JOURNAL
OF HEPATOLOGY

From the Editor's desk...

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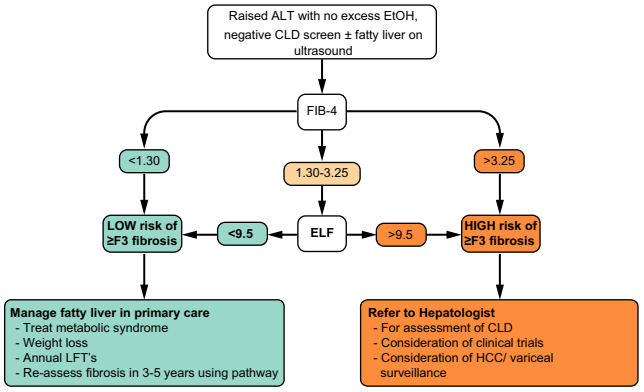
SELECTION OF THE MONTH

Novel primary care referral pathway for NAFLD

In this issue, **Srivastava *et al.*** evaluated a pathway for the management of patients with non-alcoholic fatty liver disease (NAFLD), using blood tests to stratify patients in primary care in order to improve detection of cases of advanced fibrosis and cirrhosis. They studied 3,000 patients seen in primary care using a **2-step algorithm combining the use of FIB-4 followed by the ELF™ test** if required. Use of this approach detected 5-fold more cases of advanced fibrosis and cirrhosis. **Unnecessary referrals from primary care to secondary care fell by 70–80%**. This study provides evidence that stratifying patients with NAFLD using non-invasive blood tests of liver fibrosis improves the detection of cases of advanced fibrosis and cirrhosis and reduces unnecessary referrals.



Srivastava *et al.*, 2019.
Novel primary care referral pathway for NAFLD.

MODELLING OF GENETIC LIVER DISEASES

Pluripotent stem cell-derived hepatocyte-like cells for modelling

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 polarised processes can cause severe diseases. Functional studies of autologous mutated proteins in the context of the polarised 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 could be used to study endogenous mutant proteins in liver diseases that involve hepatocyte polarity. For this, urine cell-derived pluripotent stem cells, from patients and controls, 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, enabling the investigation of 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 1,000 patients with biopsy-proven NAFLD underwent liver 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 the 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 than 20% of patients requiring a liver biopsy**. This study demonstrates that the sequential use of non-invasive tests is the best performing approach for fibrosis assessment. A rational use of these markers in the primary care setting seems appropriate.

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 an 18-month weight-loss trial in just under 300 patients with abdominal obesity/dyslipidaemia. Patients were randomised to low-fat or Mediterranean/low-carbohydrate (MED/LC) diets with/without moderate physical activity. Reduction of HFC was associated with decreases in visceral adipose tissue beyond weight loss. After controlling for visceral adipose tissue loss, the percentage decrease of HFC remained independently associated with reductions in serum gamma-glutamyltransferase and alanine aminotransferase, as well as circulating chemerin and glycated haemoglobin. **Compared to low-fat diet, MED/LC induced a greater decrease in HFC and greater improvements in cardiometabolic risk parameters**. This important study reveals that HFC is reduced by diet-induced moderate weight loss, and that this effect if more efficiently achieved by a Mediterranean diet.

Besides environmental factors, genetic factors can predispose individuals to NAFLD. In some families, a mutation in a single gene involved in fat metabolism can result in severe NAFLD. There is little information on mendelian inheritance in NAFLD families. **Youssefian *et al.*** performed whole-exome or targeted next-generation sequencing on patients with autosomal dominant NAFLD. The authors found a **heritable form of NAFLD and/or dyslipidaemia caused by 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, DAAs improve survival in successfully treated early stage HCC

Direct evidence of the impact of direct-acting antiviral (DAA) treatment-induced viral clearance (sustained virologic response [SVR]) on morbidity and mortality at the population-level is still missing, as clinical trials were not designed to evaluate potential longer-term clinical benefits of these drugs. In this issue of the *Journal*, **Alavi *et al.*** report 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 hospitalised or dying following end-stage liver disease complications increased by 2 to 3-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 a 65% reduction in HCV-related mortality is to be achieved by 2030.

Concerns were recently raised that early antiviral treatment with DAAs after curative treatment of hepatocellular carcinoma (HCC) 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 were subsequently treated with DAAs with a propensity score matched control group of DAA-untreated patients from the ITA.LI.CA. cohort. **The overall survival rate and the rate of hepatic decompensation were significantly lower in the DAA-treated group compared to 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 can never exclude DAA-induced acceleration of tumour progression in certain patients, the clear cut results of this study showing a significant survival benefit when DAAs were given to patients after successful HCC treatment should be taken as a strong argument for considering DAA treatment as an integral part of the management of early stage HCV-induced HCCs.

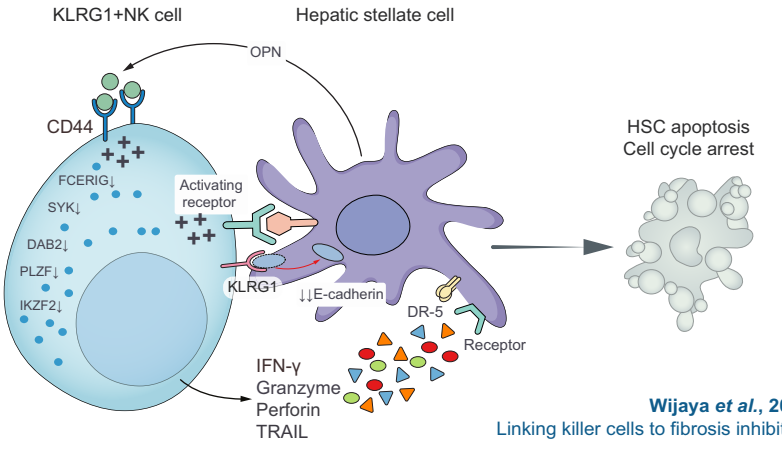
HEPATITIS B VIRUS (HBV) INFECTION

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 exists 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), providing the basis for risk-adapted HCC surveillance strategies. The evidence for risk-adapted HCC screening in individuals 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 4 large prospective European HIV cohorts, the Swiss HIV cohort study, EuroSIDA, Athena Observational Cohort Study, and the ANRS CO3 Aquitaine Cohort. The 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 initiation 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 that utilise 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 the 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 could become an important tool for future HBV drug development as it may enable researchers to study the antiviral efficacy of 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. KLRG1 may limit the antiviral activities of these T cells, 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. **The authors provide evidence for KLRG1+ NK cells limiting liver injury and fibrosis in the natural course of chronic HBV infection and thus potentially playing an**



Wijaya *et al.*, 2019.
Linking killer cells to fibrosis inhibition.

important role in controlling liver disease progression. Given their antifibrotic potential, KLRG1+ NK cells might become an interesting target for antifibrotic 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 compared to HBV monoinfection 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 that are 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 characterisation of MAIT cell phenotype and functionality in a sizeable cohort of patients with chronic HDV infection and compared the results with HBV monoinfected 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

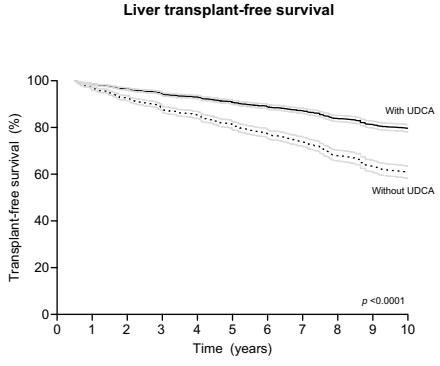
Increasing proportion of NASH patients needing liver transplants

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 in patients with PBC

At present, the lack of randomised clinical trials with long-term follow-up prevents conclusions on whether ursodeoxycholic acid (UDCA) reduces the mortality of patients with PBC. In this huge, multicentre study, **Harms *et al.*** explored the role of UDCA therapy in patients with PBC. They studied nearly 4,000 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. UDCA should remain the standard of care with which new therapies should be compared.



Harms *et al.*, 2019.
UDCA improves survival in patients with PBC.

HCC – BASIC/TRANSLATIONAL

A gene regulatory network for predicting HCC outcome, role of a key enzyme of the glycolysis in PD-L1 overexpression by peritumoral monocytes

Dedifferentiation of hepatic cells contributes to HCC progression. *LIN28B*, which encodes an RNA-binding protein, is repressed during normal hepatic cell differentiation. *LIN28B* is re-expressed in a subset of human HCCs characterised by high serum levels of alpha-fetoprotein, a finding that links 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 tumour-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 engages PD-1 (also known as CD279) on T cells, which is essential for inhibition of T cell antitumoral 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 about the mechanisms underlying PD-L1 upregulation in human tumour microenvironments. **Chen *et al.*** addressed this question by investigating monocytes/macrophages obtained from peripheral blood, non-tumour, or paired tumour tissues of patients with HCC and looked in particular at the eventual glycolytic switch in these cells. They show that tumour-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 tumour-associated monocytes. This

enzyme also mediates the increased expression of PD-L1 by activating the NF-κB signalling 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 for HCC.**

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