From the Editor's Desk August 2019

Draft 2

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SELECTION OF THE MONTH

Big Title: Novel primary care referral pathway for NAFLD

Small Title: UDCA improves survival in PBC

A new tool for studying the HBV life cycle

DAAs improve survival in successfully treated early stage HCC

sequential/combined use of non-invasive tests in NAFLD

MODELING OF GENETIC LIVER DISEASES

Pluripotent stem cell-derived hepatocyte like cells for modeling

Hepatocyte polarity is essential for the development of bile canaliculi and transport of bile and waste products such as copper safely out of the liver. Genetically inherited defects in polarized processes can cause severe diseases. Functional studies of autologous mutated proteins in the context of the polarized hepatocyte have been

challenging because of the lack of appropriate cell models. **Overeem *et al.*** aimed to obtain a patient-specific hepatocyte model that recapitulated hepatocyte polarity and to employ this model to study endogenous mutant proteins in liver diseases that involve hepatocyte polarity. For this, patients' and control subjects' urine cell-derived pluripotent stem cells were differentiated towards hepatocyte-like cells. They now show that **functional cell polarity can be achieved in patient pluripotent stem cell-derived hepatocyte like cells, and allow for the first time the investigation of the endogenous mutant proteins, patient-specific pathogenesis and drug responses for diseases where hepatocyte polarity is a key aspect.**

NON-ALCOHOLIC FATTY LIVER DISEASE

Combined use of non-invasive diagnosis tools, effects of Mediterranean diet on liver fat and a new allelic mutation.

Advanced fibrosis determines the outcome of patients with NAFLD. In this issue, **Boursier *et al.*** investigated the performance of combining different non-invasive test to assess fibrosis stage in patients with NAFLD. Almost 1000 patients with biopsy-proven NAFLD underwent stiffness measurement with vibration controlled transient elastography (VCTE), blood fibrosis tests (NAFLD fibrosis score, FIB4, Fibrotest, Hepascore, FibroMeter), and calculation of FibroMeter-VCT. For the diagnosis of advanced fibrosis, VCTE was significantly more accurate than the blood tests and among these latter, FibroMeter was the most accurate. **The sequential combination of FIB4 then FibroMeter-VCTE or VCTE then FibroMeter-VCTE provided an excellent 90% diagnostic accuracy for advanced fibrosis with less of 20% of patients requiring a liver biopsy**. This study demonstrates that the sequential use of non-invasive tests is the best performance for fibrosis assessment. A rational use of these markers in the primary care setting seems appropriate. In this issue, **Srivastava *et al.*** evaluated a pathway for management of patients with NAFLD using blood tests to stratify patients in primary care to improve detection of cases of advanced fibrosis and cirrhosis. The studied 3000 patients seen in primary care using a **two-step algorithm combining the use of FIB-4 followed by the ELF test** if required. Use of this approach detected 5 times more cases of advanced fibrosis and cirrhosis. **Unnecessary referrals from primary care to secondary care fell by 70-80%**. This study shows evidence that the use of non-invasive blood tests for liver fibrosis to stratify patients with NAFLD improves the detection of cases of advanced fibrosis and cirrhosis

and reduces unnecessary referrals. Lifestyle interventions remain the most effective approach to improve NAFLD. Whether a reduction in hepatic fat content (HFC) is a major mediator of the cardiometabolic benefit of lifestyle intervention is unknown. In this issue, **Gepner *et al.*** performed a 18-month weight-loss trial in near 300 with abdominal obesity/dyslipidemia. Patients were randomized to low-fat (LF) or Mediterranean/low-carbohydrate (MED/LC) diets with/without moderate physical activity (PA). Reduction of HFC associated with decreases in VAT beyond weight loss. After controlling for VAT loss, the percentage decrease of HFC remained independently associated with reductions in serum GGT and ALT, circulating chemerin, and HbA1c. **Compared to LF diet, MED/LC induced a greater decrease in HFC and greater improvements in cardiometabolic risk parameters**. This important study reveals that decrease HFC is reduced by diet-induced moderate weight loss, and that this effect if more efficiently achieved by a Mediterranean diet.

Besides environmental factor, genetic factors can predispose to NAFLD. In some families, mutation in a single gene involved in fat metabolism can result in severe NAFLD. There is little information in mendelian inheritance in NAFLD families. **Youssefian *et al.*** performed whole-exome or targeted next-generation sequencing on autosomal dominant NAFLD patients. The authors found a **heritable form of NAFLD and/or dyslipidemia due to monoallelic *ABHD5* mutations**, with complete clinical expression after the fourth decade of life, in 7 unrelated multiplex families encompassing 39 affected patients. This novel study describes a mendelian form of NAFLD and metabolic syndrome due to monoallelic *ABHD5* mutations.

HEPATITIS C VIRUS (HCV) INFECTION

DAA treatment-induced decline in the burden of disease, DAA improve survival in successfully treated early stage HCC

Direct evidence how direct acting antiviral (DAA) treatment-induced viral clearance (SVR) impacts morbidity and mortality on a population-level is still missing as clinical trials were not designed to evaluate potential longer-term clinical benefits of these drugs. **Alavi *et al.*** now reports in this issue of the Journal on the early impact of DAA treatments on HCV-related liver disease burden in the New South Wales, Australia. **Whereas over the decade prior to the DAA era in Australia, the numbers of HCV-infected people hospitalized or dying following end-stage liver disease complications increased by two to three-fold, the HCV-related liver disease**

burden declined significantly in the DAA era between 2015 and 2017 leading to a 21% and 17% decrease in decompensated cirrhosis diagnoses and liver-related deaths. Although this important study clearly supports a major population-level impact of DAA therapy on HCV-related liver disease morbidity and mortality, and all-cause mortality, enhanced efforts are required to continue DAA scale-up, if the WHO target of 65% HCV-mortality reduction is to be achieved by 2030 as highlighted by the authors.

Concerns were recently raised that early antiviral treatment with DAA after curative treatment of hepatocellular carcinoma may create an intrahepatic microenvironment promoting expansion of early malignant lesions followed by early HCC recurrence. In this regard the current study by **Cabibbo *et al.*** is highly important as it compared the outcome of prospectively enrolled consecutive patients who had achieved a complete radiologic response after curative resection or ablation of early Barcelona Clinic Liver Cancer (BCLC) stage 0/A HCV-induced HCC and subsequently treated with DAAs with a propensity score matched control group of DAA-untreated patients from the ITA.LI.CA. cohort. **Overall survival rate as well as the rate of hepatic decompensation were significantly lower in the DAA-treated group as compared to the controls, and no significant difference in HCC recurrence was observed between the groups**. SVR was the only variable associated with a decrease in mortality and was independently associated with a decrease in HCC recurrence risk. Although without performing a prospective study one never can exclude DAA-induced acceleration of tumor progression in certain single patients, the clear cut results of this study showing a significant survival benefit when DAA were given to patients after successful HCC treatment should be taken as a strong argument for considering DAA treatment as being an integral part in the management of early stage HCV-induced HCCs.

HEPATITIS B VIRUS (HBV) INFECTION

HCC risk estimation in HIV/HBV-coinfected patients, a new tool for studying the HBV life cycle, linking killer cells to fibrosis inhibition

Good evidence exist for calculating the individual risk for HCC development in HBV monoinfected patients under long-term antiviral therapy with second-generation HBV polymerase inhibitors like tenofovir (TDF) and entecavir (ETV), and providing the basis for risk-adapted HCC surveillance strategies. The evidence for risk-adapted HCC

screening in people being coinfected with HIV and HBV receiving anti-HBV agents as part of their antiretroviral therapy (ART), however, is limited. In the largest collaborative analysis to date, **Wandeler *et al.*** estimates HCC incidence in HIV/HBV-coinfected patients from four large prospective European HIV cohorts, the Swiss HIV cohort study, EuroSIDA, Athena Observational Cohort Study, and the ANRS CO3 Aquitaine Cohort. Main results of the study were, that **after the initiation of TDF (or ETV), the incidence of HCC remained stable over time, whereas it increased steadily among those not on TDF, suggesting that an assessment of HCC risk at TDF start would be adequate to inform long-term individual HCC screening strategies**. In those HIV/HBV-coinfected patients initiating TDF-containing ART without cirrhosis at an age <46 years, the HCC risk remained below the HCC screening threshold of 2 per 1,000 patient years.

A major drawback of current *in vitro* HBV infection systems utilizing primary human hepatocytes (PHHs) is that they do not support efficient viral amplification and spread following HBV infection. König et al. developed a new NTCP-overexpressing HepG2 derived cell line allowing a more complete study of the life cycle. The authors elegantly demonstrated by using up-to-date methodology that **their cell culture system mimics complete HBV life cycle from entry to egress with up to 1,300-fold amplification of input HBV in the supernatant over several weeks, and HBV spread to adjacent cells, forming infected cell clusters**. This new infection model can become an important tool for future HBV drug development as it may allow to study antiviral efficacy of the compounds against individual patient-derived HBV strains.

Natural Killer (NK) cells are responsible for the innate immune response to viral infection but at the same time may contribute to liver injury through sustained activation and tissue damage. Killer cell lectin-like receptor subfamily G member 1 (KLRG1) is an inhibitory receptor of the C-type lectin-like family which is expressed in approximately 20% of human CD4+ T cells and 40% of CD8+ T cells, and may limit their anti-viral activities thus contributing to viral persistence. The role of KLRG1 on NK cells particularly in chronic HBV infection, however, remains uncertain. In an elegant study, **Wijaya *et al.*** now examined the quantity, phenotype and functional characteristics of KLRG1+ NK cells in chronically HBV-infected patients and healthy controls. **Authors provide evidence for KLRG1+ NK cells limiting liver injury and fibrosis in the natural course of chronic HBV infection and thus may play an important role in controlling liver disease progression.** Given their anti-fibrotic

potential, KLRG1+ NK cells might become an interesting target for anti-fibrotic drug development strategies.

HEPATITIS D VIRUS (HDV) INFECTION

MAIT be the reason for the more aggressive course

The reasons why chronic HDV infection shows a more rapid progressive course as compared to HBV mono-infection remain largely unknown. Different patterns of immune responses against HDV in comparison to HBV, however, may contribute to these differences in disease outcome. Mucosa-associated invariant T (MAIT) cells, a group of innate-like T cells being highly enriched in the human liver, are involved in host responses towards bacterial and viral infections, but their role in chronic HDV infection is currently unknown. **Dias *et al.*** conducted the first comprehensive characterization of MAIT cell phenotype and functionality in a sizeable cohort of patients with chronic HDV infection and compared the results with HBV mono-infected patients and healthy controls. They showed that **the MAIT cell compartment is severely compromised in HDV infected patients, and cytokine driven activation-induced cell death may be involved in the observed severe loss of peripheral blood MAIT cells in HDV-infected patients**. This important observation may represent a good starting point to further explore the immune-mediated perturbations in the pathogenesis of chronic HDV infection.

LIVER TRANSPLANTATION

Increasing proportion of NASH patients needing liver transplants

With increasing rates of obesity, the proportion of patients with non-alcoholic steatohepatitis (NASH) developing decompensated cirrhosis is increasing but its impact on the need for liver transplantation in Europe is unknown. **Haldar *et al.*** explored the European Liver Transplant Registry (ELTR) between 2002 and 2016 and studied about 65,000 patients to determine the outcomes of patients transplanted with a diagnosis of NASH. **Their data clearly showed that the proportion of patients undergoing liver transplantation for NASH has increased from 1.2% in 2002 to 8.4% in 2016. Reassuringly, they showed that the mortality of patients transplanted with NASH is not different to those transplanted with other indications.** This paper confirms the worrying trend of increasing burden of

decompensated cirrhosis from NASH and further supports the current efforts focused on developing new therapies for this group of patients.

CHOLESTASIS

UDCA improves survival of PBC patients

At present, the lack of randomized clinical trials with long-term follow up does not allow conclusion on whether ursodeoxycholic acid (UDCA) reduces the mortality of patients with PBC. In this huge, multi-center study, **Harms *et al.*** explored the role of UDCA therapy in patients with PBC. They studied nearly 4000 patients with median follow up of about 8-years. **They showed that the mortality of patients treated with UDCA was about 61% compared with about 80% in those that were not treated irrespective of the severity of their underlying liver disease.** These data provide incontrovertible proof for the role of UDCA in the treatment of PBC and should remain the standard of care that new therapies need to be compared with.

HEPATOCELLULAR CARCINOMA (HCC) – BASIC/TRANSLATIONAL

A gene regulatory network for predicting HCC outcome, role of a key enzyme of the glycolysis in PD-L1overexpression by peritumoral monocytes

Dedifferentiation of hepatic cells contributes to HCC progression. *LIN28B*, which encodes a RNA-binding protein, is repressed during normal hepatic cell differentiation. *LIN28B* is re-expressed in a subset of human HCCs characterized by high serum levels of α-fetoprotein, a finding associating dedifferentiation, HCC progression and LIN28B expression. Of note, *CTNNB1*, which encodes catenin beta-1, is one of the most frequently mutated genes in HCC. **Gérard *et al.*** used elegant approaches to explore the possibility that HCC progression depends on a gene regulatory network linking *LIN28B*-dependent dedifferentiation with *CTNNB1* dysfunction. They now show that *LIN28B* and *CTNNB1* form a gene regulatory network with *SMARCA4* (encoding transcription activator BRG1), *MIRLET7B* (a microRNA), SOX9 (encoding transcription factor SOX-9), *TP53* (encoding cellular tumor antigen P53), and *MYC* (encoding myc proto-oncogene protein). The regulatory network is detected in HCC and gastrointestinal cancers, but not in other cancer types. The status of the gene network negatively correlates with HCC prognosis, and positively correlates with hyperproliferation, dedifferentiation and hepatocyte growth factor/MET pathway activation, suggesting that it contributes to a transcriptomic profile typical of the

proliferative class of HCC. The authors conclude that **identification and modelling of the gene regulatory network provides insight into prognosis and mechanisms of tumor-promoting genes in HCC.**

The B7/CD28 family of costimulatory molecules plays an important role in the regulation of the cell-mediated immunity against cancer. Expression of PD-L1 (also known as B7-H1, or CD274) on antigen-presenting cells (APCs) engages PD-1 (also known as CD279) in T cell and this engagement is essential for inhibition of T cell anti-tumoral functions. PD-L1-expressing macrophages may mechanistically shape and therapeutically predict the clinical efficacy of PD-L1/PD-1 blockade by immune checkpoint inhibitors. Little is known on the mechanisms underlying PD-L1 upregulation in human tumor microenvironments remain elusive. **Chen *et al.*** addressed this question by investigating monocytes/macrophages obtained from peripheral blood, nontumor, or paired tumor tissues of patients with HCC and looked in particular at the eventual glycolytic switch in these cells. They show here that tumor-derived soluble factors, including hyaluronan fragments, induced the upregulation of a key glycolytic enzyme, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3), in tumor-associated monocytes. This enzyme also mediates the increased expression of PD-L1 by activating the NF-κB signaling pathway. Consistently, the levels of PFKFB3+CD68+ cell infiltration in peritumoral tissues were negatively correlated with overall survival. **These findings indicate that inhibition of monocyte PFKFB3 may be a target for novel therapeutic approaches of HCC.**