells was observed in HEV-infected TX patients (chronic $p < 0.05$, acute $p = 0.06$) compared to no-TX patients (acute HEV)). When analyzing PD1 expression on MAIT cells, we observed significantly increased expression in chronic HEV compared to acute HEV, but only in TX patients. Increased functionality (significant for TNF) was observed in chronic TX patients compared no-TX patients following IL12/IL18 stimulation in vitro and likewise after stimulation with HEV protein, the latter mediated via MR1. MAIT cells showed a distinct phenotype and increased functionality in chronic HEV-infected TX patients, which may contribute to the hepatitis and progression to liver fibrosis observed in these patients.

Poster Visit Session I Basic Hepatology (Fibrogenesis, NPC, Transport) 28/01/2022, 13.55 pm – 14.40 pm

P 1.01 Augmenter of Liver Regeneration (ALR) alters IL-6 signaling in liver cells by affecting IL-6 receptor subunits gp80 and gp130

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Interleukin 6 (IL-6) is the main trigger of the acute-phase response (APR) following tissue damage, infection or inflammation. Augmenter of liver regeneration (ALR), a co-mitogen expressed in different organs, is mostly known for its hepatotrophic properties. Previously we have uncovered ALR's dual signalling impact on hepatic APR. While exogenously-applied ALR attenuates, endogenously-overexpressed ALR enhances IL-6-induced signal transduction. Here we aim to elaborate the molecular mechanism behind these observations by in vitro experiments. HepG2 cells (w/o stable sfALR expression, HepG2-sfALR) were treated with IL-6 in presence or absence of recombinant ALR (rALR). Western-blot analysis revealed, that phosphorylation of members of IL-6 receptor signalling cascade (pJAK2, pJAK1, pSHP2) was enhanced in HepG2-sfALR cells upon IL-6 induction, while treatment with rALR attenuated their activation. Furthermore, analysing cell culture supernatants performing ELISAs we found reduced levels of the soluble IL-6 receptor subunits, sgp80 as well as sgp130, in HepG2-sfALR, whereas rALR-treatment slightly increased both in HepG2 cells. Additionally, HepG2 cells revealed diminished gp80 expression after rALR treatment which might explain the attenuated IL-6 signalling. Less expression of gp80 and increased sgp80 levels might be due to enhanced shedding by metalloproteinases (ADAM17, ADAM10), but could not be confirmed for ALR-treated cells. Analysis of the cell surface expression of gp80 will be determined by FACS. IL-6 receptor unit gp130 is mainly transcriptionally regulated and therefore mRNA expression of membrane-bound and sgp130 forms will be analyzed by quantitative RT-PCR. Our preliminary results reveal that ALR impacts IL-6 signalling at least by affecting expression of IL-6 receptor subunits.

P 1.02 Is the effectivity of Schistosoma mansoni infection dependent on the host's age?

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DOI 10.1055/s-0041-1740664

Questions Schistosomiasis affects more than 230 million people worldwide. In the host the parasites produce eggs in the enteric vascular system. However, up to 50 % of S. mansoni eggs end up predominantly in the liver causing the formation of granulomas. The aim of this study was to investigate the correlation of the host's age with the infection rate, hepatic egg burden, and hepatocellular damage in a murine infection model.

Methods C57BL/6 mice were infected with S. mansoni cercariae at 8-, 14-, and 20-weeks, and infection lasted 9 weeks. The paddling-method with pre-soaking was used to infect the mice in a natural way (Dettmann 1989). Hepatic levels of TNF-α, IL-4, TGF-β, hepatic egg load (number/mg liver tissue), and the serum ALT were measured.

Results The proportion of animals that were confronted with the parasites and also showed eggs in the bowel and liver was independent of the host's age. Interestingly, the number of hepatic eggs as well as mRNA levels of TNF-α, IL-4, and TGF-β were reduced with increasing age of the infected host while serum ALT levels increased with age.

Conclusion The constant rate of successful infections of mice at the ages between 8–20 weeks demonstrated similar infection rates. This contrasts earlier studies, which indicated an age-dependent reduction of infection-effectivity. The age-dependent cytokine profiles suggest that parasite-induced tolerance might be the reason for the discrepancy between hepatic egg load and serum ALT levels.

P 1.03 Activation of the unfolded protein response (UPR) and fibrosis is associated withcholangiocellular injury in an experimental model of fibropolycystic liver disease

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Background&Aim Fibropolycistic liver disease is characterized by hyperproliferation of the biliary epithelium and the formation of multiple dilated cysts, a process recently associated with unfolded protein response (UPR). In the present study, we aimed to understand the mechanisms of cyst formation and UPR activation of hepatocyte-specific c-Jun N-terminal kinase 1/2 (Jnk1/2) knockout mice.

Methods&Results Floxed JNK1/2 (Jnkf/f) and JnkΔhepa animals were sacrificed at different time-points during progression of liver disease. Histological examination of specimens evidenced the presence of collagen fiber deposition, increased α-smooth muscle actin (αSMA), infiltration of CD45, CD11b and F4/80

cells and pro-inflammatory cytokines (Tnf, Tgfβ1), and liver injury (eg: ALT, apoptosis and Ki67 positive cells) in JnkΔhepa compared with Jnkf/f livers from 32 weeks of age. This was associated with activation of effectors of the UPR including BiP/GRP78, CHOP and spliced XBP1. Tunicamycin (TM) challenge strongly induced ER stress and fibrosis in 8 week-old JnkΔhepa animals compared with Jnkf/f littermates. Finally, thioacetamide (TAA) supplementation to JnkΔhepa induced UPR activation, peribiliary fibrosis, liver injury and markers of biliary proliferation and cholangiocarcinoma (CCA) in JnkΔhepa animals.

Conclusions These results suggest that activation of the UPR in conjuction with fibrogenesis might trigger hepatic cystogenesis and early stages of CCA.

P 1.04 Aktive Rolle von Bakterien bei der spontan bakteriellen Peritonitis: Regulation der p53 Familie in Darmepithelzellen

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DOI 10.1055/s-0041-1740666

Hintergrund Die spontan bakterielle Peritonitis (SBP) ist eine Komplikation der Leberzirrhose. Eine zentrale Rolle bei der Pathogenese spielt die bakterielle Translokation. Das intestinale Epithel stellt die primäre Barriere gegen kommensale Bakterien dar. Die p53-Familie ist eine wichtige Komponente der zellulären Stressantwort und schützt die Zelle vor Schäden, u. a. durch Induktion eines Zellzyklusarrests. Patienten mit Leberzirrhose weisen eine veränderte intestinale Expression der p53-Familienmitglieder auf. Ziel der Arbeit war die Untersuchung des Einflusses SBP-relevanter Bakterien bzw. der p53-Expression auf Zell-Zell-Kontakte und p53-Familienmitglieder in Darmepithelzellen.

Methoden HCT-116 TP53wt, HCT-116 TP53-/- und Caco-2 Zellen wurden mit Escherichia coli (E. coli;) ko-kultiviert. Die Regulation der Zell-Zell-Kontakte und der p53-Familie wurde mittels Western Blot und qPCR analysiert. Es wurde eine genomweite Arrayanalyse durchgeführt. Der Zellzyklus wurde mittels Durchflusszytometrie untersucht.

Ergebnisse Die Stimulation mit E.coli führte auf Transkriptomebene zu einer Stressantwort durch die Induktion von p53-Zielgenen und funktionell zu einem G1-Zellzyklusarrest in Epithelzellen. Zudem triggerten E.coli eine transienten Induktion von p53 und p73. Nach initialer Induktion erfolgte eine bakteriell-induzierte und MDM2-abhängige Degradation der p53-Proteine. Übereinstimmend damit wurde eine Reduktion der p53-/p73-Level in Biopsien von Patienten mit Leberzirrhose beobachtet. Darüber hinaus induziert der direkte Kontakt von Bakterien und Epithelzellen p53-unabhängig den Abbau wichtiger Zell-Zell-Kontakt-Proteine.

Schlussfolgerung Der direkte Kontakt von Bakterien und Epithelzellen beeinflusst die Stabilität des Darmepithels durch (1) den Abbau wichtiger Zell-Zell-Kontakt-Proteine und (2) die Regulation der p53-Familie: der initialen Aktivierung der p53-Familie und deren Zielfunktionen folgt eine Suppression. Zusammen begünstigen diese Effekte die bakterielle Translokation und damit die Entstehung einer SBP.

P 1.05 IGFBP2 – ein neues Zielgen der p53-Familie mit anti-proliferativen Effekten in Hepatomzellen

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Hintergrund Neue Therapieansätze für das hepatozelluläre Karzinom erfordern ein detailliertes Verständnis der molekularen Grundlagen für die Entstehung und Proliferation von Tumorzellen. p53-Proteine schützen Zellen vor DNA-Schäden unter anderem durch Induktion spezifischer Zielgene. In Vorarbeiten identifizierten wir IGFBP2 (insulin like growth factor binding protein 2) als neues Zielgen von p73. TIGAR (TP53-inducible glycolysis and apoptosis regulator) ist als p53-Zielgen beschrieben. Ziel dieser Arbeit war die Charakterisierung der molekularen Effekte von IGFBP2 auf Proliferation und Therapie-Sensitivität/Resistenz in Hepatomzellen.

Methoden Zur Expression von p53 bzw. p73 wurden Hep3B-Zellen mit adenoviralen Vektoren (rAd-Tap53, rAd-TAp73) transfiziert oder mit HCC-Therapeutika (Doxorubicin, Bleomycin, Regorafenib) stimuliert. RNA- und Proteinspiegel von IGFBP2 und TIGAR wurden mittels qPCR bzw. Western Blot ermittelt. Zelltod- und Proliferationsraten wurden mittels Durchflusszytometrie bestimmt.

Ergebnisse Die Hochregulation von p53 und p73 induzierte die Expression von IGFBP2, aber nur p73 führte zu erhöhter IGFBP2 Proteinproduktion und -sekretion. HCC Therapeutika induzierten sowohl p73 als auch die IGFBP2-Produktion. Von hoher klinischer Relevanz ist, dass endogen produziertes IGFBP2 die Sensitivität der Zellen für Therapeutika-induzierten Zelltod erhöht. Extrazellulär zugegebenes IGFB2 hingegen wirkt in moderaten Konzentrationen proliferationsfördernd während hohe Konzentrationen die Proliferation verringert. Die Expression von TIGAR wurde sowohl durch p53 als auch durch HCC-Therapeutika induziert. Eine gegenseitige Regulation von IGFBP2 und TIGAR fand nicht statt.

Schlussfolgerung Durch die Regulation von IGFBP2 und TIGAR hat die p53-Familie eine wichtige Rolle bei der Kontrolle metabolischer Funktionen und proliferativer Prozesse bei Tumoren. Die Menge von IGFBP2 wird von p73 reguliert. Endogenes intrazelluläres IGFBP2 sensitiviert die Zellen für Zelltod. Extrazelluläres IGFBP2 beeinflusst die Proliferation.

P 1.06 C5aR deficiency rescues hepatic fibrosis but hampers fibrotic resolution

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Background Complement factor C5 contributes to hepatic fibrogenesis as C5-deficient mice display less fibrosis after challenge with CCl4 (Hillebrandt et al. 2005). During inflammation C5 is cleaved and the small chemoattractive peptide C5a binds to the receptors C5aR and C5L2. Our aim was to assess the specific roles of the C5 receptors during CCl4-induced liver damage and regeneration.

Methods C5aR- and C5L2-deficient mice and wild-type (WT) controls were treated with CCl4 for 6 weeks. In addition, mice were challenged with CCl4 for 6 weeks and left untreated for another 6 weeks (regression model). Expression of Th1 and Th2 cytokines was determined by qRT-PCR, and hepatic collagen contents were measured via hydroxyproline. Mice were also objected to bile duct ligation (BDL) and unilateral ureteral obstruction (UUO) to compare fibrosis progression in liver and kidney.

Results Chronic fibrosis in liver and kidney was least pronounced in C5aR-deficient mice in comparison to the other lines. Of note, 6 weeks after the last injection C5aR-deficient mice developed highest hepatic collagen levels, indicating response and ongoing damage after cessation of fibrotic stimuli. This is resembled by cytokine profiles, with IL6, IL10, IL12, IL23 and IL27 being reduced in mice deficient for C5 receptors in the chronic model but elevated in C5aR-/- mice during fibrosis regression in the liver.

Conclusions C5aR is critical during chronic fibrogenesis. The novel observation of fibrosis progression in C5aR-deficient mice the after removal of the fibrotic stimulus points to its critical role during wound healing and fibrosis regression.

P 1.07 Enhanced expression of bone morphogenetic protein endothelial cell-precursor derived regulator in liver fibrosis

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There are conflicting data on the role of different bone morphogenic proteins (BMPs) in hepatic fibrosis; some have been shown to act act profibrogenic such as BMP4 and others rather anti-fibrogenic such as BMP6. BMP endothelial cell-precursor derived regulator (BMPER) has been shown to act as extracellular regulator of different BMP-proteins including BMP4 and BMP6. Depending on its concentration and the cellular environment, BMPER has been shown to be able to enhance but also to attenuate BMP signalling.
The aim of this study was to investigate the expression and role of BMPER in hepatic fibrosis.
Methods and results BMPER mRNA- and protein-expression is significantly increased during in vitro activation of primary human hepatic stellate cells (HSC). Furthermore, hepatic BMPER-expression is significantly increased in different mouse models of liver-fibrosis. Moreover, there is a significant correlation between the expression of BMPER and alpha-smooth muscle actin (alpha-sma) in human liver tissues and immunofluorescence analysis revealed co-localization of BMPER and alpha-sma expression in human cirrhosis.
In activated HSC, BMPER-depletion with siRNA caused a downregulation of the expression of inhibitor of differentiation 1 (ID1), a well-characterized read-out for BMP-pathway-activity. Conversely, stimulation with recombinant BMPER induced ID1-expression in activated HSCs.
Summary and conclusion Activated HSC are the cellular source of enhanced BMPER expression in hepatic fibrosis and in vitro studies indicate that BMPER causes an upregulation of BMP-activity in HSC. Further studies are required to analyse the interaction of BMPER with different BMPs and its role during the course of hepatic fibrosis.

P 1.08 Bacterial induced deradation of cell-to-cell contact proteins and loss of epithelial integrity in spontaneous bacterial peritonitis

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DOI 10.1055/s-0041-1740670

Introduction Translocation of intestinal bacteria or bacterial products from the gut to mesenteric lymph nodes is a key process in development of spontaneous bacterial peritonitis (SBP). Bacterial translocation is facilitated by increased intestinal permeability. We have shown that bacterial proteases are involved in the degradation of cell-to-cell junction proteins leading to intestinal destabilization.
Methods To analyze the pathomechanism of SBP, the integrity of intestinal epithelial cells in presence and absence of E. coli and protease inhibitors was analyzed. 3 different E. coli-strains were isolated from ascitic fluid of patients with liver cirrhosis and SBP. The laboratory strain E. coli O6:Hnt served as reference. Bacteria-dependent regulation of intestinal cell-cell contacts was evaluated by determining RNA and protein levels of the adherence junction protein E-cadherin and tight junction protein occludin. We used broad-spectrum inhibitor BB-2516 and selective metalloprotease (MMP)-8 inhibitor I to block bacterial protease activity.
Results Various E. coli-strains (O6:Hnt, Ont:H41 and O18:H7) destabilized cell-to-cell junction components of intestinal epithelial cells, indicated by reduced protein levels of E-cadherin and occludin. Protease inhibitor BB-2516 or MMP-8 inhibitor I stabilized cell-to-cell junction protein levels. Noteworthy, bacterial protease activity of E. coli O6:Hnt and patient-derived E.coli O18:H7 decreased upon addition of inhibitors.
Summary (Patient-derived) E. coli destabilize cell-to-cell junctions on protein level in a protease-dependent manner. Vice versa, protease inhibitors stabilize the epithelial barrier by targeting bacterial proteases. These data point out to bacterial proteases as potential novel targets for treatment of SBP.

P 1.09 Role of Fibroblast Growth Factor 9 in bile acid homeostasis

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Regulation of bile acid homeostasis plays an important role in chronic liver diseases. Cholesterol 7α-hydroxylase (CYP7A1) is the rate-limiting enzyme of bile acid synthesis. Fibroblast growth factor 19 (FGF19) is known to inhibit CYP7A1-expression via endocrine activation of FGF-receptor 4 (FGFR4), however, the role of other FGF-family members and FGF-receptors in bile acid homeostasis remains elusive.
The aim of this study was to analyze the role of FGF9, a paracrine FGF secreted by activated hepatic stellate cells (HSC), in bile acid homeostasis.
Methods and Results Incubation with conditioned media (CM) from activated human HSC significantly reduced the expression of CYP7A1 in human hepatoma cells, and this effect was reduced in CM from HSC with FGF9-knockdown. In line with this, recombinant FGF9 significantly reduced the expression of CYP7A1 in a dose-dependent manner as well as the bile acid secretion into the supernatant. Furthermore, FGF9 induced JNK activation in hepatocytes. Pretreatment with a specific JNK inhibitor (SP600125) abolished the FGF9-mediated suppression of CYP7A1 expression. The FGFR1/2/3 inhibitor BGJ398 almost completely abrogated the FGF9-induced JNK-activation as well as the inhibitory effects of FGF9 on CYP7A1 expression. In contrast, the selective FGFR4 inhibitor BLU9931 had no significant effect.
Summary and conclusion Our data indicate FGF9 as novel paracrine regulator of bile homeostasis in chronic liver disease. FGF9 suppresses transcription of CYP7A1 gene via binding to FGFR1/2/3 and activation of JNK signaling. Herewith, FGF9 might prevent hepatocytes from accumulating toxic bile acids during liver injury and fibrosis.

P 1.10 The role of the mechanistic target of rapamycin (mTOR) in Alpha-1 Antitrypsin Deficiency

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DOI 10.1055/s-0041-1740672

Introduction Alpha1-antitrypsin (AAT) mutations lead to the retention of the otherwise secreted protein in the endoplasmic reticulum (ER) thereby giving rise to AAT deficiency (AATD). Liver disease arising due to the proteotoxic stress is the second leading cause of mortality in AATD.
Aims & methodology Our aim was to assess the importance of mTOR signaling in AATD as the central proteostatic regulator. Animals overexpressing the characteristic AAT mutation (PiZ mice) were cross-bred with rodents harboring a hepatocyte specific-ablation of the interaction partners RAPTOR/RICTOR corresponding to mTOR complexes 1/2 (mTORC1/2) or with mice lacking mTOR.

Results At two month of age, PiZ-mTORΔhep and PiZ-RAPTORΔhep but not PiZ-RICTORΔhep mice showed signs of increased liver injury and apoptosis despite diminished AAT accumulation. While PiZ-RAPTORΔhep animals displayed increased levels of the pro-apoptotic protein CHOP, CHOP ablation did not rescue the phenotype. As a potential underlying mechanism, we observed reduced levels of several chaperones, i. e. Hsp90 or Grp94.

Conclusions mTORC1 but not mTORC2 plays an important role in PiZ induced liver injury.

P 1.11 Enhanced Expression of Fibroblast Growth Factor 9 in Hepatic Fibrosis

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Liver fibrosis can be considered as a wound healing response upon liver injury. Fibroblast growth factor (FGF) signalling plays an important role in tissue repair and regeneration but the expression and potential effects of the 22 FGF-family members in hepatic fibrosis are only incompletely understood.

The aim of this study was to analyze the expression and function of FGF9 in hepatic fibrosis.

Methods and Results A systematic screen of the 22 known FGFs revealed that FGF9 expression was most highly upregulated in different experimental models of hepatic fibrosis and during the in vitro activation of primary human hepatic stellate cells (HSC). Furthermore, FGF9 expression significantly correlated with alpha-smooth muscle actin (α-sma) in human liver tissues and immunofluorescence staining revealed colocalisation of FGF9 and α-sma in cirrhotic liver tissue indicating activated HSC as cellular source of FGF9.

Stimulation with recombinant FGF9 inhibited the in vitro activation of primary human HSC. Furthermore, FGF9 reduced the expression of collagen (COL1A1) in activated HSC. Conversely, FGF9 induced the expression of metalloproteinases MMP1 and MMP3 and the phosphorylation of JNK while knockdown of FGF9 reduced JNK-activation in activated HSC.

Summary and conclusion Our study revealed activated HSC as cellular source of enhanced FGF9 expression in hepatic fibrosis and indicates that FGF9 might exhibit anti-fibrogenic effects. Further studies are required to define the diagnostic and therapeutic potential of FGF9 in chronic liver disease.

P 1.12 Expression of liver regeneration-associated protein ALR (Augmenter of Liver Regneration) is diminished by IL-1β

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DOI 10.1055/s-0041-1740674

The liver regeneration-associated protein ALR (Augmenter of Liver Regeneration) was shown to exhibit anti-apoptotic, anti-oxidative and autophagic properties. Increased ALR levels have a beneficial impact on liver regeneration, but in chronic liver injury such as cholestasis and NAFLD (non-alcoholic fatty liver disease) expression of ALR is diminished. While several factors are known to induce ALR expression, little is known about its negative regulation. Preliminary results demonstrated that interleukin-1β (IL-1β), e. g. secreted by Kupffer cells in cholestasis, can induce ALR promoter activity. Therefore, we aimed to investigate the regulation of ALR expression by IL-1β. HepG2 and Huh-7 cells were treated with different concentrations of IL-1β and qRT-PCR as well as western-blots were performed. IL-1β treatment reduced ALR mRNA and protein expression in a time- and dose-dependent manner in both cell lines. Expression of hepcidin served as a positive control. Furthermore we analyzed potential transcription factors (TFs) mediating the effect of IL-1β on ALR expression. We found reduced mRNA expression of SP1, a known inducer of ALR, upon IL-1β treatment. In addition Egr-1, another ALR-inducing TF, was hampered to activate ALR expression after IL-1β treatment. Ongoing work elucidates a potential impact of AP1, a TF previously shown to bind onto the ALR promotor repressing its activity. Additionally, expression of HNF4α, a TF known to induce ALR expression, may be reduced by IL-1β and will therefore be analyzed. In conclusion, insights into the regulation of ALR might result in therapeutic strategies to boost its expression and increase its hepatoprotective properties.

P 1.13 Effects of regulated cell death (e. g. ferroptosis) on early hepatic ischemia reperfusion damage in steatotic donor organs

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DOI 10.1055/s-0041-1740675

Introduction During ischemia-reperfusion, regulated cell death (RCD) signaling pathways, mainly ferroptosis, are activated. In this iron-dependent form of RCD, reactive oxygen species accumulate in the cell and eventually lead to cell death. Due to shortage of donor organs, more steatotic livers are transplanted, possibly showing increased susceptibility to ischemia-reperfusion injury (IRI). Therefore, this study explores the effect of cell death mechanisms (particularly ferroptosis) on early IRI in steatotic donor livers.

Material and Methods In this study, an in vitro model of IRI was established in marginal organs. For ischemia, human HepaRG cells were hypoxically treated for 48h. Subsequently, a simulation of reperfusion followed. Differences between untreated liver cell lines and steatotic liver cells were examined, by detecting specific cell death signaling pathways (apoptosis, necroptosis and ferroptosis). Furthermore, human liver biopsies were examined before and after reperfusion.

Results In the in vitro model, significant differences in the expression of cell death-specific markers were detected between steatotic and non-steatotic liver cells.

Human graft biopsies show significant tissue damage (HE section) and differences in cell death expression, especially with respect to ferroptosis, between marginal and non-marginal donor organs.

Conclusion Ferroptosis appears to be an initial activator of hepatic IRI and affects mainly marginal organs, leading to a dramatic increase in post-translational damage. Therefore, inhibition of ferroptosis opens new therapeutic opportunities to improve liver transplantation outcomes.

P 1.14 New Rat Model of Cholestatic Liver Cirrhosis Developing Acute-on-Chronic-Liver failure

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Introduction Acute-on-chronic liver failure (ACLF) has a dramatic 28-day mortality. Several precipitating events, such as severe alcoholic injury, bacterial

translocation and infections lead via multiple organ dysfunction and failure to acute decompensation (AD) and ACLF. Validated animal models for ACLF are needed for therapeutic treatments.

Methods Bile duct ligation (BDL) induced cholestatic liver cirrhosis. Alcohol injury was mimicked by ethanol binge drinking (6g/kg). Repetitive lipopolysaccharide (LPS) injections were implemented as bacterial translocation models. Finally, transnasal stool inoculation (TNI) and cecal ligation and puncture (CLP) were used to induce pneumonia and peritonitis. Organ dysfunctions and failures were assessed using histology, invasive pressure measurements, blood biochemistry and gene/protein expression and categorized by adapted CLIF-SOFA score.

Results All precipitants induced ACLF as defined by CLIF-SOFA score with liver, coagulation and circulatory failure. Liver failure was assessed by stainings, gene expression levels and measurement of blood parameters, while coagulation failure was defined by a significant increase in International Normalized Ratio (INR). Circulatory failure was confirmed by a decrease in mean arterial pressure and reduced organ perfusion. Renal failure was induced in the models of Binge and CLP, based on gene expression levels and blood parameters. Cerebral failure was induced only by CLP, as investigated by neurological tests. Respiratory failure could be induced only in 10–20 % of the animals across the models.

Conclusion In the cholestatic liver cirrhosis model, alcoholic injury, bacterial translocations and infections induce consistent liver, coagulation and circulatory failures. Alcoholic injury and CLP are the most severe precipitants in this setting.

P 1.15 New Rat Model of Alcoholic Liver Cirrhosis Developing Acute-on-Chronic-Liver failure

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DOI 10.1055/s-0041-1740677

Introduction Alcoholic liver cirrhosis is the main etiology of decompensated cirrhosis and ACLF in Europe. However, the pathogenesis has not been completely established. Severe alcoholic hepatitis and proven bacterial infections are confirmed precipitating events for ACLF, while bacterial translocation is thought to be responsible for decompensation with unidentified precipitant. Therapies are scarce and animal models are needed.

Methods Liver cirrhosis was induced by administration of increasing concentrations of ethanol combined with phenobarbital and additional twice-weekly intraperitoneal CCl4 injection for a total of 5 weeks. Acute alcoholic hepatitis was induced by ethanol binge drinking (6g/kg), while repetitive lipopolysaccharide (LPS) injections mimicked bacterial translocation. Proven bacterial infections were induced by transnasale stool inoculation (TNI) (pneumonia) and cecal ligation and puncture (CLP) (peritonitis). Organ dysfunctions and failures were assessed using histology, invasive pressure measurements, blood biochemistry and gene/protein expression and categorized by adapted CLIF-SOFA score.

Results All precipitating events induced liver, coagulation, and circulatory failure, while kindey failure was observed after binge drinking and repetitive LPS injections, as confirmed by blood parameters and gene expression levels. The models of binge drinking and TNI induced cerebral failure as assessed by oxygen saturation levels and pathologic neurological tests.

Conclusion The model of alcoholic liver cirrhosis with the precipitating events of binge drinking, repetitive LPS injections and TNI are the most suitable models to mimic human ACLF due to bacterial translocations and infections, as well as excessive alcoholism. Thus, these models are needed to investigate the pathophysiology of ACLF in ASH and to develop pharmacologic treatments.

P 1.16 New Rat Model of Advanced Non-Alcoholic-Steatohepatitis-Cirrhosis Developing Acute-on-Chronic-Liver failure

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DOI 10.1055/s-0041-1740678

Introduction Over the last decade non-alcoholic-steatohepatitis (NASH) induced liver cirrhosis has markedly increased. Especially in the United States NASH and obesity are associated with the development of Acute-on-chronic liver failure (ACLF) and high short term mortality. In these patients especially proven bacterial infections and bacterial translocation are thought to precipitate the acute decompensation (AD) and ACLF. Thus, the aim of this study was to develop ACLF models in NASH induced liver cirrhosis.

Methods Liver cirrhosis was induced by administration of high fat western diet (WD) and additional intraperitoneal CCl4 injections twice per week combined with phenobarbital for 7 weeks. The precipitating events bacterial translocation and infections were mimicked by the repetitive lipopolysaccharide (LPS) injections and transnasal stool inoculation (TNI), respectively. Organ dysfunction and failures were assessed by histology, blood parameters, gene expression levels, invasive pressure measurement and neurological behavior tests and categorized according to the adapted CLIF-SOFA score.

Results Repetitive LPS injections and TNI induced liver, kidney and circulatory failure in NASH rats as assessed by histology, blood parameters, gene expression levels and invasive pressure measurement. Besides liver and kidney failure, NASH animals developed severe respiratory and cerebral failure, which is shown by decreased oxygen saturation and pathologic neurological tests after bacterial translocation and infection.

Conclusion The models of bacterial translocation and infection as precipitating events in NASH rats induce AD and ACLF and mimic the human situation and may be useful in testing pharmacologic treatments.

P 1.17 Dietary carbohydrate restriction inhibits carbon tetrachloride-induced murine liver fibrosis

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DOI 10.1055/s-0041-1740679

High intake of processed and simple dietary carbohydrates (especially fructose) can result in non-alcoholic fatty liver disease (NAFLD) and, ultimately, liver fibrosis. On the other hand, dietary carbohydrate restriction (DCR) can be an efficient treatment option for a subgroup of NAFLD patients. A potential beneficial effect of DCR on established liver fibrosis remains elusive. We sought to address this experimentally and applied DCR (in form of a “low carb high protein” (LCHP) chow) to mice with carbon tetrachloride-induced liver fibrosis. Laboratory parameters of hepatocyte integrity were lower in the LCHP group. Both Sirius Red staining area and abundance of alpha smooth muscle actin-pos-

itive cells displayed significantly lower values upon DRC. Finally, feeding the LCHP chow resulted in significantly reduced hepatic inflammatory cell infiltration as well as systemic levels of pro-inflammatory cytokines Il-6 and Il-1beta. Taken together, we observed a wide range of beneficial effects of the DCR on established murine liver fibrosis. The molecular mechanisms that mediate these beneficial effects are currently being evaluated by us. Low carbohydrate diets have been used safely for almost a century in humans, e. g. for the treatment of drug-resistant epilepsy. Against this background, clinical application of our results seems feasible.

P 1.18 Profibrotic cytokines in nonalcoholic steatohepatitis (NASH) liver biopsies visualized and quantified by chromogenic in situ hybridization

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DOI 10.1055/s-0041-1740680

Background Non-alcoholic steatohepatitis (NASH) leads to liver fibrosis and subsequently to liver cirrhosis and increases the risk for hepatocellular carcinoma (HCC). Chronic inflammation of liver tissue in NASH represents a key role in the development of liver fibrosis. The aim of the study is to identify pro-inflammatory pathways for potential new therapeutical interventions.

Method Formalin-fixed paraffin-embedded (FFPE) human liver biopsies from patients with NASH (n = 10) and control samples (n = 4) were analysed. Chromogenic in situ hybridization (cISH) was used to detect proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin-1-beta (IL-1B), interleukin-6 (IL-6) and transforming growth factor beta (TGF-beta) in Semi-quantitative histological scoring based on ACD criteria was used for analysis.

Results Liver biopsies were evaluated for the level of fibrosis and steatosis. NASH biopsies were scored between 1–6 using the hepatitis activity score. Steatosis was graded semiquantitatively with a score of 5 %-50 %. The control group had no or low-grade fibrosis and no steatosis. NASH samples showed in comparison to the control group significantly up-regulation for the pro-inflammatory cytokines TNF-alpha (p = 0.04) and IL-1b (p = 0.02). IL-6 (p = 0.1059) showed no difference compared to the control group. The downstream pro-fibrotic marker TGF-beta showed a significant up-regulation in the NASH group (p = 0.04). Both pro-inflammatory and pro-fibrotic cytokines were mainly expressed by immune cells in the periportal region.

Conclusion cISH allows visualization of cytokine expression on mRNA level in FFPE tissue and revealed up-regulation of pro-inflammatory and pro-fibrotic cytokines in NASH samples, mostly located in the periportal region.

P 1.19 Insulin-controlled C/EBPα expression determines the impact of TGF-β on HNF4α transcription in hepatocytes

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Background and Aims TGF-β has been thought as an HNF4α inhibitor through SMAD complex that initiates hepatocyte epithelial-to-mesenchymal transition. However, we found that SMAD complex is required for HNF4α transcription in hepatocytes. This study scrutinizes regulatory mechanisms of TGF-β signaling in HNF4α transcription.

Methods HNF4α expression was examined in 98 HBV-infected patient samples and primary human and mouse hepatocytes. The effects of TGF-β on HNF4α expression was measured by qPCR and immunoblotting. ChIP assays were used to investigate the factors, which regulates HNF4α transcription.

Results Both SMAD2/3 and C/EBPα were required for constitutively HNF4α expression in hepatocytes. Knockdown of either SMAD2/3 or C/EBPα reduced RNA polymerase II binding to the HNF4A core promoter and decreased HNF4α expression. TGF-β incubation increased HNF4α transcription in hepatocytes at 2h, however decreased its expression at 24h. In contrast to upregulating HNF4α transcription, SMAD2/3 bound to the CEBPA promoter and repressed C/EBPα transcription. Given that the half-life of C/EBPα protein was only 3h, long-term TGF-β incubation led to C/EBPα exhaustion that inhibited HNF4α expression. Impressively, SMAD2/3 binding to the CEBPA promoter was inhibited by insulin administration. Consistent with in vitro observation, 67 patients with hepatic HNF4α immune positivity expressed both p-SMAD2 and C/EBPα, whereas 22 patients without HNF4α expression lacked either p-SMAD2 or C/EBPα expression. In 18 patients lacking both HNF4α and C/EBPα expression, GLUT2 expression in hepatocytes was undetectable, indicating insulin resistance in these patients.

Conclusions Insulin-controlled C/EBPα expression is crucial to determine the effect of TGF-β on hepatic HNF4α expression.

P 1.20 Downregulation of ECM1 in hepatocytes as a damage response to liver injury

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Background Liver disease progression depends on dynamic cell-cell communication among hepatic cells. Upon hepatocytes injury, HSCs are activated and differentiate into myofibroblast-like cells, in which TGF-β plays a crucial role. In healthy liver, latent TGF-β is stored in extracellular matrix and kept quiescent by ECM1. Upon damage, ECM1 production is downregulated in hepatocytes leading to L-TGF-β activation to initiate fibrosis. The mechanism of ECM1 expression regulation is not clear yet.

Methods In silico promoter analysis and correlative datasets were used to predict pathways relevant for ECM1 regulation. Functional assays were done in HCC cell lines, AML12, mouse and human primary hepatocytes, and in mice.

Results In acute PHx and CCl4 models, ECM1 is reduced in early stages of damage/regeneration, accompanied by enhanced TGF-β signaling. ECM1 is consistently downregulated in human HCC datasets and HCC cell lines. In silico analysis gives us potential transcriptional factors. Along this line, EGF and HGF promote ECM1 expression in vitro, and downstream signaling inhibitors suggest involvement of MEK-ERK pathway. At the transcription factor level, STAT1KD decreases ECM1 expression. Furthermore, YAP activation frequently accompanies low ECM1 expression levels. Injection of EGF to WT mice does not induce ECM1 expression above homeostatic levels, suggesting that EGFR/c-MET might be the rate-limiting components. Indeed, EGFR is downregulated in the PHx model in parallel to the decrease of ECM1 expression.

Conclusion ECM1 downregulation is a robust biomarker of chronic liver disease progression and acute liver damage, and can be developed as a therapeutic target, which warrants more detailed mechanistic evaluation.

P 1.21 Multi-omics profiling identifies molecular signatures of acute-on-chronic liver failure in Abcb-4KO mice upon chemical intoxication

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Acute-on-chronic liver failure (ACLF) is a major complication in patients with chronic liver diseases. Molecular understanding of pathophysiology and dynamics of ACLF remains unclear. Therefore, we developed a mouse model for ACLF to get further insight. We treated 65 weeks-old Abcb4KO mice on Balb/c background with a sublethal dose of CCl4 to recapitulate an acute event similar to drug intoxication or binge drinking on top of an advanced hepatobiliary injury in patients. Livers and blood were collected at days zero, one, two and four after the CCl4 injection. Behavioral, histopathological and plasma analyses showed significant differences in the mice after one day. Similar to ACLF patients, we observed extrahepatic injury, i. e. in the kidney presented as histopathological changes and elevated creatinine levels. In addition to the ACLF phenotypes i. e. massive cell death, we deeply molecularly characterized each mouse by RNA-sequencing (RNA-seq), single-nucleus RNA-seq (snRNA-seq) and proteomics. Based on the omics data, we stratified the mice into distinct responder classes, and identified molecular biomarkers, paving the way for precision medicine. In particular, the time-resolved paired transcriptome and proteome profiling of 40 mice identified a molecular shift after 1 day of CCl4, which decreases towards regenerated livers. Moreover, RNA-seq and snRNA-seq data show a distinct difference between poor and good prognosed mice at day 1. Our preliminary analysis of the snRNA-seq data set shows a highly heterogenous cell population i. e. hepatocytes. In conclusion, CCl4 treatment on aged Abcb4KO mice identifies key differential cellular patterns and potential molecular biomarkers of ACLF in patients.

P 1.22 Age-related analysis of transcriptome-wide sequencing of human liver

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Background For the first time, transcriptome-wide analysis of human liver has been performed for demonstrating differences between young and old humans, identifying major age-related alterations in hepatic gene expression may pinpoint ontogenetic shifts with important hepatic and systemic consequences, provide novel pharmacogenetic information, and offer clues to more efficiently counteract symptoms of old age.
Methods We applied next-generation sequencing (NGS), normalized the expression strength to transcripts per million and carried out statistical (Mann-Whitney non-parametric test) and bioinformatic [by Ensemble Feature Selection (EFS) software] analysis. NGS results were exemplarily validated by quantitative real-time polymerase chain reaction (qRT-PCR).
Results Among 60,617 total and 19,986 protein-encoding transcripts, we identified 44 transcripts whose expressions highly significantly ($p = 0.0003$ to 0.0464) and most strikingly (EFS score >0.3: 16 transcripts; EFS score >0.2: 28 transcripts) differ between young and old livers. The genes encoding for 25 of these highly age-related transcripts could be assigned to the categories 'regulome', 'inflammaging', 'regeneration', and 'pharmacogenes', and two genes of interest did not match these categories. The differences were confirmed by qRT-PCR for 14 out of 16 selected transcripts. Our results have major implications in the area of ontogeny/aging and for the age-dependently increased occurrence of major diseases such as non-alcoholic fatty liver and steatohepatitis as well as hepatocellular carcinoma.
Conclusion Results of a transcriptome-wide analysis of human liver offer potential options towards developing future therapeutic interventions against major liver diseases and increased insight into key mechanisms underlying aging.

P 1.23 The role of PNPLA3, MBOAT7 and TM6SF2 during alcohol detoxification: different mechanisms of fibrosis and steatosis development

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DOI 10.1055/s-0041-1740686

Background and Aims PNPLA3, MBOAT7 and TM6SF2 were identified as important risk genes for the development of alcoholic cirrhosis. We here present first data on the role of PNPLA3, MBAOT7 and TM6SF2 genotypes on liver stiffness (LS), steatosis (CAP) and inflammation during alcohol withdrawal.
Method 763 patients with ALD which were hospitalized for alcohol withdrawal at Salem Medical Center between 2007 and 2018 were genotyped for PNPLA3 s738409, MBOAT7 rs626283 and TM6SF2 rs58542926 polymorphisms. All patients had routine laboratory, ultrasound and a LS measurement.
Results 71 % of the patients were male, median age was 52 years, BMI was 24.7 kg/m2 and alcohol consumption was 163 g/day. At admission, no difference between the genotypes was seen regarding age, BMI, gender, alcohol consumption or transaminase levels. Significant differences were observed for PNPLA3 and MBOAT7 during alcohol detoxification. While MBOAT7 was associated with higher LS, no differences were observed between genotypes upon alcohol detoxification. In contrast, PNPLA3 caused clearly a delayed resolution of LS during withdrawal of alcohol due to inflammation. This could be recapitulated when looking at serum markers of liver inflammation. TM6SF2 showed no effect on alcohol withdrawal. A multivariate model confirmed that PNPLA3 was associated with steatosis and inflammation but not fibrosis. MBOAT7 was only associated with fibrosis/cirrhosis but not inflammation or steatosis.
Conclusion These first genotype data on a "human alcohol knock-out" intervention underscore important differences between the three genes. PNPLA3 seems to primarily drive fibrosis through inflammation while MBOAT7 seems to have a direct effect on fibrosis.

P 1.24 Mechanically stimulated hepatic stellate cells release WNT5A that triggers apoptosis in HCC cell lines

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WNT5A is a ligand of non-canonical WNT signaling and highly expressed in liver fibrosis and cirrhosis leading to hepatocellular carcinoma (HCC). Activated hepatic stellate cells (HSC) and myofibroblasts are major sources of WNT5A in the liver. Here we report that isolated rat HSC exposed to fluid shear stress, stretching, or stiff substrate release significantly more WNT5A than the respective controls. These mechanical stimuli mimic altered blood flow and tissue stiffness of diseased liver. Among genes that show increased expression in HSC after applying mechanical forces, the mechanically gated ion channel PIEZO1 was identified. CRISPR/Cas9-mediated knockout of Piezo1 in HSC lowered WNT5A release in response to mechanical stimuli, indicating that PIEZO1 is a mechanosensor in HSC. Interestingly, α-smooth muscle actin-positive myofibroblasts that surround HCC co-expressed PIEZO1. Therefore, we tested if recombinant rat WNT5A could affect the proliferation of the rat HCC cell lines H4IIE and N1S1. Treatment of the tumor cells with 1 µg/ml WNT5A significantly reduced the cell proliferation of both HCC cell lines within 4 hours as investigated by bromodeoxyuridine assays. TUNNEL assays indicated that WNT5A triggered apoptosis in the adherent growing H4IIE line. These findings are in line with earlier studies that reported apoptosis in WNT5A-treated tumor cells from other organs. However, proliferation of tumor cells in response to WNT5A treatment has also been described. Further studies are needed to clarify whether transcript variants of WNT5A or an alternative signaling modify the outcome of non-canonical WNT pathways and may enable an escape of tumor cells from apoptosis.

P 1.25 Interference of TGFb with the activation state of liver macrophages and consequences for liver injury and regeneration

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Changes that macrophage populations of the liver undergoes in terms of composition and activation state in the context of liver regeneration are incompletely understood. This and the question of what influence cell populations that significantly influence the microenvironment of the liver, such as hepatocytes, have on the activation state of liver macrophages and which mediators are relevant in this context was the aim of the present investigations.
Analysis of the changes in the macrophage populations of the liver in vivo after partial hepatectomy (PHx) by flow cytometry and single cell sequencing. Investigation of intercellular communication by proteomic/secretomic/transcriptomic analyses using a coculture model.
In this study, a CCR2-dependently recruited macrophage subpopulation, characterized by high expression of CD11b and CD14 was identified that is rapidly recruited into the liver after PHx, reaching its maximum just one day after PHx. Under homeostatic conditions, this F4/80 + /CD11bhigh/CD14high macrophage population exhibits a particular polarization, which is temporarily lost after PHx, but reappears during the late phase of the regeneration process. Thereby, the availability of active TGFb plays a role in the intercellular communication network by which hepatocytes can influence polarization of this macrophage population. Lack of the TGFbRII in macrophages has particular influence on function and gene expression of F4/80 + /CD11bhigh/CD14high macrophages and results in prolongation of the proliferation phase of hepatocytes and accelerated regeneration after PHx.
TGFbRII mediated signaling influences in particular the activation state of macrophages CCR2 dependently recruited in the liver and thereby limits hepatocyte proliferation and aggravates injury caused by the surgical intervention.

P 1.26 KIF12 variants and disturbed hepatocyte polarity in children with a phenotypic spectrum of cholestatic liver disease

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Objectives and study Recently, KIF12 has been identified as a cholestasis-associated candidate gene. As KIF12 is a member of a microtubule-associated motor protein family involved in organizing the cytoskeleton and intracellular transport, KIF12-associated cholestasis is assumed to be a result of disturbed cell polarity.
We describe six cases with likely pathogenic KIF12 variants from four unrelated families, their different phenotypes and our investigations to study hepatocyte polarity.
Methods Children with familial cholestasis and a likely pathogenic variant in the KIF12 gene were identified by NGS screening. Parents and siblings were tested for the variants to analyze segregation. Immunofluorescence imaging of apical markers MRP2 und BSEP, basolateral marker OATP1B1, tight junction protein ZO-1 and KIF12 itself was performed on patient's liver tissue sections.
Results We detected two different homozygous KIF12 variants in five patients (4 patients: nonsense variant; 1 patient: splice site deletion). Segregation analyses confirmed autosomal recessive inheritance. The patient's clinical manifestation ranged from neonatal cholestasis with complete clinical remission, or absent clinical symptoms with the diagnosis made incidentally, to a progressive course ending in liver transplantation. Immunofluorescence imaging of liver sections of KIF12 patients revealed an ectopic cytoplasmic MRP2 staining. BSEP, and partly ZO-1 staining appeared in long clustered structures. KIF12 and OATP1B1 staining was widely unremarkable.
Conclusion Our results strongly support pathogenic KIF12 variants as cause for familial cholestatic liver disease and suggest that these variants result in functional cell polarity disturbance. Due its wide clinical presentation with even asymptomatic cases, KIF12-associated cholestatic liver diseases are potentially underdiagnosed.

Poster Visit Session ll Clinical Hepatology, Surgery, LTX
28/01/2022, 15.55 pm – 16.40 pm

P 2.01 Evaluation of Interleukin-6 for stepwise diagnosis of minimal hepatic encephalopathy in patients with liver cirrhosis

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DOI 10.1055/s-0041-1740690

Minimal hepatic encephalopathy (MHE) is associated with poor quality of life and dismal prognosis. Psychometric testing is time consuming and therefore often neglected in clinical practice. Thus, biomarkers are needed to stratify patients at risk for MHE.

This study aimed to evaluate the diagnostic accuracy of interleukin-6 (IL-6) serum levels as part of a stepwise diagnostic algorithm to detect MHE in cirrhotic patients. The development cohort comprised 197 prospectively recruited patients without evidence of HE grade 1–4. 52 patients served as the independent validation cohort. The Psychometric Hepatic Encephalopathy Score (PHES) was used for MHE diagnosis. In total, 50 (25.4 %) patients of the development cohort were diagnosed with MHE. Serum IL-6 levels were more than twice as high in patients with MHE as in patients without MHE (16 vs 7 pg/ml, $p < 0.001$). In logistic regression analysis, elevated IL-6 levels remained independently associated with MHE (OR 1.036, 95 %CI 1.009–1.064, $p = 0.008$) after adjusting for other variables such as MELD, albumin, CRP and history of ascites. Using a cut-off value of IL-6 ≥ 7 pg/ml would have avoided subsequent time-consuming psychometric testing in 38 % of all patients (sensitivity 90 %, 95 %CI 77 %-96 %; negative predictive value 93 %, 95 %CI 84 %-98 %). These results were confirmed in the validation cohort (sensitivity: 94 %, NPV: 93 %).

IL-6 serum levels may serve as biomarker in a stepwise diagnostic algorithm reducing the number of patients requiring testing with PHES. In particular, IL-6 would be helpful in patients being incapable of performing other tests e. g., due to language barriers.

P 2.02 Subclassification of human hepatic hemangiomas reveals cellular and functional heterogeneity

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DOI 10.1055/s-0041-1740691

Background and Aims Despite the high incidence, surgical resection of hepatic hemangioma (HH) is seldomly required and data on HH temporal evolution, biological function and marker expression remain relatively scarce. Limited availability of tissue and a strong bias of resected specimens towards progressive disease has made the development of a disease stage- and phenotype-specific classification difficult, which is currently still missing.

Methods A tissue microarray with 1.5 mm core size consisting of 98 HH, 69 paired tumor-liver interface regions and 66 distant liver tissues was generated. Automatic cell detection, positive cell counts of 11 immunohistochemical markers and vascular morphometry were determined using QuPath. CAT/MRI imaging data of 28 resected patients and RNA-seq data derived from experimental HH were analyzed.

Results HH were characterized by a pronounced inter- and intrapatient heterogeneity regarding macroscopic size, cellular composition, vascular architecture and flow patterns. While highly regressed HH were defined by reduced blood vessel density and flow, cavernous hemangiomas displayed a more pronounced cellular heterogeneity as seen by ERG positive cell counts and EC marker expression (CD31, CD34, THBD). Furthermore, a HH subgroup was identified, which was determined by high cell density and cellular senescence-specific marker expression. Enrichment analysis of experimental HH expression data revealed striking differences of vascular development regulating genes already in early stages of HH development.

Conclusion/Outlook HH may be classified into stage-, morphological- and phenotype-specific subgroups that may identify patients with risk of progressive disease. Current research focuses on the identification of molecular markers that promote disease onset and progression.

P 2.03 Platelet function testing in patients with liver cirrhosis and TIPS implantation

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Background Transjugular intrahepatic portosystemic shunt (TIPS) implantation is an effective treatment of portal hypertension in patients with liver cirrhosis. The use of covered stents has reduced TIPS thrombosis. However, the role of anticoagulation or platelet inhibition after TIPS implantation is not clear and different approaches are used. In order to establish strategies for platelet inhibition after TIPS, the functionality of platelets in patients with portal hypertension has to be clarified. The aim of our study was to assess platelet function before and after TIPS implantation.

Methods Platelet aggregation was tested in patients before TIPS implantation, 4 and 30 days following the procedure using light transmission aggregometry (LTA) and whole blood impedance aggregometry (WBIA). Surface platelet activation markers and platelet-neutrophil complexes (PNCs) as inflammatory marker were assessed using flow cytometry. Thrombin receptor activating peptide 6 (TRAP-6), adenosine diphosphate (ADP) and arachidonic acid (AA) were used as agonists.

Results Platelet aggregation values were reduced in WBIA, they were mostly within the normal range in LTA. P-selectin expression and GPIIb/IIIa activation were low at baseline and increased after stimulation with a diminished GPIIb/IIIa activation in response to TRAP-6. PNCs were already present at baseline with lower percentages 30 days following TIPS.

Conclusions PNCs were present in patients with TIPS implantation while platelet pre-activation was not observed. The lower percentages of PNCs 30 days after TIPS may be due to a reduced inflammatory state. This and the discrepancy of platelet function testing in WBIA compared to LTA needs to be further investigated.

P 2.04 Anwendbarkeit des EncephalApp Stroop Tests als Screening Tool für minimale hepatische Enzephalopathie bei Patienten mit Leberzirrhose in Deutschland

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DOI 10.1055/s-0041-1740693

Einleitung Die minimale hepatische Enzephalopathie (MHE) ist eine häufige Komplikation bei Leberzirrhotikern und ist prognostisch schlecht. Die EncephalApp_Stroop ist ein Smartphone-basierter und einfach durchzuführender Test, um Patienten auf eine MHE zu testen. Die EncephalApp_Stroop wurde bis dato nicht in einem deutschsprachigen Kollektiv getestet und länderspezifische Schwellenwerte fehlen.

Methods 76 Patienten mit Leberzirrhose wurden in diese prospektive Studie eingeschlossen. Patienten wurden mit der EncephalApp_Stroop und dem Psychometric hepatic encephalopathy score (PHES) getestet. Es wurde eine AUC ROC Analyze (eng. Area under the curve receiver operating characteristic) durchgeführt, um zwischen Patienten mit und ohne MHE zu unterscheiden. Es wurden Schwellenwerte für eine gleichgewichtete Sensitivität und Spezifität (Youden-Index), als auch für eine optimierte Sensitivität berechnet.

Ergebnisse Die besten Werte, basierend auf den AUROC Analysen, wurden für on Zeit (AUC 0.85, 95 % CI 0.75; 0.94) und on + off Zeit (AUC 0.85, 95 % CI 0.76; 0.94) ermittelt.

Der Youden-Index gewichtete Schwellenwert für on + off Zeit war > 224.7 s und erreichte eine Sensitivität von 71 % (95 % CI 48;88) und eine Spezifität von 87 % (95 % CI 75;94). Der für eine bessere Sensitivität (Sensitivität 2: Spezifität 1) optimierte Schwellenwert für on + off Zeit lag bei 179.8 s und erreichte eine optimierte Sensitivität von 95 % (95 % CI 74;100) und einem negativen prädiktiven Wert von 97 % (95 % CI 81;100).

Fazit Wir haben erstmalig die Anwendbarkeit der EncephalApp_Stroop zur Diagnostik einer MHE in einer deutschen Kohorte von Patienten mit Leberzirrhose validiert und bestimmten Youden-Index gewichtete als auch Sensitivität optimierte Schwellenwerte.

P 2.05 Insights into the clinical course and patterns of bacterial infections in patients with liver cirrhosis

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Background and Aims Bacterial infections play an important role in terms of mortality and morbidity in patients with liver cirrhosis. Data on prevalence of bacterial infections and the associated pathogen spectrum in Germany are scarce. The aim of this study was to analyze potential associations between infections, bacteria isolated and mortality in hospitalized patients with liver cirrhosis.

Methods Consecutive non-electively hospitalized patients between March 2019 and June 2021 with liver cirrhosis admitted to the Cirrhosis Center Mainz were included into the study. All patients underwent clinical, microbiological or laboratory testing to detect potential infections. Baseline characteristics and data regarding infections were assessed.

Results In total, 239 patients with a median age of 60 years were included into the study. The most frequent aetiology of liver cirrhosis was an alcoholic liver disease (62.3 %). A bacterial infection could be detected in 33.5 % (n = 80) at study inclusion. 69 patients (28.9 %) developed a nosocomial infection during hospital stay. The most common locations of nosocomial infections were respiratory (30.4 %) and the urinary tract (20.3 %). Bacterial infections were the most common cause of death (49.3 %) and remained an independent predictor for a higher mortality in multivariable analysis after adjusting for other variables.

Conclusion Bacterial infections are a common reason for non-elective hospitalizations of patients with liver cirrhosis and have a detrimental effect on the 30-day survival of patients with liver cirrhosis. The pathogen spectrum changed from an initial gram-negative spectrum in community acquired infections to a more gram-positive spectrum in hospital acquired infections.

P 2.06 Hepatic Sarcoidosis: Clinical Characteristics and Outcome

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Background Clinical manifestation of hepatic involvement in sarcoidosis can vary from asymptomatic disease to severe complications such as liver cirrhosis and portal hypertension. However, data on hepatic sarcoidosis are limited and evidence-based recommendations are lacking. Our study aimed to assess the features and clinical course of hepatic sarcoidosis in a predominantly Caucasian cohort.

Methods We performed a retrospective study including all patients with hepatic sarcoidosis between 2004 and 2020 in 5 German centers. The median follow-up time was 36 months (range 0.0, 195). Data on demographic parameters, clinical manifestations, treatment and outcome were collected.

Results A total of 1476 patients with sarcoidosis and 62 patients with hepatic involvement (4.2 %) were identified. 51.6 % were female and 80.6 % Caucasian. Most patients were asymptomatic and were observed to have a cholestatic pattern of liver enzyme elevations. Liver cirrhosis was detected in 9 patients (14.5 %), of whom 6 developed clinical manifestations of portal hypertension. 54 patients were medically treated, most commonly with glucocorticoids (69.4 %) or ursodeoxycholic acid (UDCA) (40.3 %). Levels of ALP decreased 60.8 % on average from baseline in patients treated with glucocorticoids and 59.9 % in patients treated with UDCA. None of the patients underwent liver transplantation or developed hepatocellular carcinoma (HCC). Three of the patients died during follow-up due to liver-related complications.

Conclusions Hepatic involvement in sarcoidosis was found in 4.2 % of patients and was clinically significant in 14.5 % of those. These findings highlight the importance of early identifying and monitoring hepatic sarcoidosis, given its increased mortality when associated with end-stage liver disease.

P 2.07 Hepatic decompensation after transarterial radioembolization (TARE) – a retrospective analysis of risk factors and outcome of patients with HCC

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Introduction Hepatic decompensation (HD) is a severe complication after TARE, associated with significant morbidity and mortality. The aim of this study was to identify prognostic factors of HD and outcome after TARE in patients with HCC.

Methods 61 HCC-patients treated with TARE were retrospectively included. HD was defined as an increase of bilirubin (minimum CTCAE grade 3) or newly developed ascites not explained by tumor progression 3 months after TARE. Logistic-regression-models were performed to analyze predictive factors of HD. Survival was assessed by Kaplan-Meier-estimator and multivariable Cox-regression-models were performed for analysis of prognostic factors.

Results 17 patients developed HD during follow-up. Patients with HD presented with higher ALBI-score (-2.1 [-1.7;-2.5] vs. -2.7 [-2.3;-3.1], p < 0.001) and CRP (16.9 mg/l [7.1;35.0] vs. 6.0 mg/l [3.8;12.1], p = 0.007) pre-treatment compared to patients without decompensation. ALBI-grade (2 vs. 1; OR 20.13 [3.21;125.9], p = 0.001) and higher Yttrium-90-activity (OR 4.60 [1.13;18.74], p = 0.033) were independent risk factors for the development of HD. The median survival of patients with HD was significantly reduced (3 ± 0.94 vs. 13 ± 6.6 months, p < 0.001). HD significantly increased the mortality-risk, adjusted for age, liver cirrhosis, portal vein thrombosis, number of HCC-lesions, Yttrium-90-activity, and creatinine (HR 3.22 (1.49;6.93), p = 0.003).

Conclusion HD after TARE leads to a 3-fold increase of mortality in HCC-patients. Thus, prevention of HD is crucial in improving outcome of these patients.

Current guidelines recommend TARE for patients with bilirubin-levels < 2 mg/dl. We suggest rather assessing liver function by ALBI-score as a valid predictor of HD in patients with HCC.

P 2.08 Novel in situ hybridization and multiplex immunofluorescence technology combined with whole-slide digital image analysis in liver tissue

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Background The elusive nature of assessing immunological processes in situ is one of the major impediments to improve diagnostics and treatment. Here, we present a proof-of-concept study using in situ hybridization in combination with multiplexed immunofluorescence (mIFISH) to detect low-abundance cytokines and perform cell phenotyping in formalin-fixed paraffin-embedded (FFPE) human liver tissue. The aim of the study was to validate novel mIFISH technology to standard assays as immunohistochemistry (IHC), chromogenic ISH (cISH/RNAscope) and qPCR.

Methods FFPE tissue of NASH liver (n = 3), non-NASH liver (n = 3) and tonsil (n = 3) were assessed for CD45 + leucocyte infiltration and CXCL9 mRNA expression levels with mIFISH, IHC, cISH and qPCR. Quantitative analysis of histological stainings was performed with an automatic machine-learning algorithm on whole-slide sections. Cell density was expressed as cells/mm^2, mRNA expression levels using ISH were expressed as the dot area in µm^2/mm^2.

Results Leucocyte density and CXCL9 expression levels were highest in tonsil (9583 cells/mm^2, 5428 µm^2/mm^2), followed by NASH liver (542 cells/mm^2, 210 µm^2/mm^2) and non-NASH liver (527 cells/mm^2, 82 µm^2/mm^2). The mIFISH assay strongly correlated with IHC for CD45 ($r = .99$, $p < .001$), with cISH for CXCL9 ($r = .99$, $p < 0.001$) and with mRNA expression levels detected by qPCR (CD45 $r = .78$, $p = .014$; CXCL9 $r = .82$, $p = .007$).

Conclusion mIFISH assay is comparable to gold standard assays as IHC or qPCR. Further, this study demonstrates the reliability of mIFISH technology in FFPE tissue to better assess immunological processes in liver tissue.

P 2.09 Successful surgical-multimodal treatment for vaccine-induced complete splanchnic vein thrombosis after ChAdOx1 nCoV-19 vaccination

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Objective Thrombotic-thrombocytopenic events are rare, but life-threatening, complications after ChAdOx1 nCoV-19 vaccination and sometimes present as symptomatic splanchnic vein thrombosis with critical illness. Life-saving aggressive and multimodal treatment is essential in these cases.

Design We report on a critically ill 40-year-old male patient with complete splanchnic (portal/mesenteric/splenic) vein thrombosis, becoming symptomatic 7 days after ChAdOx1 nCoV-19 vaccination and diagnosed on day 12. Laparotomy for abdominal compartment syndrome and repeated transjugular/transhepatic interventional and open surgical thrombectomy procedures were performed. Additional therapy consisted of thrombolysis with recombinant tissue-type plasminogen activator over 5 days, anticoagulation (argatroban), platelet inhibition (Acetylsalicylic acid /clopidogrel), immunoglobulins and steroids.

Results This aggressive treatment included 5 laparotomies and 4 angiographic interventions, open abdomen for 8 days, transfusion of 27 units of packed red cells, 9 abdominal and 4 cerebral CT scans, thrombolysis therapy for 5 days, mechanical ventilation for 15 days, and an ICU stay of 25 days. Full patient recovery and near complete recanalization of splanchnic veins was achieved.

Conclusion Without treatment, ChAdOx1 nCoV-19 vaccination-induced total splanchnic vein thrombosis has serious consequences with a high risk for death. The case described here shows that an aggressive multimodal surgical-medical treatment strategy in a specialized center can save these patients and achieve a good outcome.

P 2.10 Elevated serum levels of methylglyoxal are associated with impaired liver function in patients with liver cirrhosis

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Background Methylglyoxal (MGO) is a highly reactive dicarbonyl species that forms advanced glycation end products (AGEs). The binding of these AGEs to their receptor (RAGE) causes and sustains severe inflammation. Systemic inflammation is postulated to be a major driver in the progression of liver cirrhosis. However, the role of circulating MGO levels and its association with disease severity in patients with liver cirrhosis remains unknown.

Methods A total of 51 patients with a diagnosis of liver cirrhosis of mixed etiology were prospectively enrolled. Serum levels of MGO (ng/ml) were analyzed using high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Clinical and laboratory assessment was performed at baseline.

Results In this cohort, 51 % of patients showed a compensated (n = 26) and 49 % (n = 25) a decompensated liver cirrhosis. Patients with a Child-Pugh C liver cirrhosis showed the highest MGO levels ($p < 0.001$) than with a Child-Pugh A and B liver cirrhosis. In a multivariable regression analysis, high MGO levels remained independently associated with impaired liver function, as assessed by the model for end-stage liver disease (MELD) ($\beta = 0.448$, $p = 0.002$) and acute decompensation (AD) ($\beta = 0.345$, $p = 0.005$) scores. Furthermore, MGO was positively correlated with markers of systemic inflammation (IL-6, $p = 0.004$) and the development of ascites ($p = 0.013$).

Conclusion Circulating levels of MGO are elevated in advanced stages of liver cirrhosis and are associated with impaired liver function and liver-related parameters.

P 2.11 Partial splenic embolization used for rescue treatment for variceal bleeding - monocentric experience over four years

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Partial splenic embolisation (PSE) is a non-surgical procedure, which was first used for treatment of primary and secondary hypersplenism. Nowadays PSE is used to treat various diseases including esophageal and gastric variceal hemorrhage due to portal hypertension and/or splenic vein thrombosis.

A series of eleven patients with esophageal varices Grade III-IV is presented. At the University Hospital Regensburg all patients were successfully treated with PSE as rescue therapy. All patients had a significant portal hypertension due to various underlying diseases (e. g. factor V Leiden, JAK2 Mutation, Polycythemia vera, primary sclerosing cholangitis). Endoscopic ligature of varices was not promising due to fundal varices. Placement of a transjugular intrahepatic portosystemic shunt (TIPSS) was not possible due to local anatomy. In all eleven

cases, PSE was successfully applied. Control gastroscopy showed a significant regression of esophageal and gastric varices, now classified as Grade II or lower. No patient showed an episode of bleeding after PSE was performed. Hematologic indices improved. Serious complications of PSE were not observed.
PSE has benefited from advances in the available technology and from improvements of the applied protocol. In patients with portal hypertension it has been shown to induce significant and sustained improvements in both liver function and hematologic indices, as well as an 80 % reduction in annual bleeding episodes in patients with recurrent variceal hemorrhage.
The eleven cases of patients demonstrate that PSE is a rescue therapy option for patient with esophageal varices where endoscopic treatment or placement of a TIPSS is not promising or possible.

P 2.12 Klinischer Verlauf und Therapieadhärenz nach Ösophagusvarizenligaturen

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Einleitung Ösophagusvarizen werden bei 40–60 % der Patienten mit Leberzirrhose diagnostiziert. Neben einer medikamentösen Therapie mit nicht selektiven Betablockern ist die Therapie der Wahl die endoskopische Gummibandligatur. Nach 4–6 Wochen wird eine Kontrollendoskopie empfohlen.
Ziele Ziel der Analyse ist die Untersuchung der Therapieadhärenz und des klinischen Verlaufs von Patienten nach Ösophagusvarizenligaturen in der Primär- und in der Sekundärprophylaxe.
Methodik Es erfolgte eine retrospektive Analyse von Patienten mit Ösophagusvarizenligaturen in dem Zeitraum von 2010–2020 am Universitätsklinikum Schleswig-Holstein, Campus Lübeck.
Insgesamt wurden 140 Patienten eingeschlossen. Die Mehrheit der Patienten war männlich (72,3 %). Das mediane Erkrankungsalter war 57 Jahre (IQR: 51–68 Jahre). Bei 104 Patienten (79,4 %) war die Lebersynthese stark eingeschränkt (Child-B- bis C-Zirrhose).
Innerhalb der Erstintervention erfolgte n = 66 (46,8 %) elektiv und n = 74 (52,5 %) bei akuter Ösophagusvarizenblutung. Im Gesamtkollektiv erfolgte bei 41,6 % der Patienten (n = 32) Kontrollendoskopien nach 4–6 Wochen. Patienten mit elektiv geplanter endoskopischer Kontrolle wurden signifikant häufiger re-endoskopiert als die Notfallkohorte (75,8 % vs. 24,2 %; p < 0,001) und erhielten entsprechend signifikant häufiger eine Re-Ligaturbehandlung (53,0 % vs. 27,0 %; p = 0,002).
In der Subgruppe der elektiven Patienten war der Anteil der Patienten mit einer erneuten Ligaturbehandlung im Verlauf mit 53,0 % signifikant höher als in der Notfallgruppe (p = 0,002). Innerhalb der ersten 6 Wochen nach Ligatur sind in der Elektivgruppe 12 (18,1 %) und in der Notfallgruppe 30 (40,5 %) Patienten verstorben.
Schlussfolgerung Insbesondere Patienten mit Gummibandligatur bei akuter Varizenblutung haben eine sehr eingeschränkte Prognose.
Dieses Ergebnis betont die Wichtigkeit einer sorgfältigen Indikationsprüfung zum frühelektiven TIPS bei diesen Patienten.

P 2.13 Thyroid hormone alterations are associated with decompensated liver cirrhosis and acute-on-chronic liver failure

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Thyroid hormones (TH) are important regulators of hepatic- as well as immune-metabolism and may thereby impact the pathogenesis of liver cirrhosis and acute-on-chronic liver failure (ACLF). In the present study, we therefore determined associations between TH, clinical stages of liver cirrhosis including ACLF and TH targets in immune cells.
Patients with compensated (n = 163) or decompensated liver cirrhosis (n = 169) or ACLF (n = 36) were recruited from a prospective cohort study. TSH, FT3 and FT4 levels were determined by immunoassays. T cells were characterized for expression of TH target genes by RT-PCR.
TSH concentration in decompensated patients (2.81 ± 0.2mU/l) was increased compared compensated patients (2.01 ± 0.1mU/l, P = 0.03) and ACLF (2.18 ± 0.34mU/l, P = 0.42). Conversely, FT3 levels were significantly lower in patients with decompensated liver cirrhosis (3.80 ± 0.06pmol/l) compared to compensated liver cirrhosis (4.79 ± 0.06pmol/l, P < 0.0001), and even lower in ACLF (3.24 ± 0.15pmol/l, P < 0.01). Decreased FT3 concentrations were associated with infections in decompensated patients (3.45 ± 0.10pmol/l vs. 3.90 ± 0.07pmol/l, P = 0.0009). Furthermore, low FT3 levels and a low FT3/FT4 ratio were associated with mortality (P < 0.001). Expression of TH targets, such as TH receptor α, were altered in T cells derived from decompensated patients or ACLF.
Decompensated cirrhosis complicated with infections and ACLF with or without infections are associated with profound alterations in the TH balance resembling low T3 syndrome. Strikingly, low FT3 level and FT3/FT4 ratio are associated with increased mortality in patients with liver cirrhosis. Decreased TH receptor α expression suggests impaired TH signaling in T cells of patients with liver cirrhosis possibly contributing to reduced survival.

P 2.14 Getunnelte Peritonealkatheter als alternative Behandlungssmöglichkeit von therapierefraktärem Aszites bei Patienten mit Leberzirrhose

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Einleitung Eine Leberzirrhose kann zu refraktärem Aszites (RA) führen. Sollte keine Lebertransplantation möglich sein, stellt die Anlage eines transjugulären portosystemischen Shunts (TIPS) die Therapie der ersten Wahl dar. Jedoch kann dies nicht jedem Patienten angeboten werden, da Kontraindikationen vorliegen können. In solchen Fällen sind Patienten auf rezidivierende Parazentesen angewiesen. Eine Alternative könnte die Anlage eines permanenten, getunnelten Peritonealkatheter (PeKa) darstellen. Diese Studie untersucht den Verlauf von Patienten mit RA und PeKa-Anlage im Vergleich zu Patienten mit Standardbehandlung (SOC).
Patienten und Methoden Insgesamt wurden 223 Patienten mit RA und Kontraindikationen für eine TIPS Anlage retrospektiv erfasst. Alle Patienten befanden sich zwischen 2012 und 2020 hospitalisiert an der Medizinischen Hochschule Hannover und erhielten mindestens eine Parazentese. Endpunkte waren 90-Tages (90-d) Überleben, 90-d Inzidenz von Hyponatriämien ≤ 120mmol/l, sowie die 90-d SBP-Inzidenz.
Ergebnisse Ein PeKa wurde bei 152 Patienten implantiert, 71 Patienten erhielten SOC. Individuen mit PeKa waren älter(60 Jahre vs. 55 Jahre, p < 0.001), hatten häufiger eine SBP durchlebt(59 % vs. 25 %, p < 0.001), höhere S-Albumin-Werte (30g/l vs. 26g/l, p = 0.001) und niedrigere Thrombozyten(112.000/µl vs. 140.000/µl, p = 0.04). Insgesamt war die SBP Inzidenz im Verlauf vergleichbar (p = 0.82). Patienten mit PeKa erlitten im Verlauf eine nummerisch höhere Anzahl an Hyponatriämien(p = 0.09). Patienten mit PeKa hatten im Vergleich zu SOC ein besseres 90-d Überleben (p = 0.03). In einer Cox-Regression, welche für Alter, Thrombozyten, MELD, Z.n. SBP und S-Albumin adjustierte, war ein PeKa knapp nicht signifikant mit einem verbesserten Überleben assoziiert(HR: 0.52, p = 0.05).
Schlussfolgerung Ein PeKa kann eine Alternative für Patienten mit RA und Kontraindikation für TIPS-Anlage darstellen.

P 2.15 Safety and efficacy of the JAK-inhibitor tofacitinib in patients with primary sclerosing cholangitis: a multicentre, retrospective study

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Background/Aims We collected data on Primary Sclerosing Cholangitis (PSC) patients with associated inflammatory bowel disease (IBD) who received past or ongoing treatment with tofacitinib to assess safety and efficacy of tofacitinib on liver and bowel disease.

Method Data was collected retrospectively prior to baseline, after 3 and 12-months follow-up.

Results 42 patients with large duct PSC (69 % male) were included with a median age at diagnosis of 32 years (14–61). In 47,6 % treatment with tofacitinib was stopped, in 15/20 due to lack of efficacy, after a median treatment period of 7 months (1–21).

Colitis activity improved in 57,6 %. Faecal calprotectin dropped from a median of 858,2 ug/g (26,7 – 2000) at baseline to 508,2 ug/g (15 – 2327,p = 0.064). At follow up, one patient had developed high-grade dysplasia and one patient colorectal carcinoma.

For those still on treatment, median ALP was 150 U/l (56 – 793) at baseline and 132 U/l (47 – 558,p = 0.039) at 12-months follow-up.

Overall, there was no deterioration in liver biochemistry after commencing tofacitinib. One patient had a severe infection, no event of thromboembolism was reported during treatment with tofacitinib. No new cases of hepatobiliary malignancy occurred.

Conclusion In a retrospective analysis of 42 patients with PSC and associated colitis, treatment appeared to be well tolerated and without significant worsening of liver enzymes over a median treatment period of 13 months (1–33). The majority of patients, most of them with several prior treatment regimens, demonstrated an improvement in their colitis activity.

P 2.16 Risk factors for a suboptimal immune response to SARS-CoV-2 vaccination in liver transplant recipients

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Background Liver transplant (LT) recipients frequently show no or low response after two SARS-CoV-2 vaccinations. However, the relevance of different clinical risk factors (RF) for a suboptimal response is still unanswered.

Methods Anti-SARS-CoV-2 antibody titers of 141 LT patients determined after the second vaccination assigned them to low (< 100 BAU/ml) or high response. The relevance of previously identified clinical RF for low response (diabetes, chronic kidney injury, hypertension or age > 65y) and antiproliferative immunosuppression were now analyzed in detail.

Results The full clinical data set was available in 101 patients (55 low, 46 high responders). In total, 82 % of low and 52 % of high responders had one or more clinical RF. The risk of low response for patients having at least one, two or three clinical RF increased from 31 % (N = 10 of 32) to 65 % (N = 45 of 69), 86 % (N = 32 of 37) and 93 % (N = 13 of 14), respectively. If all four RF were present, the risk of low response increased to 100 % (N = 6 of 6). Also, a more frequent use of MMF or mTOR-inhibitors was detected in low responders (74 %) compared to high responders (37 %). Of the 26 % (N = 12) of low responders not receiving antiproliferative immunosuppression the majority had one (25 %) or more (50 %) clinical RF.

Conclusion If clinical RF are present, the risk of low SARS-CoV-2 vaccination response increases 1.6-fold and with the number of RF. These data can help to identify patients under immunosuppression with the highest risk of suboptimal response to further SARS-CoV-2 vaccinations.

P 2.17 Machine learning models predicting decompensation in cirrhosis

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Background & Aim Since decompensation of cirrhosis significantly increases patients mortality, the prevention and early treatment is paramount. We applied machine learning techniques to identify parameters predicting decompensation.

Methods Using Python, Keras, and Scikit-Learn, several machine learning techniques including Random Forests, Neural Networks and Support Vector Machines (SVM) were trained and tested with 85:15 split on the INCA trial database containing 1,415 patients with cirrhosis from three German university hospitals. In addition to laboratory values and anamnestic data, genetic data including NOD2 genotypes were analysed. Permutation features importance (PFI) as model inspecting technique evaluated the impact of features on the prediction of decompensation.

Results At the index date, 313 patients were always compensated, 354 patients were decompensated before, and 748 were currently decompensated. 825 patients (46.5 % decompensated) attended follow up. SVM showed the best performance in predicting decompensation, achieving an accuracy of 84.1 % for the training- and 77.7 % for the test data set (retrospective assessment) and 78.4 % respectively 73.8 % (prospective assessment). PFI revealed baseline levels of albumin, bilirubin and minimum serum sodium concentration were highest ranked to assess former decompensation. Maximum level of bilirubin and baseline levels of sodium and albumin were highest ranked for prospective data. In addition to parameters of established scores including MELD and Child-Pugh, NOD2 genotype and parameters related to infections were highly ranked.

Conclusions Among various machine learning models, the highest accuracy to predict decompensation was found for SVM. In addition to classical laboratory parameters, genetic factors and infections were critical parameters for individual predictions.

P 2.18 Sonographic Criteria of Budd-Chiari Syndrome

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Budd-Chiari syndrome (BCS) is a rare liver disease defined as obstruction of the hepatic venous outflow. Numerous case reports illustrate the possibilities of imaging in individual patients, but systematic data processing to optimize the diagnostic approach in BCS is lacking. This study aims at identifying diagnostic ultrasound criteria for BCS diagnosis in a multicenter-cohort with 63 patients from Germany (n = 20), Italy (n = 15) and China (n = 28).

The mean age of all patients was 43.7 years. Symptoms were mostly unspecific in the Chinese and the German cohort, but everyone was symptomatic. In the Italian group, 27 % were asymptomatic.

In the whole sample, thrombus localisation was distributed equally (thrombus in right hepatic vein 35 %, left hepatic vein 33 %, middle hepatic vein (MHV) 27 %, Vena cava inferior (VCI) 35 % and Confluence 22 %). In the Chinese sample, thrombosis of the VCI was the most frequent localisation (71 %) whereas in Italy, thrombus of the MHV (57 %) and in Germany, thrombus of the Confluence area (60 %) where the most frequent localisations. BCS mostly occurred in a chronic course (82 %). Portal venous thrombosis was present in 8 cases (15 %), none in the Chinese group. The liver presented mostly enlarged, 7 cases in China showed a smaller liver size. Vascular collaterals were visualizable in 93 %. The collaterals were localized intra-and extrahepatically in 51 %, in 36 % they were only present intrahepatically.

In this study, sonographic signs of BCS were evaluated in patients from different continents for the first time. Patients differ in symptoms and disease manifestations in the different subgroups.

P 2.19 The immunomodulatory receptor VSIG4 is released during spontaneous bacterial peritonitis and predicts short-term mortality

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Background and Aims V-set immunoglobulin-domain-containing 4 (VSIG4) is an immuno-modulatory macrophage complement receptor modulating innate and adaptive immunity and affecting the resolution of bacterial infections. Given its expression on peritoneal macrophages, we hypothesized a prognostic role of peritoneal VSIG4 concentrations in patients with spontaneous bacterial peritonitis.

Methods We isolated PM from patients with cirrhosis and analyzed VSIG4 expression and release by flow cytometry, quantitative real-time PCR, and confocal microscopy. We measured soluble VSIG4 concentrations in ascites from 120 patients with SBP and 40 patients without SBP and investigated the association of ascitic soluble VSIG4 with 90-days survival after SBP using Kaplan-Meier statistics, Cox regression, and competing-risks regression analysis.

Results VSIG4 expression was high on resting, large peritoneal macrophages, which co-expressed CD206, CD163, and MERTK. VSIG4 gene expression in PM decreased in patients with SBP and normalized after resolution. During SBP, VSIG4hi PM were decreased and soluble VSIG4 in ascites were higher than in patients without. PM activation by TLR agonists or infection with live bacteria in vitro resulted in a loss of surface VSIG4 and the release of soluble VSIG4. Mechanistically, shedding of VSIG4 from PM was protease-dependent and susceptible to microtubule transport inhibition. Soluble VSIG4 in ascites exceeded serum concentrations and correlated with serum creatinine, MELD score and C-reactive protein during SBP. Concentrations of 1.0206 µg/mL or higher indicated increased 90-days mortality.

Conclusions VSIG4 is released from activated PM into ascites during SBP. Higher peritoneal VSIG4 levels indicate patients with organ failure and poor prognosis.

P 2.20 Serum proteomic characterisation in acute liver failure

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Background and aims Acute liver failure (ALF) is defined as a rapid onset hepatocellular dysfunction with associated coagulopathy in patients without known liver disease. Liver transplantation represents the only effective treatment for life-threatening ALF, but the decision for/against transplantation remains challenging. The aim of our study was to identify potential serum prognostic factors using a systematic mass spectrometry-based approach from hospitalized ALF patients.

Methods Serum protein patterns were compared between 30 controls, 66 individuals with acetaminophen (APAP) and 53 with non-APAP-associated ALF. The latter were randomly selected from the US ALF study group database; sera were obtained within 24 hours of study admission. Non-survivors were defined as patients, who passed away or required liver transplantation within 21 days of admission. Machine-learning was applied to identify proteins differing between the groups.

Results 177 proteins were detected in at least 50 % of analysed individuals, 155 of them varied significantly between controls and ALF cases. 125 proteins differed between patients with APAP- and non-APAP related ALF. A four-protein signature discriminated well between APAP and non-APAP ALF cases (AUROCs 0.93). 25 proteins were significantly altered between ALF survivors and non-survivors. A two-protein score reached a fair discriminative power (AUROC 0.80) that surpassed the power of currently used composite scores (i. e., MELD AUROC 0.76).

Conclusion Shotgun proteomics is a promising tool to distinguish between different ALF aetiologies and help identify patients likely to require liver transplantation.

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P 2.21 MARC1 polymorphism is associated with decreased markers of liver injury and enhanced antioxidant capacity in patients with AIH

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Background The progression of autoimmune hepatitis (AIH) varies between patients, which suggests the role of genetic variants. Recent analyses demonstrated protective effects of the MARC1 p.A165T polymorphism in patients with fatty liver. Here, we analyse the MARC1, as well as the HSD17B13, PNPLA3, TM6SF2 and MBOAT7 variants in patients with AIH.

Patients and methods The study cohort was composed of 313 non-transplanted adults with AIH, as well as of a group of 30 patients who underwent liver transplantation for AIH. The MARC1, HSD17B13, PNPLA3, TM6SF2 and MBOAT7 variants were genotyped using TaqMan assays. Analysis of mitochondrial damage markers in serum was conducted in relation to the MARC1 polymorphism.

Results In patients with the minor MARC1 allele we detected lower ALT and AST activities ($P < 0.05$). After treatment for ≥ 6 months, carriers of the MARC1 polymorphism had lower AST, ALP, GGT ($P \leq 0.01$), and lower APRI ($P = 0.02$). Analyzes of patients' sera showed that patients carrying the protective MARC1 genotype had higher total antioxidant and catalase activities. Presence of the PNPLA3 polymorphism was associated with higher MELD score ($P = 0.02$), whereas the MBOAT7 polymorphism enhanced the odds of developing hepatocellular cancer (HCC; OR = 3.71). None of the variants modulated the risk of transplantation or death during the follow-up.

Conclusions Patients with AIH who carry the MARC1 polymorphism exhibit a less pronounced liver injury and improved resistance to oxidative stress. Genotyping of the MARC1, PNPLA3 and MBOAT7 polymorphism might help to stratify the risk of progressive AIH.

P 2.22 Comparative analysis of muscle quantification by sonography and computed tomography

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Background and Aims Sarcopenia is associated with frailty and reduced quality of life. Especially in patients with liver cirrhosis, sarcopenia has a high impact on mortality. Currently, cumbersome diagnostics are needed to determine low muscle quantity. Sonographic muscle assessment is a simple option, which requires evaluation to be established for diagnostic workup. Therefore, this ongoing study is designated to identify normative age-adapted values of ultrasound muscle indices and compare them with established computed tomography (CT) measurements.

Methods Between March 2020 and July 2021, 355 patients with a median age of 62 were enrolled in the study. Muscle indices, including the psoas muscle area index (PMAI) and the psoas muscle thickness height index (PMTI), were quantified by sonography. Age strata of " < 60, 61–75 and > 75 years" were assessed. In 112 patients, sonographic measurements could be compared with corresponding CT measurements from the transversal section at lumbal level L3/4.

Results Muscle index measurements identified age-adjusted values for median PMAI (" < 60 years: 487.0mm/m; 61–75 years: 433.7mm/m; > 75 years: 384.1 mm/m") and median PMTI (" < 60 years: 18.8 mm/m, 61–75 years 18.2 mm/m; > 75 years: 17.3 mm/m"). PMTI ("$r = 0.545$, $p < 0.01$") and PMAI ("$r = 0.565$, $p < 0.01$") correlated best with CT measurements obtained at the level of L4. Sonographic muscle indices showed a progressive decline throughout aging.

Conclusion Sonographic muscle quantification is simple, inexpensive and could substitute CT morphometry. Especially, high-risk patients such as those with liver cirrhosis and other wasting entities could be screened.

P 2.23 Predicting liver regeneration after major resection

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Background Disruption of synthesis, excretion and detoxification functions defines liver failure. After liver resection, post-hepatectomy liver failure (PHLF) is a rightfully feared complication due to high lethality and limited therapeutic success. Individual cytokine and growth factor profiles may represent potent predictive markers for recovery of liver function. We aimed to investigate these profiles in post-hepatectomy regeneration.

Methods A time-dependent cytokine and growth factor profiling dataset of a training (30 patients) and a validation (14 patients) cohorts undergoing major liver resection were combined with statistical and predictive models identifying individual pathway signatures. 2319 associations were tested.

Results Expression trajectories of cytokines and growth factors with strong correlation to PHLF, morbidity and mortality were identified despite highly individual perioperative dynamics. EGF drop, HGF trajectory and PLGF fluctuations were associated with mortality. PLGF fluctuations were associated with PHLF and complications. A global-association-network was calculated and validated according to the types of underlying risk-factors. Preoperative cytokine and growth factor signatures were identified for prediction of mortality following major liver resection by regularized regression modelling. Subsequently, prediction of PHLF was possible as early as on POD1 (AUC over 0.75). Elastic-net-model could predict mortality on POD1 (AUC = 0.75). Proliferation analysis of corresponding primary human hepatocytes showed significant associations of individual regenerative potential with clinical outcome.

Conclusion Prediction of PHLF and mortality is possible on POD1 with liquid-biopsy based risk profiling. Further utilization of these models would allow tailoring of interventional strategies according to individual profiles.

P 2.24 Phosphatschwankungen bei einem Patienten mit metastasiertem Hepatitis-B-Virus-assoziierten Cholangiozellulären Karzinom (CCC) mit FGFR2-Fusion unter Therapie mit Tenofovir und Pemigatinib

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Hintergrund In Deutschland gehört das CCC zur Gruppe seltener Erkrankungen (< 3 Neuerkrankungen / 100.000 Einwohner / Jahr). Die Prävalenz einer FGFR2-Fusion/Rearrangement beträgt beim intrahepatischen CCC 10–15 %. Hepatitis-B-Virus-Infektionen stellen einen Risikofaktor für intrahepatische CCC dar.

Fallbeschreibung Der 23-jährige Patient wurde 09/2020 in unsere gastroenterologisch-onkologische Ambulanz mit Erstdiagnose eines intrahepatischen CCC zugewiesen. Bei Erstdiagnose lag eine fortgeschrittene Tumorerkrankung mit pulmonaler, peritonealer, nodaler und adrenaler Metastasierung vor, sodass keine kurative Therapieoption bestand. Der Patient berichtete über rechtsseitige Oberbauchschmerzen, Rückenschmerzen und B-Symptomatik. Vorbekannt war eine chronische Hepatitis-B-Virus-Infektion, bisher ohne antivirale Therapie. Die Viruslast lag bei 1.4E3 Kop./ml. Wir begannen eine palliative Erstlinientherapie mit Gemcitabin/Cisplatin sowie eine antivirale Therapie mit Tenofovir. Nach 5 Zyklen zeigte sich ein pulmonaler und peritonealer Progress. In der molekularpathologischen Diagnostik war eine FGFR2-Fusion nachweisbar. Nach einer Bridging-Therapie mit 4 Zyklen FOLFIRI (01/2021–02/2021) erfolgte die Umstellung auf Pemigatinib (analog FIGHT-202). Es zeigte sich ein anhaltendes Therapieansprechen mit regredienter pulmonaler Metastasierung, Peritonealkarzinose und regredientem Lokalbefund. Unter antiviraler Therapie (Tenofovir) gelang eine dauerhafte Suppression der HBV-DNA-Viruslast. Therapie-assoziiert zeigten sich periodische Schwankungen der Serumphosphatkonzentration mit einem Maximum bei 1,99 mmol/l. Eine spezifische Therapie war nicht notwendig.

Diskussion Dies ist der erste berichtete sichere und effektive Einsatz einer zielgerichteten Therapie mit Pemigatinib in Kombination mit Tenofovir. Da sowohl unter Tenofovir, als auch unter Pemigatinib Veränderungen des Phosphatstoffwechsels auftreten können, ist eine engmaschige Kontrolle notwendig.

P 2.25 Bile as parameter for quality assessment during normothermic ex vivo rat liver machine perfusion

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Introduction Organ shortage for liver transplantation is a worldwide problem, necessitating the use of extended criteria donor (ECD) organs. Normothermic ex vivo liver machine perfusion (NEVLP) can be used to assess and resuscitate ECD grafts. Our aim was to evaluate bile as a parameter for quality assessment of ECD grafts in our rodent model of rat NEVLP.

Methods Liver grafts from 24 male Sprague Dawley rats were divided into four groups and underwent 6 hours of NEVLP: 45min of warm ischemia (ECD model) using either DMEM or Steen® solution as perfusate, and healthy livers without prolonged ischemia using DMEM or Steen® as controls. Bile was collected and biliary pH was assessed hourly. AST, ALT, LDH and Urea levels were measured in perfusate samples every three hours. HE- and TUNEL-staining were performed at the end of perfusion.

Results A significant increase in liver enzymes was seen in livers exposed to prolonged warm ischemia compared to healthy livers (ALT T6h: 201.6U/L vs. 821.2U/L; $p = 0.004$), along with organ damage in HE-staining. Bile secretion of ECD grafts was significantly decreased compared to healthy livers (e. g. T1h-2h: 1223mg vs 282mg; $p < 0.0001$), while bile pH was significantly increased in both ECD groups (e. g. T1h-2h: pH 7.965 vs. 8.645; $p < 0.0001$). Differences between DMEM and Steen perfusate were not found.

Conclusion Bile secretion and pH can be used as parameter of liver integrity in our rat NEVLP model. We propose to perform bile analyses to assess the quality of marginal grafts during clinical NEVLP.

P 2.26 Intraoperative Drains and Surgical Management of Biliary Complications After Endocystectomy for Hepatic Cystic Echinococcosis are Beneficial

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DOI 10.1055/s-0041-1740715

Introduction Cystic echinococcosis (CE) is a parasitic disease caused by Echinococcus granuloses. The ideal surgery method is a matter of debate. However, new studies have shown many advantages of endocytectomy. The main complication of endocytectomy is bile leak. We evaluated two different bile leak management strategies, surgical revision versus interventional therapy, comparing their outcomes, and cost/effectivity.

Methods Eighty patients (181 cysts) who underwent endocystectomy between 2005 and 2020 were investigated. Surgical procedures were performed using our standardized method and complication management were based on both hospital standards and surgeon's discretion. Perioperative data were analyzed, and results of bile leak management using different strategies were compared regarding the hospitalization and costs reimbursement through DRG-costs.

Results Postoperative complications were detected in 16 (20 %) patients . Postoperative Bile leak was seen in 12 patients with total rate of 8.1 % in operated cysts . Bile leaks were diagnosed using the intraoperative drains in ten cases (83 %). The median hospitalization was 10 days. The median DRG-cost was 8,262€. CE recurrence was reported in two patients (2.5 %).

Discussion Standardized endocystectomy is safe and efficient, with complication and relapse rate similar to radical surgery. Surgical drains led to early diagnosis of bile leak. The patients with early reoperation had a shorter hospitalization (12.5 vs 20.7) and the DRG-based cost-reimbursements were slightly higher in these patients in comparison to those with interventional therapies. Therefore, it seems that routine usage of surgical drains and prompt reoperation in cases of early bile leak are beneficial.

P 2.27 Optimising a rodent model of normothermic ex-vivo liver machine perfusion to mimic clinical application

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Background Alternative strategies to static cold storage such as normothermic ex vivo liver machine perfusion (NEVLP) have developed to address the organ shortage for liver transplantation. NEVLP enables the assessment of organ quality and allows for pharmacological intervention. To advance this field, simple and stable animal models are needed that mimic the conditions in human NEVLP as closely as possible.

Methods 36 livers from Sprague-Dawley rats were perfused for 6 hours in our dual-vessel NEVLP system using different protocols. The effect of four varying doses of Epoprostenol as vasodilator was investigated ($n = 4$), each dose with and without the addition of Glycine as Kupffer cell inhibitor. Cell culture medium supplemented with rat plasma and erythrocytes was used as perfusate. Perfusion pressures were recorded, liver enzyme secretion and tissue samples were used for assessment of graft integrity. The best-performing combination was compared to Steen®-based perfusion solution, which is used in clinical NEVLP.

Results AST, ALT and LDH levels were trending down with increasing levels of Epoprostenol as well as through addition of Glycine, while bile secretion was improved. 1000ng/h of Epoprostenol was sufficient to ensure physiological perfusion pressures in groups without Glycine. Steen®-solution decreased AST, ALT and LDH concentrations significantly compared to cell culture medium.

Conclusions Compared to human NEVLP, higher doses of vasodilation are needed to ensure adequate graft perfusion. Steen®-solution, Epoprostenol and Glycine all have beneficial effects on perfusion outcome. The optimised protocol allows for experimental research in pharmacological conditioning of marginal liver grafts.

P 2.28 Validation of the ISGLS classification of bile leakage after pancreatic surgery: A rare but severe complication

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Introduction Hepaticoenterostomy is an important step in hepatopancreatobiliary (HPB) surgery, with a subsequent bile leakage (BL) rate of up to 5 %. The International Study Group of Liver Surgery (ISGLS) proposed a grading system for BL after HPB surgery. This study aimed to validate this grading system for BL in pancreatic surgery (PS) and to investigate the postoperative outcomes of BL after PS.

Methods Data were extracted for any type of pancreatectomy with hepaticoenterostomy between 2006 and 2019. The BL was graded according to the ISGLS classification. The influence of our standardized hepaticoenterostomy technique and the complexity of the surgery on BL rate were assessed in different time frames.

Results BL was detected in 156 of 5,300 patients (2.9 %). During the study period, the overall rate of Type-B and C BL showed a slight reduction from 3.5 % to 2.4 %. Patients with Type-C BL had higher wound infection rate and longer ICU-stay. Patients with Type-C BL receiving late surgical revision (> 5th day postoperative) had higher mortality rate compared to patients with Type-B and early Type-C BL.

Conclusion The ISGLS classification is valid for classifying BL after PS. The BL rate is influenced by the complexity of surgery. Patients with early Type-C BL who undergo reoperation in the first five days have better outcomes than late onset Type-C BL patients. Although rare, BL following PS is a severe complication with a major impact on patient outcome, contributing significantly to morbidity and mortality.

P 2.29 FOXA2 inhibits hyperbilirubinemia through maintaining apical MRP2 expression in acute liver failure

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Background & Aims Multidrug resistance protein 2 (MRP2) is a bottleneck in bilirubin excretion. Its loss is sufficient to induce hyperbilirubinemia, a prevailing characteristic of acute liver failure (ALF), that is closely associated with clinical outcome. This study scrutinizes the transcriptional regulation of MRP2 under different pathophysiological conditions.

Methods Thirty-six patients, 14 with quiescent liver cirrhosis and 22 with ALF, were enrolled. Hepatic MRP2, FXR, and FOXA2 expression were examined by immunohistochemistry. Clinicopathologic association was analysed. MRP2 regulatory mechanisms were investigated in primary hepatocytes, Fxr-/- mice and LPS-treated mouse model.

Results In normal hepatocytes, homeostatic MRP2 transcription is mediated by the nuclear receptor FXR/RXR complex. Fxr-/- mice lack apical MRP2 expression and rapidly progress into hyperbilirubinemia. In ALF patients, hepatic FXR expression is undetectable, however, the patients without infection maintain apical MRP2 expression and do not suffer from hyperbilirubinemia. These patients express robust FOXA2 in hepatocytes. FOXA2 upregulates MRP2 transcription through binding to its promoter. Physiologically, nuclear FOXA2 translocation is inhibited by insulin. In ALF, high levels of glucagon and TNF-α induce FOXA2 expression and nuclear translocation in hepatocytes. Impressively, ALF patients with sepsis express few FOXA2, lose MRP2 expression, and show severe hyperbilirubinemia. In this case, LPS inhibits FXR expression, induces FOXA2 nuclear exclusion, and thus destroys the compensatory MRP2 upregulation. In both Fxr-/- and LPS-treated mice, ectopic FOXA2 expression restored apical MRP2 expression and serum bilirubin levels.

Conclusion Upregulation of FOXA2 to maintain MRP2 is an efficient strategy to prevent hyperbilirubinemia.

P 2.30 Biological abdominal wall expansion in pediatric liver recipients after transplantation with large-for-size organs.

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DOI 10.1055/s-0041-1740719

Background After pediatric split liver transplantation intraabdominal loss of domain due to a large-for-size left lateral graft is a frequent problem for fascial closure and potentially leads to reduced liver perfusion and abdominal compartment syndrome. Therefore, delayed fascial closure with use of temporary silastic meshes and reoperation or alternative fascial bridging procedures are necessary.

Methods Between 2019 and 2020 biological meshes were used for abdominal wall expansion in 4 cases of pediatric split liver transplantation. These cases were analyzed retrospectively.

Results 1 male and 3 female children with median age of 6 months (range: 0–8 months) and weight of 6.2kg (range: 3.5–8kg) received a large-for-size left lateral graft. Graft-to-recipient weight ratio (GRWR) was 5.1 % (range: 3.7–8.5 %) in median. Biologic mesh implantation for abdominal wall expansion, twice each bovine (SurgiMend 3mm, Integra) and sheep matrix (Ovitex 1s and 2s, TELA Bio), was done in median 5 days (range: 3–9 days) after transplantation when signs of abdominal compartment syndrome with portal vein thrombosis in 2 and of the liver artery in 1 case occured. Median follow up was 11.5 months (range: 9–17 months) and showed good liver perfusion by duplex sonography and normal corporal development without signs of ventral hernia. One patient died because of a fulminant graft rejection and emergency re-transplantation 11 months after initial transplantation.

Conclusion Biologic meshes can be used as safe method for abdominal wall expansion to achieve fascial closure in large-for-size children liver transplant recipients. Usage for primary fascial closure can be considered in selected patients.

P 2.31 Intraoperative Cholangioskopie mit dem SpyGlass DS II und SpyGlass Dicover: Technische Machbarkeit und Bedeutung für das chirurgische und klinische Management bei komplexen hepatobiliären Eingriffen

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Einführung Die digitale single-operator Cholangioskopie (dSOC) hat den diagnostischen und therapeutischen Horizont endoskopischer Gallengangsinterventionen erweitert. In dieser Studie haben wir die intraoperative dSCOC (intraoperative Cholangioskopie, IC) mit dem SpyGlass DS II und SpyGlass Discover bei chirurgischen hepatobiliären Eingriffen hinsichtlich der technischen Machbarkeit, biliärer Zugangswege und des Einflusses der IC auf das unmittelbare chirurgisch-operative sowie klinische Management analysiert.

Methoden Es wurden 20 Patienten (12 m, 8 w; 8 pädiatrische Patienten, Alter 33,9 Jahre (0.5–77 Jahre)) in die Analyse eingeschlossen. Bei 8 Patienten wurde zuvor eine orthotope Lebertransplantation (oLTX; 4 Kinder) durchgeführt. Eine biliodigestive Anastomose (BDA) lag in 7 Fällen vor.
Retrospektiv wurde der biliäre Zugangsweg, die erhobenen intraoperativen Diagnosen und biliäre Interventionen analysiert; zusätzlich der Einfluss der IC auf das unmittelbare operative bzw. klinische Management.

Ergebnisse Der biliäre Zugang erfolgte über die BDA (n = 5), Ductus cysticus (n = 2), Dct. choledochus (n = 3) oder einen Zugang im weiter proximalen Gallengangssystem (n = 8). Jede IC erfolgte entweder mit therapeutischer Intention (Steinextraktion, Dilatation, elektrohydraulische Lithotripsie (EHL)) bzw. mit unmittelbaren Einfluss auf das operative chirurgische Management durch Festlegung von Resektionsgrenzen, Entscheidung zur Anlage einer BDA und/oder Entscheidung für die Listung zur oLTX.

Diskussion Die Durchführbarkeit der IC mit dem SpyGlass DS II und SpyGlass Discover ist technisch mit unterschiedlichen Zugangsmöglichkeiten in das Gallengangssystem möglich. In unserer Serie war die klinische Bedeutung der IC für jeden einzelnen Eingriff sehr hoch und hatte jeweils direkten Einfluss auf das weitere chirurgische bzw. klinische Management jedes Patienten. Dies ist insbesondere vor dem Hintergrund von komplexen pädiatrischen Patienten bedeutsam.

P 2.32 Limax as a prognostic marker for survival in acute liver failure

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Background Acute liver failure (ALF) is a rare clinical syndrome with high mortality if not treated with state-of-the-art intensive care or emergency liver transplantation. Reliable tools to identify high-risk patients are still required. Thus we aimed to evaluate LiMAx, an accurate measure for liver function, as a predictor of survival in ALF.

Methods Clinical data of 35 patients with ALF or acute hepatitis (AH) was retrospectively analyzed. Liver function tests and transient elastography (TE) were analyzed for associations with clinical outcome. The LiMAx measures cytochrome P4501A2 capacity by determining CO2 in breath after 13C-Methacetin injection. Data were compared between patients with spontaneous recovery (SR) and non-spontaneous recovery (3-month mortality/LTx; NSR)

Results 35 patients (22 ALF, 13 AH; 20 male, 15 female; age 36.18 ± 14.65; 31 SR, 3 NSR) with DILI (n = 13), AIH (n = 13), AIH-DILI overlap (n = 1), viral (n = 9) and cryptogenic liver failure (n = 1) were analyzed. The LiMax was 198.47 for SR vs. 92.33 for NSR (p = 0.0135). Fibrinogen was significantly lower in patients with NSR than in SR patients (216.7 vs. 106.3; p = 0.021). Mean liver stiffness by TE was 39.3 for NSR and 16.97 for SR (p = 0.2). LiMAx results were positively correlated with serum fibrinogen and ATIII concentrations and negatively correlated with liver stiffness. Neither coagulation parameters nor the MELD Score exhibited differences between SR and NSR.

Discussion/Conclusion Decision making in ALF remains challenging. LiMAx might predict prognosis in patients with ALF and could be an objective tool to decide if liver transplantation is necessary.

P 2.33 Lack of porto-caval gradient (PCG) increase after transjugular intrahepatic portosystemic shunt (TIPS) implantation under deep sedation is associated to poor survival

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Previous data have shown that PCG measured during TIPS placement with sedation differs from PCG measured 24 hours later without sedation (Silva-Junior et al., Gastroenterology 2017). However, the association between these difference (ΔPCG) and clinical relevance remains unclear. Therefore, our aim was to investigate prospectively the influence of ΔPCG on survival.

Methods 48 patients with TIPS implantation due to refractory ascites were included. TIPS placement was done under deep sedation using propofol, midazolam and pethidine. A measurement of PCG was performed directly after TIPS placement and 24h later without sedation (24h PCG). Inclusion time 01/2018–08/2021. Variables were compared with U-Mann-Whitney. Kaplan-Meyer curves were constructed and compared with log-rank test.

Results PCG was higher without sedation (ΔPCG; mean ± SD: + 2.4 ± 3.5 mmHg, p < 0.0001). In 33 (69 %) pts ΔPCG increased (+ 4.3 ± 2.3 mmHg), in 15 (31 %) pts ΔPCG showed no increase (-1.6 ± 1.8 mmHg). There were no differences between both groups regarding age, MELD, CP score, bilirubin, creatinine, albumin, PP, and EF before or ASAT, ALAT, lactate, blood pressure, and heart frequency 24 h after TIPS placement. On survival analysis, pts without 24hPCG increase had a higher risk of death than pts with 24h PCG increase (p < 0.0009).

Conclusion Lack of increase of 24hPCG after TIPS is associated with poor survival. Clinical and hemodynamic variables evaluated at baseline were not associated to ΔPCG. No clinical or hemodynamical variables evaluated at the time of 24hPCG could further explain the association with survival. Further investigation is needed to evaluate the mechanisms involved.

P 2.34 Impact of coagulation parameters and spleen size on bleeding complications in patients with primary liver diseases undergoing mini-laparoscopic liver puncture.

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Background and Aims Mini-laparoscopic liver biopsy (LLB) is a safe procedure with a low rate of bleeding complications, primarily found in patients with abnormal coagulation parameters (CP) (Frenzel et al. 2012). But current studies question the validity of classical CP such as INR/Quick value with regard to a periprocedural bleeding risk in patients with liver disease.

Methods Retrospective analysis of patients who underwent LLB for unclear hepatopathy (UH) and/or suspected liver cirrhosis (LC). Relevant bleeding (RB) was defined as the use of argon plasma coagula-tion (APC) during LLB. Complication (C) was defined as: liver/abdominal wall hematoma (LHE/AWHE, abdominal bleeding (AB) with the need for surgical intervention.

Results In 376 patients analysed (58 % female, median age: 54years), 29 % had liver cirrhosis (LC). RB (n = 165) was found significantly more often in LC patients (LC vs. non-LC: 79 % vs. 29 %; Odds Ratio (OR): 9.1, 95 % confidence interval (95 %CI): 5.3–15.4; p = .0001). Complications (n = 6 (1.6 %): LHE n = 4, AB n = 2) were more common in LC patients (4.5 % vs. 0.4 %; OR: 10.7, 95 % CI: 1.44-126.7; p = .015). Multivariate analysis including Quick/INR, fibrinogen, aPPT, platelets, patients age, size of the biopsy needle, spleen size (cm) and presence of LC, aPTT and LC were significantly associated with RB (aPTT: OR: 1.1, 95 % CI: 1.06- 1.19; p = .000; LZ: OR: 6.5, 95 %CI: 3.17- 13.5).

Conclusion Severe bleeding complications are rare during LLB. Relevant bleeding occurs especially in LC patients and can be treated with APC. However, with the exception of aPTT, no routine coagulation parameters were associated with bleeding risk.

P 2.35 Survival in a 10 year prospective cohort of heavy drinkers: Liver stiffness is the best long-term prognostic parameter

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DOI 10.1055/s-0041-1740724

Background Alcoholic liver disease (ALD) is among the most common liver diseases in the western world. We here present first data on the prognostic impact of LS on 10 years long-term survival of Caucasian heavy drinkers.

Method Information of survival was obtained in 675 of 943 screened patients that had presented for alcohol detoxification (6.0 days) over a period from 2007–2017 with a mean daily consumption of alcohol of 178 g. Mean observation time was 3.7 years. All patients had LS measurements by transient elastography and routine laboratory tests.

Results During the observation time, 106 patients died. Overall death was highest associated with LS, followed by hemoglobin and alkaline phosphatase (AP). In a multivariate model, LS next to age, AP and hemoglobin was the most significant independent predictor of survival with a hazard ratio of 1.013 (1.003 to 1.023, P < 0.05). Using ROC analysis, LS was the best predictor of death in general with an AUROC of 0.72 and a cutoff value of 14.0 kPa, followed by AP. Moreover, LS was the top predictor of death starting from 2 to 5 years. When stratifying patients according to standard LS cut-off values < 6 kPa, 6 to 12.5 kPa and > 12.5 kPa, 3-year survival rate was 94 %, 88 % and 74 % and 5-year survival rate was 90 %, 78 % and 64 %, respectively.

Conclusion We here identify LS as the best long-term prognostic parameter in patients who heavily consume alcohol. LS measurements should become an important parameter for the screening of alcoholics.

P 2.36 Low antibody titers after second SARS-CoV-2 vaccination in patients with autoimmune hepatitis

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Aims/Background Patients with autoimmune hepatitis (AIH) require immunosuppressive treatment, which might impair the immune response to vaccination. In this prospective cohort study, we assessed the humoral immune response of AIH patients to SARS-CoV-2 vaccination.

Methods Anti-SARS-CoV-2 antibody titers of 96 consecutive patients with AIH (78 % female, median age 53y, range 19–83y, 34 % with liver cirrhosis) were included 1–6 months after the second SARS-CoV-2 vaccination. Vaccination responses were explored for their association with prescribed immunosuppression, comorbidities and laboratory values. These data/findings were compared to 56 healthy controls.

Results 93 (97 %) patients achieved a seroconversion with median anti-SARS-CoV-2 titers of 660 BAU/ml (range 20–11400 BAU/ml). A low or no response defined as antibody-titers < 100 BAU/ml was detected in 10 % (N = 10) of the patients, of which all were under immunosuppression (N = 4 azathioprine, 3 prednisone, 2 MMF + prednisone, 1 azathioprine + Tacrolimus). Antibody levels were significantly lower in AIH patients than in healthy controls (1700 BAU/ml). Interestingly, antibody-titers of AIH patients without immunosuppression (n = 10) were comparably low to AIH patients with immunosuppression. No additional, individual risk factors for impaired response to vaccination could be identified in this cohort.

Conclusion Despite high seroconversion rates, AIH patients show a significantly reduced magnitude of the humoral immune response. Therefore, these data suggest that AIH patients should be recommended an early third booster shot in agreement with recent advice by the German STIKO.

Poster Visit Session lll Metabolism (incl. NAFLD) 28/01/2022, 18.50 pm – 19.35 pm

P 3.01 Hepatic functional pathophysiology and morphological damage following severe burns: a systematic review and meta-analysis

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Introduction Severe burns are devastating injuries affecting multiple organ systems. Little is known about the influence on the hepatic system and its physiology. This systematic review aimed to assess the current state of research on morphologic liver damage and its markers following burns.

Methods Adhering to PRISMA guidelines, a search was conducted in Pubmed, Web of Science and Cochrane databases. No publication date restrictions were applied. Publications were limited to English, German and Spanish language. Outcomes included serum levels of transaminases, fatty infiltration and necrosis. Weighted individual study estimates were used to calculate pooled transaminase levels and necrosis/fatty infiltration rates using a random-effects approach. Risk ratios or Odds ratios (ORs) and 95 % confidence intervals (CIs) were used to describe pooled estimates for risk factors.

Results The literature search retrieved 2548 hits, of which 59 studies were included into qualitative synthesis, and finally ten studies were included into meta-analysis. Studies were divided into those reporting autopsies and those reporting changes of serum transaminase levels. The majority of liver autopsies showed fatty infiltration 82 %(95 % CI 39 %-97 %) or necrosis of the liver 18 %(95 % CI 13 %-24 %).

Discussion Heterogeneity in studies on hepatic functional damage following severe burns was high. Few were well-designed and published in recent years. Many studies were included because of insufficient numerical data. Many patients deceasing from burns show fatty infiltration or necrosis of hepatic tissue. Transaminases were elevated during the first days after burn. Further research on how severe burns affect the hepatic function and outcome, especially long-term, is necessary.

P 3.02 Insulin-like growth factor 1 receptor and insulin receptor mediate AP-1-activation in enterocytes stimulated with parasite antigens

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Question Schistosomiasis is a parasitic infection, which affects at least 230 million people worldwide. We have previously shown that Schistosoma mansoni soluble egg antigens (SEA) activate the proto-oncogene c-Jun in enterocytes. c-Jun belongs to the AP-1 transcription factor family. We aimed to investigate receptors, pathways, and gene regulation involved in AP-1 promotor activation.

Methods The promotor activity of AP-1 in SEA-stimulated SW620 cells was analyzed via luciferase AP-1 reporter gene assays. Insulin-like-growth-factor1-receptor (IGF1-R), insulin receptor (IR), and downstream kinases such as Akt 1/2/3 and ERK 1/2 were specifically inhibited under SEA incubation. Additionally, the effect of androgen receptor inhibition on AP-1-induction was investigated in the presence of SEA stimulation.

Results SEA stimulation induced a concentration-dependent AP-1 activity in the reporter gene assay. SEA induced activation of the AP-1 transcription factor was reduced by the inhibition of IGF1-R/IR (corr. sig. p = 0,014) and the inhibition of the androgen receptor (corr. sig. p = 0,030). Also the inhibition of downstream kinases Akt 1/2/3 and ERK 1/2 led to a decrease of AP-1 promotor activity (corr. sig. p = 0,000). The inhibition of both receptors simultaneously potentiated this reduction.

Conclusion With our results we identified two receptors (androgen and insulin receptor) as well as downstream pathways (AP-1 activation), which are functionally activated by SEA in colon epithelial cells. These findings might be useful for new therapeutic approaches.

P 3.03 Mechanisms of increased triglyceride synthesis and lipolysis induced by Fatty acid Transport Protein 4 deficiency in hepatocytes

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Background Increased expression of adipose Fatty Acid Transport Protein 4 (FATP4) is observed in obese individuals. FATP4 mutations are associated with blood triglycerides (TG) and insulin resistance. FATP4 deficiency may cause metabolic risks. We have reported an increase in secreted TG, glycerol, and non-esterified free fatty acids (NEFA) in liver-specific Fatp4-deficient (L-FATP4-/-) mice fed with high-sugar and high-fat diets. These lipids were also elevated in FATP4-deficient HepG2 (HepKO) cells. Here, we investigated the mechanisms for these increases.

Methods Two models of FATP4 deficiency were used including HepKO cells generated by CRISPR/Cas9 technology and hepatocytes isolated from L-FATP4-/- mice. These cells were treated with 600 microM oleate or control BSA for 4 h and subjected to lipid and expression analyses.

Results Both HepKO and L-FATP4-/- hepatocytes showed an increase in TG, glycerol, and NEFA in cells and supernatants. This TG increase led to an increase in VLDL and HDL secretion as well as cellular MTTP and ApoB mRNA expression. TG and lipoprotein levels were further increased upon oleate treatment. Expression of CD36, FATP5, FATP2, and fatty acid synthase (FAS) was also increased in HepKO and L-FATP4-/- hepatocytes suggesting increased fatty acid uptake and de novo synthesis. TG lipolysis in FATP4-deficient cells was likely due to the action of plasma-membrane hepatic lipase (HL) but not cytosolic ATGL and HSL.

Conclusions Hepatocyte FATP4 deficiency induced an elevation of CD36, FATP5, FATP2, FAS, and HL resulting exaggerated secreted lipids. Our study may provide mechanistic insights for metabolic abnormalities seen in individuals with FATP4 mutations.

P 3.04 Metabolic dynamics of sexual dimorphism in primary mouse hepatocytes in vitro

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Background and Aims The liver is one of the most sexually dimorphic organs. Sex-specific differences are evident in metabolism as well as in the development and progression of liver diseases, e. g. non-alcoholic fatty liver disease and hepatocellular carcinoma. Despite these differences, sex of cells is rarely considered in cell culture or development of pharmacological agents. The current project aims to provide a better understanding of molecular mechanisms in sexual dimorphism and their relevance in cell culture experiments.

Method Primary hepatocytes from 12 weeks old female and male C57BL6/N mice were isolated and cultured in collagen-coated plates. Transcriptome, proteome and extracellular metabolome analyses were conducted after 0, 24, 48, 72 and 96 h in vitro cultivation.

Results The sex-specific gene expression of drug metabolism is altered during cultivation. The total amount of sex-specific expressed proteins is greatly reduced with progressing cultivation and partly restored after 96 h. There is significant reduction of female-specific gene expression of Cyp2b13 and Cyp2b9. Sex-dependent differences of several signalling pathways increased, including genes related to serotonin and melatonin degradation. For pathways such as amino acid degradation, beta-oxidation, androgen signalling and hepatic steatosis, the ratios of male and female gene expression were inversed during cultivation.

Conclusion Our results showed large differences in the sex-specific gene and protein expression and the dynamics during cultivation of primary hepatocytes. In consequence of measured signalling pathways, the cultivation time should be adapted to the experimental focus and the dimorphic changes need to be borne in mind for the interpretation of results.

P 3.05 Circulating miR-148a is a precipitant independent biomarker of acute-on-chronic liver failure (ACLF)

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Background/aim Bacterial infection (BI) is a common trigger of ACLF. We aimed to identify differentially expressed genes (DEGs) and miRNAs in a BI-ACLI model mimicking ACLF.

Methods Either saline or LPS (4 mg/kg) was intraperitoneally injected to C57BL/6J (n = 16) and Abcb4-/- (n = 16) mice. Constructed mRNA libraries (NebNext Ultra-II RNA kit) were used to reveal DEGs (NextSeq500, Illumina). Samples were screened for miRNAs (miScript, Qiagen). Identified miRNAs were evaluated in serum of chronic liver disease (CLD) patients with (n = 10) or without BI (n = 37), ACLF patients (n = 9 grade I and n = 26 grade II; n = 7 BI-ACLF, n = 28 nonBI-ACLF) and healthy controls (HC) (n = 7) by TaqMan assays.

Results A total of 145 DEGs (127 overexpressed; 18 repressed) were identified in LPS-treated KO mice. qRT-PCR confirmed upregulation of Rantes, Il-22, Il-2 and Il-6. Significant upregulations and repressions of M1 and M2 macrophages respectively were observed. Among 7 identified miRNAs, overexpression was present only in mir148a-3p in CLD with concurrent BI (time of BI detection < 2 weeks, n = 15), compared to those without BI ($p < 0.05$, n = 37), and HCs ($p < 0.01$). miR-148a upregulation was also evident in ACLF patients, regardless of whether acute trigger was BI or not. Elevation was more prominent in grade II patients compared to grade I (26.9 vs 7.44-fold, $p < 0.05$). None of the identified DEGs was among up-to-date targets of miR-148a, suggesting the presence of a previously unidentified target.

Conclusions Circulating miR-148a can be a trigger-independent biomarker for ACLF, the target of which is yet to be identified.

P 3.06 Effects of a one-month religious fast on metabolism and steatotic liver injury in type 2 diabetes

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DOI 10.1055/s-0041-1740731

The fasting month of Ramadan can be regarded as a modified form of interval fasting. However, in contrast to the usual recommendations, religious fasting in this form also requires abstaining from liquids during the day, while food being consumed in defined periods after sunset, generally resulting in a reduced and timed intake of total calories. Type 2 diabetes (T2D) is associated with non-alcoholic fatty liver disease (NAFLD). The aim of this study was to characterize the impact of a one-month interval fasting period on liver health, glucose and lipid metabolism in a cohort of T2D patients. Patients' blood samples were collected at the beginning and end of the four-week fasting period. The status of the liver was examined by fibroscan including measurement of the controlled attenuation parameter (CAP). Following the fasting period, the patients experienced a significant weight loss. While fibroscan (8.0 ± 1.2 vs. 8.6 ± 1.6 before/after fasting) and CAP (311.4 ± 9.6 vs. 302.6 ± 10.4 before/after fasting) showed modest changes, transaminase levels and the apoptosis marker M30 as well as adiponectin were significantly decreased after fasting. Serum levels of triglycerides were significantly decreased after the interval fasting. Glucose levels did not change significantly but serum levels of C-peptide and insulin increased following the interval fasting period. In this cohort of T2D patients, we demonstrated that a 4-week intermittent fasting period resulted in an improvement in different serum parameters associated with glucose, liver, and lipid metabolism.

P 3.07 A potential role for bile acid signalling in celiac disease-associated fatty liver

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Background Recently, liver injury emerged as a severe but rare extraintestinal manifestation of celiac disease. The spectrum ranges from simple steatosis with or without hepatic inflammation to liver failure. However, the mechanisms of celiac disease-associated steatosis and liver injury are not entirely understood. We and others have previously identified alterations in bile acid (BA) metabolism and gut-liver interactions as pivotal players in the pathogenesis of hepatic steatosis and its complications. Therefore, we here aimed to investigate the role of the gut-liver-axis with a focus on BA metabolism, hepatic steatosis, and liver damage in celiac disease.

Methods We included 20 patients with known celiac disease and 20 healthy volunteers. We analyzed liver function tests, cell death markers, and markers of BA and fatty acid metabolism (GLP1, FGF19, FGF21, Serum BAs). Hepatic steatosis was determined using a controlled attenuated parameter (CAP) via Fibroscan® and MRI-HFF. Findings: FGF19 levels were suppressed in celiac disease patients compared to controls, although all patients were in clinical remission. Intriguingly, an inverse association of FGF19 with the degree of steatosis was found in the celiac cohort. Subgroup analysis was performed comparing patients with measurable serum levels of anti-tTG to those without detectable anti-tTG levels. Correlation analyses of the subgroups indicated that especially patients with transglutaminase antibody levels > 1U/ml present an invert association between FGF19 and the extent of steatosis.

Conclusion FGF19 is repressed in celiac disease. Low levels of FGF19 are associated with higher grades of steatosis, especially in patients with increased levels of anti-tTG antibodies.

P 3.08 Gender-related differences in response to DUAL diet in murine model of steatohepatitis

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DOI 10.1055/s-0041-1740733

Background and Aims Chronic alcohol intake is an important risk factor for Metabolic Associated Fatty Liver Disease (MAFLD) progression. However, the patients with dual clinical features of MAFLD and ALD (alcohol-associated liver disease) represent a large, understudied area in hepatology with a huge unmet need in preclinical and clinical studies. In the present study we evaluated and compared the extent of liver damage, steatosis, inflammation, and fibrosis in DUAL murine model with main focus on gender related differences.

Methods 10 weeks old C57BL6/J male and female mice were treated with DUAL diet (WD with sweetened drinking water and 10 % alcohol) for 23 weeks. Mice receiving standard chow diet and tap water were used as control. Serum markers of liver damage, immunohistochemistry (IHC), immunofluorescence (IF), histopathology and gene expression were analyzed.

Results Feeding with DUAL diet caused significantly faster and stronger weight gain in male mice compared to females. Male rodent displayed more prominent obesity, hypercholesterolemia and elevated plasma levels of AST, ALT, LDH. The liver of male mice was characterized by more significant liver damage and more prominent hepatomegaly, profound liver steatosis and positive TUNEL staining. DUAL feeding in male mice resulted in significant lobular inflammation. Notably, DUAL male mice exhibited stronger collagen accumulation and more progressive hepatic fibrosis compared to females. **Conclusions** Our study confirmed that the gender differences in patients with DUAL chronic liver diseases (MAFLD + ALD) have to be taken under consideration for clinical practice to improve diagnostic and therapeutical approaches in the future.

P 3.09 Epigenomic and transcriptional profiling identifies impaired glyoxylate detoxification in NAFLD as a risk factor for hyperoxaluria

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Epigenetic modifications (e. g. DNA methylation) in NAFLD and their contribution to disease progression and extrahepatic complications are poorly explored. Here, we use an integrated epigenome and transcriptome analysis of mouse NAFLD hepatocytes and identify alterations in glyoxylate metabolism, a pathway relevant in kidney damage via oxalate release - a harmful waste product and kidney stone-promoting factor. Downregulation and hypermethylation of alanine-glyoxylate aminotransferase (Agxt), which detoxifies glyoxylate, preventing excessive oxalate accumulation, is accompanied by increased oxalate formation after metabolism of the precursor hydroxyproline. Viral-mediated Agxt transfer or Inhibition of hydroxyproline catabolism rescues excessive oxalate release. In human steatotic hepatocytes, AGXT is also downregulated and hypermethylated, and in NAFLD adolescents, steatosis severity correlates with urinary oxalate excretion. Thus, this work identifies a reduced capacity of the steatotic liver to detoxify glyoxylate, triggering elevated oxalate, and provides a mechanistic explanation for the increased risk of kidney stones and chronic kidney disease in NAFLD patients.

P 3.10 Exploring the role of IL-1, IL-33, and IL-36 signaling in hepatocytes and metabolic liver disease

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Background IL-1, IL-33, and IL-36 are members of the IL-1 family of cytokines, which mediate their effects by heterodimeric receptors made up of a cognate receptor subunit (IL-1R1, IL1RL1/ST2, and IL1RL2 resp.) and the IL-1 receptor accessory protein (IL1RAcP). This study aims to elucidate their effect on hepatocytes and in NAFLD.

Methods Human HepG2 cells and primary hepatocytes were exposed to recombinant human IL-1a/b, IL-33 or IL-36a/b/g protein (≤ 100ng/ml). Different readouts were assessed after 3, 6 or 18h.

Results IL-1a/b efficiently increased basal IL-1R1 and IL-1RAcP expression in HepG2s at 3–18h. By contrast, we were unable to detect mRNA expression of IL-1RL1 and IL-1RL2 in HepG2s by RT-PCR, and IL-33 and IL-36a/b/g stimulation failed to induce expression of their cognate receptors and IL-1RAcP at the indicated time points. Likewise, only IL-1a/b rapidly increased the expression of RELA (NF-kB p65), pro-inflammatory cytokines, and chemokines (TNF-a, TGF-b, IL-6, IL-8, and CCL2) and induced IL-8 release. Remarkably, in contrast to HepG2s, primary hepatocytes showed an increased mRNA expression of TNF-a and IL-8 following IL-1a/b- and IL-36a/b/g-stimulation, albeit IL1RL2 and IL-1RAcP remained unaffected with IL-36 treatment. Oleate treatment of HepG2s, which is less cytotoxic than palmitic acid and strongly steatogenic by upregulating especially SREBP-1 signaling, also increased IL-8 and CCL2 mRNA expression and production.

Conclusion In contrast to IL-33, IL-1a/b and IL-36a/b/g appear to mediate pro-inflammatory transcriptional changes in human hepatocytes. These might amplify the severity of liver disease in NAFLD in concert with fatty acids, which utilize similar pro-inflammatory downstream signaling pathways.

P 3.11 Four-and-a-half LIM-domain protein (FHL2) affects diet induced obesity, diabetes and hepatic steatosis and inflammation

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The four-and-a-half LIM-domain protein 2 (FHL2) has been described to interact with more than 50 different proteins involved in various signaling pathways and cellular functions. FHL2 is ubiquitously expressed, but it exerts tissue- and cell context-specific functions that are often opposing. The role of FHL2 in non-alcoholic fatty liver disease (NAFLD) has been unknown. The aim of this study was to investigate the expression and function of FHL2 in NAFLD.

Methods and Results FHL2-wildtype (wt) and knockout (FHL2-/-) mice were fed with a NAFLD-inducing Western-type diet (WTD). Interestingly, WTD-induced weight gain was decreased in FHL2-/- compared to wt mice, although food-consumption and fatty acid (FA) concentration in faeces did not differ, indicative for similar FA-uptake. Furthermore, WTD-fed FHL2-/- mice did not develop insulin resistance and diabetes in contrast to their wt-counterparts. Also hepatic triglyceride (TG) levels were significantly decreased in FHL2-/- compared to wt-mice. Furthermore, WTD-induced hepatic pro-inflammatory gene expression and immune-cell infiltration were lower in FHL2-/- mice.

Knockdown of FHL2 caused reduced cellular TG-levels in an in vitro model of hepatic steatosis. Interestingly, in vitro as in vivo in mice lipid droplets appeared significantly smaller in FHL2-depleted hepatocytes. Furthermore, a decreased activation of the AKT-pathway was detected in the livers of WTD-fed FHL2-/- mice as well as in FHL2-depleted hepatocytes after FA-stimulation.

Conclusion FHL2 quantitatively and qualitatively affects diet-induced (hepatic) steatosis. Analysis of the underlying mechanisms may lead to the identification of new prognostic markers or therapeutic targets for the prevention and treatment of the metabolic syndrome and NAFLD.

P 3.12 Common MARC1 and HSD17B13 polymorphism have protective effects on liver injury in obese patients undergoing bariatric surgery

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Background The severity of liver steatosis is modulated by the PNPLA3 p.I148M, TM6SF2 rs58542926 and MBOAT7 rs641738 polymorphisms. Recently two genetic variants, namely MARC1 rs2642438 and HSD17B13 rs72613567, were shown to have protective effects in patients with chronic liver diseases. Here we analyse these variants in patients undergoing bariatric surgery.

Methods A total of 165 obese individuals (mean BMI 43.8 kg/m2) who underwent laparoscopic sleeve gastrectomy were prospectively recruited. Genotyping of the MARC1, PNPLA3, TM6SF2, MBOAT7 and HSD17B13 polymorphisms was performed using TaqMan assays. Liver biopsies were performed intraoperatively in all patients.

Results Overall, 70.3 % of operated patients had hepatic steatosis but none had cirrhosis at liver biospy. The MARC1 minor allele had protective effects on hepatic fibrosis whilst variant PNPLA3 increased the risk of developing steatosis and fibrosis as well as non-alcoholic steatohepatitis (NASH) and was associated with higher serum glucose. On the other hand, the harmful effects of the PNPLA3 p.I148M variant were lower among carriers of the MARC1 polymorphism. Multivariate analysis showed that variant MARC1 was an independent protective factor against liver fibrosis. The HSD17B13 polymorphism was protective against liver injury as reflected by lower AST and ALT activities. The TM6SF2 polymorphism was associated with increased risk for S3 steatosis and increased serum ALT.

Conclusion Hepatic steatosis is frequent among obese patients but presence of the MARC1 and HSD17B13 polymorphisms lowers liver injury in these individuals. Of note, variant MARC1 reduces the harmful effects of the major genetic risk factor for progressive fatty liver, namely PNPLA3 p.I148M.

P 3.13 Uncontrolled hypertension: a neglected risk in patients with NAFLD

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Background Growing evidence indicates a bi-directional relationship between non-alcoholic fatty liver disease (NAFLD) and hypertension. Although hypertension affects up to 50 % of all NAFLD patients and is a major risk factor for adverse cardiovascular events, integrative management programs as well as valid information on hypertension control among NAFLD patients are lacking. Thus, we integrated a 24-h-ambulatory-blood-pressure monitoring (24-h-ABPM) based hypertension screening and control program into NAFLD outpatient-care.

Methods 226 NAFLD patients were recruited during regular follow-up visits and underwent office-blood-pressure (OBP) measurements as well as 24-h-ABPM following a standardized protocol. Uncontrolled hypertension status was defined as elevated out-of-office BP in treated or untreated individuals.

Results 218 data sets were eligible for final analysis. At the time of ABPM, 101 NAFLD patients had a medical history of hypertension, of whom 93 (92.1 %) were treated. Controlled hypertension (OBP and ABPM normotension) was confirmed in only 23 of 93 (24.7 %) NAFLD patients with known and treated hypertension, while 44 (47.3 %) participants on antihypertensive treatment revealed sustained uncontrolled hypertension (OBP and ABPM hypertension). Masked hypertension (normal OBP with elevated out-of-office BP) was identified in 42 (19.3 %) NAFLD patients. A new diagnosis of sustained hypertension was established in 33 NAFLD patients. Overall, 127 of 218 (58.3 %) NAFLD patients showed an uncontrolled hypertension status.

Conclusions Given the high rate of uncontrolled hypertension among NAFLD patients, the implementation of hypertension management programs including 24-h-ABPM into patient-centered care is urgently needed. Improving hypertension control could help to reduce the risk of cardiovascular events in patients with NAFLD.

P 3.14 Metabolic syndrome and hepatectomy - a meta-analysis with subgroup analysis for NAFLD

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DOI 10.1055/s-0041-1740739

Metabolic (dysfunction) associated fatty liver disease (MAFLD) a progression of the metabolic syndrome (MetS), is a vast-growing cause of primary liver tumors - major indications for liver surgery. The aim of this meta-analysis was to investigate the impact of MetS on complications and long-term outcomes after hepatectomy.

Methods The meta-analysis protocol was registered at PROSPERO before data extraction. MEDLINE, Web of Science and Cochrane Library were queried for publications on liver resections and MetS. Data were pooled as odds ratio (OR) with a random-effects model. Risk of bias was assessed using Quality in Prognostic Studies tool (QUIPS) and certainty of evidence was evaluated using GRADE. Subgroup analyses for patients with histopathologically confirmed non-alcoholic fatty liver disease (NAFLD) versus controls were performed when possible.

Results Fifteen comparative studies were included. Patients with MetS suffered significantly more overall complications, major complications, postoperative hemorrhages and infections. There were no significant differences in mortality, recurrence, 1- or 5- year overall or recurrence-free survivals. Patients with histologically confirmed NAFLD did not have significantly more overall complications; however, PHLF rates were increased . Recurrence and survival outcomes did not differ significantly. The certainty of evidence was generally low.

Conclusion Patients with MetS suffer more complications after haptectomy, especially hemorrhage and infection but not liver-specific complications. Histologically confirmed NAFLD is associated with significantly higher PHLF rates, yet, survivals of these patients are similar to control. Further studies should focus on markers of MAFLD stages and early markers of PHLF.

P 3.15 Role of endurance training in diet-induced steatohepatitis in rats

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Background Metabolic-associated fatty liver disease (MAFLD) and non-alcoholic steatohepatitis (NASH) are considered hepatic manifestations of metabolic syndrome for which no effective pharmacological treatment exists. Dietary intervention with more than 10 % weight loss is effective but often fails due to low patient compliance. Alternatively, an increased physical activity is considered to improve fatty liver disease even without weight loss. The underlying mechanisms are unclear and cannot be studied in humans.

Methods Wistar rats were fed a standard or NASH-inducing high-fat diet with cholesterol and fructose for 7 weeks. Both diet groups were divided into a sedentary and a running exercise group.

Results Animals fed the high-fat diet gained more weight than standard diet-fed animals, got glucose intolerant, and developed a liver pathology with steatosis, inflammation and fibrosis similar to human NASH in the metabolic syndrome. While the endurance training did not reduce body weight or improve the NASH activity score, it significantly reduced the hepatic overload with dietary cholesterol and the resulting oxidative stress. In addition, endurance training improved the diet-induced glucose intolerance, possibly through exercise-induced generation of the hepatokine FGF21, which increased fatty acid utilization in muscle.

Conclusion Endurance training failed to ameliorate diet-induced hepatic fatty liver disease in rats but reduced hepatic cholesterol accumulation and oxidative damage of hepatocytes, as well as high-fat diet-induced glucose intolerance possibly in part by production of the hepatokine FGF21.

P 3.16 Elevated serum bile acids in NASH patients with fibrosis in the context of their cholestatic genetic predisposition

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Background Current drug development in NAFLD show promising results for FXR-agonists against NAFLD progression, but bile acid (BA) retention could also promote liver injury. Genetic variant c.1331T>C of the bile-salt export pump (ABCB11) as the most frequent pro-cholestatic polymorphism represents a predisposition factor for bile salt retention under pathologic condition.
To analyze whether the relationship between serum BAs and NAFLD depends on c.1331T>C variant.

Method 70 NAFL, 124 NASH and 165 clinically diagnosed NAFLD patients were included in this study. The c.1331T>C variant was genotyped using TaqMan assays. Serum BAs were analyzed by mass-spectrometry in 33 NAFL, 58 NASH, and 146 NAFLD patients.

Results 69 % of NAFLD patients presented a cholestatic pattern of serum enzymes with (ALT/ALTULN)/(AP/APULN) < 2. Total serum BAs were significantly higher in NASH compared to NAFL (2.6 ± 2.2 vs. 1.8 ± 1.6µM p = 0.02). No significant association between overall BAs and steatosis, inflammation, or ballooning in histology was found. Histology proved NAFLD patients with F3/F4 had highest BAs, and F1/F2 patients still had higher BAs compared to F0. All NAFLD patients with cholestasis (BAs > 10µM) had significantly higher liver stiffness compared to non-cholestatic NAFLD. No significant elevation in serum BAs was observed in TT carriers compared to CT + CC. A significant correlation between liver stiffness and BAs was observed for CT + CC patients.

Conclusion NASH patients are characterized by higher serum BAs, and BAs are associated with advanced fibrotic disease. The c.1331T>C variant might be a co-factor for cholestasis in NAFLD, but no significant impact was found in the present cohort.

P 3.17 Loss of Oncostatin M receptor aggravates dyslipidemia, hepatic lipid accumulation and adipose tissue inflammation in Apoe-deficient mice fed a Western diet

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Oncostatin M (OSM) is a member of the interleukin-6-type family and plays a pivotal role not only in inflammatory processes, but also in the regulation of metabolism. Previous studies revealed metabolically deleterious characteristics in mice lacking the OSM receptor beta gene (Osmr). Therefore, protective properties of OSM were suggested. To further investigate OSM-mediated effects on the lipid metabolism, we fed apolipoprotein E-deficient (Apoe-/-) and Apoe-/-Osmr-/- mice a Western-type diet for twelve weeks, and evaluated weekly weight gain, and serum lipid levels, lipoprotein fractions and hepatic lipid content after sacrifice. Furthermore, qPCR analyses of duodenal, hepatic and adipose tissue sample were performed. Although deficiency in Osmr did not result in body weight differences in the course of the diet, Apoe-/-Osmr-/- mice had increased serum concentrations of total and VLDL cholesterol, triglycerides, and free fatty acids when compared to Apoe-/- mice, in concordance with down-regulated hepatic lipoprotein receptor expression and up-regulated mRNA levels of lipases in adipose tissue. Moreover, most likely due to a repressed bile acid synthesis, Apoe-/-Osmr-/- mice showed an elevated liver cholesterol content. Furthermore slightly higher hepatic triglyceride levels were observed in Apoe-/-Osmr-/- mice, in line with down-regulated Cpt1a expression. In mice lacking the Osmr, qPCR analyses indicated a loss of anti-inflammatory macrophages in adipose tissue, but unaltered cytokine expression patterns. In light of our results, we hypothesize that OSM/OSMR/gp130 receptor complex conveys protective effects on lipid metabolism in Apoe-deficient mice.

P 3.18 H2O2-mediated autophagy during ethanol metabolism

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DOI 10.1055/s-0041-1740743

Background Alcoholic liver disease (ALD) is the most common liver disease worldwide and its underlying molecular mechanisms are still poorly understood. Moreover, conflicting data have been reported on potentially protective autophagy, the exact role of ethanol-metabolizing enzymes, and ROS.

Methods Expression of LC3B, CYP2E1, and NOX4 was studied in a mouse model of acute ethanol exposure. Autophagy was further studied in primary mouse hepatocytes and huh7 cells in response to ethanol and acetaldehyde. Experiments were carried out in cells overexpressing CYP2E1 and NOX4 silencing. The response to external H2O2 was studied by using the GOX/CAT system. Autophagic flux was monitored using the mRFP-GFP-LC3 plasmid, while rapamycin and chloroquine served as positive and negative controls.

Results Acute ethanol exposure of mice significantly induced LC3B expression but also induced the ROS-generating CYP2E1 and NOX4 enzymes. Notably, ethanol but not its downstream metabolite acetaldehyde induced autophagy in primary mouse hepatocytes. In contrast, autophagy could only be induced in CYP2E1 overexpressed huh7 cells. In addition, overexpression of NOX4 also significantly increased autophagy, which could be blocked by siRNA silencing. The antioxidant N-acetylcysteine (NAC) also efficiently blocked CYP2E1- and NOX4-mediated induction of autophagy. Finally, specific and non-toxic production of H2O2 by the GOX/CAT system as evidenced by elevated peroxiredoxin (Prx-2) also induced LC3B which was efficiently blocked by NAC. H2O2 strongly increased the autophagic flux as measured by mRFP-GFP-LC3 plasmid

Conclusion We here provide evidence that short-term ethanol exposure induces autophagy in hepatocytes both in vivo and in vitro through the generation of ROS.

P 3.19 Liver iron overload in alcoholic liver disease: Crosstalk between endothelial cells and hepatocytes in iron regulation

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DOI 10.1055/s-0041-1740744

Background and Aims Liver-secreted hepcidin is considered the systemic master switch of iron homeostasis with liver sinusoidal endothelial cells (LSECs) derived bone morphogenetic protein 6 (BMP6) and the BMP6/SMAD signaling pathway being essential for hepcidin expression. However, there are continued controversies about the strong and direct suppressive effect of iron on hepatocellular hepcidin in vitro in contrast to in vivo conditions. We here directly study the crosstalk between endothelial cells (EC) and hepatocytes and Huh7 cells using in vitro co-culture models that mimic the hepcidin signaling in vivo.

Methods Huh7 cells were direct co-cultured with ECs (HUVECs and SK hep). EC-conditioned media (CM) were also employed to culture Huh7 cells and primary mouse hepatocytes. To explore the reactions of ECs to surrounding iron, they were put under ferric ammonium citrate (FAC) and hemin. Intravenous iron injection in mice was used to study hepcidin signaling in vivo.

Results Both direct co-culture with ECs or EC-CM significantly increased hepcidin in Huh7 cells. The upstream SMAD pathway of hepcidin, including pSMAD1/5/8, SMAD1, or Id1, were induced by EC-CM. Efficient blockage of this EC-mediated hepcidin upregulation by an ALK2/3 inhibitor or BMP6 siRNA identified BMP6 as a major hepcidin regulator in this co-culture system, which highly fits the hepcidin regulation by iron in vivo. In addition, EC-derived BMP6 and hepcidin responded highly sensitive to levels of ferric iron but also heme as low as 500 nM.

Conclusion We here establish a hepatocyte-endothelial co-culture system to fully recapitulate iron regulation by hepcidin through EC-derived BMP6.

P 3.20 Evidence for alcohol-mediated masked hemolysis in ALD patients and a vicious cycle of hemolysis and iron-mediated liver damage

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Background and Aims Our recent study indicates that red blood cells (RBC) are also an important target in alcohol-related injury. We here studied the injury of hematologic system in ALD patients and its translational relation with liver injury.

Methods Based on a large prospectively patient cohort of heavy drinkers, we identify a subgroup of 25 % with high ferritin and low hemoglobin levels that show the worst outcome. RBC fragility in the ALD patients with the normal people by the hemolytic agent phenyl hydrazine (PHZ). The ALD mouse model was applied to observe the damage caused by alcohol in bone marrow and erythropoiesis. In addition, hemolysis and iron overload models were used to verify the vicious effect of iron accumulation on liver function.

Results In the subgroup with high ferritin and low hemoglobin, the large erythrocytes, elevated unconjugated bilirubin and LDH are highly suggestive of enhanced hemolysis. This is further confirmed by the high expression of CD163. Moreover, despite iron overload in these patients, serum levels of the iron master switch hepcidin were suppressed ultimately causing further iron uptake. ALD mouse showed less hematopoietic cells, and acute ethanol binge even caused quick erythroid inhibition. In both iron overload model and hemolytic model, serum iron and hepcidin were increased, while the hemolytic and iron overloading combined mouse model showed no further hepcidin upregulation with even higher serum iron.

Conclusion Our translational findings suggest a novel role of heme turnover in hemolytic damage of patients with ALD.

P 3.21 In vitro erythrophagocytosis model to study alcohol-mediated heme turnover

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DOI 10.1055/s-0041-1740746

Background & Aims The accumulation of hepatic iron still remains poorly understood in alcoholic liver disease (ALD) patients. Our preliminary clinical data suggest that ethanol promotes red blood cell (RBC) turnover. The overexpression of the erythrophagocytosis marker CD163 and other findings also suggest that ethanol causes enhanced sequestration of RBCs rather than direct hemolysis.

Methods RBCs were taken from healthy donors and incubated with oxidizing CuSO4 or other ALD mimicking conditions for 2 hours following co-incubation with differentiated THP-1 macrophages. Heme oxygenase-1 (HO-1), hepcidin and CD163 levels were measured by q-PCR or/and western blotting. Hemin was used or hypo-osmotically lysed RBCs. Finally, we also tested the effects of heme-scavenging haptoglobin (Hp) and hemopexin (Hpx).

Results 24 hours 0.01 % to 1 % hematocrit (Htc) of lysed RBCs significantly induced HO-1 in THP-1. Hepcidin and CD163 were induced in parallel by lysed RBCs in a dose-dependent manner. In addition, 1 % oxidized RBCs induced HO-1 in a time-dependent manner. Hepcidin and CD163 were up-regulated in line with HO-1 induction. Furthermore, HO-1 was gradually induced by different dosages of hemin for 24 hours. Heme degradation production bilirubin and bile acids also enhanced erythophagocytosis while induction of HO-1 was blunted both by Hpx ad Hp.

Conclusion We here establish an in vitro erythrophagocytosis model using differentiated THP-1 macrophages to study in detail enhanced RBC turnover by ALD-mimicking conditions. First data suggest enhanced erythrophagocytosis by bilirubin and bile acids and protection of uncontrolled HO1-mediated heme degradation by Hpx and Hp.

P 3.22 Deletion of the thrombin receptor PAR4 reduces diet-induced liver damage

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Fatty liver, one of the most common liver diseases, is often caused by excessive intake of high- calorie food. The course of the disease ranges from simple hepatic steatosis to an inflammatory reaction to liver fibrosis and cirrhosis. Both, patients with and without cirrhosis have an enhanced risk to develop a hepatocellular carcinoma.

For the study of liver disease progression under high-calorie diet, wt and Par4 knock-out mice were fed with western diet for up to 50 weeks.

In the longitudinal analysis of the progression of fatty liver disease wt mice show a significant increase the concentration of the serum parameter AST, ALT and LDH, especially after 16 and/or 26 weeks. After 30 weeks, there was a significant increase in RNA expression of TGFb1, 2, and 3 and increased collagen formation in liver tissue, accompanied by increased expression of various chemokines and the formation of so-called crown-like structures mainly from recruited macrophages. After 40 weeks of feeding, 25 % and after 50 weeks of feeding, 60 % of the animals developed macroscopically visible liver tumors. In comparison, the Par4 knock-out animals showed significantly reduced concentrations of AST, ALT, and LDH during the course of feeding, and fibrosis of the liver is significantly reduced. In addition, no PAR4 knock-out animal showed tumors after 50 weeks and only 1 animal had developed a tumor after 40 weeks of feeding.

In comparision to the wt mice, the depletion of Par4 appears to lead to a reduction in liver injury induced by a high-calorie diet.

P 3.23 Vaccination against upper respiratory infections is a matter of survival in Alcoholic Liver Disease

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Background Chronic liver disease (CLD) patients, including those with ALD, are particularly susceptible to infections. Thus, respiratory infections such as seasonal flue or COVID 19 may be speculated to be a major threat to these patients. However, despite general recommendations for seasonal flu vaccinations systematic evaluations of the efficacy of such vaccinations are widely lacking. In the current pandemic, substantial evidence on the efficacy on vaccination against upper airway respiratory infection is essential to improve the outcome of patients with Alcoholic liver disease.

Methods Evaluating a large cohort of patients with alcoholic liver disease form the USA with a total of 4667 patients, we investigated the efficacy of vaccination of patients with ALD. As quality of hepatological treatment may have significantly changed and improved over the past several decades the analysis was limited to the years 2000 to 2020.

Results During the last decade vaccinations against several seasonal influenza A variants (H1N1 (p = 0.000), H3N2 (p = 0.000)), influenza B virus (p = 0.000), Massachusetts-2–2010-liver variant (p = 0.000), and B-Wisconsin-1-2010 (p = 0.006) variant all demonstrated a highly significant survival benefit for these patients.

Discussion and Conclusion Vaccination against ongoing or seasonal viral upper airway infections improves survival of patients with ALD and should therefore be recommended and carried out consistently.

P 3.24 Adult cholesteryl ester storage disease (CESD): Three case reports

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DOI 10.1055/s-0041-1740749

Introduction Adult cholesteryl ester storage disease (CESD) is characterized by an autosomal-recessive deficiency of lysosomal acid lipase (LAL), which leads to accumulation of cholesteryl esters/triglycerides in macrophages with consecutive hepatosplenic involvement. In contrast to infantile Wolman disease, CESD follows a more benign clinical course. Sebelipase-α (Kanuma®, Alexion) is a recombinant form of LAL approved in 2015 as enzyme replacement therapy (ERT) for CESD.

Methods Single-center retrospective study of CESD patients between 1999-2021.

Results Three patients were diagnosed with CESD. Patient #1 was a 36-year-old woman with hepatosplenomegaly and anemia, suspected for Gaucher´s disease. Palpebral xanthelasmata, enlarged supraclavicular lymph nodes and severe splenomegaly were noted. LDL/HDL was 211/25 mg/dl. Bone marrow aspirates revealed sea-blue histiocytes. Plasma chitotriosidase activity (CTA) was 827 nmol/ml/h (Ref. 20-100) and fibroblasts exhibited a 15-fold increase of cholesteryl esters. LAL activity was reduced and mutational analysis confirmed the diagnosis of CESD.

Pats. #2/#3 were siblings aged 30/32 with severe hepatomegaly and xanthelasmata. Total cholesterol was 480/500 mg/dl, livers were massively enlarged, with only slight splenomegaly. CTA was elevated 2-3 fold in both cases. Therapies with statins/colestyramin/colesevelam and ERT with sebelipase alpha decreased liver volume.

Conclusion In patients with liver steatosis, hepatosplenomegaly and elevated LDL/HDL ratio/hypercholesterolemia, CESD should be considered. Sebelipase-α improves visceral manifestations in this lysosomal storage disease.

P 3.25 Niemann-Pick type B as ultra-rare differential diagnosis in hepatomegaly, steatohepatitis, low HDL and increased plasma chitotriosidase activity: three case reports

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Introduction Niemann-Pick type B (NPB) is a usually benign lysosomal storage disorder, based on autosomal-recessively inherited deficient acid sphingomyelinase (ASM) activity, leading to accumulation of sphingomyelin in lipid-laden macrophages, coined NPB cells. Olipudase (Sanofi-Genzyme) has been shown to improve visceral manifestations and is currently being licensed as enzyme replacement therapy (ERT) for ASM deficiency.

Methods Two-center retrospective study of NP-B patients between 2014-2021.

Results Three patients were diagnosed with NP-B. Patient #1, female, * 1962, had had posttraumatic splenectomy at age 22 and presented slightly hyperpnoic with mild hepatomegaly, total cholesterol (TC) of 254, LDL-C of 195 and HDL-C of 15 mg/dl. Chitotriosidase activity (CTA) was 4800 nmol/ml/h (ref. 10-150). On body plethysmography, carbon monoxide transfer factor (COTF) was 26 % of predicted. Pat. #2, male, * 1999, had hepatosplenomegaly, CTA of 3037 nmol/ml/h, spleen of 23 x 14 cm, low HDL and COTF of 49 %. Pat. #3, a female child, * 2011, showed hepatosplenomegaly and dyslipidemia. CTA and COTF were not available. All three diagnoses were confirmed by diagnostic molecular genetic testing. In addition to supportive therapies, initiation of ERT in the two adult patients is currently being established by an individual approach of the manufacturer.

Conclusion In all patients with hepatosplenomegaly, low HDL and increased CTA, NP-B should, besides Gaucher (GD) and cholesterol ester storage disease (CESD), be a differential diagnosis. Pulmonary CO transfer factor must necessarily be determined.

P 3.26 Liver-specific depletion of an endosomal regulator causes apoptosis, cell death and liver failure

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The endosomal regulators play a key role in signal transduction and the recycling of plasma membrane proteins. However, little is known about the function of these regulators in physiology in the liver. To identify the role of one of the regulators in liver we did the adenovirus mediated liver-specific KD in adult mice. Loss of this regulator induces severe liver inflammation and organ damage leading to animal lethality after 10 days of RNAi. This is associated with significantly decreased blood glucose and elevated aspartate aminotransferase and alanine aminotransferase levels in the serum. Reduction of this regulator in the liver causes a time dependent activation of liver inflammation, apoptosis and cell death, accumulating into a lethal phenotype in mice. Moreover, we find its expression is decreased in patients with NASH and to negatively correlate with the inflammation markers IL6 and IL1b in humans, highlighting its potential regulatory function in liver diseases.

P 3.27 Novel function for endosomal trafficking adaptors in hepatic metabolic disease

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DOI 10.1055/s-0041-1740752
In recent years, membrane trafficking through the endosomal transport system has received increasing attention for the regulation of metabolism. Quick responses of metabolically active tissues such as the liver to alterations in the nutrient status are highly dependent on the timely shuttling of transporters and growth-hormone receptors to the plasma membrane. Moreover, in highly polarized cells – as are hepatocytes –, transport and secretion of proteins occurs under an additional level of complexity, where certain proteins are required at apical (biliary) membranes while other proteins must be delivered to basolateral (sinusoidal) membranes. Bridging the two fields of endocytosis and liver metabolism is therefore an important step towards a better understanding of metabolic associated liver disease.
Here, we are reporting a thus far unknown role for an endocytic membrane trafficking adaptor protein in NASH development and progression. We found our protein to be upregulated in livers of various mouse models of NASH, as well as in human NASH patients. Interestingly, protein levels are correlating positively with degree of fibrosis in mice fed a NASH-inducing diet. When depleted of this protein, livers of mice fed two different NASH-inducing diets show increased fibrosis, compared to control mice. Using several proteomics approaches, we are currently investigating potential cargo proteins to dissect the underlying mechanism of enhanced disease progression.
Taken together, our data indicate a novel trafficking route regulating NASH disease progression. As hampering with this route seems to worsen disease outcome, we are hoping to identify mechanisms that could possibly improve disease outcome if increased.

Poster Visit Session lV Tumors 29/01/2022, 09.45 am – 10.30 am

P 4.01 Bone morphogenetic protein 13 is expressed by activated hepatic stellate cells and promotes tumorigenicity of hepatocellular carcinoma

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Activated hepatic stellate cells (HSC) play a key role in hepatic fibrosis, and herewith, build the soil for hepatocarcinogenesis. Furthermore, HSC are known to promote the progression of hepatocellular carcinoma (HCC) but the molecular mechanisms are only incompletely understood. Following activation, HSC produce bone morphogenetic proteins (BMPs) to support liver-regeneration and some BMP-family have been shown to affect also HCC-development and progression. So far, BMP13 has mostly been studied in the context of cartilage and bone repair.
The aim of this study was to analyze the expression and function of BMP13 HCC.
Methods and Results RT-qPCR and Westernblot analyses revealed high BMP13-expression in activated human HSC but not in human HCC-cell-lines. Furthermore, analysis of human HCC-tissues showed a significant correlation between BMP13 and alpha-smooth muscle actin (alpha-sma) and immunofluorescence staining confirmed co-localization of BMP13 and alpha-sma, indicating activated HSC as cellular source of BMP13 in HCC. Stimulation of HCC-cells with recombinant BMP13 dose-dependently increased expression of Inhibitor of Differentiation 1 (ID1), a known target of BMP-signaling and cell-cycle promotor. In line with this, BMP13-stimulation caused induced Smad 1,5,9-phosphorylation as well as reduced expression of cyclin-dependent kinases (CDKs) and increased proliferation and colony size formation of HCC-cells in clonogenicity assays. Interestingly, BMP13 also induced pro-tumorigenic ERK-activation, i. e. non-canonical BMP-signaling, in HCC-cells.
Summary and conclusion This study newly identified stroma-derived BMP13 as tumor promotor in HCC and indicates this secreted growth-factor as potential novel prognostic marker and therapeutic target in HCC.

P 4.02 Cdk5 as a possible new target in biliary tract cancer: preliminary in-situ findings

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Objective Biliary tract cancer (BTC) still shows a poor survival prognosis due to the lack of sufficient therapies and the observed chemoresistance. Cyclin-dependent kinases (Cdks) have gained increasing interest as potential cancer therapeutic targets in the last few years. The current study evaluates the protein expression of Cdk5 and its biomarker potency in a clinico-pathologically well-characterized patient cohort of BTCs.

Methods For this study, a comprehensive cohort of resected human BTC specimen (n = 119) was retrospectively evaluated. The immunohistochemical scores of Cdk5 (including intensity and extensity) in the tumor centre and margin were related to clinico-pathological characteristics as well as to markers (E-cadherin and vimentin) of epithelial-mesenchymal differentiation (EMT).

Results The patient cohort consists of mostly intrahepatic BTC (n = 58 (48.7 %) with mass forming (n = 56 (47.1 %) or periductal growth (n = 59 (49.6) pattern, mostly low grade differentiation (n = 75 (63 %)) and homogenous distribution of UICC stages (I-II: n = 62 (52.1 %); III-IV: n = 57 (47.9 %). Cdk5 was differentially more expressed at the tumor centre (mean (confidence interval) 40.1 (30.3-50.0)/median (min-max) 10.0 (0/250)) compared to the tumor margin (23.5(16.2-30.7 / 6.6 (0-230). Furthermore, the statistical analysis revealed an association of the expression pattern of Cdk5 with clinico-pathological parameters (including overall survival) and markers of EMT.

Conclusion and Outlook Our preliminary data show (i) that Cdk5 is generally expressed in BTC using immunohistochemistry and (ii) that protein expression of Cdk5 correlates with clinic-pathological characteristics including prognostic aspects, Therefore, in future experiments, we will evaluate cellular and molecular effects of pharmacological Cdk5 inhibition on BTC cells.

P 4.03 Variants APOE (rs429358) and TM6SF2 (rs187429064) modify the risk of hepatocellular carcinoma

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Background & Aims The host genetic background for hepatocellular carcinoma (HCC) is incompletely understood. We aimed to determine if four germline genetic polymorphisms, rs429358 in APOE, rs2642438 in MARC1; rs2792751 in GPAM and rs187429064 in TM6SF2, previously associated with progressive alcohol-related and non-alcoholic fatty liver disease are also associated with HCC.

Patients & Methods Four HCC case-control datasets were constructed, including two mixed etiology datasets (UK Biobank and FinnGen); one HCV cohort (STOPHCV) and one alcohol-related HCC cohort (Dresden HCC). Cases with HCC were compared against cirrhosis controls (i. e. cirrhosis patients without HCC). Adjusted odds ratios (OR) reflecting variant frequency in cases versus controls were calculated using multivariate logistic regression under an additive genetic model. Fixed-effect meta-analysis was used to determine the average effect size across all datasets.

Results Across four case-control datasets, 2,070 HCC cases, 4,958 cirrhosis controls. The rs429358:C allele (APOE) was significantly less frequent in HCC cases versus controls OR: 0.68; 95 %CI: 0.58-0.79; P = 9.30 × 10-7). Rs187429064:G (TM6SF2) was significantly more common in HCC cases versus controls and exhibited the strongest effect size (OR: 2.61;95 %CI:1.97-3.39; P = 1.4 × 10-11). In contrast, rs2792751:T (GPAM) was not associated with HCC (OR:0.99; 95 %CI:0.90-1.08; P = 0.85), whilst rs2642438:A (MARC1) was less frequent in cases versus controls, but narrowly missed statistical significance (OR: 0.89; 95 %CI:0.80-0.98; P = 0.02).

Conclusion This study associates carriage of rs429358:C (APOE) with a reduced risk of HCC, whereas carriage of rs187429064:G in TM6SF2 is associated with an increased HCC risk. No association with HCC was found for GPAM and MARC1 variants.

P 4.04 Lenvatinib as First-line Treatment of Hepatocellular Carcinoma in Patients with Impaired Liver Function in Advanced Liver Cirrhosis: Real World Data and Experience of a Tertiary Hepatobiliary Center

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Background Lenvatinib is a multikinase inhibitor approved for systemic first line treatment of hepatocellular carcinoma (HCC) in patients with compensated liver cirrhosis and unaltered liver function. We evaluated efficiency and tolerability of lenvatinib in patients with HCC, also including patients with advanced liver cirrhosis and impaired liver function.

Methods Retrospectively, 35 patients with HCC BCLC stages B, C and D were screened. After drop-out and exclusion of patients not receiving active treatment for > 2 weeks, 28 patients (27 male; median age 64.7) with advanced HCC and liver cirrhosis were included.

Results Fourteen patients (male, median age 62.7) treated had Child-Pugh class B liver cirrhosis, while 12 patients had Child-Pugh class A (male, median

age 68.8). Two patients had advanced Child-Pugh class C liver cirrhosis. The patients received an escalating dosing scheme of lenvatinib up to 12 mg/d. Tolerability of lenvatinib was similar in most of the patients, with no significant difference between the subgroups. Median survival was better in patients with Child-Pugh A liver cirrhosis (p = 0.003). More than 60 % of the patients with Child-Pugh A were still on treatment at the time of data analysis with a median follow-up of 274 ± 117.5 days compared with 153 days (95 %CI: 88.3–217.7) in patients with Child-Pugh B and 30 days in Child-Pugh C. Survival benefit significantly correlated with less impaired liver function (p = 0.003).

Conclusion Tolerability and toxicity of lenvatinib are similar in patients with Child-Pugh class A and B liver cirrhosis, but patients with less impaired liver function have an improved survival benefit.

P 4.05 Dimethyl fumarate (DMF) inhibits proliferation and migration and induce cell death in solid tumor cells

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Introduction Recently, we have shown in mouse models that dimethyl fumarate (DMF) treatment led to reduced metastasis formation and induction of cell death in malignant cells e. g. T-cell lymphoma. Solid tumors including hepatocellular carcinoma a characterized by a rather high ability to form metastasis. Therefore, we analyzed the effects of DMF on solid tumors.

Methods Various human cancer cell lines were treated with DMF and cellular ATP content was measured using a luminescence-based assay. Scratch assays with different tumor cell lines were performed to investigate whether DMF affects migration. In addition, cell proliferation of was determined either by FACS analysis or a colorimetric based assays. Flow cytometry was applied to analyze cell death induction.

Results DMF application resulted in a dose-dependent depletion of up to 89 % of cellular ATP content depending on the cell line tested. Thus, we analyzed whether the decrease in ATP level induced cell death and/or inhibited proliferation and migration. In accordance to ATP-depletion the tumor cell lines showed a strongly diminished proliferation (up to 89 %) and migration (up to 100 % inhibition). Furthermore, DMF application resulted dependent on the dose and cell line in up to 46 % cell death.

Conclusion We could show that DMF is capable to induce energy depletion resulting in a reduced migration and proliferation in various tumor cell lines. Furthermore, DMF was able to induce cell death. DMF is clinical approved and could be used as a basis to develop novel treatment options for treatment of metastasis formation.

P 4.06 Introduction of an novel exon-specific isoform expression reporter system (EXSISERS) into p53 for the study of differential protein-isoform expression

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Introduction p53 enhances cell survival by promoting the transcription of DNA repair enzymes. However, it also promotes the expression of pro-apoptotic genes leading to cell death. These differential cell fates are dependent on the interplay of different p53 protein isoforms. Here we present a state of the art method replacing differential expression analysis by mass spectrometry.

Methods The exon-specific isoform expression reporter systems (EXSISERS) were introduced into coding sequences, (exon 2, 4, and 7) of TP53. The genetic integration was conducted via CRISPR/Cas9. To determine the differential p53 protein isoform expression, we used treatments like chemotherapeutics and targeted therapies.

Results We established a cell line carrying two EXSISERS in TP53 and we were able to distinguish between different p53 isoforms. We demonstrated correct transport of p53 into the nucleus and preservation of its role as a transcription factor. The integration of EXISERS at multiple loci did not affect p53 structure and integrity. We conducted a real-time luminescence assay with stable modified cells exposed to different therapeutics. We confirmed that chemotherapeutics such as 5-FU and Oxaliplatin reduce cell proliferation by upregulating the FLp53 isoform and induce cell death in case of irreparable DNA damage. Regorafenib and Sorafenib immediately induce cell death in a p53 dependent manner by upregulating the Δ40p53.

Conclusion This novel technique enables detection expression profiles in real time. Thus, implementing this novel state of the art technology into various cell lines would allow research and optimized cancer treatment in different tumor entities.

P 4.07 Combination of Mcl-1 (Myeloid-Cell Leukemia 1) and Bcl-2 (B-Cell Lymphoma 2) inhibition – A novel treatment approach for Hepatocellular Carcinoma (HCC)

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Introduction Hepatocellular Carcinoma is one of the most common malignancies worldwide and has the third-highest mortality rate of all cancerous diseases. Late diagnosis and resistance towards medication often prevent successful therapy. Therefore, new systemic treatment alternatives are needed. The use of BH3-mimetics constitutes a novel approach. These inhibitors bind pro-survival-proteins of the Bcl-2-family resulting in induction of apoptosis.

Aims & Methods The HCC cell line Hep3B was treated with MIK665 and ABT199 (Venetoclax) respectively inhibiting Mcl-1 and Bcl-2. Cells were stimulated separately or with a combination of both drugs. Cell viability and caspase activity were measured via luminescence-based assays. Flow cytometry was used to analyse cell death induction. Cleavage of caspases as well as PARP were determined by Western blot.

Results Combination treatment with MIK665 and ABT199 resulted in strongly reduced cellular ATP content, compared to single drug treatment. Assays showed an increase of activity of caspases 8, 9, 3/7 after 4h. Accordingly, Western blots revealed cleaved caspases after 4h. Flow cytometry confirmed an induction of cell death by combination of both drugs. The strongest effects were detected after treatment with a combination of 5μM ABT199 and 6,25μM MIK665. In addition, the apoptosis marker PARP was detected.

Conclusion The development of resistances to approved HCC therapeutics is still a major challenge in successful treatment. Bypassing upstream apoptosis activators that often mutate during treatment by directly targeting Bcl-2-proteins constitutes a novel approach in HCC therapy.

P 4.08 Biliary Rhabdomyosarcoma in Pediatric Patients: A Systematic Review and Meta-Analysis of Individual Patient Data

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Background The biliary tree is a rare location of pediatric rhabdomyosarcoma. Due to the low incidence, there is a lack of evidence concerning therapeutic guidelines for this tumor location. In particular, the impact of surgery is discussed controversially.

Purpose Objective is to generate evidence-based treatment guidelines for pediatric biliary rhabdomyosarcoma (BRMS). All available published data on therapeutic regimens and important prognostic factors are investigated with a focus on the role of surgery.

Methods A systematic literature search of MEDLINE, Web of Science, and CENTRAL was performed. Patient data were entered individually. Data was pooled and qualitative and quantitative analyses of demographic data, therapy, post-operative/interventional outcomes, relapse, and survival were conducted. In an individual patient data analysis, cox regression was applied to identify key factors predicting the outcome of patients with BRMS.

Results 65 studies met the inclusion criteria, providing data on 176 patients with BRMS. Individual patient data analysis showed a 5-year overall survival of 51 % for the total study population. For patients treated after 2000, 5-year OS was 65 %. Absence of surgical tumor resection was an independent risk factor for death (Hazard ratio 8.9, 95 %-CI 1.8-43.6, p = 0.007) and significantly associated with recurrent disease.

Conclusion This analysis provides comprehensive information on the largest number of patients with hitherto reported in the literature. BRMS is still associated with high morbidity and mortality. Surgical tumor resection is essential for appropriate oncological treatment of BRMS. International cooperation studies are needed to enhance evidence and improve the outcome of his orphan disease.

P 4.09 Novel short-termed mouse model of intrahepatic cholangiocarcinoma by orthotopic transplantation of Hep-55.1C in mice with human homology

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Background Intrahepatic cholangiocarcinoma (ICC) is a highly malignant and progressive cancer that ranks the second most common liver cancer. To further understand molecular mechanisms of rise and progression of cholangiocarcinoma animal models are needed. Next to carcinogen-induced ICC-models or genetically engineered models, there are only few allograft or xenograft transplant models that claim challenging operative techniques or longer time periods for tumor development.

Method Hep55.1C cells were injected in the liver of 6-8 weeks old C57/B6 male mice. Tumor growth was followed by weekly ultrasound. After two or four weeks respectively mice were sacrificed. ICC and the tumor microenvironment were characterized via qRT-PCR, histological characterization/ multiplex imaging, flow cytometry and RNAseq. Murine data were comprehensively correlated with human data.

Results Tumor size was 0.6cm^2 or 1cm^2 on average after two or four weeks after transplantation with a prevalence 100 %. Tumor tissue in comparison to surrounding tissue showed high expression of cholangiocyte markers but not hepatocyte markers or markers for hepatocellular carcinoma. Immunohistological staining revealed a strong and ubiquitous signal for Pan-Cytokeratin within the tumor but not the surrounding tissue in contrast to HNF4α that was upregulated in the surrounding tissue. The analysis of the tumor microenvironment revealed a strong human homology regarding proliferation, gene expression pattern, tumor-associated immune response and stromal composition.

Conclusion We here present a new intrahepatic cholangiocarinoma mouse model with human homology. Tumor induction shows a 100 % success rate with macroscopic tumor growth within few weeks. Our model is therefore most suitable to evaluate interventional therapies.

P 4.10 Pathogenetic role of aberrant fucosylation in intrahepatic cholangiocarcinoma

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Background Intrahepatic cholangiocarcinoma (iCCA) is a lethal malignancy with limited therapeutic options. Aberrant protein glycosylation is a hallmark of cancer. Here, we investigated the levels of protein fucosylation and its role in iCCA development.

Methods Global protein fucosylation was determined using lectin histochemistry and Western blotting. The GDP-L-fucose synthetase (FX) and the GDP-fucose transmembrane transporter (SLC35C1), both major players of cellular fucosylation, were silenced via small interfering RNA. Moreover, iCCA cell lines were treated with 6-Alkynylfucose (6AF), a fucosylation inhibitor. In these cells, the fucosylation effects on the NOTCH and NF-kB pathways, two predominant cascades in cholangiocarcinogenesis and fucosylation targets, were also investigated.

Results Levels of global fucosylation and members of the fucosylation pathway were upregulated in human iCCA tissues compared to corresponding non-tumorous surrounding livers. Fucosylation inhibition following 6AF administration resulted in a dose-dependent decrease of proliferation and migration of iCCA cell lines. These effects were annulled by adding fucose to the cell medium. At the molecular level, 6AF administration or FX/SLC35C1 silencing led to the decrease of Notch receptors and related target genes in iCCA cell lines. In the same cells, NF-κB p65 and Bcl-xL protein levels diminished, whereas IκBα (a critical NF-kB inhibitor) increased after FX/SLC35C1 knockdown and drug addition.

Conclusions The present findings indicate that elevated global fucosylation characterizes iCCA. In this disease, fucosylation is involved in cell growth and migration and the upregulation of the NOTCH and NF-kB pathways. Thus, aberrant fucosylation is a novel pathogenetic player and a potential therapeutic target for human iCCA.

P 4.11 14-3-3 scaffold protein family as a potential molecular driver of sorafenib resistance in HCC patients

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Introduction The hallmarks of many cancers, including hepatocellular carcinoma (HCC), are apoptosis resistance, poor response to the drug treatment or quick relapse after initial remission.

Aim To dissect molecular drivers of drug resistance frequently observed in HCC, particularly in the patients who demonstrated the worst response to sorafenib.

Methods Integrative RNA sequencing and whole-exome sequencing analyses were employed to identify predictive markers of sorafenib resistance based on our cohort of 19 HCC patients. Potential drivers of drug resistance were evaluated by IPA and GSEA. Validation was performed in our in vitro model of sorafenib resistance by western blot and in publicly available data sets (GEPIA), followed by siRNA or peptide (R18) inhibition of selected molecular target candidates.

Results Patients with worst response (n = 7) were characterized by significantly shorter treatment duration and poor overall survival than good responders (n = 12) (66,6 months and 133,3 months, respectively; p < 0,0004). Molecular

analyses revealed that the worst subgroup was associated with activation of ERK and hypoxia-related scaffold proteins from the 14-3-3 protein family. Further, in our focus group, analysis based on gene expression signature showed significant enrichment of gene sets associated with Hippo/YAP signaling, ERK signaling and hypoxia. From hypoxia-related targets, we could observe that 14-3-3 zeta and sigma proteins might play a significant role in the acquisition of drug resistance.

Conclusion Defining the actionable targets of resistance and subsequent inhibition, e. g. 14-3-3 zeta and/or sigma protein might be of great help to delineate distinct molecular alterations driving sorafenib resistance.

P 4.12 Intraepithelial TIRC7 + immune cells are positive prognosticators in cholangiocarcinoma and represent a potential target for immunotherapy

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Cholangiocarcinoma is a heterogeneous malignancy with a detrimental prognosis. Therapeutic options are largely limited to surgery and conventional chemotherapy has very limited prospects of success. As immunotherapy has proven highly effective in various cancer types, we here assessed the quantity of immune cells expressing the T-cell immune response cDNA 7 (TIRC7), an emerging immunoinhibitory receptor, in a cohort of 135 cholangiocarcinoma (CCA) patients. Immunohistochemical staining of TIRC7 was correlated with clinicopathological criteria and patient survival. TIRC7 + immune cells were present in both the tumor epithelia and stroma in the majority of CCA cases with the highest levels found in intrahepatic CCA. While intraepithelial density of TIRC7 + immune cells was decreased compared to matched non-neoplastic bile ducts, stromal quantity was higher in the tumor samples. Tumors exhibiting signet ring cell or adenosquamous morphology were exclusively associated with an intraepithelial TIRC7 + phenotype. Survival analysis showed intraepithelial TIRC7 + immune cell density to be a highly significant favorable prognosticator in intrahepatic, but not proximal or distal CCA. Furthermore, intraepithelial TIRC7 + immune cell density correlated with the number of intraepithelial CD8 + and with the total number of CD4 + immune cells. Our results cofirm the presence of TIRC7 + immune cells in CCA and highlight TIRC7 as a potential new target for CCA immunotherapy.

P 4.13 Role of enhanced bone morphogenetic protein-endothelial cell-precursor derived regulator in hepatocellular carcinoma

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The role of bone morphogenic protein (BMP) signaling in hepatocellular carcinoma (HCC) is complex. BMPs have been shown to act as tumor-suppressors but also oncogenic factors. BMP endothelial cell-precursor derived regulator (BMPER) has been shown to act as extracellular regulator of different BMPs. Depending on its concentration and the cellular environment, BMPER can enhance or attenuate BMP signalling.

The aim of this study was to investigate the expression and function of BMPER in HCC.

Methods and results BMPER mRNA and protein are significantly increased in human HCC-cells compared to primary human hepatocytes. Furthermore, enhanced BMPER-expression in HCC-tissues correlates inversely with patients' survival. Stimulation with low concentrations of recombinant BMPER-protein (rBMPER; 1ng/ml) had no or slightly enhancing effects on the expression of inhibitor of differentiation 1 and 3 (ID1, ID3), which are well-characterized readouts for BMP-pathway activity. At higher concentrations, rBMPER dose-dependently downregulated ID1- and ID3-expression. In line with this, smad 1/5/8 phosphorylation was induced by stimulation with low but reduced with higher BMPER-doses. Interestingly, also ERK-phosphorylation was reduced after BMPER-stimulation. Furthermore, BMPER-treatment inhibited expression of Smad7, indicating additional indirect regulation of BMP signalling by BMPER.

Summary and conclusion We newly identified enhanced BMPER expression in HCC, and its correlation with pure patients survival suggest a pro-tumorigenic role in HCC. In vitro data indicate stimulatory as well as inhibitory effects of BMPER on BMP-activity in HCC-cells. Further analyses are need to reveal BMPER's molecular mechanisms of action and its functional relevance in HCC-development and -progression.

P 4.14 Interprofessionelle Ausbildungsstation Regensburg (A-STAR) mit gastroenterologisch-hepatologischem Schwerpunkt – Versorgungsqualität aus Sicht der Patienten

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Hintergrund Exzellente Versorgung von Patienten erfordert eine gute Zusammenarbeit aller Berufsgruppen der Medizin. Die WHO konstatiert, dass die interprofessionelle Zusammenarbeit bereits in der Ausbildung vermittelt werden sollte. Am Universitätsklinikum Regensburg werden in der Klinik und Poliklinik für Innere Medizin I Studierende der Humanmedizin im Praktischen Jahr und Auszubildende der Pflege im 2. und 3. Ausbildungsjahr auf der Ausbildungsstation Regensburg (A-STAR) ganzjährig ausgebildet. Die Station hat einen gastroenterologisch-hepatologischen Schwerpunkt. Wir untersuchten, ob Patienten sich durch PJ-Studierende und Auszubildende der Pflege auf der A-STAR ebenso kompetent betreut fühlen wie Patienten auf unseren Normalstationen vom hier tätigen professionellen medizinischen Personal.

Methoden Im Zeitraum 01.03.-31.08.2021 wurden alle Patienten der A-STAR und Normalstationen am Entlasstag auf einen Studieneinschluss gescreent. Patienten, die ihre Zustimmung gaben, erhielten einen Fragebogen mit 38 Fragen zu Aufenthalt, Organisation, Betreuung, gesundheitlichem Befinden. Diese wurden anonymisiert ausgefüllt.

Ergebnisse Es konnten 42 Patienten der A-STAR und 63 Patienten der Normalstationen in die Studie eingeschlossen werden. Es zeigten sich keine signifikanten Unterschiede hinsichtlich Aufklärung, Behandlung, Organisation, Fachwissen, Kompetenz, Gesprächsführung, Auftreten und Empathie des medizinischen Personals, dass Patienten auf der A-STAR im Vergleich zu Patienten auf den Normalstationen erleben.

Schlussfolgerung Patienten auf der A-STAR, die von Medizinstudierenden der Humanmedizin und Auszubildenden der Pflege betreut werden, fühlen sich ebenso gut aufgehoben und behandelt wie Patienten auf regulären Stationen mit professionellem medizinischen Personal. Dies spricht dafür, dass Ausbildungsstationen ein tragfähiges Konzept in der medizinischen Ausbildung darstellen, die aus Sicht der Patienten sowohl die Beziehungsebene als auch die subjektiv wahrgenommene Versorgungsqualität sicherstellen.

P 4.15 Hepatitis B surface antigen induces nuclear accumulation of YAP, thereby driving BMI1-associated hepatocarcinogenesis

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Background and aim Chronic hepatitis B virus (HBV) infection is one of the foremost sources of hepatocellular carcinoma (HCC). Efficient restraint of HBV viremia and necroinflammation via nucleos(t)ide analogue treatment could reduce the HCC incidence. However, hepatocarcinogenesis still occurs in the absence of active hepatitis, correlating with high hepatitis B surface antigen (HBsAg) serum levels. Nevertheless, the molecular mechanisms leading to neoplastic transformation remain elusive.

Methods Hemizygous HBsAg-transgenic mice (tg(Alb1HBV)44Bri) were investigated as a model of hepatocarcinogenesis driven by accumulation of HBsAg.

Results Gene set enrichment analysis suggested that signatures in HBsAg-transgenic mice correlated with YAP-downstream, cell cycle, DNA damage and spindle events (GSE84429). Flow cytometry revealed that polyploidy and aneuploidy frequently occurred in hepatocytes of these mice. Quantitative PCR, western blot and immunohistochemical staining revealed the downregulation of MST1/2, loss of YAP phosphorylation and the induction of BMI1 expression. Bioinformatics analysis of the Bmi1 promoter indicated the presence of several TEAD4 bind sites. Chromatin immunoprecipitation and the analysis of mutated binding site in DLR assays confirmed that the YAP/TEAD4 transcription factor complex was able to bind and activate the Bmi1 promoter. Loss and gain of function of Yap or Bmi1 in vitro confirmed the Yap-regulated Bmi1 expression, which led to suppression of p16INK4a, p19ARF, p53 and induction of Cyclin D1, here a CCK-8 assay indicated the direct proliferative effect.

Conclusion Our findings reveal an HBsAg-mediated inactivation of Hippo pathway, resulting in increased BMI1 expression, thereby promoting proliferative hepatocarcinogenesis through alterations in the cell cycle and chromosomal stability.

P 4.16 Identification and validation of a plasticity driver of Combined Hepatocellular-Cholangiocarcinoma using functional interspecies comparison

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Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) represent the two most common types of primary liver cancer, while combined HCC-CCA (cHCC-CCA) is rarely observed. Lineage-tracing studies in mice have shown that cholangiocarcinoma may develop from hepatocytes, while ductular cells may also give rise to HCC as a result of their plasticity. The aim of this project was to identify molecular mechanisms leading to a phenotypic switch in tumor cells resulting in cHCC-CCA formation.

Exome and transcriptome sequencing of HCC and CCA components of fifteen human cHCC-CCA samples was conducted. Data integration identified 57 potential phenotypic driver genes which were altered between both tumor compartments. In vivo RNAi screening was used to validate the candidate genes using two transposon-based mosaic mouse models (HCC model: MYC-AKT1 in C57BL/6 mice; iCCA model: KRASG12V in p19-/- mice). Individual tumor nodules displaying a phenotypic switch in tumor type based on histopathological evaluation were sequenced to identify the expressed shRNA.

One candidate gene (to be disclosed during the meeting) was identified and validated. It was upregulated in the CCA compartment of human cHCC-CCA and shRNA-mediated knockdown of gene expression resulted in a mixed HCC-CCA in the iCCA mouse model, while its overexpression led to cHCC-CCA formation in the HCC mouse model. Functional analyses in vitro revealed a protumorigenic impact on clonogenicity and cell viability of isogenic cell lines derived from our HCC mouse model compared to the controls.

Altogether, we discovered a new driver of cellular plasticity in liver cancer. Further mechanistic characterization is ongoing.

P 4.17 Hepatitis B surface antigen induces endoplasmic reticulum stress, impairs autophagy and promotes proliferation, thereby driving hepatocarcinogenesis

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Background & Aims Hepatitis B surface antigen (HBsAg) has been identified to increase the risk and contribute to hepatocellular carcinoma (HCC). However, factors and mechanisms that drive HBsAg-induced hepatocarcinogenesis remain poorly defined, thus hindering the development of new therapeutic strategies.

Methods Data mining of the microarray set GSE84429 indicated the potential candidate signatures of HBsAg-driven intracellular events. Hemizygous tg(Alb1HBV)44Bri/J mice were investigated for this HBsAg-driven carcinogenesis by western blotting, immunohistochemical and immunofluorescence staining. Finally, the findings were verified by HBsAg overexpression in Hepa1-6 cell line. Functional analysis was performed to study the contribution of these events.

Results Gene set enrichment analysis suggested signatures in HBsAg-transgenic mice correlated with endoplasmic reticulum (ER) stress, unfolded protein response (UPR), autophagy, cell cycle and proliferation. These events were investigated in 2-, 8- and 12-month-old HBsAg-transgenic mice. In HBsAg-transgenic mice the UPR was induced. Interestingly, our study indicates that HBsAg impaired the autophagic flux. In HBsAg-transgenic mice autophagy was enhanced at the early stage (increased Beclin1) and blocked at the late stage (increased p62 and LC3B-II). These findings were verified in HBsAg-overexpressing Hepa1-6. In addition, HBsAg changed lysosomal acidification, visualized by acridine orange staining and promoted proliferation, indicated by CCK-8 staining and colony formation assay.

Conclusion Our findings revealed the HBsAg directly induces ER stress, impairs autophagy and promotes proliferation thereby driving hepatocarcinogenesis. Moreover, this study expanded the understanding of HBsAg-mediated intracellular events in carcinogenesis.

P 4.18 Non-canonical NF-κB signaling induces proliferation in primary liver cancer

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Background and Aims Primary liver cancer is the third leading cause of cancer related death with increasing incidence and mortality worldwide. Recent studies demonstrate a role of non-canonical nuclear factor kappa B (NF-κB) signaling in various chronic liver diseases. Here, we aimed to further elucidate the role of non-canonical NF-κB signaling and its key transcription factor RELB in hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA).
Methods Non-canonical NF-κB signaling was manipulated in human HCC and iCCA cell lines by treatment with recombinant human Lymphotoxin α1/β2 ([LTα1/β2] pathway inducer). Subsequently, protein expression, cell death and proliferation were determined by Western blot analysis, flow cytometry and continuous impedance measurement, respectively. Murine HCC and intrahepatic CCA (iCCA) models were generated by hydrodynamic tail vein injection of plasmids carrying cMET/β-Catenin (HCC) or AKT/NOTCH1 (iCCA). Non-canonical NF-κB signaling in murine liver tissue was assessed by immunohistochemistry and qRT-PCR.
Results In both murine models a significant upregulation of RELB has been observed in tumor cells. Furthermore, Lymphotoxin-β has been identified, among other possible ligands, as being the prevailing pathway inducer. In line thereto, LTα1/β2 treatment led to enhanced RELB expression and its nuclear translocation in vitro and induced proliferation of all HCC cell lines and one iCCA cell line that showed low basal RELB expression.
Conclusion Lymphotoxin-β as the predominant ligand induces non-canonical NF-κB signaling in primary liver tumors, leading to a significant induction of tumor cell proliferation.

P 4.19 Galectin-1 upregulation in PIK3CA induced hepatocarcinogenesis- therapeutic cooperativity

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DOI 10.1055/s-0041-1740771

Background Aberrant activation of the PI3K/AKT/mTOR pathway is a hallmark of hepatocarcinogenesis. In mice, hydrodynamic transfection of phosphatidylinositol 3-kinase catalytic alpha polypeptide (PIK3CA) mutant forms elicits hepatocellular carcinoma development. The induced tumors are characterized by an early and consistent upregulation of Galectin1 (Gal1). This study aimed to elucidate putative Gal1 effectors in addition to strategies for therapeutic intervention.
Methods PIK3CA H1047R and E545K mutant forms were delivered to the mouse liver by tail vein hydrodynamic injection. Gene expression microarray analyses identified Gal1 as a key effector in hepatocarcinogenesis. A compound library including 315 approved anti-cancer drugs identified drugs synergistic with the Gal1 inhibitor OTX008. Gal1 function was studied in vitro and by the use of proteomics.
Results Gal1 was commonly overexpressed in PIK3CA-driven preneoplastic and neoplastic liver lesions. The induced HCCs displayed a strong lipogenic phenotype, and concordantly, expression of the master regulator of lipogenesis Stearoyl-CoA desaturase-1 was found to depend on Gal1. Moreover, combinatory drug screenings revealed synergy between the Gal1 inhibitor OTX008 and PIK3CA- or JAK/STAT-inhibitors. To complement these findings, both STAT1 and 2 were recognized as Gal1 effectors by proteomics.
Conclusions Overall, our research indicates that PIK3CA-induced hepatocarcinogenesis is characterized by elevated Gal1, which functionally regulates both lipogenesis and STAT proteins. Furthermore, these findings uncover a specific vulnerability, which can be targeted by Gal1- and receptor tyrosine kinase-inhibitor combinatory treatment regimens offering potential therapeutic venues for human HCC.

P 4.20 Expression of fibroblast growth factor receptors in hepatocellular carcinoma

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Fibroblast growth factor receptors (FGFRs) are generated from four genes (FGFR1/2/3/4). Alternative splicing of FGFR1-3 creates b- and c-isoforms that differ in ligand-binding and thus further enhance the complexity of FGF-signalling. FGFR-inhibition has been shown to inhibit progression of hepatocellular carcinoma (HCC) in experimental models. However, existing clinical studies with mostly unspecific FGFR-pan-inhibitors in non-selected patient-cohorts have not shown beneficial effects on survival.
The aim of this study was to gain insights into the expression of different FGFR-variants in HCC.
Methods and Results COSMIC database analysis revealed that genetic FGFR-aberrations are rare in HCC (less than 4 % in 2090 patients). Still, FGFR1, FGFR3 and FGFR4 are upregulated in human HCC-cell-lines compared to primary human hepatocytes and most HCC-tissues compared to corresponding non-tumorous liver. Expression of different FGFRs significantly correlated with each other pointing to common transcriptional regulation. In search for the molecular mechanisms, we analyzed histone acetylation and identified histone deacetylase 7 (HDAC7) as negative regulator of FGFR-expression in HCC-cells. Regarding variant-specific FGFR-expression, we analyzed the effects of siRNA-mediated knockdown of epithelial splicing regulatory protein 1 (ESRP1) in HCC-cells. ESRP1-suppression led to significant upregulation of FGFR2c and FGFR3c but downregulation of the corresponding b-isoforms. Surprisingly, ESRP1-knockdown significantly inhibited expression of both FGFR1b and FGFR1c.
Summary and conclusion Our study provides novel insights into the transcriptional regulation of FGFR-variants in HCC that might be exploited for new therapeutic strategies or prognostic parameters. The observed variation in the FGFR-expression pattern might also be utilized for individualized FGFR-inhibitory strategies.

P 4.21 Crosstalk of hepatic stellate cells and uveal melanoma cells in the liver metastatic niche

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Hepatic metastasis is the critical factor determining tumor-associated mortality in different types of cancer. This is particularly true for uveal melanoma (UVM) that almost exclusively metastasizes into the liver. Hepatic stellate cells (HSC) are the precursors of tumor-associated fibroblasts and support growth of metastases. However, the underlying mechanisms are widely unknown. Fibroblast growth factor (FGF) signalling is dysregulated in many types of cancer. The aim of this study was to analyze the protumorigenic effects of HSC on UVM cells and the role of FGFs in this crosstalk.
Methods and Results Conditioned medium (CM) from activated human HSC significantly induced proliferation combined with enhanced ERK and JNK-activation in UVM-cells. High expression levels of FGF receptor 1 (FGFR1) significantly correlated with poor survival of UVM patients, while FGFR2/3/4 expression did not significantly influence survival. Protumorigenic effects of HSC-CM on UVM-cells were abrogated by the FGFR1/2/3 inhibitor BGJ398, whereas the selective FGFR4 inhibitor BLU9931 had no significant effect. Expression analysis revealed that the vast majority of the different paracrine FGFs is only expressed by HSC but not by UVM-cells, including FGF9. Immunofluorescence analysis confirmed HSC as cellular source of FGF9 in hepatic metastases of UVM-patients. Treatment with recombinant FGF9 significantly enhanced the

proliferation UVM-cells and this effect was efficiently blocked by the FGFR1/2/3 inhibitor BGJ398.

Summary and conclusion Our study indicates that FGF9 released by HSC promotes the tumorigenicity of UVM cells, and thus, suggests FGF9 as a promising therapeutic target in hepatic metastasis.

P 4.22 Equal overall survival in elderly patients with hepatocellular carcinoma and liver cirrhosis receiving palliative treatment.

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Background Treatment of hepatocellular carcinoma (HCC) in elderly patients is often complicated by comorbidities and frailty. In this study at a tertiary referral center we have assessed the outcome and overall survival (OS) in elderly HCC patients receiving palliative therapy Method: Retrospective study: Tumor stage and liver function were rated according to the Barcelona Clinic Liver Cancer (BCLC) classification and Child-Pugh-Turcotte score (CPS). Patients were grouped as: young (< 60 years; YP), intermediate (60-70 years; IP) or elderly (> 70 years; EP). Administration of tyrosine kinase inhibitor (TKI) and transarterial chemoembolization (TACE) was defined as palliative treatment.

Results Out of 987 patients n = 657 received palliative treatment: YP n = 194; IP n = 241; EP n = 222. 82.5 % (n = 542) were male, median age: 67 (range 23 – 87) years. All patients had underlying liver cirrhosis with a larger proportion of impaired liver function in the YP cohort (CPS A/B/C [%]: YP 53/28/18 vs. IP 61/26/12 vs. EP 66/28/6; p = 0.01). OS in patients (CPS A and CPS B) receiving TACE was 16 vs. 16 vs. 20 months for YP, IP, and EP, respectively; p = 0.38. In TKI-treated patients, OS was 13 vs. 15 vs. 13 months; p = 0.73. The rate of adverse events (AE) in TKI-patients was comparable with 47 vs. 43 vs. 49 %.

Conclusion In this study, OS was equal in elderly patients receiving palliative treatment for HCC. Furthermore, the rate of AE was comparable in younger and elderly patients. Therefore, we propose regular palliative treatment stratification in spite of high age of patients.

P 4.23 Apoptosis sensitivity of hepatocellular carcinoma to sorafenib-based treatment combinations depends on the expression pattern of BH3-only proteins

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Background Anti-angiogenic immune checkpoint inhibitor-based combination therapy is currently used for treatment of progressed HCC, but improves survival only in a subset of patients. Tyrosine-kinase inhibitors (TKI) such as sorafenib represent an alternative treatment option but have only modest efficacy. Using different HCC cell lines and HCC tissues from various patients reflecting HCC heterogeneity, we investigated whether sorafenib response could be enhanced by the combination with pro-apoptotic agents, such as TNF-related apoptosis-inducing ligand (TRAIL) or the BH3-mimetic ABT-737, which target the death receptor and mitochondrial pathway of apoptosis, respectively.

Methods Cell death and apoptosis were assessed by crystal-violet staining and a luminometric caspase assay. Expression of apoptosis regulators was determined by RT-PCR and Western blot analyses. In addition, siRNA-mediated knockdown experiments were performed.

Results We found that both agents could enhance sorafenib-induced cell death which was, however, dependent on specific BH3-only proteins. TRAIL augmented sorafenib-induced cell death only in NOXA-expressing HCC cells, whereas ABT-737 enhanced the sorafenib response also in NOXA-deficient cells. Accordingly, HCC tissues sensitive to apoptosis induction by sorafenib and TRAIL revealed enhanced NOXA expression compared to HCC tissues resistant to this treatment combination. NOXA-knockdown in HCC cells significantly impaired cell death induction by sorafenib and TRAIL. Sorafenib cytotoxicity could, however, not be enhanced by ABT-737 in the absence of BIM, even when NOXA was strongly expressed.

Conclusion BH3-only protein expression determines the treatment response of HCC to different sorafenib-based drug combinations. Individual profiling of BH3-only protein expression might therefore assist patient stratification to certain TKI-based HCC therapies.

P 4.24 Expression of growth differentiation factor 5 in liver disease and cancer

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Growth differentiation factor 5 (GDF5) belongs to the TGF-β/BMP-superfamily. Several members of this growth factor-family play a critical role in the progression of liver disease and hepatocellular cancer (HCC). So far, GDF5 has been mostly studied in the context of cartilage development and homeostasis. The aim of this study was to assess the expression and function of GDF5 in liver disease and cancer.

Methods and results Screening of different liver cell types revealed high expression of GDF5 mRNA and protein in activated human hepatic stellate cells (HSC) compared to primary human hepatocytes and different human HCC-cell lines. GDF5 expression levels increased during in vitro-activation of HSC and in different murine models of hepatic fibrosis. Furthermore, there is a significant correlation between the expression of GDF5 and alpha-smooth-muscle-actin in non-tumorous human liver tissues as well as in clinical HCC-samples.

Treatment of human HCC cell-lines with recombinant GDF5 led to a dose-dependent induction of the expression of the transcription factors inhibitor of differentiation 1 (ID1) and 3 (ID3), which are well-characterized read-outs for BMP pathway activity and known promoters of HCC-progression. In line with this, GDF5 induced the phosphorylation of Smad1/5/8 and the proliferation and colony formation of HCC-cell lines.

Summary and conclusion Our study newly identifies activated HSC as major cellular source of enhanced GDF5 expression in fibrotic liver disease and HCC and indicates its pro-tumorigenic effect. Future analyses will reveal the potential of this soluble growth factor as therapeutic target or prognostic marker for the development and progression of HCC.

P 4.25 Direct interaction of the oncogenes YAP and TAZ with the transcription factor HNF1B in hepatocellular carcinoma

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Background Yes-associated protein (YAP) and WW domain containing transcription regulator 1 (WWTR1, TAZ) are transcriptional co-activators and main effectors of the Hippo pathway. Nuclear YAP or TAZ contribute to liver tumor formation and progression via induction of proliferation, cellular stemness and chromosomal instability. However, how YAP and TAZ support tumorigenesis

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at the molecular level is not fully understood. Here, we investigate the YAP-/TAZ-specific interactome in hepatocellular carcinoma (HCC) with focus on transcriptional regulators.

Methods HCC cell lines with inducible expression of YAP and TAZ fused to the biotin ligase BirA were used for BioID proximity-dependent labeling. Samples were subjected to mass spectrometry. Potential binding partners were confirmed using co-immunoprecipitation (co-IP) and proximity ligation assay (PLA). Gene silencing was performed using transient transfection of gene-specific siRNAs. Mice expressing constitutively active YAP_S127A were investigated.

Results Mass spectrometry revealed 88 YAP and TAZ interacting proteins. Interestingly, the cholangiocyte-specific marker hepatocyte nuclear factor 1β (HNF1B) was identified. HNF1B expression and binding with YAP and TAZ was confirmed in HCC cell lines. Spatial analysis illustrated prominent nuclear interactions of HNF1B with YAP/TAZ as well as with TEAD4, a known YAP/TAZ-binding transcription factor. YAP/TAZ silencing did not affect HNF1B expression; however, an induction of HNF1B is detectable in YAP_S127A-induced hepatomegaly and tumors.

Summary/Conclusion HNF1B is expressed in HCC cells and interacts with YAP and TAZ. Thus, elevated expression of HNF1B upon hepatocyte dedifferentiation/malignant transformation and binding to YAP/TAZ may represent a mechanism how the Hippo pathway contributes to tumor expansion with stemness characteristics in hepatocarcinogenesis.

P 4.26 Comparative response of HCC cells to sorafenib, lenvatinib, cabozantinib and regorafenib; descriptive expression analysis

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Introduction There are several tyrosine kinase inhibitors (TKI) currently approved for treatment of hepatocellular carcinoma (HCC), which will represent the future backbone of HCC treatment alone or in combination with immune checkpoint-inhibitors (CPI). The use of TKIs and their sequence of application in different lines of treatment is currently determined by empirical evidence, and no established biomarker capable of predicting the likelihood of response of one specific treatment exists. Thus, we examined differential sensitivity and investigated potential transcriptomic predictors of sensitivity to different TKIs.

Methods To this aim, the sensitivity of nine HCC cell lines to sorafenib, cabozantinib, lenvatinib and regorafenib was evaluated by proliferation assay to determine their respective growth rate inhibition concentrations (GR50). Subgroups discriminated by GR50 values underwent differential expression and gene set enrichment analysis (GSEA).

Results The nine cell lines showed broadly different sensitivity to different TKIs. GR50 values of sorafenib and regorafenib clustered closer in all cell lines, whereas treatment with lenvatinib and cabozantinib showed diversified GR50 values. GSEA showed the activation of specific pathways in sensitive vs. non-sensitive cell lines. A signature consisting of 15 biomarkers discriminates the cell lines' response into three distinct treatment profiles: 1) equally sensible to sorafenib, regorafenib, and cabozantinib, 2) sensible to lenvatinib and 3) more sensible to regorafenib than sorafenib.

Outlook Different sensitivities to TKIs are connected to distinct transcriptomic profiles and signaling pathways. This prompts larger studies to validate this expression signature for predicting a treatment's response for a personalized HCC treatment approach.

P 4.27 Relevance of MEK/ERK signaling in biliary differentiation in murine liver cancer

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Introduction Activation of oncogenic RAS signaling is commonly observed in human cholangiocarcinoma (CC), but less frequently in hepatocellular carcinoma (HCC). In mice, activation of oncogenic RAS together with genetic inactivation of two important tumor suppressor genes (RPK; Rblox/lox;p53lox/lox;Kras/lsl-KrasG12D) in the liver results in rapid development of aggressive cholangiocarcinoma characterized by activation of PI3K/AKT and MEK/ERK signaling. To date, the relevance of these RAS-dependent signaling pathways in CC is not fully understood.

Methods To investigate RAS-dependent signaling pathways, animals with activation of oncogenic Kras (RPK) and genetic inactivation of either PI3K-activated AKT (RPK;Pdk1lox/lox) or MEK/ERK signaling (RPK;Map2k1lox/lox;Map2k2-/-) were generated. All mice harbored a liver-specific inducible CreER (AlbCreER) and tumor development was induced by tamoxifen. Tumors were analyzed by histopathology and determination of mRNA and protein expression levels.

Results Genetic inactivation of PI3K/AKT or MEK/ERK signaling both result in delayed tumor development and prolonged survival of RPK mice. While tumors in RPK;Pdk1lox/lox animals were more well differentiated than those in RPK control mice, RPK;Map2k1lox/lox;Map2k2-/- tumors were more poorly differentiated despite longer time to tumor development. Strikingly, genetic inactivation of MEK/ERK signaling resulted in a change in tumor differentiation towards the hepatocyte lineage, with 33 % HCC and 29 % mixed HCC/CC. This differentiation shift correlated with an activation of Wnt/beta-catenin signaling, a commonly activated pathway in human HCC.

Conclusion In cholangiocarcinoma, RAS-dependent signaling pathways seem to have distinct functions in tumorigenesis. While activation of PI3K/AKT signaling was associated with more poorly differentiated tumors, MEK/ERK signaling was relevant driver of biliary differentiation in murine CC.

P 4.28 Cold shock protein YB-1 upregulates MDR1 transcription and thus contributes to cholangiocarcinoma chemoresistance to cisplatin

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Background & Aim Cholangiocarcinoma is a liver cancer with high mortality. Chemotherapy with cisplatin or gemcitabine is a main approach to treat cholangiocarcinoma patients at advanced stage. However, efficiency of these drugs is poor due to chemoresistance. To date, detailed mechanisms underlying chemoresistance in cholangiocarcinoma remain largely unknown. In this study, we demonstrate a crucial role for the cold shock protein Y-box binding protein-1 (YB-1) in cholangiocarcinoma chemoresistance to cisplatin.

Methods Expression of YB-1 was examined by immunohistochemistry in 28 cholangiocarcinoma patients receiving surgery. Among them, 10 patients received chemotherapy following operation. The function and relevant mechanisms of YB-1 in cholangiocarcinoma were investigated in vitro.

Results YB-1 expression in cancer cells, in particular in nuclei, was closely associated with survival of patients. From 10 patients receiving chemotherapy, 2 patients without YB-1 expression, but only 3 out of 8 patients with YB-1

expression survived a 5-year follow-up. In vitro, immunofluorescence staining showed that cisplatin administration resulted in YB-1 nuclear translocation. ChIP assays further demonstrated YB-1 binding to the multidrug resistance gene MDR1 promoter prior to expression induction. Impressively, administration of cisplatin significantly increased YB-1 binding to the MDR1 promoter. Functionally, knockdown of YB-1 by RNAi inhibited cancer cell proliferation and increased cisplatin-dependent apoptosis.

Conclusions YB-1 nuclear expression plays a crucial role in cholangiocarcinoma chemoresistance to cisplatin treatment through upregulating MDR1 expression. YB-1 expression in cancer cells might be a useful biomarker to determine chemotherapy in cholangiocarcinoma. Disruption of YB-1 is a potential approach to treat cholangiocarcinoma in clinical

P 4.29 Atezolizumab and bevacizumab in patients with advanced hepatocellular carcinoma with impaired liver function and prior systemic therapy – a real-world experience

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Aim Evaluation of efficacy and safety of atezolizumab + bevacizumab in real-world patients with impaired liver function and prior systemic therapy.

Methods 79 patients treated with atez/bev at two german university hospitals were retrospectively analyzed.

Results Almost half of the cohort (46.8 %) met at least one major exclusion criteria of IMbrave150-trial (hereafter referred to as IMbrave-OUT, n = 37), whereas 53.2 % were eligible (IMbrave-IN, n = 42). The overall response and disease control rates were 19.0 % and 53.1 %, respectively. Patients in IMbrave-IN group had longer median overall survival (not reached vs 4.4 months, 95 % CI 1.05 – 7.58; p = 0.008) and a trend for longer median progression-free survival (5.7, CI 95 % 2.3 – 9.1, months vs 3.3, 95 % CI 2.0 – 4.5, months; p = 0.068) than the IMbrave-OUT group. Prior systemic treatment had no significant impact on OS (p = 0.68). mOS was not yet reached for patients with ALBI grade 1 and 4.4 months for patients with ALBI grade 2/3 with no difference for ORR, DCR and mPFS. Absence of baseline ascites and portal vein invasion (PVI) were independent prognostic predictors [HR 0.37 (95 % CI 0.17 – 0.77; p = 0.008)] and [HR 0.46 (95 %CI 0.23 – 0.93; p = 0.030)], respectively. 39 patients (41.8 %) had a CTCAE grade ≥ 3 and patients in the IMbrave-OUT group were at higher risk for hepatic encephalopathy (18.9 % vs 0 %, p = 0.003) and ascites (32.4 % vs. vs. 14.3 %; p = 0.01).

Conclusion Efficacy in this real-world cohort was comparable to the results of IMbrave150-trial, regardless of prior systemic therapy . Presence of PVI and ascites at baseline independently impacted survival.

P 4.30 In-depth analysis of High-mobility group A proteins in NASH-HCC

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Liver cancer is the sixth most common type of cancer worldwide and the fourth leading cause of cancer-related death. In contrast to other cancer types, the mortality of liver and biliary tract cancer increases dramatically, but the molecular causes are largely unknown. Therefore, understanding the underlying molecular mechanisms and identifying new therapeutic targets is important. In the past decades, infections with HBV and HCV or intoxications with alcohol or aflatoxins were the main causes of HCCs. Nowadays the global obesity epidemic is closely associated with the rising prevalence of NASH-HCC. Dysregulated epigenetic factors are promising therapeutic targets for liver cancer patients. High mobility group-A proteins are small non-histone chromatin-associated proteins involved in the modulation of transcription. Previously we have shown the mechanism by which HMGA proteins regulate transcription. Here we investigate the role of HMGAs in liver cancer. We use RNA-seq and corresponding clinicopathological data from publically available databases and tissue microarrays for expression and survival analysis of human HCC patients. To elucidate the underlying epigenetic mechanisms we perform in-depth analysis combining immunohistochemistry, metabolomics, and proteomics of hepatocyte-specific single and double-knockout mice in a NASH mouse model, resembling the human pathology. Furthermore, to analyze the impact on cellular transformation and signaling we perform in-vitro assays using human and murine HCC cell lines. In this study, we report that aberrant expression of HMGAs correlates with poor overall survival and advanced tumor grade of HCC patients. This indicates that the dysregulation of epigenetic factors potentially might contribute to NASH-HCC development.

P 4.31 Tumour-suppressive BMP-9 signalling in HCC

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Bone Morphogenetic Protein (BMP)-9, a member of the TGF-β family of cytokines that is constitutively produced by hepatic stellate cells of the liver, is a high-affinity ligand of the type I receptor activin receptor-like kinase1 (ALK1). BMP-9 is a strong enhancer of bone formation, plays an important role in vascular and hepatic homeostasis and it affects glucose metabolism and insulin resistance. In addition, we recently found that BMP-9 enhances pro-inflammatory responses of macrophages.

Regarding its role in tumorigenesis and liver fibrosis, the results have been controversial. It seems that BMP-9 can be both, a pro- as well as anti-fibrogenic factor and it can act pro- or anti-proliferative on cancer cells.

We found that in human liver ALK1 is highly expressed on sinusoidal endothelial cells and Kupffer cells/macrophages but not hepatocytes (HC). In healthy HC BMP-9 therefore signals via alternative type I receptors, like ALK2, and mediates maintenance of a differentiated, non-proliferative cellular phenotype via activation of the Smad-1 pathway. In many HCC patient samples, in contrast, ALK1 expression is upregulated and correlates with tumour-promoting expression signatures. We found that in highly malignant HC BMP-9 does not efficiently activate the Smad-1 pathway anymore and ID1 induction is strongly dampened. Obviously, in such cancer cells ALK1 acts like a ligand trap, preventing the anti-cancerous BMP-9 signalling via ALK2.

In conclusion, BMP-9 targeted therapy acting via specific blockage of the BMP-9/ALK1 interaction on HC but leaving BMP-9/ALK2 signalling intact, could be a promising new approach to treat HCC patients.

P 4.32 Deep view on HCC gene signatures: and their comparison with other cancers

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DOI 10.1055/s-0041-1740784

Background Throughout the past two decades, numerous HCC-dependent gene signatures have been published, but none of them made it into the clinical routine application. Setting up a comparative transcriptomics profiling approach, we, therefore, explored the specificity of these signatures to HCC as this may be critical with respect to clinical usage.

Methods From the existing publications, we collected HCC gene signatures and evaluate them in independent genome-wide gene expression profiles of patients suffering from HCC. Evaluations were performed using four different (commercial and non-commercial) gene expression profiles, particularly a modified version of the ProfileChaser, Oncomine Premium, GENEVA, and SigCom LINCS for assigning similar pre-analyzed profiles to our signatures of interests. These results were screened for different categories (e. g.: tumor, benign liver disease).

Results and Discussion Although the specificity of the investigated HCC signatures varied significantly, none of the signatures exhibited strict specificity to HCC. Many signatures expressed similar profiles in other tumors, especially to renal, breast, and colorectal cancers, and particularly those signatures designed to distinguish between good and poor survival of patients. Since signature specificity proved difficult we furthermore evaluated overlapping pathways among signatures and evaluated a significant heterogeneity in signature generation approaches that may account for this phenomenon. Hence, our study demonstrates that the clinical use and independent validation of HCC genetic signatures remain challenging. Overall, our data suggest the necessity of standards in tissue preparation and storage as well as signature generation and statistical methods.

Poster Visit Session V Viral Hepatitis and Immunology 29/01/2022, 12.15 pm – 13.00 pm

P 5.01 Infection grade determines intestinal immune reaction in Schistosomiasis

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Introduction Schistosomiasis is one of the most common parasitic diseases worldwide with over 200 million infections. It is caused by trematodes of the genus Schistosoma, that mature, pair and lay eggs into intestinal veins (S. mansoni) of the host. The eggs can be trapped in capillary vessel, where they cause inflammatory reactions, predominantly in the liver and intestine. We aimed to analyze the effect of S. mansoni infection on the immune response in intestinal tissues.

Methods Female hamsters were infected with S. mansoni cercariae of both sexes (bisex) or with cercariae of one sex (monosex control without egg deposition). The worms were rinsed out of the hosts circulation by perfusion and subsequently counted, while the egg-load was counted in KOH-digested tissue. Th1 and Th2 specific cytokines were analyzed by RT-qPCR.

Results Compared to the control-groups (monosex and not infected), IL-4, IL-10, and IFNγ were induced in bisex-infected hamsters. In this group a positive correlation between the Th1 cytokine IFNγ and worm burden was observed. Interestingly, IL-5 mRNA expression levels did not show any group differences, but an inverse correlation between egg load and IL-5 expression.

Conclusion Our results suggest that the bisex-infection with S. mansoni caused a Th1 response and Th2 response in the intestine. There is evidence that the Th1 response is associated with the number of worm burden, whereas the inverse correlation of IL-5 with egg load may suggest that the Th2 response depends on the time of egg production and development of immune tolerance.

P 5.02 Contribution of the Cellular Lipid Kinase PI4KA to HCV-induced Liver Pathogenesis

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DOI 10.1055/s-0041-1740787

Phosphatidylinositol-4-phosphate (PI4P) generated by phosphatidylinositol-4-kinase IIIα (PI4KA) plays a direct role in cellular trafficking and provides substrates for the synthesis of other phosphoinositides which are largely involved in signal transduction. Hepatitis C virus (HCV) is known to activate PI4KA. Recent studies showed elevated PI4KA expression in hepatocellular carcinoma, particularly associated with poor prognosis. Therefore changes in PI4KA activity and abundance might be a critical determinant in regulating tumor progression.

In this study we found that PI4KA silencing or inhibitor treatment in hepatoma cells induced changes in cell morphology due to reorganization of cytoskeletal structures. Phosphorylation of paxillin and cofilin, two important actin cytoskeletal regulators, was reduced under these conditions, resulting in less focal adhesions and lower invasiveness. The PI4KA activation upon HCV infection or expression led to opposite phenotypes with increased p-paxillin and p-cofilin, elevated focal adhesions numbers and enhanced cell invasiveness, suggesting PI4P concentration as the driving force. Evaluation of PIP synthesis pathways revealed that silencing of PIK3C2G, a lipid kinase converting PI4P to PI(3,4)P2, led to similar phenotypes observed in PI4KA-knockdown cells. Knockdown of PI4KA or PIK3C2G reduced PI(3,4)P2-containing podosome-like structures at cell plasma membrane, dampening p-AKT2. HCV infection or expression in contrast stimulated p-AKT2. Key findings were validated using immortalized hepatocytes, PHH and mice.

In conclusion, our data suggested that elevated PI4KA expression or activity promotes cellular pathways governing cell morphology, cytoskeleton dynamics and cell invasiveness, favorable for cancer progression via PI(3,4)P2 and its downstream mediator AKT2. HCV therefore potentially contributes to liver pathogenesis via activating PI4KA.

P 5.03 3D spatially resolved HCV replication models simulated at realistic cell geometries

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The Hepatitis C virus steadily jeopardizes the health of the host during chronic infection.

The HCV viral RNA replication cycle is a dynamic process occurring in three dimensional space (3D). HCV-generated replication factories are housed within virus-induced intracellular structures termed membranous webs (MW) which are derived from the Endoplasmatic Reticulum (ER).

We present 3D spatio-temporal resolved diffusion-reaction models of the HCV RNA replication cycle by means of partial differential equation (PDE) descriptions. We present models of different complexity evaluated each upon realistic reconstructed intracellular compartments (ER/MW).

While the most simple model describes the dynamics of the basic components of the HCV RNA replication cycle, namely HCV RNA, non-structural viral proteins (NSPs) and a host factor, extended models are based upon two additional parameters: Different aggregate states of HCV RNA and NSPs, and population dynamics inspired diffusion and reaction coefficients instead of multilinear ones. The combination of both aspects enables realistic modeling of viral replication at all scales.

In particular, the replication complex state we introduce consists of HCV RNA together with a defined amount of NSPs. Indeed, the combination of spatial resolution and different aggregate states allows to mimic a cis requirement for HCV RNA replication. While in part our model still uses heuristic parameters, our simulations allow fitting core aspects of virus reproduction at least qualitatively. Our spatio-temporal resolved ansatz paves new ways for understanding intricate spatial-defined processes central to specific aspects of virus life cycles.

P 5.04 Association between low anti-HBc level and lower risk of virological relapse after nucleos(t)ide analogue cessation in HBe antigen negative chronic hepatitis B patients

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Background Relapse must be expected in > 50 % of HBeAg negative patients after nucleos(t)idanalogue (NA) cessation. Reliable biomarker for a safe therapy discontinution, next to HBs-Ag are missing. Recently, a low anti-HBc-level was linked to a higher relapse-risk in a cohort of predominantly HBeAg positive patients. This study investigated the association of serum anti-HBc-level with relapse after NA cessation in HBeAg negative patients.

Methods Before NA cessation level of anti-HBc, HBsAg and HBcrAg were determined in 136 HBeAg negative patients, participating in a therapeutic vaccination trial (ABX-203,NCT02249988). Importantly, the partly performed concomitant therapeutic vaccination showed no impact on relapse. Relapse was defined as HBV DNA > 2,000 IU/ml. Anti-HBc-level were compared between relapsers and off-treatment responders using the Mann-Whitney U test. Optimal anti-HBc threshold and correlation between the biomarker was determined by the Youden-index and Spearman-correlation, respectively.

Results No correlation was found between baseline anti-HBc and HBsAg (r = 0.016,p = 0.85) or anti-Hbc and HBcrAg (r = -0.011;p = 0.90). Relapse occurred in 68 patients (50 %). Median anti-HBc-level was significant higher compared to off-treatment responders (520 IU/ml vs. 330 IU/ml,p = 0.0098). The optimal anti-HBc cut-off to predict relapse was determined as 325 IU/ml. 35 % of patients with an anti-HBc-level < 325 IU/ml relapsed, while this was the case in 60 % of those with values ≥ 325 IU/ml (p = 0.0103; sensitivity 50 %,specificity 75 %).

Conclusion In contrast to a cohort of predominantly HBeAg positive patients, lower anti-HBc-level are associated with a significant lower relapse-risk after NA cessation in HBeAg negative patients. Anti-HBc-level should be further explored for the etablishment of prediction models in the future.

P 5.05 Production and release of hepatitis B virus particles in a 1.4-transgenic mouse model lead to increased phagocytic activity in Kupffer cells

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Background and Aims Pathogenesis of Hepatitis B virus (HBV) infection is driven by the adaptive as well as the innate immune system. The present project aims to investigate how HBV surface antigen (HBsAg) affects Kupffer cell function, utilising different HBV-transgenic mouse strains.

Method F4/80-positive KC were prepared after two-step perfusion of murine livers (wild type, tg(Alb1HBV)44Bri (Alb/HBs), tg1.4HBV-s-mut and tg1.4HBV-s-rec [F1 generation of Alb/HBs × tg1.4HBV-s-mut]) followed by MACS bead separation. Phagocytic activity (PA) was analysed by flow cytometry. F4/80 was stained in Eci-cleared mouse liver and visualised by Light sheet fluorescence microscopy (LSFM).

Results Two populations of F4/80-positive KC (dim and bright) could be identified and were independently analysed. F4/80-bright KC derived from tg1.4HBV-s-rec mice and Alb/HBs-derived F4/80-dim KC showed decreased PA. Interestingly tg1.4HBV-s-rec-derived F4/80-dim KC showed significantly increased PA. Although the KC counts were significantly increased in tg1.4HBV-s-mut mice, F4/80-dim and -bright populations showed phagocytic activities and capacities related to wild type KC. Furthermore, in Eci-cleared liver tissue of tg1.4HBV-s-mut mice, compared to wild type samples, LSFM visualised a distinct distribution and signal intensity of F4/80 + cells within the liver lobular structure. The F4/80-related signal intensity was significantly decreased on KC in HBV-s-mut mice and further indicated a migration from peripheral/portal area towards the central area of liver lobules.

Conclusion The production and release of viral particles in tg1.4HBV-s-rec mice possibly lead to an increased phagocytic activity in F4/80-dim KC. Assuming, that KC function is not generally impaired in HBV-replicating murine models.

P 5.06 Hepatitis B virus-replicating transgenic mice exhibit a functional but altered responsiveness to Tlr3 and Rig-I activation

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DOI 10.1055/s-0041-1740791

Background and Aims Pathogenesis of Hepatitis B virus (HBV) is driven by the adaptive as well as the innate immune system. The present project aims to investigate how HBV surface antigen (HBsAg) affects hepatic responses to poly(I:C), utilising HBV-transgenic mouse strains.

Method Liver tissue from C57/Bl6 and HBV-transgenic mouse strains (tg(Alb1HBV)44Bri (Alb/HBs), tg1.4HBV-s-mut and tg1.4HBV-s-rec [F1 generation of Alb/HBs × tg1.4HBV-s-mut]) was analysed using transmission electron microscopy (TEM). All-in-one liver cell preparation was performed, obtaining primary murine hepatocytes (PMH), liver sinusoidal endothelial cells (LSEC) and Kupffer cells (KC) and remaining NPC (rNPC). Responsiveness to poly(I:C) was determined using quantitative RT-PCR and multiplex-based cytokine assay.

Results TEM visualised viral particles (tg1.4HBV-s-rec), nuclear circular formations (tg1.4HBV-s-mut) and pathologic changes in the endoplasmic reticulum (Alb/HBs). Response to poly(I:C) treatment (Tlr3) and transfection (Rig-I) was investigated. A distinct cell type-dependent and mouse strain-dependent gene expression pattern for interferon (Ifnb, Ifiti1), cytokine (Tnf, Il1b, Il10) and chemokine (Ccl2, Ccl5) was observed by quantitative RT-PCR and validated using a multiplex-based cytokine assay. Emphasizing that tg1.4HBV-s-rec-derived liver cells responded to poly(I:C) with massively suppressed gene expression, when compared to the other mouse strains. Contrary, rNPC of tg1.4HBV-s-rec mice showed the highest gene induction for Il10, Tnf, Il1b and Ccl5 in response to poly(I:C). In all HBV-transgenic mice, the induction of Ccl2 was significant lower in PMH but increased in rNPC.

Conclusion Our data indicates that PMH of tg1.4HBV-s-rec mice produce viral particles and all liver cell types exhibit a functional but suppressed Tlr3 and Rig-I signalling.

P 5.07 Colonisation of bile ducts with Enterococcus sp. associates with the prognosis of Primary Sclerosing Cholangitis

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Background and Aims We have recently described an altered biliary microbiota composition in Primary Sclerosing Cholangitis (PSC) with a high abundance of Enterococcus sp. Little is known about the impact of these findings on the clinical course of disease.

Method We here investigated the association of bile fluid culture results obtained routinely during ERCP on major clinical endpoints in a large single center cohort of PSC patients. Peri-interventional i. v. antibiotics were given as standard after the sampling of bile fluid, and procedures with overt bacterial cholangitis and receiving antibiotic treatment prior to ERCP were excluded enabling the analysis of biliary colonization on disease course.

Results Microbiological culture results from 591 ERCP were included. Bacteria grew in 75 % of biliary cultures and in 32 % of first time ERCP procedures. Positive bile cultures were significantly associated with development of cirrhosis and its complications and survival free of liver transplantation. The presence of Enterococcus faecalis and/or faecium in bile fluid conferred risk of disease progression with a HR of 2.28 (95 % CI 1.32-3.9.2, $p < 0.005$) for achieving clinical endpoints and HR of 1.9 (95 % CI 1.05-3.41, $p < 0.05$) for survival free of liver transplantation.

Conclusion The strong association between bile duct colonization with Enterococcus sp and disease progression highlights the importance of microbiota-mucosal interplay for the pathogenesis of PSC. These results should stimulate further mechanistic studies on the role of microbiota for biliary inflammation in PSC.

P 5.08 Liver fibrosis does not impair tolerance induction by liver sinusoidal endothelial cells in vivo

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Background Liver sinusoidal endothelial cells (LSECs) contribute to the liver's ability to induce immune tolerance. We have previously shown that LSECs can be harnessed for therapy of autoimmune disease by targeted delivery of autoantigen-peptides with nanoparticles (NPs). Yet, in liver fibrosis, LSECs undergo capillarization and acquire enhanced immunogenicity. Here we explore whether fibrotic LSECs maintain their ability to induce tolerance to NP-delivered peptides.

Methods Liver fibrosis was induced in C57BL/6 mice using biweekly injections of CCL4 for 4 weeks; alternatively, spontaneous fibrosis in 8-12 weeks old MDR2-/- mice was examined. Tolerance induction by LSECs was tested in CD4 + T cell-driven myelin oligodendrocyte glycoprotein (MOG)-induced neuroinflammation (MOG-EAE), or in OT-1 CD8 + T cell-induced cholangitis in K14-OVAp mice. Autoantigen-peptide-loaded NPs or unloaded NPs were injected one day before induction of autoimmune disease.

Results

In fibrosis, MOG-EAE was effectively ameliorated by NP-mediated targeting of MOG-peptide to LSECs (mean clinical disease score 0,42 vs.2,57; P = 0,0006). Likewise, cholangitis was prevented in fibrotic mice by treatment with SIINFEKL-loaded NP (reduced hepatic infiltration of antigen-specific CD8 + T cells (52,35 % vs.71.7 %; P = 0,0123) and reduced weight loss (-1,92 %vs.-12,44 %; P = 0,0072).

Conclusion The ability of LSECs to induce immune tolerance to ingested autoantigen-peptides in vivo was not impaired in two models of liver fibrosis. As in non-fibrotic mice, selective delivery of autoantigen-peptides to LSECs using NPs in fibrotic livers protected both from CD4 + T cell-driven and CD8 + T cell-driven autoimmune disease. Thus, the in vivo tolerance-inducing function of LSECs seems to be more robust to fibrotic changes than previously thought.

P 5.09 Acute Cytomegalovirus (CMV) Hepatitis in an Immunocompetent Female, A Case Report and Review of Literature

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Cytomegalovirus (CMV) is a common pathogen with a worldwide seroprevalence ranging from 40-100 %. Hepatic involvement varies depending on the patient's immune condition with severe disease being usually encountered in immunocompromised patients. On the other hand, most cases among immunocompetent subjects are asymptomatic and CMV hepatitis requiring hospitalization is very rare. Thus, it's not uncommon for such diagnosis to be delayed, especially among immunocompetent patients, leading to an extensive and unnecessary diagnostic workup. This case report describes a 26-year-old immunocompetent female with an acute CMV hepatitis presenting with a one-week history of headache and nausea. The CMV-Infection was diagnosed based on positive serology and polymerase chain reaction (PCR). The patient was treated conservatively with documented spontaneous viral elimination and consequent clinical improvement and normalization of liver enzymes.

P 5.10 Comprehensive analysis of different phases of chronic Hepatitis B Infection

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Chronic Hepatitis B virus (HBV) infection was classified into four active phases by the EASL: HBeAg-positive chronic infection, HBeAg-positive chronic hepatitis, HBeAg-negative chronic infection and HBeAg-negative chronic hepatitis. Recently, it was observed that released HBsAg amounts, ratios of L-, M- and S-proteins and morphology of subviral particles varied by genotype (Gt). Here, we characterized the different phases of chronic HBV infection regarding HBsAg, HBcAg and particle assembly in vivo and in vitro.

Sera of patients infected with GtA or GtD of different disease phases were analyzed by HBsAg-specific Western Blot (WB) and density gradient centrifugation. HBV genomes of GtA, GtB, and GtD of representative HBeAg-negative patients with precore mutation G1896A were cloned into pUC-18 and expressed in Hepatoma cells. Corresponding wildtype genomes were used for comparison. In sera of patients, ratios of HBsAg proteins differed among genotypes but not among different disease phases. In addition, density of HBsAg-particles varied among the genotypes but not in regard to stage of infection. In vitro, HBeAg-negative genomes showed a reduction in HBsAg expression. WB anal-

yses indicated an accumulation of LHBs and core protein within cells. Furthermore, immunofluorescence microscopy analyses showed a perinuclear accumulation of HBsAg and a strong intranuclear localization of assembled/dimeric core protein.
In conclusion, consistent ratios of HBsAg protein and similar particle densities were observed among different phases of infection. In contrast to HBeAg-positive genomes, HBeAg-negative genomes are characterized by an intranuclear localization of assembled/dimeric core protein and a perinuclear accumulation of HBsAg in vitro. This might affect novel antiviral strategies.

P 5.11 Th2 immune response correlates inversely with the egg load in S. mansoni infection

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Question Infection with Schistosoma mansoni, a worm parasite, results in deposition of parasitic eggs in the liver where they cause granuloma formation. Adult worms induce a hepatic Th1 immune response, shifting towards a Th2 immune response after egg production. We aimed to investigate whether the hepatic Th2 immune response is correlated with egg infestation.
Methods Twenty-two female hamsters were infected with S. mansoni bisex (both sexes), ten with monosex cercariae (control without egg deposition), and six were not infected (super control). After 6-8 weeks, liver tissue was examined by qRT-PCR for expression of Th2 inflammatory interleukins, such as IL-4, IL 5, and IL-13. The extent of hepatic infection with S. mansoni eggs and worms was determined by counting.
Results The mRNA expression of IL-4, IL-5, and IL-13 were significantly upregulated in bisex-infected hamsters. Within the bisex-infected group, expression of IL-4, IL-5, and IL-13 correlated inversely with the number of eggs. There was no strong correlation between the examined inflammatory markers and the total number of worms.
Conclusion The increased expression of Th2 interleukins in bisex-infected animals confirms that Th2 response is triggered by the parasitic eggs. The inverse correlation of egg load with Th2 response, however, remains elusive. The connection between a decrease in the Th2 immune response and an increasing egg load has never been described before. A possible explanation could be an acquired immune tolerance due to severe infection. Understanding the underlying mechanisms in detail could help to provide new therapeutic approaches.

P 5.12 A patient with congenital hepatic fibrosis, after living-donor liver transplantation, developed a systemic IgG4-related disease following infection with SARS-CoV-2

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Introduction Congenital hepatic fibrosis (CHF) is an autosomal recessive disease characterized by hepatosplenomegaly and portal hypertension. IgG4-related disease is considered a systemic and inflammatory syndrome characterized by enlargement of involved organs, elevated IgG4, dense lymphoplasmacytic infiltrate, and fibrosis. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-related coronavirus disease 2019 (COVID-19) is associated with significant morbidity and mortality in liver disease. While immunosuppression after liver transplantation (LT) may attenuate inflammatory response to COVID-19, it may also increase virological injury and prolong viral shedding.
Case report A 23-year-old Caucasian male, diagnosed with CHF in 1998, underwent living-donor LT after multiple bleeding complications. In March 2020, patient was hospitalized with SARS-CoV-2 infection. In December 2020, MRI demonstrated a progressive, elongated tissue plus retroperitoneally along the abdominal aorta to the common iliac artery. Pathologically enlarged lymph nodes were detected (mesenteric, peritoneal, iliac, and inguinal). Whole-body PET confirmed these findings matching to a post-transplant lymphoproliferative disease in 2021. However, EBV-DNA and skewed kappa:lambda immunoglobulin free light chain ratio in peripheral blood were not detectable. Colonoscopy with biopsies demonstrated > 40 positive plasma cells/hpf rich in IgG4-immunostaining. Biopsy of retroperitoneal lymph nodes revealed IgG4-positive plasma cells. In addition, highly elevated IgG4 with 45'300 mg/l along with an IgG of 53'500 mg/l, highly elevated sIL2 receptor with 2'246 U/ml, and increased eosinophils with 11.8 % were present. Treatment of this underlying IgG4-RD with rituximab is scheduled.
Conclusion Diagnosis of IgG4-RD is challenging. This disease may also occur despite immunosuppressive therapy, in our case, probably induced by the coronavirus.

P 5.13 Enolase represents a metabolic checkpoint controlling the differential exhaustion of virus-specific CD8 + T cells in viral hepatitis

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Exhausted T cells (TEX) accumulating in patients with chronic Hepatitis B and -C virus (HBV/HCV) infection are functionally impaired and contribute to insufficient virus elimination. There is recent evidence that a dysregulated energy metabolism is a major driver in the development of T cell exhaustion. However, the connection of the metabolic profiles and antiviral function of HBV- and HCV-specific CD8 + T cells in chronic infection remains poorly adressed. Therefore, we analyzed the metabolic determinants of HBV- and HCV-specific CD8 + T cells by flow cytometry and peformed microarray analysis of sorted HBV- and HCV-specific CD8 + T cells to analyze key metabolic pathways. We found that in chronic infection, HCV-specific CD8 + T cells exhibit reduced mitochondrial polarization associated with increased expression of inhibitory receptors, suggesting that these cells are more profoundly exhausted compared to HBV-specific CD8 + T cells. Metabolic pathway analysis revealed downregulation of the ENO1 mRNA transcripts in HCV-specific CD8 + T cells, suggesting that diversion of metabolic flux during glycolysis represents a metabolic checkpoint for exhausted CD8 + T cells. Bypassing this bottleneck by supplementation of pyruvate bolsters antiviral function of HCV-specific CD8 + T cells. Taken together, these results reveal distinct metabolic programs of exhausted HBV- and HCV-specific CD8 + T cells. They demonstrate that metabolic interventions are useful strategies to improve T cell function in chronic viral infections.

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P 5.14 A polymorphism at the BACH2 locus associates with skewed T cell differentiation in patients with primary sclerosing cholangitis

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Background Genome-wide association studies (GWAS) associated primary sclerosing cholangitis (PSC) to several polymorphisms in immune-related genes. We here addressed the question whether specific polymorphisms may affect T cell differentiation, immune cell phenotype and clinical course of patients with PSC.

Methods In an ongoing study, patients with PSC (n = 270) were genotyped for the disease-associated risk variants rs56258221 (BACH2), rs80060485 (FOXP1), rs4147359 (IL2RA) and rs7426056 (CD28). Comprehensive immunophenotyping and functional experiments on naive CD4 + T cells from peripheral blood were performed.

Results Carriers of the risk variant rs56258221 (BACH2) showed an increased frequency of naive CD4 + (34,6 % vs. 24,6 %, p = 0,048) with concomitant decrease in memory CD4 + T cells (49,6 % vs. 61,8 %, p = 0,024) in the peripheral blood, compared to non-carriers. Functional in vitro experiments with naive CD4 + T cells revealed an increased capacity of these cells to differentiate into T Helper 17 cells (TH17) (5,5 % vs. 2,2 %, p = 0,042), whereas the conversion into induced regulatory T cells (iTREG) was decreased in carriers of rs56258221 (9,6 % vs. 17,3 %, p = 0,022). In order to assess a potential clinical impact of the risk variant rs56258221, we compared the prevalence of liver transplantation between the genotypes and observed a higher rate in the cohort of rs56258221-carriers, compared to non-carriers (17,5 % vs. 12,2 %).

Conclusion The gene polymorphism rs56258221 (BACH2) associates with skewed T cell differentiation in patients with PSC and thereby might contribute to the immune-related pathogenesis in PSC. Potential implications for the course of disease warrant further investigation.

P 5.15 MicroRNAs modulate SARS-CoV-2 infection in primary human hepatocytes by regulating the entry factors ACE2 and TMPRSS2

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Infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused the current coronavirus disease 2019 (COVID-19) pandemic. Despite a preferential respiratory tropism of SARS-CoV-2, multi-organ involvement has been described. SARS-CoV-2 entry into host cells is mediated by the entry factors angiotensin converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2). Recent studies suggest that SARS-CoV-2 causes direct hepatic impairment in COVID-19 patients. Interestingly, ACE2 and TMPRSS2 are also expressed in primary human hepatocytes (PHH). Despite this evidence, data on infection and factors modulating functional regulation of SARS-CoV-2 infection in PHHs are scarce. MicroRNAs (miRNAs) are a class of small non-coding RNAs that have been described to modulate various cellular processes and have been implicated as potential therapeutic target. We aimed to study the infection of PHHs with SARS-CoV-2 and to evaluate the potential of miRNAs for modulating viral infection. We could demonstrate that PHHs can be readily infected with SARS-CoV-2. Bioinformatics analyses revealed miR-200c-3p, miR-429, let-7c-5p and miR-141-3p as candidate miRNAs targeting ACE2 and TMPRSS2. All miRNAs were able to reduce SARS-CoV-2 burden in PHH by supressing ACE2 and TMPRSS2. Our findings provide the first evidence of the applicability of miRNA molecules in reducing SARS-CoV-2 viral loads.

P 5.16 Reality Check: Bulevirtide for HBV/HDV coinfection, dynamics of virological parameters and liver-stiffness

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Background HDV is the most aggressive form of chronic viral hepatitis with a high risk for the development of liver cirrhosis and its complications. Until 2020 treatment with interferon-alpha was the only therapeutic-option for HBV/HDV-coinfection, with serious side effects and limited efficacy. Bulevirtide is a synthetic lipopeptide that blocks HBV/HDV uptake into hepatocytes by binding to the NTCP-receptor. There are few reports of its clinical use from Germany and little experience on treatment dynamics and efficacy in a real-life settings. We present clinical and virological data on our first 12 patients treated with bulevirtide.

Methods Since 11/2020 12 HDV-infected patients with active hepatitis started bulevirtide-treatment. Median age (4 female, all HDV-genotype 1) was 43. Five patients had compensated liver cirrhosis, ten patients received NUC-therapy and eight patients had a history of previous interferon treatment. Every 30 days HDV-RNA, HBV-DNA, HBsAg, ALT/AST, and bile-acids as well as liver-stiffness were tested.

Results Median baseline-parameters were: HDV-RNA 597.000 IU/ml, ALT 117 U/l, AST 64 U/l, bile acids 11,5 umol/l, platelets 160x1000/ul, HBsAg 23.074 IU/ml, liver-stiffness 10kPa. At the time of analysis (range of treatment-duration two-ten months) five patients achieved normalisation of transaminases, . Median HDV-RNA was reduced by 1,35 log (2,8 % of baseline HDV) - or 28 % after three months of treatment and median liver- stiffness decreased to 7,7KPa.

Conclusion Real- life data in patients with advanced liver disease demonstrate a favorable safety profile, good patient compliance, and improvement of biochemical and virological parameters as well as liver damage regression for most patients.

P 5.17 Exploring the potential of multiplex immunohistochemistry to unravel histological and immunological changes during non-alcoholic steatohepatitis and primary sclerosing cholangitis in human and mouse liver

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Background Non-alcoholic fatty liver disease (NAFLD) roughly affects a quarter of the world population and is mainly characterized histologically by lipid deposition in hepatocytes. In some cases, NAFLD may evolve to non-alcoholic steatohepatitis (NASH) with immune cell recruitment and hepatocyte ballooning. Primary sclerosing cholangitis (PSC) is a progressive cholangiopathy with onion-skinning fibrosis around injured bile ducts and inflammatory infiltrates. The goal of this study is to apply a novel multiplex immunostaining approach optimized in our laboratory, for comparing morphological changes in liver histology of NAFLD, NASH and PSC patients, and identifying similarities and discrepancies between immune cell populations.

Methods Liver FFPE sections from normal, NAFLD, NASH and PSC patients, and from mouse NASH and PSC models were used. We recently implemented in our laboratory a method of sequential immunostaining that allows to

stain >15 markers on a singular FFPE tissue section. This protocol was combined with digital image analysis tools.

Results We successfully stained and analyzed archival FFPE samples from normal, NAFLD, NASH and PSC patients. In NASH and PSC, disease stage is associated with remarkable loss of lobular areas, increased fibrosis and defined infiltration of myeloid and lymphoid cell populations. NASH patients predominantly exhibit myeloid-cell infiltration, whereas PSC patients had a pronounced lymphoid cell response. Nonetheless, both diseases displayed intense monocyte accumulation in the perilobular areas, and this finding was confirmed on murine models of liver disease.

Conclusions Monocytes/macrophages infiltration and accumulation represent the most common histological feature associated with the progression of NASH and PSC.

P 5.18 Auto-aggressive CXCR6+ CD8 T cells cause liver immune pathology in NASH

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Non-alcoholic steatohepatitis (NASH) is characterized by chronic sterile inflammation that causes liver disease but the mechanisms of immune-mediated liver damage in NASH remain incomplete. An important feature of tissue-resident T cells in the liver is to integrate extracellular factors such as cytokines, nutrients and cell stress molecules into transcriptional networks to balance effector functions. Here, we identify murine and human CXCR6+CD8+ T cells as critical tissue-resident T cells causing liver damage in NASH after IL-15 and metabolic activation.

In NASH mice, we detected hepatic accumulation of CD8 T cells with phenotypes that combined tissue-residency (CXCR6) with effector (Granzymes) and exhaustion (PD-1) characteristics. Liver CXCR6+ CD8 T cells were characterized by low FOXO1 transcription factor activity and were abundant in mouse and human NASH. Mechanistically, IL-15 induced FOXO1 down- and CXCR6 up-regulation, which rendered CXCR6+ CD8 T cells susceptible to metabolic stimuli in NASH livers, including acetate and extracellular adenosine triphosphate, and collectively triggered auto-aggression through TNF and FasL.Treatment of NASH mice with antibodies blocking the activity of TNF, FasL or IL-15 significantly ameliorated liver damage. Importantly, CXCR6+ CD8 T cells from mouse and human NASH livers had similar transcriptional signatures and showed auto-aggressive killing of cells in an MHC-class-I-independent fashion after signaling through P2X7 purinergic receptors.

In summary, killing by auto-aggressive CD8 T cells fundamentally differed from that by antigen-specific cells, thus mechanistically distinguishing auto-aggressive from protective T cell immunity.

P 5.19 Expression of inflammatory, immune regulatory and tissue restorative genes by both Ly6Chi and Ly6Cint/- monocyte/macrophage subsets in acute and chronic liver injury in mice

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Background Autoimmune liver diseases (AILD) may lead to flares and chronic liver inflammation mediated by the innate and the adaptive immune system. Here we phenotypically characterized monocyte/macrophage subsets in murine models of AILD, based on previous definitions of inflammatory M1 (Ly6Chi/CCR2+) and anti-inflammatory/restorative M2 (Ly6Cint/low/CX3CR1+) subsets, to analyse monocyte/macrophage differentiation during acute and chronic phases of inflammatory liver injury.

Methods To induce anti-inflammatory/restorative monocyte/macrophage subsets, C57BL/6 mice were treated with IL-33 on three consecutive days. Acute immune-mediated liver injury was induced by administration of ConA to C57BL/6 mice. Mdr2-/- mice develop sclerosing cholangitis and served as chronic model. Simultaneous analysis of mRNA and protein expression by flow cytometry was performed using the PrimeFlow RNA assay.

Results IL-33 pre-treatment prevented acute hepatitis and resulted in elevated frequencies of Ly6Cint monocytes/macrophages expressing genes associated with anti-inflammatory/restorative function (Mmp9, Chil3, Tgfb1). However, all subsets showed increased expression of the M1 marker CCR2. In acute hepatitis, frequencies of Ly6Chi and Ly6Cint monocytes/macrophages increased. Both subsets expressed inflammation-associated (Nos2, Tnf) as well as anti-inflammatory/restorative genes (Il10, Arg1, Chil3, Tgfb1). In Mdr2-/- mice, Ly6Cint and Ly6C- macrophages were increased while Ly6Chi monocytes were decreased. They exhibited an un-specific gene expression profile by up-regulating Il12, Tnf, Il10, Chil3 and Areg.

Conclusion M1/M2 classification based on expression of Ly6Chi/CCR2+ and Ly6Cint/low/CX3CR1+ seems not to be appropriate for characterization of hepatic monocyte/macrophage subsets in acute versus chronic liver inflammation as they expressed a mixed pro- and anti-inflammatory/restorative gene profile.

P 5.20 Tim4high expression identifies a Kupffer cell subset with enhanced catching proficiency

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Background Kupffer cells (KCs) are tissue macrophages residing in liver sinusoids with critical functions to maintain liver homeostasis. Recent single-cell data in health and disease have expanded our understanding of the complexity of KCs and at least two subsets have been identified, however, a functional correlate of different KC subsets is largely unclear.

Methods Using a combination of intravital microscopy and spectral flow cytometry, we analyzed liver macrophages in healthy mice, mice with chronic liver injury and injury regression induced by CCl4 and human liver tissue.

Results We identified two subsets of F4/80+ KCs based on the expression of scavenger receptor Tim4. A subset of F4/80+Tim4high KCs with increased cell volume localized in the periportal region and efficiently filtered particulate targets out of the circulation. Flow cytometric analysis revealed expression of Tim4 by 70 % within the KC population. Following chronic liver injury, we observed a marked loss of Tim4+ KCs correlating with a reduction of the KC filter function. Regression of liver damage led to normalization of transaminase levels and reversion of the histopathological phenotype, however, KC catching ability was still impaired as assessed by particle uptake. Importantly, the subset of Tim4high KCs was significantly lower during injury regression and catching was restricted to the remaining Tim4high KCs. Flow cytometry of human tissue revealed Tim4 expression by CD14+ KCs.

Conclusion Our results identify Tim4 as a marker indicating proficiency of KCs to filter particles. Chronic injury and regression are accompanied by loss of Tim4 and correlate with impaired liver clearance.

P 5.21 The IL-33/amphiregulin axis mediates immunoregulation in acute and chronic liver disease

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Background In autoimmune liver diseases, the alarmin IL-33 is released from necrotic hepatocytes. IL-33 binds to the ST2 receptor thereby activating group 2 innate lymphoid cells (ILC2) and regulatory T cells (Tregs). Both cell types express amphiregulin (AREG), which has been associated with tissue repair, immunosuppression, fibrogenesis, and liver carcinogenesis. Here we investigated the immunosuppressive role of AREG in acute and chronic liver inflammation.

Methods Acute immune-mediated hepatitis was induced by ConA administration to WT or Areg-/- mice. To study IL-33-mediated immunosuppression mice received IL-33 three days before ConA challenge. 12 weeks old Mdr2-/- mice were used as a model for chronic liver inflammation resembling PSC.

Results Hepatic expression of Il33 and Areg was elevated in acute and chronic hepatitis, in which Areg was the most up-regulated EGFR ligand. ILC2 and ST2 + Tregs showed increased expression of AREG in acute hepatitis, whereas Tregs expressed less AREG in chronic liver inflammation. IL-33 pre-treatment resulted in strong AREG expression by ILC2 and ST2 + Tregs and prevented acute hepatitis. Interestingly, IL-33-induced ST2 + Tregs exhibited an immunosuppressive phenotype. Areg-/- mice were more sensitive towards ConA hepatitis, which corresponded to stronger activation of ILC2 and a reduced frequency of ST2 + Tregs in the liver.

Conclusion In acute hepatitis, IL-33 seems to mediate immunoregulation by expansion and activation of AREG-expressing ILC2 and ST2 + Tregs. In chronic liver disease, immunosuppressive function of Tregs might be attenuated because of reduced AREG expression, critically involved in maintenance of Treg function particularly under inflammatory condition.

P 5.22 Serological and clinical features of primary biliary cholangitis in first degree relatives: a prospective cohort study.

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Background/Aims First degree relatives (FDR) of patients with primary biliary cholangitis (PBC) frequently display anti-mitochondrial antibodies (AMA). However, the prevalence of PBC-specific anti-nuclear antibodies is unknown, nor have FDR been followed up systematically in order to assess clinical/serological features of PBC over time.

Methods A cohort of 231 FDR of PBC patients was prospectively screened for PBC-specific autoantibodies (AMA, MIT3, gp210, sp100) and biochemical makers of cholestasis. FDR with features of PBC were further examined and all participants were followed-up after 2 and 4 years. All participants completed a comprehensive survey to assess environmental risk-factors for the development of PBC.

Results At baseline, 5 of 231 (2.2 %) FDR were diagnosed with PBC. Sixteen (7 %) additional cases with PBC-specific antibodies were detected without signs of cholestasis. Notably, in 4 of the 5 identified cases with PBC, the affected family-member was a sister. The prevalence of disease specific autoantibodies was as follows: AMA: 5 % (10/231), MIT3: 3 % (8/231), SP100: 2 % (4/231), and gp210: 0 %. 80 % and 33 % of the 231 FDR completed the 2- and 4-year follow-up, respectively. No additional diagnosis of PBC was identified. However, 7 additional cases with PBC-specific antibodies were detected. Overall, 28/231 (12 %) FDR demonstrated PBC-specific antibodies, with the highest prevalence among sisters (23 %) of PBC patients. No environmental risk factors could be identified.

Conclusions Overall, the prevalence of PBC among FDR was 2 %, whereby the prevalence of PBC and PBC-specific antibodies was particularly increased in sisters of PBC patients. Thus, screening in this group can be considered.

P 5.23 CD5-mediated mTOR inhibition on T cells through binding of soluble CEACAM1

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DOI 10.1055/s-0041-1740808

Introduction Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) controls immunity via self- or heteroligation. Its soluble isoform (sCC1) is elevated in sera of patients with obstructive and autoimmune liver diseases. In ConA-mediated hepatitis, Ceacam1-/- mice show exacerbation and persistence of hepatitis as a result of impaired Treg function and stability. T cell activation and Treg induction use the short CEACAM1 isoform (CECAM1-S), whereas its ITIM-bearing long isoform (CEACAM1-L) is required to impose T cell inhibition. Recently, we identified the Treg regulator CD5 as a novel binding partner for sCC1.

Objectives Confirm the role of sCC1-CD5 interaction in immune homeostasis.

Materials & Methods sCC1 was detected in serum samples of humans and mice by Western Blotting. LC-MS/MS identified sCC1-CD5 interactions. iTregs were induced by sCC1, sCD5, or §CC1 and §CD5 antibodies, or TGFb, and tested in suppression assays. Activation of signaling pathways critical for Treg induction (via pSTAT5, Foxp3, pSmad2/3, mTOR/pS6) were monitored in FACS and Western Blots.

Results sCC1 was detectable in sera of patients and mice with advanced PSC or ConA hepatitis. CEACAM1-CD5 interactions were confirmed and functionally tested in cell binding and Treg induction assays. sCC1 binding to activated T cells induced pSTAT5 and Foxp3 concomitant with reduction in mTOR activity. These effects were modulated by §CD5 antibodies. sCC1-induced Tregs imposed suppression on T effector cells.

Conclusion Binding of sCC1 to activated T cells supports Treg induction by integrating CD5 mediated mTOR inhibition. Further experiments will reveal the role of CEACAM1-CD5 interaction in context of Treg homeostasis.

P 5.24 Immune cell composition in cholangiopathies: Target and modulator of gut-to-liver signals in cholestatic liver injury

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Although inflammation appears to be a driver of cholestatic liver disease progression, the exact mechanisms linking biliary injury, inflammation and fibrosis remain poorly understood. Remarkable changes in the composition of the gut microbiota in patients with primary sclerosing cholangitis (PSC) and other biliary diseases suggest have been reported, suggesting that dysbiosis may be crucial for cholangiopathy pathogenesis or progression. Moreover, approximately 80 % of PSC patients suffer from inflammatory bowel disease. In this study, using mouse models of biliary and/or intestinal injury as well as human samples, we aimed at identifying potential cellular actors implicated in cholangiopathy progression. We investigated the consequences of dextran sulfate sodium (DSS)-induced colitis on liver, colon and blood immune cell populations in a murine model of PSC (Mdr2-/- mice). We characterized the in situ expression of pattern recognition receptors (PRRs) in various cell populations with an innovative multiplex immunostaining protocol. Moreover, we validated a 27 marker panel for flow cytometry to further define these populations. Similar approaches were applied to PSC patient blood samples to correlate changes in immune cell populations with the severity of the disease. So far, we evidenced

that PRRs are expressed by virtually all immune cells and some othert cells, notably toll-like receptor 4 (TLR4) by endothelial cells. In the liver, concomitant colitis induces changes in neutrophil infiltration, which particularly express TLR9, and higher TLR4 expression is observed on infiltrating monocytes. Our findings may imply that the gut-liver axis modulates hepatic inflammation and disease progression in cholangiopathies.

P 5.25 Immune-mediated Hepatitis associated with SARS-CoV-2 mRNA vaccination

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In a fraction of SARS-CoV-2 vaccinees, hepatitis compatible with features of autoimmune hepatitis (AIH) have been observed. However, it remains unclear whether the association is coincidental, reflects drug-induced liver injury, or involves vaccine-induced antigen-specific immune activation. Here, we report a case of a 52-year-old male developing a transient hepatitis after the first mRNA vaccination and severe AIH-compatible hepatitis after the second.

The intrahepatic immune cell infiltrate was analysed by highly multiplexed imaging mass cytometry broadly covering key immune cell populations. Liver and longitudinal blood samples were analysed for the presence and phenotype of SARS-CoV-2 Spike-specific CD8 T cells using MHC class I tetramer technology. Additionally, Serum titers against SARS-Cov2-Spike antibodies were assessed.

We identified a panlobular CD8 T cell dominant immune cell infiltrate in the liver without significant plasma cell components. Spike-specific CD8 T cells were highly enriched within the intrahepatic CD8 T cell population expressing activation markers and a tissue-resident phenotype. The activation phenotype correlated with the circulating Spike-specific CD8 T cell profile and longitudinal analysis revealed a rapid decline of T cell activation after the initiation of budesonid therapy. However, the patient experienced a mild relapse under therapy that was paralleled by the peripheral activation of Spike-specific CD8 T cells and was controlled under systemic steroid therapy.

Collectively, our results indicate that an immune-mediated hepatitis after COVID19 vaccination can present with typical clinical features of an AIH but can be pathophysiologically separated from a classical AIH. Whether a long-term immunosuppressive regimen will be required remains to be determined.

P 5.26 Dysfunctional liver-resident CXCR6 + CD8 T cells during persistent viral liver infection

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Background A strong CD8 T cell response is crucial to eliminate hepatotropic viral infections such as hepatitis B virus infection. Contrastingly, a weak T cell response characterizes persistent viral infection of the liver. We aimed at pinpointing the mechanism mediating dysfunctionality in liver-resident CD8 T cells during persistent viral liver infection.

Methods Adenovirus-based gene transfer (coding for ovalbumin or the HBV genome) was used to establish persistent or acute-resolved infection. Naïve (CD44negCD62Lhi) virus-specific CD8 T cells bearing a congenic marker were transferred before infection. Cells were analyzed at different time points for phenotypic and functional characterization and RNAseq.

Results We identified two distinct virus-specific hepatic T cell populations after acute-resolved infection, i. e. CX3CR1 + and CXCR6 + CD69 + CD8 T cells. In persistent infection, mainly CXCR6 + CD69 + liver-resident CD8 T cells were found. Liver CXCR6 + CD69 + CD8 T cells expressed a tissue-residency gene signature, but in the persistent infection lacked anti-viral effector functions, i. e. determined by loss of effector cytokine production and cytotoxicity. Using RNAseq and bioinformatic analyses, we defined a single transcriptional pathway distinguishing dysfunctional CXCR6 + CD69 + T cells in persistent infection from effector memory T cells after resolved infection.

Conclusion The identification of a single transcription factor distinguishing effector memory from dysfunctional tolerized virus-specific resident CD8 T cells in preclinical models of persistent expression of ovalbumin and HBV points towards a liver-specific mechanism mediating immune tolerance during persistent hepatocyte infection.

P 5.27 Pre-clinical characterization of an HBsAg-specific monoclonal antibody preventing HBV spreading and reducing HBV, HDV and HBsAg in serum of humanized mice

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Treatment using monoclonal antibodies targeting the HBV surface proteins has the potential to induce functional cure not only in chronic HBV (CHB) patients but also in individuals coinfected with HDV, which depends on HBsAg for its life cycle. HBC34, the parental molecule of VIR-3434, is a human monoclonal antibody targeting the antigenic loop present in all HBV surface proteins (L-/M-/S-HBsAg). VIR-3434 is currently in Phase 1/2 clinical development.

Methods 3-weeks post HBV inoculation (spreading model) human liver chimeric mice received either HBC34 (1mg/kg/2-times/week), control antibodies, or entecavir for 6 weeks. Chronic HBV infected mice received HBC34, lamivudine or both drugs in combination (6-weeks). HBV/HDV coinfected mice received HBC34 (4-weeks). Viral markers were measured by ELISA, RT-qPCR and immunofluorescence.

Results HBV spreading and increase of intrahepatic viral markers were efficiently blocked in mice receiving HBC34 or entecavir. In chronic HBV monoinfected mice, HBC34, Lam or combination treatment caused 0.7log, 1.3log and 2.4log viremia reduction, respectively. HBsAg dropped 1.3log in mice receiving HBC34 alone and 2.6log in the combination group, but not in Lam treated mice. HBC34 administration in HBV/HDV coinfected mice efficiently reduced HBV (2.1log) and HDV (2.8log) viremia and HBsAg levels (2.7log), while HBeAg and intrahepatic viral loads remained unchanged.

Conclusion HBC34 potently blocked intrahepatic HBV spreading and lowered HBV and HDV viremia in chronically infected mice. The strong HBsAg reduction indicates that HBC34 could accelerate HBsAg clearance both in HBV and HBV/HDV infected patients. These data support the development of VIR-3434 for treatment of chronic HBV and HBV/HDV infection.

P 5.28 Reverse Inflammaging: Biological age is accelerated in chronic HCV patients and decelerates after HCV cure

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Background and AIM Recently, HIV and HBV infections have been shown to cause significant biological age acceleration. Accelerated epigenetic or biological age may be a predictor of all-cause and cancer mortality. Thus, we analyzed if HCV is associated with biological age acceleration and if this is reversible after HCV cure.

Methods We included well-characterized 54 patients (mean age 56 years) with chronic hepatitis C at three time points (baseline, end of treatment and follow-up 24-240 (mean 96 weeks) weeks after the end of treatment). Genome-wide DNA methylation data were generated using the illuminaEpic array on PBMC and were used to calculate 2 measures of epigenetic age acceleration (Horvath and Hannum AgeAccel).

Results Our results show that chronic HCV infection leads to a significant acceleration of epigenetic age ($p = 0.0015$). While chronic HCV patients without evidence of cirrhosis have no significant age acceleration, patients with cirrhosis have a significantly higher biological age (3.15 yrs, $p = 0.0009$). Treatment of cirrhotic patients with DAA results in reversal of age acceleration, but this effect is only detectable at long-term follow-up. There are no associated cell composition changes.

Interestingly, patients who developed HCC after SVR ($n = 8$) have the highest age acceleration and show no evidence of reversion after treatment.

Conclusion Hepatitis C patients with cirrhosis show biological age acceleration that may be reversible after HCV cure. Lack of reversibility appears to be associated with HCC risk. Our findings underline the need for more individualized infectious disease medicine based on biological rather than chronologic age in the future.

P 5.29 Imprint of unconventional T cell response in acute hepatitis C persists despite successful early antiviral treatment

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Unconventional T cells (UTCs) are a heterogenous group of T cells that typically exhibit rapid responses towards specific antigens from pathogens. Chronic hepatitis C virus (HCV) infection causes dysfunction of several subsets of UTCs. This altered phenotype and function of UTCs can persist over time even after direct acting antiviral (DAA) mediated clearance of chronic HCV. However, it is less clear if and how UTCs respond in acute, symptomatic HCV infection, a rare clinical condition, and if rapid DAA treatment of such patients reverses the caused perturbations within UTCs. Here, we comprehensively analyzed the phenotype and reinvigoration capacity of three major UTC populations, mucosal associated invariant T (MAIT) cells, γδ T cells, and CD4 and CD8 double-negative αβ T cells (DNT cells) before, during, and after DAA-mediated clearance of acute symptomatic HCV infection. Among that, MAIT cell functionality was also systematically analyzed. We observed a reduced frequency of MAIT cells. However, remaining cells presented with a near-to-normal phenotype in acute infection, which contrasted with a significant dysfunction upon stimulation that was not restored after viral clearance. Notably, DNT and γδ T cells displayed a strong activation ex-vivo in acute HCV infection, that subsequently normalized during the course of treatment. In addition, DNT cell activation was specifically associated to liver inflammation. Altogether, these data provide evidence that UTCs respond in a cell type-specific manner during symptomatic HCV infection. However, even if early treatment is initiated, long-lasting imprints within UTCs remain over time.

P 5.30 SARS-CoV-2-specific adaptive immune response after mRNA vaccination in liver transplanted patients

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Background and aim Liver transplant recipients have an increased risk to develop severe or prolonged COVID-19. Therefore, these individuals particularly benefit from prophylactic vaccination. Recent studies demonstrated reduced antibody response rates upon COVID-19 mRNA vaccination in immunosuppressed individuals, however, little is known about the role of cellular immunity in this setting.

Methods We analyzed T cell responses after bnt162b2 vaccination using overlapping peptides spanning the SARS-CoV-2 spike protein and a set of pre-described epitopes located in different SARS-CoV-2 proteins, as well as the humoral response (serology and neutralizing antibodies) in 10 patients following liver transplantation.

Results Importantly, all patients showed T cell and/or antibody responses after vaccination. In line with previous studies, detectable levels of S1-specific IgG and titers of neutralizing antibodies were lower compared to the general vaccinated population. Similarly, the CD4 + and CD8 + T cell epitope repertoire was narrow and cytokine production was reduced. Hypothesizing that the phenotype of T cells is different under immunosuppression with imminent implications for the long-time immunity, we currently perform in-depth phenotypical profiling of the detectable CD8 + T cells using tetramer-based enrichment and multi-parameter FACS analysis.

Conclusions Our data suggest an impaired immune response after SARS-CoV-2 vaccination in the vulnerable cohort of individuals after liver transplantation. Cellular immune responses may however compensate for lacking antibody responses. Our data support the notion that immunocompromised patients may benefit from an early third vaccination.

P 5.31 Therapeutic shutdown of HBV transcripts promotes reappearance of the SMC5/6 complex and cccDNA silencing in vivo without affecting posttranslational modifications of cccDNA-bound histones

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We previously showed that treatments with siRNA targeting all HBV transcripts or pegylated interferon-α (peg-IFNα) strongly reduce all HBV markers, including HBx levels, thus enabling the reappearance of the host restriction factor "structural maintenance of chromosome 5/6 complex (SMC5/6) (Allweiss/Giersch Gut 2021).

Aim Understanding the mechanism of SMC5/6-mediated transcriptional silencing by investigating the epigenetic landscape of cccDNA-bound histones after siRNA or peg-IFNα treatment.

Methods HBV-infected human liver chimeric mice received siRNA or peg-IFNα for 4 or 6 weeks, respectively, or were left untreated. Active and repressive posttranslational modifications (PTMs) of cccDNA-bound histones and SMC5/6 occupation were analysed by chromatin immunoprecipitation-qPCR (ChIP-qPCR) and compared with occupation on host genes.

Results In untreated mice with high HBV replicative activity, active PTMs (H3K27ac, H3K4me3) were highly enriched on the cccDNA minichromosome, while the repressive mark H3K27me3 was undetectable. Notably, these treatments did not provoke significant changes for the analysed PTMs, despite strong reduction of cccDNA transcription. Consistent with previous results, only NSE4 occupancy became detectable after siRNA and peg-IFNα treatment. Interestingly, the heterochromatin mark H3K9me3 displayed intermediate levels on the cccDNA as compared to host heterochromatin (MYT-1 promotor) regardless of the treatment.

Conclusion The pattern of cccDNA-bound histone PTMs is consistent with active cccDNA transcription. The role of H3K9me3 deserves further investigation as it is linked with constitutive heterochromatin. In line with the function of SMC5/6 as a DNA micro-compaction machinery (Serrano, Molecular Cell 2020), the data suggest that SMC5/6 mediates transcriptional silencing through physical cccDNA compaction rather than through epigenetic changes.

P 5.32 HCV modifies the expression of the ErbB ligands NRG1 and EGF in a Sp1-dependent manner

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Background & aims The Hepatitis C Virus (HCV) is one of the leading causes for chronic liver disease worldwide. The fact that an infection with HCV remains asymptomatic over decades suggests that the virus is able to efficiently subvert antiviral immunity and to utilize the host cell infrastructure without affecting cell viability. As our group described previously, the growth factors EGF and NRG1 are both overexpressed in the presence of HCV, either interfering with chemokine expression or rearranging the ErbB receptor expression pattern. This project focuses on the mechanism by which the expression of growth factors is altered in HCV infected cells.

Methods Experiments were performed in Huh9-13 replicon or Huh7 control cells. Inhibitors were used and ErbB ligand expression was determined by rtPCR. Binding of the transcription factors Sp1 and STAT1 to the respective promoter region was determined by chromatin immunoprecipitation (ChIP).

Results Inhibitor experiments provided evidence for an important role of Sp1 and STAT1 in the regulation of both EGF and NRG1 transcription, especially in the presence of HCV. Consistently, enhanced Sp1 and STAT1 DNA binding to the respective promoter could be demonstrated in Huh9-13 replicon cells by ChIP.

Discussion It could be shown that the transcription factor Sp1 as well as STAT1 are involved in the HCV-dependent upregulation of both NRG1 and EGF. While Sp1 and STAT1 expression is not enhanced in the presence of HCV, its DNA binding capacity to EGF and NRG1 promoter is significantly increased in Huh9-13 replicon cells compared to control.

P 5.33 Unravelling the role of hepatocytes in hepatitis B virus specific immunotolerance

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HBV persistence is enabled by dysfunctional antiviral immunity. CD8 T cells are key mediators of virus control but fail to clear the virus from chronically infected hosts. The role of hepatocytes during this process is less clear. We addressed the hypothesis that HBV infection alters antigen presentation by hepatocytes and mitigates cytotoxic T cell responses.

Primary human T cells were grafted with cloned T-cell receptor (TCR) recognizing either Glypican 3 (GPC3) as a typical liver cancer antigen, or myeloperoxidase (MPO), a neoantigen on leukemic cells. HepG2- or HuH7-cells grafted with the HBV receptor (NTCP) were used as target cells. T-cell mediated killing of hepatoma cells was monitored in real-time using the xCELLigence real-time cell analyzer. T cell activation was determined by cytokine release.

In a first set of experiments, HBV-permissive HepG2-NTCP cells were infected with HBV and co-cultured with T cells engrafted with a GPC3-specific TCR. As GPC3 is highly expressed on hepatoma cells, the GPC3-specific T cells killed all HepG2-NTCP cells. This was neither altered by HBV-infection nor in HBV-transgenic hepatoma cell lines HepG2-H1.3, HepG2-2.2.15 and HepG2-tgHBV-tRFP. Killing capacity correlated with IFN-γ-levels released as a marker for T-cell activation and remained unchanged irresepctive of HBV infection or replication.

In a second set of experiments, the results were confirmed using HuH7-Lunet cells expressing MPO after mRNA-transfection. Again, HBV infection did not alter T-cell activation or T-cell mediated killing. In conclusion, HBV infection did not alter the hepatocyte microenviroment in our experiments in order to prevent T-cell activation or cytoxicity.

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