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FINAL

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SELECTION OF THE MONTH

Big title: Guideline for 'Clinical Practice Guidelines'

Small title (cirrhosis): Pocket device to test organs for steatosis severity

LIVER INJURY

Autophagy in liver sinusoidal endothelial cells (LSEC) protects the liver

Liver sinusoidal endothelial cells (LSECs) are highly specialized endothelial cells representing the interface between blood cells on the one side and hepatocytes and hepatic stellate cells on the other side. Inductive angiocrine signals released by LSECs are required for liver regeneration. LSECs also play a key role in the initiation and progression of chronic liver diseases. They become capillarized and lose their protective properties, and they promote angiogenesis and vasoconstriction. Autophagy

is an endogenous protective system whose loss could affect LSEC integrity. Ruart *et al.* investigated the role of autophagy in the regulation of endothelial dysfunction and the impact of its manipulation during liver injury. Using elegant rodent models, they now show that **pharmacological and genetic inhibition of endothelial autophagy increases oxidative stress in vitro. During liver injury in vivo, the selective loss of endothelial autophagy is associated with cellular dysfunction, reduction in intrahepatic NO, impaired ability to scavenge oxygen reactive species and aggravates fibrosis.** Potentiation of autophagy selectively in LSEC may be a target for novel therapeutic approaches at early stages of liver diseases.

NON-ALCOHOLIC STEATOHEPATITIS (NAFLD)

A new genetic polymorphism linked to hepatic steatosis

It is well known that genetic factors influence fibrosis progression in NAFLD. However, the genetic predisposition to develop hepatic steatosis is largely unknown. In this issue, Metwally *et al.* studied the role of irisin, the cleaved extra-cellular fragment of the fibronectin type III domain-containing protein 5 (encoded by *FNDC5*). Irisin modulates different metabolic activities. The authors identified rs3480 in the 3′ untranslated region of *FNDC5* and studied its role in 987 Caucasian patients with NAFLD. The rs3480 (G) allele was associated with advanced steatosis (OR: 1.29), but not with other histological features. Interestingly, this effect was additive to variations in the two main genes that influence NAFLD (i.e. *PNPLA3* and *TM6SF2*). *In vitro* studies showed that the rs3480 polymorphism influenced *FNDC5* mRNA stability and the binding to regulatory microRNA, that would result in decreased mRNA translation into protein. Finally, elevated serum irisin was associated with reduced steatosis. This genetic study reveals that carriage of the *FNDC5* rs3480 minor (G) allele is associated with more severe steatosis. The study also identified irisin as a factor that exerts favorable effects on NAFLD. Whether this protein has therapeutic potential deserves further investigation.

HEPATITIS B VIRUS (HBV) INFECTION

The impact of HBsAg loss on top of viral suppression, human cytomegalovirus (HCMV) co-infection alters NK-cell phenotype and effector function

The loss of HBsAg, termed functional cure, is regarded as the optimal treatment endpoint because it allows safe discontinuation of antiviral therapy. As chronic HBV

infection cannot be completely eradicated due to the persistence of cccDNA and integrated HBV DNA, it is however debated whether HBsAg loss adds to the prevention of the long-term consequences of chronic HBV infection in patients in whom viral replication is completely suppressed under antiviral therapy. Wong *et al.* now performed a large-scale territory-wide retrospective cohort study to explore the risk of HCC and hepatic events in tenofovir- and entecavir-treated patients with and without HBsAg seroclearance. Out of 20,263 entecavir or tenofovir-treated patients 17,499 had complete viral suppression, and 376 (2.1%) further achieved HBsAg loss. **Complete viral suppression was associated with a lower risk of HCC and hepatic events in patients receiving antiviral treatment, and HBsAg loss further reduced the risk of HCC on top of complete viral suppression** but not the risk of hepatic events and liver-related mortality. This study points to the importance of intrahepatic transcriptional activity of HBV infection as a relevant driver of hepatocarcinogenesis in virally suppressed HBV infected patients, and supports our attempts in aiming for HBsAg loss as the main endpoint of current and future antiviral strategies.

Natural killer (NK) cells mediate anti-HBV immunity by direct and indirect mechanism and its phenotype and function is altered in chronic HBV infection in comparison to healthy donors. The elegant study by Schuch *et al.* evaluated whether co-infections with HCMV, which are quite common in HBV-infected patients and associated with the emergence of a distinct NK cell subset (memory-like NK cells) displaying superior CD16-mediated effector functions, triggers alterations of the NK-cell repertoire. In-depth analyses of circulating NK cells in chronically HBV-infected patients and controls with respect to their HCMV serostatus showed that based on mutual effects of HCMV infection and HBV chronicity the NK-cell repertoire in HBV-/HCMV-coinfected patients is biased towards CD16-mediated effector functions. **This study underpins that co-infections especially with HCMV can shape the immune repertoire and consequently affects the immune response in chronic HBV infection** and therefore needs to be considered with respect to the design and application of new immunotherapeutic approaches in HBV cure involving NK cells.

HEPATITIS C VIRUS (HCV) INFECTION

The long-term sequels of HCV infection in childhood, first real-world evidence of G/P`s safety and efficacy, protease position 80 substitution Q80K - not an innocent bystander

Little is known about the development of the long-term liver disease outcome in patients infected with HCV in childhood, and how antiviral treatment impacts the natural history of chronic HCV infection in this population. Modin *et al.* enrolled 1049 patients of the HCV Research UK cohort who all were infected with HCV in childhood. Serious long-term complications of chronic hepatitis C (cirrhosis, HCC, need for liver transplantation and death) developed in one third of the patients. **A critical finding in the analysis was that the long-term development of progressive liver disease was independent of the age or route of acquisition, with a median time to diagnosis of 32-36 years**. The study also demonstrates for the first time in patients infected in childhood, that early treatment, especially before development of cirrhosis, significantly decreases HCV-related morbidity and mortality. Authors conclude that HCV infection in childhood causes serious long-term liver-related complications which can now be prevented with antiviral therapy. Based on this evidence, treatment of chronic HCV in childhood should be provided to children by health authorities.

The second generation protease- and NS5A-inhibitor regimen glecaprevir plus pibrentasvir (G/P) showed improved pangenotypic efficacy with a high barrier against resistance in numerous phase 3 trials and was approved by EMA in July 2017. The first large real-world cohort assessment of the safety and efficacy of G/P for the treatment of chronic HCV infection was now presented by D'Ambrosio *et al.* in this issue of the journal. **In their cohort of 793 patients, the overall SVR rate across all genotypes was 99% and hence was comparable with the results of controlled clinical trials**. Only five patients suffered from a post-treatment virologic relapse, and these were infected with either HCV type 2 or 3. Intriguingly, the high SVR rates were achieved by treating most of the patients for only 8 weeks. The low premature G/P withdrawal rate of 0.7% also highlights to the overall safety of this regimen.

The naturally occurring amino acid changes at NS3 protease position 80 (Q80K) have been shown to influence sensitivity to the protease-inhibitor simeprevir in HCV type 1-infected patients. However, little is known about the impact of Q80K on the efficacy of other protease inhibitors, and also the molecular mechanisms underlying treatment failure mediated by baseline protease substitutions have not been studied. In the study by Pham et al. the effect of NS3 substitutions at position 80 on viral fitness and resistance to first- and second-generation protease inhibitors for HCV genotypes 1-6 was investigated by using infectious cell culture systems as well as next generation sequencing. In classical short-term resistance assays, Q80K conferred simeprevir

resistance across genotypes, but no-to-little resistance to other protease inhibitors. **However, Q80K had the potential to promote accelerated viral escape from other protease inhibitors in long-term treatments.** Carrying pre-existing Q80K, genotype 3a appeared to be more prone to escape from glecaprevir and voxilaprevir than genotype 1a. Thus, the study describes for the first time how position-80-substitutions impact on fitness and resistance to all 6 clinically relevant protease inhibitors and reveal that pre-existing position-80-substitutions facilitate accelerated escape from protease inhibitors.

CIRRHOSIS

Multidrug resistance (MDR) in hospitalized cirrhotic patients, non-invasive predictors of response to carvedilol

Infection is the commonest precipitating event leading to the hospitalization of cirrhosis patients and also complicates the course. Epidemiological data about the resistance patterns, risk factors and outcomes in patients with acute decompensation and acute on chronic liver failure is not clear. Fernandez *et al.* examined the CANONIC dataset and also a second clinical dataset from Europe and showed alarming statistics. **The data suggested that infection was identified in about 40% of patients in both series and about half of these were culture positive. Of these, about 30% were infections with MDR organisms, which was associated with poor prognosis and its occurrence was significantly more prevalent in those that had recent hospitalization, admission to intensive care unit and nosocomial infections.** Novel strategies aimed at preventing the spread of MDR infections are urgently needed.

The gold standard to define the hemodynamic response to the administration of non-selective beta-blockers is invasive measurement of hepatic venous pressure gradient (HVPG) and an adequate non-invasive marker is an unmet need. Kim *et al.* describe the results of an extremely important study where they measured several parameters and studied patients with HVPG on two separate occasions. **They showed that an adequate hemodynamic response was observed in about 55% patients and only the change in splenic stiffness was an independent predictor of response. They generated a new mathematical model and validated this in a second cohort with a high degree of accuracy (AUC of 0.848).** These data are important and is likely to change clinical practice.

LIVER TRANSPLANTATION

Rescuing severe heatstroke associated acute liver failure with transplantation, pocket device to test organs for steatosis severity

Severe acute liver injury (sALI) is a rare but a serious complication of exertional heat stroke. Criteria and timing for transplanting these patients is unknown as some of these patients will recover spontaneously and others will be too sick to transplant. Ichai *et al.* describe important results from studying 26 patients with sALI from exertional heat stroke that were admitted to 7 tertiary centers. **Of these, 15 recovered rapidly and 9 patients were listed for transplantation. Five were withdrawn from the list, as on days 2 and 3 as there was an improvement of in their prothrombin time to >10%. The other 4 (15%) required a liver transplant and 3 of these patients are alive after 1 year.** The study provides the first data that the vast majority of patients with sALI are likely to improve and only those in whom the PT is <10% on day 3 should undergo a liver transplant.

At present, selection of organs for transplantation, especially if they are steatotic is based upon clinical judgement, which means that some organs are selected for transplantation that are too steatotic and often organs are turned down, when they could be used, as frozen sections are rarely available. **Golse *et al.* describe the results of an innovative study where they evaluated the performance of a commercially available pocket spectrometer (PSM), that provides instantaneous read out on the severity of steatosis. Their data show a high degree of accuracy amounting to over 91% and a reproducibility of about 85% in mild-moderate steatosis.** This device if validated further could add considerable value to selection of organs for transplantation increasing the donor pool and improving outcomes.

PRIMARY SCLEROSING CHOLANGITIS (PSC)

Phase 2 trial of NGM282 fails to meet the primary end point

Treatment of PSC is an unmet need. NGM282 is an FGF19 analogue that is known to regulate CYP7A1-mediated bile acid homeostasis. Hirschfield *et al*. performed a placebo-controlled trial of NGM282 in PSC patients with a change in alkaline phosphatase as the primary end point. **Their data showed that NGM282 did not meet the primary end point but achieved its pharmacological effect, significantly reduced bile acids and surrogate markers of fibrosis including the enhanced**

liver fibrosis score and Pro-C3. The drug was safe and well tolerated apart from more frequent gastrointestinal symptoms. The authors discuss issues around identifying appropriate end points for clinical trials in PSC.

HEPATOCELLULAR CARCINOMA (HCC) – BASIC

Increased receptor tyrosine kinases (RTKs) promote HCC, myeloid-derived suppressor cells (MDSCs) inhibit cytokine-induced killer (CIK) cells

The variety of alterations found in HCC challenges the identification of functionally relevant genes and their combinatorial actions in tumorigenesis. Deregulation of receptor tyrosine kinases (RTKs), such as MET (a receptor for hepatocyte growth factor), is frequent in HCC. However, little is known about the molecular events that cooperate with RTKs and whether these cooperative events play an active role at the root of liver tumorigenesis. To address these questions Fan *et al.* used Sleeping Beauty transposon insertional mutagenesis to accelerate liver tumor formation in a genetic context resulting in slightly increased MET levels. **Their results reveal unexpected genetic interactions underlying gene cooperativity with RTKs in HCC.** Moreover, this study shows that moderately increased levels of wild-type RTKs result in permissive context allowing a large spectrum of deregulated mechanisms to initiate liver cancer.

CIK cells are a mixed cell population of effector cells with diverse TCR specificities that also possess non-MHC-restricted cytotoxic activity against tumor cells. CIK cells, which comprise cytotoxic T cells, natural killer (NK) cells, and NK-like T cells that expressing both NK– and T–cell markers are expanded ex vivo using recombinant interferon (IFN)-γ, interleukin (IL)-2 and anti-CD3. CIK-cell-based immunotherapy is effective as adjuvant therapy in early stage of HCC but lacks efficacy in advanced HCC. Here, Yu *et al.* show that **adoptive cell transfer of CIKs into tumor bearing mice induces inflammatory mediators (e.g., IL-13) in the tumor microenvironment and an increase in tumor infiltrating MDSCs leading to impaired anti-tumor activity in two different HCC tumor models.** MDSCs efficiently suppresses the cytotoxic activity of CIKs in vitro. In contrast, treatment with an inhibitor of the phosphodiesterase PDE5 reverses the MDSC suppressor function via blockade of arginase 1 and inducible nitric oxide synthase (two well-known executors of MDSCs). The Authors suggest that targeting MDSCs may be an efficient strategy to enhance the antitumor efficacy of CIKs for the treatment of patients with HCC.

HCC – CLINICAL

Spleen stiffness cancer recurrence after surgical HCC resection

Hepatic resection can be used for treating HCC. However, cancer recurrence occurs in at least 60% of cases, 5 years after surgical resection. Predictors of late recurrences (24 months after surgery) are not known. Marasco *et al.* aimed to evaluate these predictors, using measurements of liver and spleen stiffness. They prospectively enrolled 175 patients who had underwent hepatic resection for HCC and followed them for up to 30 months or until HCC recurrence. Using multivariate analyses, they show that **higher spleen stiffness was the only significant independent predictor for late HCC recurrence (hazard ratio, 1.046; 95% confidence interval 1.020 to 1.073).** Late HCC recurrence-free survival was significantly different when a cut-off of 70 kPa was used for spleen stiffness. These promising results should be confirmed in larger cohorts.