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SELECTION OF THE MONTH

Big title: Simple, accurate and validated algorithm for hepatic fibrosis

Small titles:

HCV direct acting antivirals going generic HBVcircle: a novel tool to study cccDNA

Cirrhosis/cholestasis: New biomarkers for disease severity in PSC

Non-invasive fibrosis estimation algorithm

With the introduction of non-invasive tests, the reliance on liver biopsy to stage liver disease has declined substantially.

LIVER INJURY

RIPK1 activity in the liver does not mean R.I.P.

Upon ligand binding, receptor-interacting protein kinase–1 (RIPK1) is recruited to tumor necrosis factor receptor superfamily (TNFRSF) and Toll-like receptor (TLR) complexes promoting prosurvival and inflammatory signaling. RIPK1 also directly regulates caspase-8-mediated apoptosis or, if caspase-8 activity is blocked, RIPK3-mixed lineage kinase domain–like protein (MLKL)-dependent necroptosis (a form of programmed cell death). Necroptosis is characterized by early loss of plasma membrane integrity, leakage of intracellular contents, and organelle swelling. The cells dying through necroptosis lack the typical apoptotic characteristics, such as membrane blebbing, chromatin condensation, and intranucleosomal DNA cleavage into 180 bp DNA laddering, but may show TUNEL positivity. Here, Filliol *et al.* show that, in the liver, lipopolysaccharide (LPS, a Gram-negative bacteria byproduct which a pathogen-associated molecular pattern (PAMP) is recognized by its TLR and this recognition stimulates Kupffer cells to produce and secrete tumor necrosis factor α (TNF-α) which engages its cognate TNFRSF. In the absence of RIPK1, TNFRSF engagement by TNF-α causes hepatocyte apoptosis. **These data reveal the pivotal function of RIPK1 in maintaining liver homeostasis in conditions of macrophage-induced TNF-α burst in response to LPS (and perhaps other PAMPs) sensing.** Since RIPK1 inhibition or invalidation can lead to necroptosis and subsequent release of danger-associated molecular patterns resulting in systemic inflammation, future studies should investigate whether RIPK1 activity is decreased in liver diseases and could contribute to the development of systemic inflammation in these diseases.

HEPATOCELLULAR CARCINOMA (HCC)

Erbin promotes HCC, differences between human FGF-19 and its murine ortholog FGF-15, DDA failure in active HCC

Aberrant estrogen receptor-α (ERα) expression and signaling are implicated in the development of HCC, but its regulation in HCC remains unknown. Wu *et al.* were interested in the Erbin protein which contains 17 leucine-rich repeats and one PDZ domain. They show that Erbin expression is significantly elevated in human HCC tissue. This elevated expression of Erbin contributes to tumorigenesis of HCC by negatively regulating ERα signaling. **Restoring ERα signaling by inhibiting Erbin expression enhances the sensitivity of HCC cells to tamoxifen treatment, providing a new approach for tamoxifen treatment in HCC.**

The bile acid receptor (also known as farnesoid X-activated receptor) is encoded by *NR1H4* (also known as *FXR*). This receptor is involved in many metabolic processes, including the regulation of bile acid, lipid and glucose homeostasis. A significant component of bile acid receptor-mediated events is related to induction of the enteric endocrine hormone Fibroblast growth factor 19 (FGF-19) or its rodent ortholog, FGF-15. Zhou *et al.* compared the properties of human FGF-19 and murine FGF-15 in the regulation of hepatocarcinogenesis and metabolism in various mouse models of disease. They reveal that, although both hormones repress bile acid synthesis, murine FGF15 lacks the profound, weight-independent HbA1c-cell-protective effects characteristic of human FGF19 in *db/db* mice with overt diabetes. More strikingly, unlike FGF19, FGF15 does not induce HCC in three mouse models of metabolic diseases (*db/db*, diet-induced obese, and *Mdr2*-deficient mice). **They reveal striking species-associated differences between FGF-19 and FGF-15 that may restrict the relevance of mouse models for the study of the bile acid receptor/FGF19 pathway, and underscore the importance of clinical assessment of this pathway, with respect to both safety and efficacy, in humans.**

Patients with hepatitis C virus (HCV)-related cirrhosis and HCC can be treated with oral direct acting antiviral (DAA) regimens. However, data on the use of DAA's in HCV-positive patients with HCC are scarce. Prenner *et al.* here how that **the presence of active HCC tumor at the initiation of HCV therapy is significantly associated with all-oral DAA treatment failure. HCV treatment after curative therapies for HCC resulted in excellent sustained virologic response.**

NON-ALCOHOLIC FATTY LIVER DISEASE

Role of Integrin beta-7, MAdCAM-1 and dendritic cells in the pathogenesis of NASH.

There is an urgent need to develop targeted therapies for non-alcoholic steatohepatitis (NASH). In particular, it is important to identify drivers of immune cell infiltration in order to attenuate the inflammatory response to fatty liver. Two interesting studies in this issue of the *Journal* have identified new molecular and cellular drivers of NASH. In a first study, Drescher *et al.* investigated the role of two molecules potentially implicated in neutrophil infiltration as a response to fatty liver (i.e., Integrin beta-7 and Mucosal addressin cell adhesion molecule 1 [MAdCAM-1]). By using several transgenic mice subjected to animal models of NASH, the authors eloquently demonstrated that **while**

MAdCAM-1 (encoded by *Madcam1*) promotes steatohepatitis, integrin beta-7 (encoded by *Itgb7,* also known as *Ly69*) unexpectedly exerts protective effects. *Itgb7*$^{-/-}$ mice showed earlier steatohepatitis initiation and significantly stronger fibrosis progression while *Madcam1*$^{-/-}$ mice showed less severe steatohepatitis. The interaction of integrin beta-7 and their receptor MAdCAM-1 provides novel targets for therapeutic interventions in NASH. In a second study, Heier *et al.* studied the role of CD103^{+} dendritic cells (DCs). These cells represent a heterogeneous cell population among which CD103^{+} DCs play a significant role in immunity and tolerance. The authors used several transgenic animals that lack CD103^{+} DCs. **Metabolic challenge to mice lacking CD103^{+} DCs resulted in the progression of steatosis towards steatohepatitis, manifesting in increased influx of inflammatory cells into the liver and elevated inflammatory cytokine production of myeloid cells upon innate stimuli**. Conversely, the adoptive transfer of CD103^{+} cells to DCs deficient animals reversed these observed changes and more importantly could attenuate cellular damage and inflammation in established murine steatohepatitis. This important study has identified the murine CD103^{+} cells as a protective DCs subtype that influences the pro-anti-inflammatory balance and protects the liver from metabolic damage.

HEPATITIS C VIRUS (HCV) INFECTION

Direct antiviral agents (DAA) in real world revisited, DAA going generic, HCV-induced NK cell activation results in altered NK-mediated antibody-dependent killing

Evaluation of DAA in the real world setting where patient populations are more diverse and complex may provide significant additional information with respect to safety and efficacy of the regimens. Calleja and Crespo *et al.* investigated the two oral DAA combination regimens, ombitasvir/paritaprevir/ritonavir plus dasabuvir and ledipasvir/sofosbuvir, in a large Spanish national database, which includes almost 4,000 HCV type 1-infected patients (approximately half of them with cirrhosis). Cure rates were high with 96.8% and 95.8%, respectively after 12 weeks of therapy, and a similar efficacy rate was seen in the subgroup being eligible for a shorter 8-week treatment duration. **This real-world study – being one of the largest to-date - provides relevant and detailed new information regarding treatment efficacy and safety in certain subgroups which includes SAEs and SAE-associated treatment**

discontinuation rates as well as renal safety comparisons of both regimens but also addresses the controversial issue of HCC risk in DAA treated patients with cirrhosis.

Price and access currently limit broadening treatment rates for chronic HCV infection worldwide as only few patients from developing countries can afford brand name DAAs. Generic DAAs may become a solution for this demanding problem. Controversies, however, exist concerning the bioequivalence of generics, and relevant studies are missing describing the safety and efficacy of generic ledipasvir-sofosbuvir regimens. In this issue of the journal, Zeng *et al.* reported for the first time the safety and efficacy of 8 or 12 weeks of generic ledipasvir-sofosbuvir ± ribavirin for 187 Chinese patients with chronic genotype 1b HCV infection in an observational real-world study setting. **Although not studied head-to-head, SVR rates of approximately 97%, which were achieved with the generic ledipasvir-sofosbuvir regimen in all treatment groups, were comparable to SVR rates reported with the same brand name regimen**. Undoubtedly, the broad implementation of low pricing and accessible generic DAAs (approximately USD $300 per 28 generic tablets) can dramatically affect the global burden of HCV-related diseases, and future validation studies with larger number of patients but also shorter treatment durations are warranted.

The mechanisms involved in immune escape as well as immune control of HCV infection are still not well defined. The dual function of natural killer (NK) cells, e.g. recognition and killing of target cells, and the coordination of the adaptive immune response, made them important players in orchestrating immune responses in chronic viral infections, including HCV. The present study by Oliviero and Mantovani *et al.* elegantly examined NK cell CD16 expression and function in patients with chronic HCV infection and healthy controls in order to get new mechanistic insights into the effect of HCV on its functional modulation. The present study provides for the first time evidence that HCV-infection induces NK cell activation, which leads to shedding of their Fc receptor for IgG (CD16), an essential molecule for antibody binding, hence resulting in altered NK-mediated antibody-dependent killing of target cells. This occurs through the action of enzymes named metzincins. This study has important translational implications, as it provides clear evidence that virus-induced NK cell activation not only impairs IFNγ production but also alters the efficiency of antibody-dependent killing in chronic HCV infection. The new mechanism described here may contribute to HCV

persistence and may represent a general phenomenon whereby some viruses can escape host's immune responses.

HEPATITIS B VIRUS (HCV) INFECTION

HBVcircle: a novel tool to study cccDNA

Hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) persists as a stable episome in infected hepatocytes and serves as a template for the transcription of all viral genes and has become an interesting target for novel therapeutic strategies to cure HBV infection. The establishment of an immune-competent mouse model that supports persistent cccDNA-dependent HBV replication would greatly facilitate preclinical studies aiming for destabilizing cccDNA, and to study HBV-related immunopathogenesis. Yan *et al.* used minicircle technology to generate cccDNA-like molecules (called HBVcircle), and characterized the performance of HBVcircle in *in vitro* transfection studies as well as in immunocompetent mice after hydrodynamic injection. **HBVcircle formed authentic cccDNA-like molecules *in vitro* and *in vivo*, and supported high levels and persistent HBV replication**. Different factors affecting *in vivo* HBV replication and persistence were also studied, including different classes of anti-HBV drugs. HBVcircle, a close mimic of cccDNA, represents an appealing novel tool for addressing HBV cccDNA-related biological questions, and for anti-HBV drug discovery.

NON-INVASIVE FIBROSIS ESTIMATION

Simple, accurate and validated algorithm for hepatic fibrosis using biochemical and fibrosis tests

With the introduction of non-invasive tests, the reliance on liver biopsy to stage liver disease has declined substantially. Boursier *et al.* performed a hugely important study in over 3000 patients to develop and validate an algorithm for assessment of the severity of fibrosis using routine biochemical data and many tests of liver fibrosis. The newly developed e-LIFT test, which combines routine biochemical data performed the best as the first line test. The FibroMeterVCTE (FMVCTE) was the most accurate among the eight, fibrosis tests evaluated. **The sensitivity of the eLIFT-FMVCTE algorithm (first-line eLIFT, second-line FibroMeterVCTE) was 76.1% for advanced fibrosis and 92.1% for cirrhosis. The test was also able to provide accurate prognostic information.** The data would allow to further reduction in the

need for liver biopsy.

NOVEL BILIARY PROTEINS IN PRIMARY SCLEROSING CHOLANGITIS (PSC)

Newly discovered proteins in bile and serum define prognosis, which may serve as future therapeutic targets

Treatment options for PSC patients are limited and the prognosis is also extremely difficult to define. Vesterhus *et al.* performed a study in patients with PSC and measured 63 key proteins in the serum and bile of patients with PSC. Twenty-four proteins in the bile derivation panel were significantly ($P<0.05$) different between PSC patients with mild compared to severe cholangiographic changes. **They observed that serum IL-8 provided excellent discrimination for transplant-free survival both in the serum derivation and validation cohort.** This provides important pathophysiological information that may be useful in prognostic models and for therapeutic targeting.