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From the Editor's Desk...

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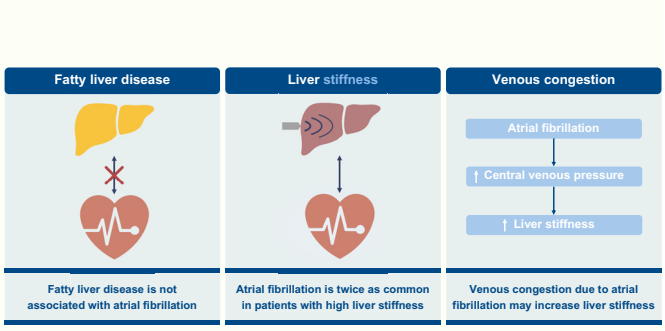
SELECTION OF THE MONTH

Liver stiffness not fatty liver disease is associated with atrial fibrillation

The association between fatty liver disease and atrial fibrillation has been suspected in the recent past. In the large Rotterdam population-based study, **van Kleef and coworkers**, showed that hepatic steatosis was not associated with a higher prevalence nor incidence of atrial fibrillation but there was an association between the latter and liver stiffness >8 kPa, even after adjustment for alcohol intake, smoking, and components of the metabolic syndrome. The association was significant whether chronic heart disease or heart failure were present. Interestingly, the association was stronger and not confounded by any metabolic parameters in the subpopulation (n = 2,830) without steatosis. The association between liver stiffness and atrial fibrillation might be explained by hepatic congestion driven by (subclinical) venous congestion. Basically, this study **debunks the concept of a causal association between fatty liver and atrial fibrillation and suggests reverse causality, with cardiovascular conditions causing atrial fibrillation and increasing liver stiffness (via hepatic congestion).**



van Kleef *et al.*, 2022
Liver stiffness not fatty liver disease is associated with atrial fibrillation

EXPERIMENTAL AND TRANSLATIONAL HEPATOLOGY

A new flavour of innate T cells in the hepatic sinusoid

The liver - as an immunological organ - is enriched with many "unconventional" lymphocytes, including T cells with innate-like functions. **Koh, Rha, Choi and coworkers** have used multi-omic single-cell techniques to analyse the immune cell composition of liver perfusate samples from healthy individuals and patients with HBV-associated liver disease. In this granular analysis of liver-infiltrating lymphocytes, liver-resident CD69+CD56hiCD161-CD8 T cells that express high levels of natural killer (NK) cell markers, respond to innate cytokines, and even show NK cell-like effector functions, are expanded in diseased liver in the absence of classical T-cell activation pathways. This study sheds light **on a potentially interesting CD8 T-cell subset in HBV-infected patients,** whose functional involvement in disease progression is enigmatic.

Liver-specific mitochondrial carrier protein SLC25A47 controls metabolic health

Hepatic mitochondrial stress is regularly observed in non-alcoholic fatty liver (NAFLD) and impacts whole-body homeostasis. **Bresciani, Demagny and coworkers** now identified that the mitochondrial carrier SLC25A47 is selectively expressed in hepatocytes, prompting them to investigate its function using conditional *Slc25a47* knock-out mice. Slc25a47 deficiency in hepatocytes results in impaired mitochondrial oxidative pathways and a sustained mitochondrial stress response, leading to liver injury, hepatocytic lipid accumulation, decreased gluconeogenesis and a predisposition to hepatic fibrosis. At the same time, hepatic mitochondrial stress drives the secretion of the mitokine FGF21, **which is responsible for the systemic effects on metabolism in the knock-out mice.** This central pathway of mitochondrial homeostasis in the liver suggests that the carrier or its metabolites could be targeted to prevent NAFLD-related sequelae such as liver fibrosis.

NAFLD

Ethyl glucuronide in hair detects a high rate of harmful alcohol consumption in presumed non-alcoholic fatty liver disease

Every clinician faces the difficulty of assessing alcohol consumption as reliably as possible. **Staufer and coworkers**, in a population of 184 patients (114 with NAFLD on usual alcohol consumption criteria, and 70 with alcohol-related liver disease) measured hair and urine ethyl glucuronide (longer term and shorter term quantitative indicators of alcohol consumption, respectively) and recorded results of AUDIT and SIAC (systematic inventory of alcohol consumption) questionnaires. Other measurements included the ANI score. In patients originally classified as having NAFLD, hair ethyl glucuronide measurements detected 10.5% with excessive alcohol consumption and 19% with moderate alcohol consumption within the past 3-6 months. All patients with excessive alcohol consumption according to hair ethyl glucuronide admitted to excessive alcohol consumption after confrontation with the test results. In the alcohol-related liver disease cohort, 29% of the patients who indicated alcohol abstinence in the past 6 months tested positive for hair ethyl glucuronide. Hair ethyl glucuronide measurements were clearly more accurate than mean corpuscular volume, carbohydrate deficient transferrin, ANI score, and urine ethyl glucuronide for diagnosing both moderate and, especially, excessive alcohol consumption. **Interestingly,**

patients with NAFLD and moderate alcohol consumption had less metabolic dysfunction than those with low or no alcohol consumption. This important study underlines an epidemiological reality of the overlap of these conditions and a reliable way to diagnose them.

CHOLESTATIC DISEASE

TREM-2 exerts protective effects against cholestasis and participates in UDCA-mediated anti-inflammatory responses in Kupffer cells

Inflammation plays a crucial role in the progression of cholestatic diseases, mainly triggered by bacterial products binding to toll-like receptors (TLRs) expressed on Kupffer cells (KCs), monocyte-derived macrophages, and hepatic stellate cells. TREM-2 belongs to the family of triggering receptors expressed on myeloid cells and is a negative regulator of the TLR-mediated inflammatory response, thus having a protective role during chronic liver injury. In this study, **Labiano, Agirre-Lizaso and coworkers** demonstrated that TREM-2 expression is upregulated in the livers of patients with primary biliary cholangitis and primary sclerosing cholangitis, correlating positively with markers of inflammation, fibrosis and overall severity. TREM-2 was also upregulated in mouse models of obstructive or chemically induced cholestasis. TREM-2 expression is low in hepatocytes but high in KCs and stellate cells. TREM-2 knockout mice subjected to bile duct ligation or to chemical cholestasis exhibited exacerbated necroptosis and inflammatory gene responses, which were partly abrogated after administration of antibiotics. Conversely, TREM-2 overexpression protected mice from chemically induced cholestasis. Finally, the authors showed that **UDCA counteracted the inflammatory response triggered by lipopolysaccharide by increasing the expression of TREM-2, specifically in KCs.** The hepatoprotective effects of TREM-2 against cholestatic injury, particularly through its effects on non-parenchymal cells, are thus shown to go beyond those already described in carcinogenesis or other forms of liver injury.

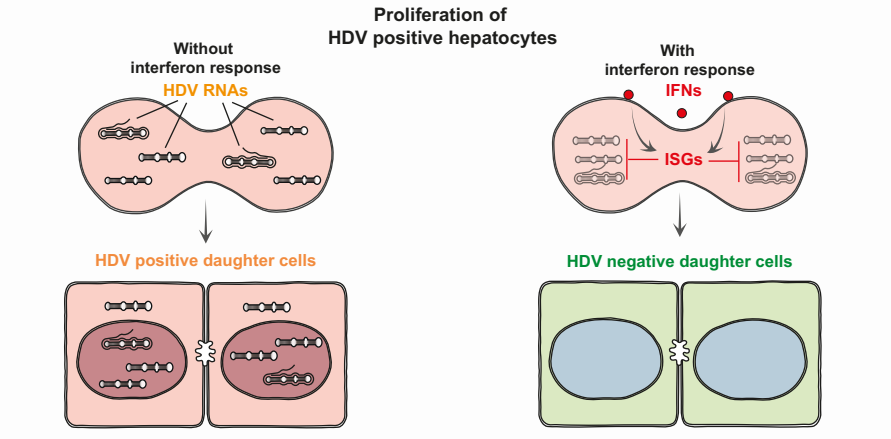
VIRAL HEPATITIS

Phase IIa, randomised, double-blind study of GSK3389404 in patients with chronic hepatitis B on stable nucleos(t)ide therapy

GSK3389404, an antisense oligonucleotide targeting HBV pregenomic and mRNA transcripts, is conjugated to N-acetyl galactosamine for enhanced hepatocyte delivery. In a phase IIa study led by **Yuen and coworkers**, patients with chronic hepatitis B on nucleos(t)ide analogue therapy were randomised to GSK3389404 (30, 60, 120 mg weekly or 120 mg bi-weekly) or placebo until day 85. One patient each in the 60 mg weekly, 120 mg weekly and 120 mg biweekly arms achieved a HBsAg response. HBsAg reductions were dose-dependent (day 85: mean 0.34 [60 mg weekly] to 0.75 $\log_{10}$ IU/ml [120 mg weekly]) and occurred in HBeAg-positive and -negative patients. **No patient achieved HBsAg seroclearance**. Alanine aminotransferase flares (>2x upper limit of normal) occurred in 2 GSK3389404-treated patients (120 mg weekly, 120 mg bi-weekly); both were associated with decreased HBsAg, but neither was considered a responder. While no clear optimal dose was identified, the findings from this study may help in the development of improved treatment options for patients with chronic HBV.

HDV-induced IFN response and administered IFNs control cell division-mediated virus spread

Besides HBV-dependent *de novo* infection, cell division-mediated spread contributes to HDV persistence and dampens the effect of antivirals abrogating the *de novo* infection. Antivirals and interferons (IFNs) showed strong synergism in recent clinical trials, implying a complementary mode-of-action of IFNs. Therefore, **Zhang and coworkers** investigated the effect of IFN response on cell division-mediated HDV spread. Cell division-mediated HDV spread was highly efficient following infection of HuH7NTCP65 cells (defective in IFN production), but profoundly restricted in infected IFN-competent HepaRGNTCP66 cells. Treatment with IFN-α/-λ1 inhibited HDV spread in dividing HuH7NTCP67 cells **but exhibited a marginal effect on HDV replication in resting cells**. The authors conclude that both HDV-induced IFN response and exogenous IFN treatment suppress cell division-mediated HDV spread, presumably through acceleration of HDV RNA decay.



Zhang *et al.*, 2022
HDV-induced IFN response and administered IFN control cell division-mediated virus spread

Impact of HBsAg seroclearance on late recurrence of HBV-related HCC after surgical resection

Yoo, Kim and coworkers investigated the impact of HBsAg seroclearance on the

recurrence of hepatocellular carcinoma (HCC) in 2,520 consecutive patients after curative liver resection. To focus on late recurrence, patients with recurrence or a follow-up duration less than 2 years were excluded. A total of 891 patients developed HCC recurrence at rates of 11.2%, 25.5%, and 46.8% at 3, 5, and 10 years after resection, respectively. HBsAg seroclearance was achieved in 172 patients during a median follow-up duration of 6.9 years after resection. **HBsAg seroclearance, compared with persistent HBsAg positivity, was associated with a lower risk of late HCC recurrence.** Patients without HBsAg seroclearance also transitioned to HCC recurrence more rapidly than patients who experienced HBsAg seroclearance.

Mechanisms of CD8+ T-cell failure in chronic HEV infection

In immunosuppressed patients, persistent HEV infection is observed and may lead to cirrhosis and liver failure. **Kemming and coworkers** studied HEV-specific CD8+ T-cell responses in patients with self-limiting (n = 34) or chronic HEV infection (n = 12). They identified **HEV-specific CD8+ T-cell epitopes restricted by different HLA class I alleles.** In self-limiting HEV infection, HEV-specific CD8+ T cells were vigorous, contracted after resolution of infection, and formed functional memory responses. In contrast, in chronic infection, the HEV-specific CD8+ T-cell response was diminished, declined over time, and displayed phenotypic features of exhaustion. Improved proliferation of, and IFN-γ production by, HEV-specific CD8+ T cells and evolution of a memory-like phenotype were observed upon reduction of immunosuppression and/or ribavirin treatment and were associated with viral clearance. In 1 patient, mutational viral escape in a targeted CD8+ T-cell epitope contributed to CD8+ T-cell failure.

Is hepatitis B elimination feasible in high-income countries with ongoing immigration?

Tian and coworkers developed an agent-based model determining which integrated strategies involving vaccination, screening, and treatment would achieve the WHO's goals of HBV elimination in a high-income country with ongoing immigration. The authors predict that under the current strategies, the incidence of acute hepatitis B, and HBV-attributable decompensated cirrhosis and HCC would decrease by 64.5%, 9.4%, and 10.5% between 2015–2030, respectively. However, **the incidence of chronic hepatitis B and liver-related deaths would increase by 26.6% and 1.0% between 2015–2030**, respectively. Results were sensitive to the number of immigrants and HBV prevalence in the immigrants. The authors conclude that even with extensive integrated scale-up in vaccination, screening, and treatment, the WHO's morbidity and mortality targets may not be reachable.

CIRRHOSIS AND LIVER FAILURE

Carvedilol reduces the risk of decompensation and mortality in patients with compensated cirrhosis in a competing-risk meta-analysis

Carvedilol might be particularly effective since its intrinsic vasodilatory activity may ameliorate hepatic vascular resistance, a major mechanism of portal hypertension in early cirrhosis. In this meta-analysis, **Villanueva and coworkers** aimed at clarifying whether carvedilol can prevent decompensation and improve survival in patients with compensated cirrhosis and clinically significant portal hypertension (CSPH) without previous bleeding. Only randomised-controlled trials (RCTs) comparing carvedilol *vs.* control therapy (no-active treatment or endoscopic variceal ligation [EVL]) were included in the meta-analysis. Primary outcomes were prevention of decompensation (liver transplant and death were competing-events) and death (liver transplant, competing-event). A competing-risk time-to-event meta-analysis was performed using individual patient data. Models were adjusted using propensity score for baseline covariates. Among 125 full-text studies evaluated, 4 RCTs were eligible, comprising 352 patients with compensated cirrhosis, 181 treated with carvedilol and 171 controls (79 received EVL and 92 placebo). Baseline characteristics were similar between groups. The risk of developing decompensation of cirrhosis was lower with carvedilol than in controls, mainly due to a reduced risk of ascites. **The risk of death was also lower with carvedilol.** Thus, long-term carvedilol therapy reduced decompensation of cirrhosis and significantly improved survival in compensated patients with CSPH. This suggests that screening patients with compensated cirrhosis for CSPH to ensure the early initiation of carvedilol could improve outcomes.

Gadoxetic acid-enhanced MRI-derived FLIS and spleen diameter predict outcomes in ACLD

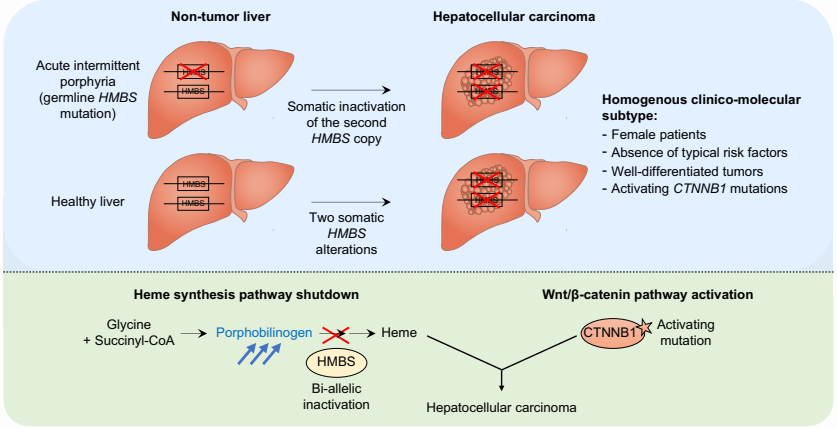
The aim of this study was to investigate the accuracy of a combination of the functional liver imaging score (FLIS) – derived from gadoxetic acid-enhanced MRI – and splenic cranio-caudal diameter (SCCD) for predicting hepatic decompensation and acute-on-chronic liver failure (ACLF) in patients with chronic liver disease (CLD). **Bastati, Beer and coworkers** included in the study 397 patients with CLD. The FLIS was calculated by summing the points (0-2) of 3 hepatobiliary-phase features: hepatic enhancement; biliary excretion; and portal vein signal intensity. Patients were stratified into 3 groups according to liver fibrosis severity and presence/history of hepatic decompensation. SCCD showed excellent intra- and inter-reader agreement. Importantly, SCCD was an independent risk factor for hepatic decompensation in patients with compensated advanced CLD (ACLD). Patients with compensated ACLD and a FLIS of 0-3 points and/or a SCCD of >13 cm were at increased risk of hepatic decompensation. In decompensated patients, a FLIS of 0-3 was independently associated with an increased risk of ACLF, even after adjusting for other prognostic factors. Finally, **a FLI and SCCD-based algorithm was independently predictive of transplant-free mortality and stratified the probability of transplant-free survival in patients with ACLD.** Thus, FLIS and SCCD are simple imaging markers that provide complementary information for risk stratification in patients with compensated/decompensated ACLD.

HEPATIC AND BILIARY CANCER

Unravelling a cause of liver cancer in non-fibrotic livers

Heterozygous germline mutations in hydroxymethylbilane synthase or *HMBS* cause acute intermittent porphyria (AIP), a condition associated with an increased incidence of HCC for which a clear biological explanation is lacking. **Molina and coworkers** have explored the role of HMBS alterations in the development of HCC in a very large series including 4 patients with

HCC and AIP. Recurrent bi-allelic *HMBS* inactivation was detected in patients with AIP acquiring a second somatic *HMBS* mutation as well as in sporadic HCC with 2 somatic hits. Bi-allelic *HMBS* inactivation results in a massive accumulation of porphobilinogen and synergises with *CTNNB1*-activating mutations to drive the occurrence of well differentiated HCC with a transcriptomic signature of Wnt/β-catenin pathway activation. ***HMBS* is therefore a tumour suppressor gene whose bi-allelic inactivation defines a homogenous clinical and molecular subtype of HCC that appears most frequently in females without fibrosis or other usual risk factors.**



Molina *et al.*, 2022
Unravelling a cause of liver cancer in non-fibrotic livers

A new category of aggressive primary liver cancer in children

Primary liver cancer in children is rare and most frequently it is either hepatoblastoma or HCC. Recently, another category provisionally named hepatocellular neoplasm not otherwise specified (HCN NOS) was described as showing intermediate or combined hepatoblastoma and HCC histological features. **Sumazin, Peters, Sarabia and coworkers** have for the first time characterised molecularly a group of HCN-NOSs by comparing their molecular features with those in other paediatric liver cancers. Based on the finding of common underlying biological features that were previously observed in HCCs they **propose and describe histologically and molecularly a new category of hepatoblastoma with carcinoma features** that will cover both HCN-NOSs and hepatoblastoma with focal atypia or pleomorphism. **These are aggressive tumours, regardless of patient age or resectability, and would likely benefit more from liver transplantation** than from standard chemotherapy and surgery. Altogether, their findings emphasise the importance of molecular testing and early therapeutic intervention for aggressive paediatric liver cancer.

A potential diagnostic aid in BTC based on miRNA quantification in blood

Biliary tract cancer (BTC) often presents diagnostic challenges in part due to considerable heterogeneity across patients. **Høgdall and coworkers** hypothesised that systemic reprogramming as a result of cancer occurrence could help in the diagnosis of BTC and prospectively studied peripheral whole-blood microRNAs (miRNAs) in a large cohort of patients with BTC. They found 4 miRNAs whose abundance in whole blood differed between patients with BTC and healthy individuals; 2 of them also showed increased expression in BTC compared to tumour-adjacent tissues. Then they constructed **2 miRNA scores that were able to differentiate patients with BTC from healthy individuals, with a very high performance when the elevation of CA 19.9 was integrated into the model**. However, the scores were not able to differentiate patients with BTC from those with conditions such as pancreatic and duodenal cancer or chronic pancreatitis. The expression of individual miRNAs in tumour tissue was associated with different immune features including T-cell dysfunction or exclusion, and other pathobiological features. The utility of miRNA quantification in whole blood as a diagnostic aid in BTC should now be confirmed prospectively.

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