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VIRAL HEPATITIS –
FROM BENCH TO BEDSIDE

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Molecular virology defines new therapeutic targets
Non-cytopathic degradation of HBV cccDNA

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Current antivirals can control but not eliminate hepatitis-B-virus (HBV), because HBV establishes a stable nuclear cccDNA. Interferon-α treatment can clear HBV but is limited by systemic side effects. We described how interferon-α, interferon-γ and tumor necrosis factor-α can induce specific degradation of the nuclear viral DNA without hepatotoxicity and propose lymphotoxin-β-receptor activation as a therapeutic alternative. Interferon-α, interferon-γ, tumor necrosis factor-α and lymphotoxin-β-receptor activation up-regulated APOBEC3A and 3B cytidine-deaminases in HBV-infected cells, primary hepatocytes and human liver-needle biopsies. HBV-core protein mediated the interaction with nuclear cccDNA resulting in cytidine-deamination, apurinic/apyrimidinic site formation and finally cccDNA degradation that prevented HBV-reactivation. Genomic DNA was not affected. Thus, inducing nuclear deaminases – e.g. by lymphotoxin-β-receptor activation – may allow development of new therapeutics that combined with existing antivirals may cure hepatitis B.
Hepatitis C virus entry inhibition

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Chronic hepatitis C virus infection is a major cause of liver disease and hepatocellular carcinoma world-wide. While the development of direct-acting antivirals is expected to cure the large majority of patients, patients with defined genotypes, advanced liver disease or transplantation may need alternative or complementary therapies. Furthermore, recent data indicate patients with liver cirrhosis remain at risk for the development of liver cancer despite viral cure. Therapeutic intervention using entry inhibitors prevents viral resistance and can clear viral infection in state-of-the-art animal models in vivo. In this presentation we will review the recent discoveries of virus-host interactions during viral entry and their impact as therapeutic targets for HCV and other pathogens.

References:


Molecular targets of antiviral therapy for hepatitis C

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Therapy of chronic hepatitis C that is caused by infection with the hepatitis C virus (HCV) is a success story. The virus was discovered in 1989, which lead to the implementation of blood tests causing a dramatic decline of transfusion-associated hepatitis C. However, it took another 10 years to develop the first cell culture model that recapitulates virus replication in cultured cell lines: engineered HCV mini-genomes (so-called replicons). With the advent of this system, and multiple improvements thereof, two viral drug targets, the NS3/4A protease and the NS5B RNA-dependent RNA polymerase, have been validated and a new one, the NS5A replicase/assembly factor has been identified. NS3/4A, targeted e.g. by Telaprevir, Boceprevir or Simeprevir, is a heterodimeric complex containing in the N-terminal NS3 domain a serine-type protease that is responsible for cleavage of the HCV polyprotein. NS5B, targeted e.g. by Sofosbuvir, is the catalytic core of the viral replicase and responsible for the amplification of the viral RNA genome. NS5A, targeted e.g. by Daclatasvir and Ledipasvir, is an unusual drug target as it lacks enzymatic activity. NS5A binds viral RNA and multiple viral cellular proteins, including cyclophilin A. It is thought that NS5A promotes HCV replication and virus production by interaction with these factors. While the molecular mechanisms how NS5A-targeting DAAs block HCV replication, remain unclear two antiviral activities have been identified for this inhibitor class: first, blocking the biogenesis of the HCV replication factory (called membranous web); second, blocking production of infectious virus particles. The availability of these highly potent DAAs allowed establishment of interferon-free antiviral treatment modalities that are well tolerated and lead to high sustained viral response rates in the vast majority of patients. These results illustrate the great progress that has been made in the HCV field and how fundamental research has laid the ground for innovative strategies to counteract this pathogen.
HCV mouse models in antiviral and vaccine development

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At least 150 million people are chronically infected with hepatitis C virus (HCV) and is a major causative agent of liver disease including fibrosis, cirrhosis and liver cancer. Development of more effective clinical therapies has been delayed by the lack of robust and suitable animal models. HCV has an almost unique and mechanistically unexplained host tropism limited to robust infections in humans and chimpanzees. We are pursuing three independent but possibly complementary approaches to overcome current species barriers and generate a small animal model for persistent HCV: 1. Adaptation of HCV genomes to infect hepatocytes of non-human primates, with the long-term goal of a simian tropic HCV strains. 2. Humanization of the mouse liver and immune system by transplanting human hematopoietic stem cells and hepatocytes into a single murine recipient, thus allowing studies of pathology, immune correlates, and mechanisms of HCV persistence. 3. Genetic host adaptation to create an inbred murine model for HCV. Through the development and use of these platforms, we aim to shed light on HCV molecular virology, to understand the associated liver disease, and to uncover novel avenues for therapeutic intervention.
Session II

Immunological determinants of persistence and clearance
Contrasting innate immune response to hepatitis A and hepatitis C viruses, positive-strand hepatitis viruses with divergent infection outcomes

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Host recognition of viral RNAs by RIG-I-like receptors (RLRs) and Toll-like receptors (TLRs) activates signaling pathways that induce interferons and proinflammatory cytokines that typically limit virus replication. Hepatitis C virus (HCV), an enveloped flavivirus that frequently causes persistent infection and chronic liver disease, disrupts both RIG-I and TLR3 signaling by targeting essential adaptor proteins, MAVS and TRIF respectively, for cleavage by its NS3/4A serine protease. Hepatitis A virus (HAV), a unique hepatotropic picornavirus that never establishes long-term persistent infection, similarly disrupts RIG-I/MDA5 and TLR3 signaling through cleavage of MAVS and TRIF by virally-expressed proteases. The targeting of these two adaptor proteins by distinctly different proteases expressed by HAV and HCV provides a unique example of convergent evolution in viral immune evasion, and is indicative of the importance of these host signaling pathways to control of RNA virus infection in the liver. However, since HAV infections never persist, additional immune escape mechanisms must underlie HCV persistence. Surprisingly, HCV typically evokes robust intrahepatic interferon-stimulated gene (ISG) expression, while HAV is extraordinarily stealthy, inducing minimal ISG expression despite a 100-fold greater abundance of viral RNA in the liver.

Since plasmacytoid dendritic cells (pDCs) play a dominant role in production of type I interferons in many virus infections, and produce IFN-α when placed in co-culture with HCV-infected hepatoma cells, we asked whether the lack of ISG expression in acute hepatitis A might reflect an inability of pDCs to sense HAV. However, freshly isolated human pDCs produce substantial quantities of IFN-α through a TLR7-dependent pathway when co-cultured with HAV-infected cells. As with HCV, this requires direct contact between pDCs and infected cells. Remarkably, pDC stimulation results from a novel HAV particle that is released from infected cells completely cloaked in host membranes, while high titer challenge of pDCs with standard non-enveloped HAV particles does not induce IFN-α production. The biogenesis of these quasi-enveloped HAV virions ('eHAV') results from the hijacking of cellular membranes through interactions with host proteins associated with late components of the ESCRT (endosomal-sorting complexes required for transport) system, ALIX and VPS4B. These particles are fully infectious and are the only detectable form of the virus in the blood during acute HAV infection.
Collectively, our studies reveal a surprising reverse correlation between acute intrahepatic interferon responses and clearance versus persistence of HAV and HCV, and show how studies of HAV pathogenesis can provide a unique perspective on host responses and mechanisms of persistence of HCV.
Cellular immune responses

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Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections account for 57% of cases of liver cirrhosis and 78% of cases of primary liver cancer worldwide and cause a million deaths per year. Although HBV and HCV differ in their genome structures, replication strategies and life cycles, they have common features, such as their non-cytopathic nature and their capacity to induce chronic liver disease. However, the rate of disease progression from chronic hepatitis to cirrhosis varies greatly among infected individuals, and the factors that regulate it are largely unknown.

Liver injury and disease progression are thought to be driven by host immune responses in both infections, and previous research has mostly focused on the role of virus-specific T cells in this process. However, research over the past 5 years revealed several non-redundant mechanisms that drive attenuation and exhaustion of HBV- and HCV-specific T cells in chronically infected patients. The few virus-specific T cells that remain tend to target sequences in which the virus has mutated and, thus, cannot eliminate infected target cells. Given these observations, it is plausible that most of the immune-mediated liver injury in chronic HBV and HCV infection is mediated by immune cells other than virus-specific T cells.

This presentation summarizes our current understanding on the role of innate immune cells in the pathogenesis of chronic hepatitis B and C, in particular natural killer (NKs) cells, which constitute the largest innate immune cell population in the liver. Data on mechanisms of NK cell activation by virus-infected hepatocytes and the functional consequences of this activation – an altered functional profile with increased cytotoxicity and decreased production of antiviral cytokines – will be presented. Importantly, normal NK cell function can be restored by therapy with direct acting antivirals, which may result in better immunosurveillance and possibly prevent relapse.
Virus and host contributions to the outcome of hepatitis virus infection

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The viral and host factors determining the outcome of adult HBV infection are not well understood. To determine the impact of the size of the viral inoculum on the outcome of HBV infection, HBV naïve chimpanzees were inoculated with virus doses ranging from $10^{10}$ to $10^{1}$ genome equivalents (GE) of a monoclonal HBV inoculum. Virological, immunological and disease profiles were monitored throughout the infections. Animals receiving $\geq 10^{4}$ GE developed typical self-limited infections resolving in 12–16 weeks following an early ($\leq 3$ weeks) peripheral HBCAg-specific CD4 T cell response and coinciding with a highly synchronized appearance of HBV-specific CD8 T cells in the liver and sharply elevated serum ALT activity. Unexpectedly, chimpanzees inoculated with $< 10^{4}$ GE of HBV developed greatly prolonged or chronic HBV infection. The immunological correlates of these unexpected results were the absence of early CD4 T cell responses to HBCAg and the appearance of a poorly synchronized intrahepatic CD8 T cell response. These results suggest that early CD4 T cell priming determines the outcome of HBV infection. Consistent with this hypothesis, a chimpanzee depleted of CD4 T cells prior to and for several months after inoculation, with a dose of HBV that typically results in a self-limited infection, became persistently infected. These results suggest that a very early (i.e. preceding spread of the infection) CD4 T cell response, probably induced by subviral antigens that are present in a $10^{4}$-fold excess over the infectious virus in HBV positive sera, is required for the development of optimal CD8 T-cell responses which then determine the outcome of HBV infection.
Innate regulation of T cell responses

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It is increasingly evident that innate immune responses are not only important in the early stages of acute infections, but can also serve as key regulators of adaptive immunity in persistent viral infections. I will illustrate this with our recent findings from patients chronically infected with HBV (CHB), revealing novel mechanisms by which innate populations contribute to the profoundly depleted antiviral T cell response.

We have found that NK cells can exert an immunoregulatory function in patients with CHB (Peppa et al, JEM 2013), in line with recent evidence for a rheostat role for NK cells in the mouse LCMV model of viral persistence (e.g. Waggoner et al Nature 2011). NK cells constitute 30–40% of intrahepatic lymphocytes; in CHB they are highly activated and an increased proportion express the death ligand TRAIL. We have found that they can kill HBV-specific T cells, that upregulate the TRAIL-R2 death receptor. Our recent data show that HBV-specific T cells also upregulate NKG2D-ligands, allowing them to activate NKG2D+ intrahepatic NK cells as part of their bidirectional cross-talk.

Another innate cell type that can potently down-regulate T cells is the myeloid-derived suppressor cell (MDSC). We have identified an expansion of granulocytic MDSC in patients replicating HBV without hepatic immunopathology. These MDSC express high levels of arginase and can inhibit the proliferative expansion of functional T cells (virus-specific and bystander) by depriving them of the conditionally essential amino acid arginine.

The implications of these pathogenic innate/adaptive immune interactions for understanding HBV pathogenesis and optimising antiviral therapy regimens will be discussed.
Session III

Bringing novel therapies into the clinics: HBV
Entry inhibition of hepatitis B and D virus: from basic in vitro findings to novel therapies

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For almost three decades after the discovery of hepatitis B virus (HBV) the early events of infection (attachment, receptor binding and fusion) remained entirely unresolved. Although the extraordinary hepatotropism of HBV and its peculiar host specificity were supposed to be linked to specific receptor binding, it remained unclear which determinant(s) within the viral envelope protein(s) are essential for mediating HBV and hepatitis D virus entry and which cellular receptor(s) are addressed. One reason was the lack of suitable in vitro infection systems. Following the establishment of the susceptible HepaRG cell line and primary hepatocytes from humans (PHH) as reliable cell culture infection systems, systematic analyses allowed the identification of a myristoylated preS1-subdomain as essential for specific hepatocyte binding.

Using two different approaches sodium taurocholate co-transporting polypeptide (NTCP/SCL10A1) could recently be identified as this highly specific hepatic receptor for the myristoylated preS1 subdomain. The identification of peptidic ligands of this receptor acting as potent inhibitors of viral entry opened a novel therapeutic option to prevent infection and treat chronically infected patients. The lead substance of such a NTCP-ligand (Myrcludex B) displayed remarkable liver tropism and inhibitory potential at picomolar concentrations. Following proof of principal studies in HBV and HDV mouse models Myrcludex B was transferred into clinical development and successfully passed a phase Ia clinical safety trial in 2013. Following these initial safety studies Myrcludex entered two phase IIa efficacy trials in chronically HBV and HBV/HDV co-infected patients, which are currently running. Interim results of these still ongoing clinical trials show safety in all applied doses and remarkable virologic and biochemical effects in both HBV and HBV/HDV infected patients. As expected from targeting NTCP, bile salt levels are influenced by Myrcludex B therapy. In addition to its direct action resulting in in vivo inactivation of HBV receptor function Myrcludex B administration induced HBV-preS-specific antibody responses that might contribute to a sustained suppression of de novo infection following Myrcludex B withdrawal. Thus entry inhibition, in addition to its expected preventive effects, has been demonstrated to be effective in chronically HBV and HDV infected patients and might contribute as one important pillar in future curative therapeutic regimens for both viruses.
Cellular therapies for HBV

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Cancer immunotherapy using a patient’s own T cells redirected to recognize and kill tumor cells has achieved promising results in metastatic melanoma and leukemia. This technique involves harnessing a patient’s T cells and then delivering a gene that encodes a new T cell receptor or a chimeric antigen receptor that allow the cells to recognize specific cancer antigens. The potential for development of redirected T cell therapy for persistent viral infections like HBV and their associated malignancies has started to be explored by different groups and we recently demonstrated in a first-in-man clinical trial that the adoptive transfer of HBV-specific TCR redirected T cells in a patient with HBsAg-productive HCC cause a profound inhibition of HBsAg production. However, adoptive T cell therapy for CHB is still regarded with skepticism because HBV-specific T cells can potentially attack infected hepatocytes that are not cancerous, possibly leading to liver damage. The challenge to design TCR redirected T cells that can cure virally infected cells without damaging the normal liver is open. We will discuss new concepts and methods that might lead to the clinical use of TCR redirected T cells that can cure virally infected cells without targeting the normal tissue.
Therapeutic vaccines in treating chronic hepatitis B: from bench to bedside and back

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The antiviral treatment of chronic hepatitis B virus (HBV) infection has greatly improved over the last 20 years since it has allowed a disappearance of cirrhosis decompensation and a significant reduction of the incidence of hepatocellular carcinoma. However, a complete HBV cure has not been achieved and alternative treatments are still needed to optimize the current treatments. Therapeutic vaccination is a promising new strategy for controlling persistent infections and tumors. However, this approach has not been as successful as initially anticipated for chronic hepatitis B. General impairment of the immune responses generated during persistent HBV infection, with exhausted T cells not responding correctly to therapeutic vaccination, is most likely responsible for the poor clinical responses observed to date. We will present results from previous clinical trials of therapeutic vaccination, in the hope that useful lessons will emerge, and novel immunotherapeutic strategies either tested in clinic or at preclinical stages in animal models for chronic HBV infection.
Session IV

Bringing novel therapies and vaccines into the clinics: HCV
Vector based vaccines in clinical trials

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The global burden of HCV infection is immense with 180 million people infected worldwide and 4 million people newly infected each year. New directly acting antivirals are now available associated with cure rates of > 90%. Whilst these represent remarkable progress, they are unaffordable even in developed countries (£30–70,000/person), do not prevent reinfection, and may be associated with viral resistance. Therefore, a vaccine to prevent or treat HCV infection targeted to “at risk” populations, or more widely in high prevalence countries would be of enormous global benefit.

HCV should be particularly susceptible to a T cell strategy, since immune mediated viral eradication, maintained indefinitely, occurs spontaneously in 20% of people following primary infection. We and others have shown that this is crucially dependent on effective T cell immunity and an appropriate host immune genetic background.

In recent years we have, in collaboration with Okairos, developed highly immunogenic HCV T cell vaccines in Phase-I experimental medicine studies that include both healthy volunteers and HCV infected patients. We have used simian and human adenoviral (Ad) vectors derived from rare serotypes, in addition to Modified Vaccinia Ankara (MVA) vectors encoding the entire non-structural (NS) HCV proteins in heterologous prime/boost regimens. In healthy volunteers we have shown that these vaccines are highly immunogenic generating very high levels of functional CD4+ and CD8+ HCV specific T cells targeting multiple parts of the HCV genome. The detailed assessment of T cell function and phenotype has employed both traditional methodologies and novel CyTOF technology.

However, we have also shown that responses are generally attenuated in people with persistent infection and that intra-host and inter-host viral diversity, in combination with host HLA heterogeneity, may present a major challenge to the development of a fully protective HCV vaccine. Currently HCV T cell vaccines based on Chimpanzee Ad/MVA prime boost strategies are undergoing efficacy testing for HCV prevention, in intravenous drug using populations in the USA with outcome data expected in 2016.

Moving forward we are now assessing these vaccines in people with HIV infection on HAART, in addition to developing genetically adjuvanted class-II Ii chain vaccines as part of an EU Fp7 funded program-PEACHI (http://www.peachi.eu/), and new vaccines based on conserved HCV segments to tackle the thorny issue of HCV diversity.

Reference:

Optimisation of a recombinant gpE1/gpE2 vaccine component for future clinical trials

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A prophylactic vaccine to prevent chronic, persistent infection by the hepatitis C virus (HCV) is an urgent unmet medical need, particularly in individuals at high risk of infection. Previously, a recombinant gpE1/gpE2 vaccine candidate derived from a single 1a strain has been shown to be protective in chimpanzees, to elicit strong T helper responses and anti-gpE1/gpE2 antibodies in humans and able to elicit broad cross-neutralising antibodies in mice, guinea pigs, chimps and humans. Data obtained from passive immunisation studies conducted in mice and chimps has also demonstrated the protective efficacy of various neutralising monoclonal and polyclonal antibodies. We now show that anti-gpE1/gpE2 antisera derived from immunised goats and humans inhibits the binding of a large panel of cross-neutralising monoclonal antibodies targeting both gpE2 and gpE1 thus confirming that this vaccine presents a multiplicity of highly conserved, cross-neutralising epitopes. While it does elicit broad cross-neutralising antibodies, further optimisation of this vaccine preparation has been performed by testing the immunogenicity of a variety of vaccine antigens. HCV 1a-derived rec gpE1/gpE2 or rec gpE2 but not rec gpE1 elicited broad, cross-neutralising antibodies in goats but with only weak titers observed against genotypes 2 & 3 (as found in vaccinated humans). Rec gpE2 derived from genotype 2 failed to elicit strong neutralising titers against genotype 3 indicating that an optimum global vaccine should consist of antigens prepared from genotypes 1, 2 & 3. We anticipate developing a similar vaccine cocktail for clinical testing in the near future which will be combined with components to elicit broad CD4+ & CD8+ T cell responses to the virus. Since HCV is known to be complexed with apolipoproteins in vivo, we have also tested the ability of rec gpE1/gpE2 antisera to neutralise free and complexed HCV. Both are neutralised and detailed comparative data will be presented.

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Antiviral therapies for chronic hepatitis C

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The establishment of a robust HCV cell culture system and subsequent detailed characterization of the HCV life cycle was a key step towards the identification of putative antiviral targets and respective antiviral drugs. As of today, these include direct-acting antiviral agents (DAAs) such as NS3/4A protease inhibitors (PI), NS5A inhibitors, nucleos(t)ide (NI) and non-nucleoside polymerase (NNI) inhibitors, as well as host-targeting agents (HTAs) directed against cellular factors involved in viral entry or replication. Trials investigating different drug classes with and without pegylated interferon (PegIFN) and/or ribavirin (ribavirin) have rapidly evolved, yielding highly promising sustained virologic response (SVR) rates.

In regard to the enormous public health impact of HCV infection with an estimated 180 million anti-HCV positive persons worldwide, the recent advances in HCV drug development, harbor, together with implementation of improved HCV screening programs, the potential to substantially reduce the global burden of HCV-associated advanced liver disease including hepatocellular carcinoma. Eradication of HCV only with antiviral drugs and without a vaccine, however, is a challenging task.

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Diagnostic discrepancy for hepatitis C virus infection in haemodialysis patients in Bulgaria

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Introduction: Patients receiving haemodialysis are at higher risk for acquiring hepatitis C virus (HCV) infection than general population. Routine screening and adherence to standard infection control practices are the key strategies for preventing HCV transmission in haemodialysis units.

Objectives: The objective of this study was to assess the prevalence of HCV in a cohort of dialysis patients in Plovdiv region, Bulgaria.

Methods: HCV status of patients receiving maintenance haemodialysis in University Hospital dialysis center, Plovdiv, Bulgaria, was measured by HCV antibody (HCV Ab) testing by third generation ELISA and HCV RNA detection in plasma and peripheral blood mononuclear cells (PBMCs) using commercial real-time PCR assays. A total of 93 patients were enrolled in this longitudinal study conducted between May 2013 and 30 October 2014. HCV RNA was tested on two occasions and HCV Ab, liver function tests (ALAT, ASAT and GGT) every six months during the study period. The patients were evaluated clinically every six months.

Results: Sixty eight out of 93 patients met the inclusion criteria. Mean age was 57.12 years (range 18–84) and men (58.8%) were more often on dialysis than women (41.1%). Mean duration on haemodialysis treatment was 54.34 months (range 9–365). Ten patients were both HCV PCR (+) and HCVAb (+), giving the prevalence of 14.7%. One patient seroconverted in the study period. The duration of haemodialysis was risk factor for hepatitis C (p < 0.05). None of the 38 patients tested were positive for HCV RNA in PBMCs. Four patients were persistently HCV RNA (-) tested both in plasma and in PBMCs but HCVAb (+). Two of them were previously HCVAb (+) when their charts were reviewed retrospectively. Considering their immunodeficiency these results were more likely to be biological false positive rather than a sign of resolved past infection.

Discussion/Conclusion: HCV infection is common in our study. Further studies with serial testing for both HCVAb and HCV RNA are needed for complete assessment of HCV status of dialysis patients in Bulgaria.
The disease course of chronic HBV infection among pediatric patients

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Introduction: Natural history studies show that the risk of development chronic HBV infection is inversely proportional with age of acquisition: 25% of children infected at < 5 years develop chronic infection, versus < 10% adults. In Romania, treatment of HBV infection in children is limited to lamivudine and alpha2b-interferon. Treatment of all children with chronic HBV must be tempered by studying the natural history in children. Spontaneous HBeAg clearance is influenced by mode of transmission/viral load/necroinflammatory activity/immunocompetence/genotype.

Aim: To investigate the rate of HBe/HBsAg seroconversion during childhood, spontaneous and after antiviral treatment.

Methods: We retrospectively studied 176 children with chronic HBV and +HBeAg. The route of transmission was vertical in 92 and parenteral/unknown in 84 children. The mean age at diagnosis was 7.5 years. In the lot with vertical transmission, 64 children emerged from HBe+ Ag mothers with high viral load and 28 from HBe-/HBs+ Ag mothers with low viral load. 112 children were diagnosed during the first 4 years of life. HBV markers, viral load, clinical and liver function were tested every 6 months.

Results: 24 (21%) of the 112 infected infants seroconvert in “e” system before 4 years without treatment. 3 (12%) with spontaneous HBeAg seroconversion emerged from HBe+ Ag mothers compared to 6 (25%) from HBe- Ag mothers and 15 (63%) from noninfected mothers (p < 0.005). No spontaneous “s” system seroconversion was detected. 58 children > 4 years (HBs+ Ag/HBe+ Ag, hypertransaminasemia, detectable viral load, necroinflamatory activity) started antiviral treatment. HBe+ Ag seroconversion and virusologic respond rate were 48% after 12 months of interferon and 24%, respectively 45% after 12 months, respectively 24 months of lamivudine (p < 0.005 at one year). HBsAg clearance was obtained in 2 cases (3%) after one year of interferon and in 1 case (1.7%) after 2 years of lamivudine.

Conclusions: Maternal carrier status is very important: children of HBeAg seropositive mothers have lower rates of HBeAg seroconversion. Due to the possibility of spontaneous HBeAg seroconversion, antiviral treatment shouldn’t be initiated in the first 4 years of life. HBsAg clearance rate was higher but not statistic significant in children who had anti-HBeAb achieved under interferon treatment.
Bispecific antibody constructs mediate immunotherapeutic retargeting of effector cells towards HBV infected target cells

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Introduction: Retargeting of effector cells is a promising immune-therapeutic approach to circumvent the immunotolerant state found in malignancies and chronic viral infections. Effector cells are supplied with designed specificities allowing redirection, and can be further equipped with co-stimulatory signals, together reverting the immunocompromised situation. To achieve retargeting against HBV-infected cells, we constructed tetravalent bispecific antibody constructs harboring two different binding domains of immunoglobulins. The first binding site was designed to target HBV-infected cells by binding the S domain of HBV envelope proteins on the plasma membrane of infected hepatocytes. The second binding motif engages immune effector cells using specificities for T cells (CD3-T cell receptor and CD28 for co-stimulatory signals). These binding sites are connected via an Fc-linker, also providing for dimerization. We furthermore generated variants of bispecific antibody constructs containing mutations in the Fc-spacer, abrogating Fc-receptor binding, to control for unwanted activation through antibody-dependent cell-mediated cytotoxicity.

Methods/Results: Confocal microscopy showed that all bispecific constructs bound specifically to the surface of HBV-producing cell lines. Through the engagement of HBV-positive target and immune effector cells, the bispecific constructs induced a specific activation of effector cells, which secreted high amounts of IFNγ, IL-2 and TNFα. In deep multispectral flow cytometry was used to measure phenotypic changes of retargeted effector cells by intracellular cytokine staining. This showed, that up to 50% of effector cells were polyfunctionally activated by the bispecific antibody constructs upon coculture with HBV positive target cells. Co-administration of CD3- and CD28-specific constructs had a synergistic effect and mediated up to 98.5% specific cytotoxic elimination of HBV S-protein positive cells in co-cultures with PBMC. MACS sorted CD4 and CD8 positive cells could also individually be retargeted to specifically kill target cells. Importantly, bispecific antibody constructs were also able to redirect effector cells to HBV-infected HepaRG cell resulting in specific killing of HBV-infected cells. Furthermore, immobilized HBsAg was sufficient for immunotherapeutic retargeting, while soluble HBsAg, mimicking the high viral loads in patient serum failed to induce effector cell activation. First preliminary in vivo experiments with transplanted HBV-positive tumors showed a marked decrease in tumor size upon treatment with bispecific constructs.

Discussion/Conclusion: Thus, retargeting of immune effector cells towards HBV-infected cells using bispecific constructs is a promising new immune-therapeutic approach against chronic Hepatitis B.
Viral replication in HBV transgenic mice lacking the surface antigen is accompanied by Toll-like receptor 3-mediated antiviral responses

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Introduction: Chronic hepatitis B virus (HBV) infection is an important cause of liver-related morbidity and mortality worldwide. Impairment of Toll-like receptor (TLR) signalling by the hepatitis B surface antigen (HBsAg) attenuates both the innate and the adaptive immune responses, thereby facilitating chronicity of infection.

Methods: This study was designed to analyze immune activation in HBV transgenic mice lacking HBsAg (Tg1.4HBV-S-Mut3). We injected HBxAg-targeting small interfering RNAs (siRNAs) or TLR3 ligand polyinosinic-polycytidylic acid (Poly[I:C]) intravenously into Tg1.4HBV-S-Mut3 mice, their HBV-negative littermates, and TLR3-/- and Tg1.4HBV-S-Mut3/TLR3-/- animals. We then measured the levels of immune genes, HBV DNA, HBeAg, and HBcAg in liver tissue from these mice.

Results: Interestingly, Tg1.4HBV-S-Mut3 mice exhibited increased and hepadnaviral replication-dependent expression of interferon beta (IFN-β), interferon-sensitive gene 15 (ISG15), and interferon-induced protein with tetratricopeptide repeats 1 (IFIT1). This antiviral response could be reduced by treatment with HBxAg-targeting siRNA and was completely abrogated in Tg1.4HBV-S-Mut3/TLR3-/- animals. Suppression of these TLR3-mediated immune responses led to increased HBV replication. Interestingly, administration of Poly(I:C) significantly induced the expression of IFN-β, ISG15, and IFIT1 and thereby suppressed HBV replication in vivo and in vitro. However, induction of these immune genes was significantly lower in Tg1.4HBV-S-Mut3 mice than in control animals, a finding indicating HBs-independent immune evasion.

Discussion/Conclusion: In contrast to HBV-infected patients, HBV transgenic mice that lack HBsAg do not exhibit total abrogation of hepatic TLR signalling; instead, HBV replication induces TLR3-mediated antiviral responses in non-parenchymal liver cells. We therefore hypothesize that HBsAg attenuates TLR signalling, thereby impairing innate and adaptive immune responses in the liver.
HBsAg-specific humoral and cellular immune memory after hepatitis B booster vaccination in adolescents 10–15 years after immunization in infancy

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Introduction: Up to 50% of individuals lose protecting antibodies within 10 to 15 years after primary vaccination against hepatitis B. However, the presence of immune memory should protect these individuals against hepatitis B disease.

Methods: To analyze the quality of HBsAg-specific immune memory we studied the kinetics of humoral and cellular immune responses after a booster dose of hepatitis B vaccine in adolescents with anti-HBs below 10 IU/l. Adolescents 14 to 18 years old were tested for anti-HBs 10 to 15 years after infant immunization. Individuals with anti-HBs concentrations below 10 IU/l were offered a booster dose and the analysis of humoral and cellular responses followed at day 0, 3, 7, 14 and 28 after revaccination.

Results: Of the 77 study participants, 67.5% showed anti-HBs levels ≥ 10 IU/l and 32.5% had anti-HBs < 10 IU/l. 23 of the latter were revaccinated. Cellular immune responses were analyzed by measuring the HBsAg-specific IL5- and IFNγ-secreting cells in enriched CD4+ peripheral blood mononuclear cells. Considering both, anti-HBs and cellular responses, four booster patterns have been observed: no or extremely week anti-HBs and cellular response (3 out of 19 subjects), only anti-HBs response (10 subjects), strong anti-HBs and cellular response (4 subjects) and high intrinsic production of IL5 (2 subjects).

Discussion/Conclusion: In conclusion, our data indicate that with the available techniques humoral response is the dominant immune response after hepatitis B revaccination in adolescents.
Performance of the aspartate aminotransferase-to-platelet ratio index for predicting advanced liver fibrosis in chronic hepatitis C Tunisian patients

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Introduction: Liver biopsy is still considered to be the gold standard for assessment of liver fibrosis. The aspartate aminotransferase-to-platelet ratio index (APRI), a tool with limited expense and widespread availability, is a promising noninvasive alternative to liver biopsy for detecting hepatic fibrosis. The aim of this study was to evaluate the accuracy of APRI for predicting advanced liver fibrosis in chronic hepatitis C (CHC) Tunisian patients.

Methods: Retrospective study including patients with chronic hepatitis C who underwent liver biopsy in our department. APRI was calculated using the following formulae: \([\text{aspartate aminotransferase/upper limit of normal}/\text{platelet count}] \times 100\) for assessment of liver fibrosis. Areas under summary receiver operating characteristic curves, sensitivity, specificity, positive predictive value and negative predictive value were used to examine the APRI accuracy for the diagnosis of advanced fibrosis.

Results: Overall, 45 patients were collected, 17 men (38%) and 28 women (62%). The mean age was 52 years (range: 24–71 years). A statistically significant positive correlation was found between the APRI score and severe fibrosis F3–F4 \((p = 0.05)\). Area under the receiver operating characteristic curves was 0.82 \([0.673–0.967]\). The cut-off value of APRI score for advanced fibrosis (F3–F4) was 0.91 \((\text{OR} = 1.38 \,[1.088–1.773]; \, p = 0.017)\). An APRI threshold of 0.91 was 87% sensitive and 75% specific. Positive predictive value was 40% and negative predictive value was 96%.

Discussion/Conclusion: In our series, an APRI score lower than 0.91 can rule out certainly advanced fibrosis. Thus, APRI score is an accurate and affordable tool to identify advanced fibrosis in CHC patients. Application of this index may decrease the need for staging liver biopsy specimens among CHC patients.
Interest of liver biopsy in chronic hepatitis B patients with ALT ≥ 2N and HBV DNA ≥ 20000 IU/ml. Results of a Tunisian survey

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Introduction: Liver biopsy is the gold standard for assessing the activity and liver fibrosis in chronic hepatitis B. Patients with alanine aminotransferase (ALT) ≥ 2N and HBV DNA ≥ 20000 IU/ml have significant fibrosis in 90%. In this setting, EASL 2012 recommended to start antiviral therapy without histological study of the liver. The aim of this study was to evaluate the interest of liver biopsy in these patients.

Methods: This was a retrospective study including chronic hepatitis B patients seen in our department with ALT ≥ 2N and HBV DNA ≥ 20000 IU/ml. Liver biopsy was performed in all patients. Metavir scoring was used to evaluate severity of necroinflammatory activity and fibrosis.

Results: A total of 20 patients were enrolled in the study. They were 15 men (75%) and 5 women (25%). The mean age was 36 years [range: 22–60]. None of included patients had clinical, endoscopic nor radiologic features of portal hypertension. Liver function was preserved in all patients. Most of patients were infected with mutant virus (90%). Mean viral load was 4 × 10^6 IU/ml [range: 32300–4 × 10^8]. Ten patients (50%) had a necroinflammatory activity ≥ A2 and 13 patients (65%) had fibrosis ≥ F2.

Discussion/Conclusion: In our series, chronic hepatitis B patients with ALT ≥ 2N and HBV DNA ≥ 20000 IU/ml had a significant fibrosis in two third of cases. Thus, liver biopsy should not be considered in this setting.
Delta like ligand 4 ameliorates liver fibrogenesis through inhibition of inflammatory chemokines

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Introduction: Humans have five Notch ligands (Jagged1, Jagged2, Delta like ligand [Dll]1, Dll3 and Dll4) and four receptors (Notch1, 2, 3 and 4). Mutation of Jagged1 or Notch2 causes Alagille syndrome, a disease characterized by defective development of intralobular bile ducts and liver injury, but no overt fibrosis. If and how Notch ligands participate in liver fibrogenesis is not known.

Methods: Immunohistochemical and immunofluorescent staining were used to investigate the potential association between Notch ligands and liver fibrosis in patients with chronic HBV infection. Notch ligands were functionally examined in carbon tetrachloride (CCl4) mediated liver damage in mice and cultured mouse hepatic stellate cells (HSC), Kupffer cells (KC) and hepatocytes.

Results: Only Dll4 expression was found in myofibroblasts of cirrhotic livers with chronic HBV infection. Dll4 immunohistochemical score correlated with inflammatory grades and fibrotic stages in these patients. Administration of recombinant Dll4 protein (rDll4) remarkably ameliorated hepatocyte apoptosis, inhibited inflammatory cell infiltration, decreased expression of cytokines and chemokines, and improved fibrosis in mice that underwent a 4 weeks CCl4 challenge. In vitro, incubation with rDll4 did not directly influence apoptosis of hepatocytes, collagen I production in HSCs, and lipopolysaccharide (LPS)-induced fibrogenic cytokines in KCs. However, rDll4 administration remarkably decreased LPS-induced chemokines (e.g. CCL2 and CCL5) in both KCs and HSCs.

Discussion/Conclusion: Dll4 exerts a compelling anti-inflammatory and anti-fibrotic role in CCl4-induced liver fibrosis via inhibiting expression of chemokines. Upregulated expression of Dll4 may play a compensatory role in preventing liver fibrogenesis in patients with HBV infection.
Submassive hepatic necrosis distinguishes HBV-associated acute-on-chronic liver failure from cirrhotic patients with acute decompensation

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Introduction: Distinguishing between acute-on-chronic liver failure (ACLF) and decompensated liver cirrhosis is difficult due to a lack of pathological evidence.

Methods: In a prospective single-center study, we investigated 174 patients undergoing liver transplantation due to acute decompensation of hepatitis B virus (HBV)-associated liver cirrhosis. Two groups were identified, distinguished by the presence or absence of submassive hepatic necrosis (SMHN). Core clinical features of ACLF were compared between these groups. Disease-severity scoring systems were applied to describe liver function and organ failure. Serum cytokine profile assays, gene expression microarrays and immunohistochemical analyses were used to study systemic and local inflammatory responses.

Results: SMHN was identified in 69 of 174 patients who were proven to have cirrhosis by histological means. Characteristic features of SMHN were extensive necrosis along terminal hepatic veins and spanning multiple adjacent cirrhotic nodules accompanied by various degrees of liver progenitor cell-derived regeneration, cholestasis, and ductular bilirubinostasis. Patients with SMHN presented with more severely impaired hepatic function, a higher prevalence of multiple-organ failure (as indicated by higher CLIF-SOFA and SOFA scores) and a shorter interval between acute decompensation and liver transplantation than those without SMHN (p < 0.01 for all parameters). Further analyses based on serum cytokine profile assays, gene expression microarrays and immunohistochemical analyses revealed higher levels of anti-inflammatory cytokines in patients with SMHN.

Discussion/Conclusion: These data suggest that SMHN is a critical histological feature of HBV-associated ACLF. Identification of a characteristic pathological feature strongly supports the notion that ACLF is a separate entity in end-stage liver disease.
Genotypic and phenotypic analysis in hepatitis C virus infected patients, the continuous need for resistance testing

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Introduction: The addition of the hepatitis C virus (HCV) protease inhibitors telaprevir and boceprevir to peginterferon α with Ribavirin therapy has increased cure rates in HCV infected patients. Clinical studies of the nonstructural protein (NS3) protease inhibitors (PIs) telaprevir and boceprevir, each used alone over 2 weeks, showed rapid emergence of resistant mutants. Simeprevir, the potent protease inhibitor was recently approved by FDA for treatment of chronic HCV infection of genotype 1 to 4.

Methods:
Genotyping: Baseline prevalence and kinetics of resistant mutations appearance, or treatment failure under interferon based triple therapy with (telaprevir or boceprevir) were subjected to RNA extraction, RT-PCR and then analyzed by population based sequencing. Changes at NS3 aminoacid sequences were analyzed by alignment to geno2pheno (hcv).
In patients who failed treatment, baseline samples were subjected to next generation sequencing (NSG) to detect minority polymorphisms.
Phenotyping: A panel of replicons harboring NS3 from patient samples were generated in Con1 (1b) based shuttle replicon. Susceptibility to protease inhibitors were detected by subjecting replicons to serial dilutions of the protease inhibitors.

Results: Clinically failed to treat samples showed (Resistant mutations), either pre-existing mutations or acquired under PIs treatment. One relapse patient with susceptible (new) mutations was identified. Phenotypic testing of resistant samples, confirmed the phenotype of “known” resistance mutations and identified the resistance profile of novel substitutions
Frequency of Q80K, a mutation associated with simeprevir resistance was high at baseline samples for HCV patients, with high prevalence in genotype 1a.

Discussion/Conclusion: Analysis of HCV patients at baseline is important tool to predict the response or failure of treatment, this suggests the clinical need for genotypic resistance test.
The predictive factors in the response to antiviral therapy in chronic hepatitis C

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Introduction: The purposes of this research was the evaluation of biological response rates and sustained virological response in patients with chronic hepatitis C and the identification of predictive factors for a favourable response to antiviral therapy in patients with chronic hepatitis C.

Methods: There were selected to take part to the research 210 patients with chronic HCV who have fulfilled all inclusion and exclusion criteria and were treated with: pegylated interferon plus ribavirin. Patients progress has been monitored by determining next parameters: age, sex, weight, height, body mass index; biochemical tests: alanine aminotransferase, aspartate aminotransferase, fasting glucose, fasting insulin, total cholesterol and triglycerides, serum iron and serum ferritin; insulin sensitivity using HOMA-IR; Serological assays – detect antibody to hepatitis C virus (anti-HCV) and molecular assays – detect quantify and/or characterize HCV-RNA; liver histopathological examination.

Results: These parameters were included in an analysis of AUC (area under curve) in order to estimate their degree of influence on getting EVR (early viral response) and SVR (sustained viral response). Based on the obtained results, it appears that only the values of HOMA index, those of insulinemia values, alongside initial value of HCV RNA, dVL parameter value (low relative percentage of viral load during the first 12 weeks of treatment), mean blood glucose values at baseline, as well as values of histological scores of fibrosis (Ishak), steatosis and hepatic iron loading, may be predictive in the early viral response in chronic hepatitis C.

Discussion/Conclusion: Our research demonstrates that all the parameters defining insulin resistance are negative predictors for achieving both EVR and SVR.
Efficiency of treatment with peginterferon alfa-2a in patients with Ag HBe positive chronic liver disease

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Introduction: Currently there are two major groups of antiviral therapy in HBV chronic liver disease: peginterferon alfa-2a and nucleoside/nucleotide agents. The aim of this study was to evaluate the response to treatment with peginterferon alfa-2a of patients with Ag HBe positive chronic liver disease.

Methods: In this retrospective study were included 40 patients with Ag HBe positive chronic liver disease. Only patients treated for 48 weeks with peginterferon alfa-2a and in which a viral load for at least 6 months after the end of treatment was available, were included in our study. We defined as sustained viral response as a viral load less than 2000 IU/mL (10000 copies/ml) at 6 months or more after the end of treatment. All subjects were assessed for loss of hepatitis B e antigen (HBeAg), presence of hepatitis B antibody (anti-HBe), suppression of hepatitis B virus (HBV) DNA after follow-up.

Results: Of the 40 patients with Ag HBe positive chronic hepatitis B 52.5% (21) were naive and 17.5% (7) pretreated (with standard interferon or nucleoside/nucleotide agents). Of all patients, 25% (10) had SVR and the loss of HbeAg was observed in 10% (4) of patients. Of the 7 pretreated AgHBe positive patients neither one presented SVR.

Discussion/Conclusion: Naive patients responded better to antiviral therapy than pretreated patients, but the number of patients included in the study is low and further studies are required.
Loss of HBsAg-expression ameliorates liver serum parameters and reduces ER-stress in HBV-transgenic mice

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Introduction: Liver cirrhosis and hepatocellular carcinoma are the most important reasons for morbidity and mortality in hepatitis B infection. Much is known about the immune-mediated destruction of hepatocytes but there are not much data about direct effects of the HBV surface protein on liver cells. The present study investigates the mechanism of age dependent loss of HBsAg-expression as well as the consequent positive effects of the liver of HBV transgenic mice.

Methods: Liver tissue of HBV surface protein transgenic mice (pAlb-PSX [2]) were analysed by Western blots, immohistochemical stainings, serum analyses and RT-PCR.

Results: At 4 moth mice start loosing the expression of the HBsAg in the liver. Some animals show HBsAg-free areas, others only express the surface protein in a few hepatocytes. There is a correlation between the loss of HBsAg-expression and ameliorated liver serum parameters. In addition the lower HBsAg-expression correlates with less ER-stress as shown in Western blots with ER-stress markers. We demonstrated that the mice close down the surface protein expression on a transcriptional level. The later is regulated by genome-methylations. The amount of the HBsAg-expression correlates negatively with the amount of methylations in the mouse genome.

Discussion/Conclusion:
1. The HBV transgenic mice loose the surface protein expression on a transcriptional level, regulated by genome-methylations.
2. The spontaneous loss of HBsAg-expression has a protective effect.
3. It is necessary to find out if this effect also appears in human hepatocytes.
4. A therapeutic applicability of the effect has to be evaluated.

References:
Sperm protein 17 and AKAP-associated sperm protein cancer-testis antigens are expressed in ciliated hepatic foregut cysts

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Introduction: Ciliated hepatic foregut cyst (CHFC) represents a rare type of cyst lined by a layer of ciliated columnar cells and containing mucoid material and debris. CHFCs are retained benign lesions of the liver. However, a case of squamous cell metaplasia and five cases of squamous cell carcinoma arising from a CHFCs have been described. We aimed to investigate the expression of the cancer-testis antigens Sperm protein 17 (Sp17) and AKAP-associated sperm protein (ASP) in four surgically resected CHFCs.

Methods: Formalin-fixed and paraffin-embedded CHFC specimens were taken from two patients who went to the Medical College of Wisconsin, Milwaukee, USA and two patients who went to the Fundación Jiménez Díaz, Madrid, Spain. Two-micrometers thick sections were processed for immunohistochemistry.

Results: CHFCs were found immunopositive for Sp17 and ASP. Both proteins were localized to the cytoplasm of ciliated cells lining the cysts, and in their cilia. Confocal microscopy demonstrates that both Sp17 and ASP overlap in the same region of the cell. No immunoreactivity was recognized at the level of sub-epithelial connective tissue, smooth muscle layer, or of the outer fibrous capsule.

Conclusions: Sp17 and ASP cancer-testis antigens were found in ciliated cells of four surgically resected CHFCs. The expression of both Sp17 and ASP in CHFCs might suggest that these two proteins play a role in the development, growth and progression of these cysts. Further characterization of Sp17 and ASP in CHFCs may provide significant clues for understanding the molecular mechanisms underlying their predisposition to develop squamous cell carcinomas.
Identification of a new TCAB1 mutation in hepatitis C-induced liver cirrhosis

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Introduction: Telomere dysfunction due telomerase mutations has been associated with liver cirrhosis development and hepatocarcinogenesis. Telomerase Cajal body protein 1 (TCAB1) missense mutations have recently been shown to cause dyskeratosis congenita by the disruption of telomerase trafficking. Whether TCAB1 mutations are associated with cirrhosis formation is currently unknown.

Methods: The TCAB1 gene at the WRAP53 gene location on chromosome 17 was sequenced in 48 patients with cirrhosis induced by hepatitis C, 48 patients with cirrhosis induced by alcohol and 48 non-cirrhotic controls. Patients with nucleotide variants within the TCAB1 gene were screened for demographic and clinical characteristics, such as progression of disease, overall survival or tumorigenesis.

Results: We detected 9 different nucleotide variants within the TCAB1 gene, including a newly identified c.663G>A p.V221V mutation in the group of hepatitis C-induced cirrhotics (allele frequency 0.010). Furthermore, the group of homozygote mutation carriers in the alcohol-induced cirrhosis group was found to significantly correlate with a more rapid progression of liver cirrhosis (p value 0.0139). Survival data of this group showed a significant correlation between the presence of homozygote TCAB1 nucleotide variants and liver failure (p-value 0.0352).

Discussion/Conclusion: Taken together, these data provide the first experimental evidence that TCAB1 gene mutations are present in a subset of liver cirrhosis patients. Furthermore, we were able to show that TCAB1 mutations lead to an accelerated cirrhosis formation in chronic liver disease and a decreased survival of affected patients. These data provide a potential starting point for the development of new therapeutic strategies for the treatment or prevention of liver cirrhosis.
Screening for hepatitis B in inflammatory bowel disease patients: Are recommendations already applied?

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Introduction: Screening for HBV infection in patients with inflammatory bowel disease (IBD) is already recommended by international guidelines because of virus reactivation risk under immunosuppressive therapy. The goal of this study was to assess the application of HBV screening in IBD patients and to describe management of those having positive HBV serologic markers.

Methods: This single centre retrospective and descriptive study included 250 IBD patients, recruited in our department between 2010 and 2014. Patients who were screened for the following HBV infection were included (hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb) and HBcAb).

Results: HBV serologic markers were assessed in 168 patients IBD among the 250 included patients. Positivity of at least one marker was noted in 8.9 % of them (HBsAg 0.06%, anti-HBc 6.5%). Effective vaccination (anti-HBs, without anti-HBc) was present in only 2 patients, younger than 20 years; resolved HVB infection was present in one patient. Eight of our patients were inactive carriers of HVB; among whom 4 patients were under immunosuppressive drugs and none of them presented viral reactivation. We diagnosed 2 patients with active HBV infection. Both of them were undergoing an immunosuppressive treatment and were receiving antiviral therapy (lamivudine and entecavir).

Discussion/Conclusion: This descriptive retrospective study shows that international recommendations for HVB screening in IBD is already applied as well as pre emptive antiviral treatment for those receiving immunosuppressant treatment but not systematic vaccination.
H. pylori eradication (sequential) therapy influence on motor function of the stomach in patients with overlap-syndrome, functional dyspepsia combined with non-erosive reflux disease

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Introduction: Functional dyspepsia (FD) combined with non-erosive reflux disease (NERD) known as overlap-syndrome (FD/NERD) is one of the most common disease of upper digestive tract, which has a variety of options related to the gastroesophageal reflux (GER), presence of Hp infection, stomach motility dysfunction and the psycho-emotional condition.

The purpose of the study was to determine the effect of anti H. pylori (sequential) therapy on motor function of the stomach in patients with overlap-syndrome (FD/NERD).

Methods: The study material was the results of clinical examination and treatment of 139 Hp positive patients with FD/NERD.

Rome III criteria were used to diagnose the functional dyspepsia and Whistler consensus – for non-erosive reflux disease.

There were done laboratory, endoscopy and morphological examination, abdominal ultrasound, 13C octanoic acid breath test, stomach and esophagus 24-hour pH monitoring and impedancemetry for each patient. All of them filled short form Leeds dyspepsia questionnaire (SF-LDQ). The urea breath test and fecal antigen test were used to determine H. pylori. The patients with any organic or concomitant diseases were excluded from the study.

Results: Successfully sequential eradication therapy significantly reduces clinical manifestation of overlap-syndrome (FD/NERD) in Hp positive patients. However, determining the motor function of the stomach, there were shown that sequential therapy actually has no significant effect on it. Gastric emptying coefficient and the elimination half-life of solid food from the stomach before treatment was 2.87 ± 0.17 and 151.57 min ± 6.55 respectively, and after completion of anti Hp therapy – 2.35 ± 0.19 (p > 0.05) and 159.6 min ± 7.2 (p > 0.05), respectively.

Successful eradication of sequential therapy was observed in 95% of cases.
Discussion/Conclusion: There were no significant differences comparing motor-evacuation function of the stomach in Hp positive patients with FD/NERD before and after sequential eradication therapy. However, clinical symptoms of FD and NERD greatly reduced, according to SF-LDQ. So motility dysfunction isn't the principal reason for clinical manifestation of upper functional disorders, such as functional dyspepsia and non-erosive reflux disease. Sequential therapy has no effect on the motor-evacuation function of the stomach, namely on 13C octanoic acid breath test indicators (gastric emptying coefficient and the elimination half-life of solid food from the stomach).
The relationship between acid gastroesophageal reflux and depression level in Hp negative patients with overlap-syndrome (functional dyspepsia/non-erosive reflux disease)

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Introduction: In many countries gastroenterological diseases have a steady trend to increasing prevalence of functional disorders. Leading position among these pathologies takes functional dyspepsia (FD), which can be combined with other disorders – non-erosive reflux disease (NERD), known as overlap-syndrome. The purpose of this study is to identify the relationship between acid gastroesophageal reflux (GER) and the level of depression in Hp negative patients with overlap-syndrome.

Methods: The study materials were the results of treatment and clinical examination of 136 Hp negative patients with FD/NERD. Patients were divided depending on the presence (AET+SI+) and absence (AET-SI+) of pathological acid GER. For the validity, the results were compared with the control group (healthy volunteers) parameters. Rome III criteria were used to diagnose the functional dyspepsia and Whistler consensus – for non-erosive reflux disease.

There were done laboratory, endoscopy and morphological examination, abdominal ultrasound, 13C octanoic acid breath test, stomach and esophagus 24-hour pH monitoring and impedancemetry for each patient. All of them filled dyspepsia questionnaire (SF-LDQ’) and anxiety/depression questionnaire (HADS’’). The patients with non-acid GER and any organic or concomitant diseases were excluded from the study.

Results: Depending on the presence or absence of pathological acid GER, in our study was found that the average depression scale by HADS in patients with FD/NERD & AET-SI+ was 10.43 ± 0.79 (clinical expressed depression), which is significantly higher than in the group of patients with AET+SI+ (p < 0.05) and the control group (p < 0.05). In the group of patients with FD/NERD & AET+SI+ average depression scale by HADS was 6.53 ± 0.97, which corresponded to the normal parameters and not significantly differed from the control group (p > 0.05).
Discussion/Conclusion: Depression level in Hp negative patients with overlap-syndrome (FD/NERD) depends on the type of GER (physiological or pathological). Hp negative patients with FD/NERD & AET-SI+ have expressed visceral hypersensitivity and significantly higher depression level than the patients with FD/NERD & AET+SI+.

* AET+SI+ Acid exposure time is pathology and symptoms coincide with episodes of GER
** AET-SI+ Acid exposure time is normal and symptoms coincide with episodes of GER
‘ SF-LDQ – Short form Leeds dyspepsia questionnaire
” HADS – Hospital anxiety and depression scale
A diagnostic algorithm of overlap-syndrome’s (functional dyspepsia/non-erosive reflux disease) different variants

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Introduction: The terminological similarity between functional dyspepsia (FD) and non-erosive reflux disease (NERD), as well as clinical similarity (sense of discomfort, epigastric pain after eating and etc.) and instrumental data (no damage of esophageal and stomach mucosa according to routine endoscopy) are not only make difficulties to diagnose them in clinical practice, but also complicate the evaluation and interpretation of the results in clinical studies, examining these diseases. The purpose of our study was to develop an algorithm for the diagnosis of overlap-syndrome’s (FD/NERD) various options, depending on gastroesophageal reflux (GER) and H. pylori infection.

Methods: The study material was the 175 patients’ clinical findings with the diagnosis of FD/NERD. There were highlighted clinical variants of overlap-syndrome: Hp positive and Hp negative options and types of GER, which were determined on esophagus 24-hour impedance- pH monitoring – when patients’ clinical symptoms been associated with pathological acid GER (AET+SI+*) or with physiological acid GER (AET-SI+**). Rome III criteria were used to diagnose the functional dyspepsia and Whistler consensus – for non-erosive reflux disease. There were done laboratory, endoscopy and morphological examination, abdominal ultrasound, 13C octanoic acid breath test, stomach and esophagus 24-hour pH monitoring and impedancemetry for each patient. The urea breath test and fecal antigen test were used to determine Hp infection. The patients with non-acid GER and any organic or concomitant diseases were excluded from the study.

Results: Our study results allow us to propose a diagnostic algorithm for overlap-syndrome’s (FD/NERD) various options.
Clinical manifestation according to: Rome criteria III - FD
Montreal consensus - GERD

FD/GERD ?

General examination + Abdominal Ultrasound + upper endoscopy with biopsy

- Presence of organic pathology
  - Appropriate treatment
- Absence of organic pathology
  - Esophagus pH-monitoring
  - Test on Hp

FD/NERD !

- AET+ SI+
- AET- SI+
- AET+ SI+ Hp+
- AET+ SI+ Hp-
- AET-SI+ Hp+
- AET-SI+ Hp-

Discussion/Conclusion: So we have 4 various groups of patients with overlap syndrome based on GER type and H. pylori infection. In the future, we will continue complete the patients’ treatment methods for different variants of overlap-syndrome (FD/NERD) on the base of our study results.

*AET+SI+ Acid exposure time is pathology and symptoms coincide with episodes of gastroesophageal reflux

**AET-SI+ Acid exposure time is normal and symptoms coincide with episodes of gastroesophageal reflux
The interaction of hepatitis B virus infection and Schistosomiasis in chronic pathogen-induced liver inflammation

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Introduction: One of the main complications of acute Hepatitis B virus infection (HBV) is the development of a chronic infection. Interestingly, chronicity develops at a higher frequency in developing countries in which co-infections with several helminth species, such as S. mansoni, are common. The underlying mechanisms leading to HBV chronicity are only partly understood but compromise CD8⁺ and CD4⁺ T cell responses, which might be affected in helminth co-infected patients due to dynamic schistosome-driven immune responses. These range from an initial Th1- to Th2-immunity and eventually to long-term immunosuppression. Thus, the effects of pre-existing helminth infections on concomitant HBV infection are hard to predict and need clarification.

Methods: In our studies we investigated the impact of acute schistosomiasis on the outcome of an additionally acquired, acute or pre-existing, chronic HBV infection in mice by monitoring serum viral antigen levels, viral load and virus-specific T cell responses within liver-associated lymphocytes and splenocytes. Liver pathology was assessed by H&E- and immunohistological stainings for viral antigens. Schistosome infection assessment included parasite egg counts in stool and liver, Masson’s stainings of liver sections and measurement of helminth-specific immune responses upon antigen-specific stimulation of splenocytes and mesenteric lymph nodes.

Results: Acute HBV infection acquired during the Th1 phase of schistosomiasis was suppressed in co-infected animals when compared to HBV mono-infected mice, since Hepatitis B surface antigen and Hepatitis B early antigen were significantly reduced in co-infected mice. Additionally, less HBsAg- and Hepatitis B core antigen-positive hepatocytes were detected in liver sections in co-infected animals, indicating that viral elimination occurs at a very early timepoint. Furthermore, Treg frequency was elevated within liver-associated-lymphocytes of co-infected mice. Simultaneously, frequencies of IFN-γ producing CD8⁺ cells within the liver were significantly increased in these co-infected mice. To determine whether liver disease in chronic HBV infection is altered by a concomitant helminth infection, HBV-transgenic mice were infected with S. mansoni. Here, HBV replication in terms of viral antigen levels were again significantly decreased in co-infected mice with higher frequencies of CD8⁺ IFN-γ⁺ T cells in LALs and splenocytes.

Discussion/Conclusion: Taken together, our data indicate a causal relationship between schistosome-induced IFN-γ production within the liver, the induction of antiviral T cells and clearance of HB Virus.
Non invasive serum fibrosis markers in comparison with grading and staging in chronic hepatitis B

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Introduction: Chronic hepatitis is defined as a necroinflammatory disease of the liver continuing for at least six months. The aim of this study was to evaluate the role of noninvasive fibrosis markers by assessing their correlation with histological grading and staging of the disease in patients with chronic hepatitis B.

Methods: We retrospectively studied data of 145 patients with chronic hepatitis B. Routine biochemical indices and serum fibrosis indexes such as aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR), AST to platelet ratio index (APRI) and Fibrosis 4 score (FIB-4) were determined, and the histological grade and stage of the liver biopsy specimens were scored according to the Metavir scoring system. Receiver operating characteristic curve (ROC) analysis was conducted to compare diagnostic accuracies of these markers for prediction of significant fibrosis.

Results: We identified 62 liver biopsies from chronic hepatitis B patients with contemporaneous laboratory values for imputing AAR, APRI and FIB-4. From all, 38 males (61%) and 24 females (39%), with the mean age of 39 years were studied. FIB-4, APRI and AAR were significantly correlated with the stage of fibrosis. FIB-4 (AUROC = 0.84) and APRI (AUROC = 0.78) was superior to AAR at distinguishing severe fibrosis from mild-to-moderate fibrosis and gave the highest diagnostic accuracy.

Discussion/Conclusion: Application of these markers was good for distinguishing significant fibrosis which can lead to a decrease for the need for staging liver biopsy specimens among patients with chronic hepatitis.
Previous interferon therapy does not lead to a better virological response in patients treated with entecavir

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Introduction: Entecavir (ETV) is a potent inhibitor of HBV replication. The aim of the study was to explore if previous interferon (IFN) therapy might influence response to ETV in chronic hepatitis B.

Methods: Monocentric retrospective study was performed, including all subjects who received ETV for chronic hepatitis B. We assessed viral and biochemical response. Comparison of categorical data was performed by c2-test or Fisher’s exact were applicable.

Results: Forty seven patients were followed for a median period of 24 months. The study population was 70% male, 100% HBeAg-negative, 32% IFN-pretreated, 8% lamivudine-pretreated, 51% cirrhotics. At baseline, the median hepatitis B virus DNA was 5.95 (interquartile range = 1.08–9.97) log10 IU/ml. At week 48, 80% of the patients achieved a virological response and 91% of those with elevated baseline alanine aminotransferase showed a biochemical response. Positive predictive factors for virologic response are: low score of fibrosis ($p < 0.05$), low level of HBV DNA ($p < 0.05$). Baseline level of ALT, age, sex, previous lamivudine or IFN therapy had no impact on virologic response. Virological breakthrough was found in 4% of patients.

Discussion/Conclusion: ETV maintained and even increased the high initial response rate. Low score of fibrosis, low level of HBV DNA, HBe antigen negative status, predict a good virologic response. Lamivudine and IFN-resistant patients usually respond well to ETV.
The inactive HBV-carrier profile: the long-term outcome

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Introduction: Inactive HBV profile is one of the aspects of natural history of chronic hepatitis B (HVB). We aimed to define epidemiological, clinical and virological features of inactive HVB-carriers and to evaluate their long-term outcomes.

Methods: This monocentric and descriptive study included 145 chronic HVB-carriers – over 10 years old –, followed since 2004. The inactive HBV profile was defined by normal serum aminotransferases, HBeAg-negative state and DNA levels < 2000 IU/ml. We excluded patients with HIV and/or HCV co-infection and alcohol consumers. HBV-reactivation was defined by an HVB DNA load up to 2000 IU/ml. All clinical, biological and serological data were collected. Serum HBV DNA levels were measured using real-time PCR quantification assays. Liver biopsy was indicated according to international guidelines.

Results: On 145 considered patients, the inactive HVB-carriers represented 52% (n = 76). Mean age was 41.8 years old [18–71], male gender was prominent (sex-ratio = 1.2). Mean time of follow-up was 5 years [1–12]. The most probable ways of HVB transmission were unprotected sexual practices and unsafe tooth-care (respectively 57% and 34.5%). Ultrasoundography found a heterogeneous hepatic parenchyma in 12% and steatosis in 4%. HVB-reactivation was reported in 5 patients (6.5%) which indicates antiviral treatment. Spontaneous HBsAg loss was achieved in 2 patients. No case of hepatocellular carcinoma was reported.

Discussion/Conclusion: Our “real-life” experience confirms that the inactive HVB profile is associated to less complications and better long-term outcome
Sustained virological response is low in diabetic Tunisian chronic hepatitis C patients

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Introduction: Diabetes mellitus (DM) is common in chronic hepatitis C (CHC) patients and reduces the therapeutic effectiveness of pegylated interferon (peg IFN), ribavirin therapy.

Aim: To study the effect of DM on the sustained virological response (SVR) after peg IFN, ribavirin in Tunisian CHC patients and the incidence of glucose abnormalities developed in non diabetic patients and its relation to the SVR.

Methods: One hundred sixty one Tunisian CHC patients were divided into Group (1): 75 diabetic patients, and Group (2): 86 non-diabetic patients. All received peg IFN and ribavirin for 48 weeks and monitored for SVR and glucose abnormalities.

Results: SVR of the study group was 68.3% (64% for diabetics and 72% for non-diabetics, p < 0.05). On multivariate analysis, diabetic patients were significantly older (p 0.008), had higher BMI (p < 0.05) and steatosis (p < 0.05). Low SVR was associated with higher fasting blood sugar (p < 0.05), steatosis (p < 0.05), grade 3 fibrosis (p < 0.05) and high viral load (p < 0.005).

Discussion/Conclusion: DM significantly decreases the SVR in Tunisian CHC patients (40.5% in diabetic and 59.3% in non diabetic patients).
The anti-TNF-α antibody infliximab inhibits the expression of fat-transporter-protein FAT/CD36 in a selective hepatic-radiation mouse model

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Background: Previously, we reported a radiation-induced inflammatory response triggering fat accumulation through fat-transporter-protein FAT/CD36 in rat liver. Furthermore, inhibition of radiation-induced FAT/CD36 expression by anti-TNF-α (infliximab) was shown in vitro. The current study investigates hepatic fat accumulation and fatty acid transportation in a mouse model of single-dose liver-irradiation (25-Gy) and the effect of anti-TNF-α therapy on gene expression of FAT/CD36 in vivo.

Methods: Mice livers were selectively irradiated (25-Gy) in vivo in the presence or absence of infliximab (IFX). Serum- and hepatic triglycerides (TG), mRNA, and protein were analyzed by colorimetric assays, RT-PCR, and Western-Blot, respectively. Sudan staining was used to demonstrate fat accumulation in liver tissue.

Results: In mice livers, early (1–3 h) induction of TNF-α expression, a pro-inflammatory cytokine, was observed after irradiation. It was followed by elevated hepatic TG levels (6–12 h), compared to sham-irradiated controls. In contrast, serum TG levels of the same mice were decreased at these time points. Similar to TG levels in mice livers, Sudan staining of liver cryosections showed a quick (3–6 h) increase of fat-droplets after irradiation. Furthermore, expression of the fat-transporter-protein FAT/CD36 was increased at protein level caused by radiation-induced TNF-α. TNF-α-blockage by anti-TNF-α prevented the increase of FAT/CD36 in mice livers. Immunohistochemistry showed basolateral and cytoplasmic expression of FAT/CD36 in hepatocytes. Moreover, FAT/CD36 was detected in CK-19+-billary cells, SMA+-myofibroblasts and F4/80+-macrophages.

Conclusion: In summary, selective liver radiation triggers fat accumulation in mice livers, involving acute-phase processes. Accordingly, anti-TNF-α therapy inhibited radiation-induced expression of FAT/CD36 in vivo.
Peroxisome proliferator activated receptor gamma agonists are less effective in chronic hepatitis C (CHC) compared to non-alcoholic steatohepatitis (NASH)

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Introduction: Peroxisome Proliferator Activated Receptor Gamma – NR1C3 (PPARG) plays an important role in various biological processes including lipid and glucose metabolism. Previous studies showed that PPARG agonists have been used in treatment of different metabolic disorders related to hepatic injury decreasing steatosis, inflammation, and fibrosis, but reports related to CHC are contradictory. The aim of the study was to find out the perspectives for metabolic therapy of CHC with thiazolidinediones.

Methods: Thirty-nine patients with hypertension, dyslipidemia, metabolic syndrome participated in the study. Study group formed by 21 CHC patients and 18 NASH patients formed control group. PPARG agonist Pioglitazone administered 15–30 mg daily during 50–51 weeks in both groups. Insulin resistance, lipid metabolism, inflammation markers, liver function and hemostasis markers as well as liver biopsies and all routine tests including HCV RNA titration (CHC group) performed prior and after study.

Results: Pioglitazone improved glycemic control and glucose tolerance in both groups (P < .001), normalized liver aminotransferase levels, decreased hepatic fat and increased hepatic insulin sensitivity. Administration of pioglitazone caused improvement in histologic findings with regard to steatosis, ballooning necrosis, and inflammation in both groups. Expression in fibrosis did not change in CHC group. While positive influence on metabolic and hemostatic aspects was observed, no significant influence on HCV RNA titer (P = .109) observed in study group. Significant individual differences of response were marked in both groups. Adverse effects included two cases of edema and one case of anemia in study group.

Discussion/Conclusion: Administration of thiazolidinediones leads to metabolic and histologic improvement in most patients with both CHC and NASH. However, thiazolidinediones cause almost no influence on HCV infection itself.
Transcriptional phenotype of virus-specific CD4+ and CD8+ T-cells during acute-resolving and chronic hepatitis B virus infection

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Introduction: Growing evidence suggests a critical role of transcription factors for effector and memory T-cell differentiation and the generation of protective T-cell responses. Gene expression profiling studies have revealed, that exhausted T-cells develop a transcriptional state distinct from that of functional effector and memory CD8+ T-cells. A few transcription factors have been found to be functionally important in this process, including Blimp-1, T-bet and Eomes. Recently, we were able to show, that dysfunctional CD8+ T-cells in HBV and HCV are characterized by a lack of antigen-specific T-bet induction and additionally found that restoration of IFN-γ release is achieved by IL-12 stimulation.

Methods: In this study, we have extended the phenotypic and functional analysis of T-bet and Eomes co-expressing virus-specific CD8+ and CD4+ T-cells in 10 acutely infected HBV patients and 10 chronically infected subjects. HLA-A*0201- and DRB1*0101-restricted MHC class I and II Tetramers were used for detailed phenotyping of HBV-specific T-cells. PBSE-based proliferation assay and intracellular cytokine assay were used to characterize CD8+ T-cell function.

Results: Our analysis reveal several interesting insights: (1) Tetramer-based analysis of HBV-specific CD8+ and CD4+ T-cells co-expressing T-bet and Eomes demonstrate distinct phenotypical signatures in acute-resolving versus chronic HBV infection. (2) In contrast to T-bet single positive CD8+ T-cells co-expression of T-bet and Eomes is strongly associated with vigorous antiviral effector function characterized by increased IFN-γ and Perforin release. (3) On contrary, CD8+ T-cells lacking both molecules virtually lose their ability to produce cytokines and to proliferate.

Conclusion: Taken together our results suggest that T-bet and Eomes expressing T-cells represent a unique and highly functional T-cell subset, which induction will reflect a promising approach for further therapeutic interventions in chronic persisting HBV infection.
**Specific and non-hepatotoxic degradation of nuclear hepatitis B virus cccDNA by lymphotoxin-beta receptor activation**

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**Introduction:** Current antiviral agents can control but not eliminate hepatitis B virus (HBV), because HBV establishes a stable nuclear covalently closed circular DNA (cccDNA). Interferon-α treatment can clear HBV but is limited by systemic side effects.

**Methods:** Using HBV-infected, differentiated HepaRG cells, primary human hepatocytes and HBV1.3 transgenic mice we investigated the effects of host cell specific lymphotoxin-beta receptor (LTβR) signaling on HBV infection in vitro and in vivo. By Affymetrix array analyses, qRT-PCR and Western blot we found that LTβR signaling lead to an up-regulation of the cytidine deaminase APOBEC3B which mediated deamination, apurinic/apyrimidinic site formation, and finally degradation of cccDNA. Hence, APOBEC3B actively prevented HBV reactivation. (Lucifora, Xia et al. 2014)

**Results:** Looking for therapeutic alternatives for HBV infection we found that by activation of the LTβR we can induce specific degradation of the nuclear viral DNA without hepatotoxicity. LTβR signaling up-regulated and stabilized the expression of the cytidine deaminase APOBEC3B in HBV-infected differentiated HepaRG cells and primary hepatocytes resulting in cytidine deamination, apurinic/apyrimidinic site formation, and finally degradation of cccDNA. Hence, APOBEC3B actively prevented HBV reactivation. (Lucifora, Xia et al. 2014)

**Discussion/Conclusion:** The exact molecular mechanisms, how short-term LTβR activation leads to a long-lasting and stable up-regulation of APOBEC3B, its catalytic activity and finally to cccDNA degradation without affecting genomic DNA are currently under investigation. Here I will present data that reveal some of the mechanistic underpinnings of APOBEC3B regulation and function.

Targeting active demethylation; a new possible mechanism in treatment of hepatocellular carcinoma

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Introduction: Global deregulation of methylation status is one of the crucial causes of hepatocellular carcinoma (HCC). It has been reported that the anti-cancer drug 5-Azacytidine (5-Aza) mediates the activation of tumor suppressor genes through passive demethylation by inhibiting DNMT1. Our aim was to ascertain if 5-Aza also induces active demethylation by increasing the expression of 5hmC, which is considerably depleted in various types of cancer, may lead to new approaches in cancer therapy.

Methods: HCC cells (Huh-7, HLE, HLF) and tissue sections from healthy and HCC patient cohorts (55 patients) were studied by immunofluorescence (IF) staining and immunohistochemistry staining for 5hmC and 5mC, and via Real-time PCR for TETs. HCC cells were stimulated with different concentrations of 5-Aza (0-20 µM). Viability and toxicity were measured after 24 and 48 hours via Resazurin conversion and LDH release. The expression of TETs, 5hmC-5mC, and PCNA (as a proliferation marker) were measured via Real-time PCR, IF staining, and Western blotting, respectively.

Results: The expression of 5hmC was lower in HCC tissue than in healthy tissue samples. This finding was confirmed by comparing HCC cells to hHeps. The expression of TET2 and TET3 was lower in HCCs than in non-tumor liver tissues and hHeps. 5-Aza inhibited the proliferation of HCC without causing any significant LDH release. Moreover, it increased the expression of TET2 and TET3 at m-RNA level, as well as of 5hmC after 48 hours.

Discussion/Conclusion: Our data exhibit a decrease of 5hmC and an increase of 5mC in HCCs through down-regulation of the TETs. However, the expression levels of 5hmC and the TETs can be re-induced by 5-Aza through active demethylation, which is a novel function of the drug. We suggest that the loss of the expression of 5hmC is an additional marker for HCC diagnosis.
Hepatoprotective role of Nerium oleander in thioacetamide induced chronic inflammation

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Introduction: Many plant extracts and their bioactive substances are well recognized for their potential chemoprotective role in the treatment of liver injury induced by viruses, chemicals and drugs. This study is an attempt to evaluate the hepatoprotective potential of Nerium oleander leaves extract against thioacetamide (TAA) induced liver damage.

Methods: Fifteen Wistar rats of either sex were randomly divided into three groups. Group I is provided with normal drinking water while group II and III were orally given TAA (200 mg/l) in drinking water for 18 weeks. Additionally Group III was treated with Nerium oleander leaves extract for seven days. Liver damage was assessed by hematological, biochemical parameters and histopathological observations. Data were analyzed by using one way ANOVA test with Tukey’s post hoc test and Prussian blue staining.

Results: Significant variations were observed in TLC, hematocrit, hemoglobin, and MCHC with $P = 0.009$, $P = 0.0001$, $P = 0.01$ and $P = 0.03$, respectively. Serological analysis also showed significant variations in total lipids, triglycerides, H.D.L., L.D.L, & BSR with $P = 0.0001$, $P = < 0.0001$, $P = 0.02$, $P = 0.002$ and $P = 0.03$ respectively. Blue stained hemosiderin granules were observed in the peripheral area of hepatic lobules and mainly in Kupffer cells in group II while lesser degree of iron deposition was observed in group III. Meanwhile, there was no noticeable hemosiderin granule in the group I.

Discussion/Conclusion: These finding indicate that TAA disturbs hematological, serological and histological aspects whereas Nerium oleander leaves extract has potential activity against TAA-induced liver damage.
A simple and rapid plate-based LC-MS/MS assay for the analysis of serum 25-hydroxyvitamin D levels in patients with chronic liver diseases

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Introduction: Vitamin D deficiency occurs frequently in patients with chronic liver disease (CLD) and is inversely related to CLD progression. We previously showed vitamin D deficiency to be associated with increased mortality in CLD patients. Currently the influential role of other vitamin D metabolites remains unknown. We compared serum 25-hydroxyvitamin D (25(OH)D) concentrations in patients with CLD, as assessed by standard clinical immunoassay with a rapid Liquid Chromatography-Mass Spectrometry (LC-MS/MS)-based method, with potential for rapid quantification of all vitamin D metabolites.

Methods: Serum samples were obtained from a subset of patients (n = 14, 43% men, age 41–74 years) belonging to a larger cohort with CLD of various viral and non-viral etiologies. Serum 25(OH)D levels were assessed at baseline with a routine clinical immunoassay (Liaison chemiluminescent immunoassay, DiaSorin). The results were compared with the LC-MS/MS micro-extraction plate assay, in which commercial 96-well micro-extraction plates (AC Extraction Plate, Tecan) were combined with 25(OH)D isotope calibration and quality control kits (Recipe). Patients received vitamin D (20,000 IU cholecalciferol/week) for six months. Serum 25(OH)D levels were monitored at 3, 6 and 12 months with both methods.

Results: Both assays accurately captured the time-dependent changes of 25(OH)D levels during the course of vitamin D substitution, yielding very similar area-under-the-curve values. The results of the LC-MS/MS assay correlated well with the chemiluminescent immunoassay, with no significant bias (r2 = 0.76). None of the samples exceeded the upper limit of the calibration range for vitamin D. The mean 25(OH)D concentration levels measured by LC-MS/MS and the immunoassay were 26.7 and 25.9 ng/mL, respectively, corresponding to an overall delta of 3.1% between assays.

Discussion/Conclusion: The high correlation between the two assays supports use of the simple LC-MS/MS assay that can be rapidly established in non-specialized laboratories, without laborious sample preparation. Importantly, the method can be readily extended to assess downstream vitamin D metabolites such as 1.25(OH)2D or 24.25(OH)2D in patients with CLD.
Is systemic inflammation, liver cirrhosis and cancerogenesis in chronic hepatitis C patients associated with T894G polymorphism of endothelial nitric oxide synthase gene and vascular injury?

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Introduction: Chronic hepatitis C (CHC) is considered the major cause of hepatic cancerogenesis. Whilst the role of proangiogenic and proinflammatory factors is well established in hepatic cancerogenesis, the association of T894G polymorphism of endothelial nitric oxide synthase (eNOS) gene with cytokines, cardiovascular injury in patients with hepatic carcinoma due to CHC is unclear.

Methods: Study group included 9 patients with liver malignancies due to CHC cirrhosis (4 female, 5 male, mean age 67.2 ± 7.31); control group included 12 healthy volunteers. All patients had vascular injury in a form of concomitant Arterial Hypertension (AH) and Chronic Heart Failure (CHF). IL-4, TNF-α, TGF-β1, pro-Atrial Natriuretic Peptide (proANP) plasma concentrations were defined in ELISA; eNOS (T894G) gene polymorphism was assessed with PCR.

Results: Difference in genotypes distribution groups was not significant. Presence of T-allele in patients with liver cirrhosis was associated with increase of AST activity (27.4%, p < .05), urea concentration (33.3%, p < .05), creatinine (22.2%, p < .05) compared to GG-carriers. In T-allele carriers concentration of proANP was higher (89.2%, p < .001), than in patients with GG-genotype. IL-4, TNF-α and TGF-β1 did not differ reliably between genotypes, but TNF-α significantly higher in research patients (p < .001). In research group males T-allele also combined with the increased LVMI (by 12.2%, p < .05) compared to GG-genotype patients.

Discussion/Conclusion: eNOS is connected with hepatic circulation via changes of endothelial and metabolic functions, inflammation reflects widespread vascular damage as proved by cardiovascular changes. We hypothesize that under Hep C infection and cirrhosis oxidation, systemic inflammatory reaction and unregulated cellular proliferation depends on eNOS gene expression. T-allele associates with cytolysis, indirect fibrotic liver changes, cardiovascular failure progression (proANP) and may be a risk factor for liver cancer in CHC patients.
Therapeutic (pro-metabolic) influence on peroxisome proliferator activated receptor gamma in chronic hepatitis C (CHC) and nonalcoholic steatohepatitis (NASH) patients is determined by PPAR-G Pro12Ala gene polymorphism

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Introduction: Multiple studies showed that peroxisome proliferator activated receptor gamma – NR1C3 (PPARG) plays an important role in various biological processes including lipid and glucose metabolism. PPARG agonists have been used in treatment of different metabolic disorders decreasing steatosis, inflammation, and fibrosis. The aim of the study was to clarify the perspectives for individualized metabolic therapy of CHC and NASH with thiazolidinediones.

Methods: 53 patients with hypertension, dyslipidemia, metabolic syndrome participated in the study. Study group formed by 15 CHC patients and 38 NASH patients were selected to form control group. PPARG agonist Pioglitazone administered 30 mg daily during 50–51 weeks in both groups. Genetic polymorphism (Pro12, Pro12Ala, Ala12Ala) of PPARG gene determined by PCR. Genotypes were: Pro12 (n = 34, 64.2%); Pro12Ala (n = 15, 28.3%); Ala12 (n = 4, 7.6%) Liver biopsies and all tests performed prior and after study.

Results: Pioglitazone improved glycemic control and glucose tolerance (P < .001), normalized liver aminotransferase levels as it decreased AST by 40.2 ± 1.39%, P = .015; ALT by 53.8 ± 1.63%, P < .001; decreased hepatic fat by 51.4 ± 3.01%, P < .001; and increased hepatic insulin sensitivity by 52.1 ± 2.14% P < .001. Administration of pioglitazone caused improvement in histologic findings with regard to steatosis, ballooning necrosis, and inflammation in both groups. In 4 (7.6%) Ala12 patients no reliable changes were observed, except glycemic control and glucose tolerance. Reduction in fibrosis did not change in CHC group. Statistically insignificant weight gain and mild lower-extremity edema developed in 2 subjects (NASH group) with Pro12Ala genotype, no other side effects were observed.

Discussion/Conclusion: Administration of thiazolidinediones leads to metabolic and histologic improvement in most patients with both CHC and NASH. However, individual response may be affected by Pro12Ala polymorphism of PPARG gene. This study shows that carriers of Ala genotype whilst comparatively rare among these patients are much less sensitive to PPARG agonists' therapy.
HCV infection and relationship between gut microflora, antiendotoxin core antibodies and nitric oxide levels

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Introduction: Gut microflora translocation of as well as translocation of endotoxins into the systemic circulation might increase because of impaired intestinal permeability, bacterial overgrowth, or defective clearance of microbial products in hepatocytes under HCV infection. Relationship between gut microflora, antiendotoxin Core antibodies (EndoCAb) and nitric oxide (NO) levels are interesting in relation to hepatic dysfunction, hepatocellular carcinoma and cirrhosis in pathogenesis of chronic hepatitis C (CHC).

Methods: Study included 27 patients with clinically proven CHC (7 [25.9%] with minor fibrosis, 20 [74.1%] with cirrhosis), mean age – 57.03 ± 6.29. Twenty-five practically healthy volunteers of respective age and gender formed control group. EndoCAb assessed by ELISA, NO (nitrite/nitrate) by IEA.

Results: Colonic flora changes dramatically in CHC patients with respect to hepatic dysfunction: significant decrease (P < .05) or elimination of autochthonic anaerobic microorganisms and hyperproliferation of conditionally pathogenic Enterobacteriacea: E. coli, including Hly+ – 9.47 ± 0.58 lg CFU/g against 7.39±0.56 lg CFU/g in control; Klebsiellae – 5.26±0.39 lg CFU/g against 3.48±0.49 lg CFU/g in control, Proteus – 6.35 ± 0.31 lg CFU/g, and Serratia – 4.86 ± 0.61 lg CFU/g (not found in control). EndoCAb changes were not uniform. In patients with cirrhosis, EndoCAb IgM (1.07 ± 0.03 MMU/ml) and IgG (2.49 ± 0.14 GMU/ml) were lower than in control (P < .01). Patients with minimal fibrosis give higher figures of IgM (2.96 ± 0.21 MMU/ml) and IgG (9.57 ± 0.84 GMU/ml). Significant (p < .05) EndoCAb growth was observed only in one (3.7%) patient with minor fibrosis. In 4 (14.8%) cases only IgM increased, while IgG level remained low. NO levels rose reliably (P < .05) in all patients (43.52 ± 2.68 mmol/l vs 34.61 ± 3.07 mmol/l in healthy subjects). Strong correlation (r = -0.76, P < .05) between EndoCAb and NO levels found only in cirrhosis group.

Discussion/Conclusion: Excessive growth of conditionally pathogenic Enterobacteriacea and endotoxin release under CHC is combined with suppressed production of antinuclear anti-endotoxin antibodies. NO hyperproduction aggravate status by means of decreased motility and increased intestinal permeability.
Myrcludex-B inhibits hepatitis delta superinfection and spreading in HBV stably infected humanized mice

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Introduction: Because of the lack of direct anti-HDV agents, therapeutic strategies affecting not only HBV but also HDV infection are urgently needed. We previously demonstrated prevention of de novo HBV/HDV co-infection with the entry inhibitor Myrcludex-B in naïve-humanized mice (Lütgehetmann et al., Hepatology 2012). Aim of this study was to assess whether Myrcludex-B administration could hinder the establishment of HDV infection also in mice already infected with HBV.

Methods: One group of HBV infected mice received Myrcludex-B (2 mg/kg; daily) 2 days before until 5 days after HDV inoculation, while in the second group treatment was prolonged for 5 weeks. Viral loads were quantitated in serum and liver by qRT-PCR and visualized by immunohistochemistry.

Results: All mice receiving Myrcludex-B up to day 5 p.i. became HDV infected, although development of viremia was delayed at week 2 (Δ2Log) and week 4 (Δ1Log) post-HDV inoculation, while viremia levels became comparable to untreated control mice at week 6 post-infection (7x10E7 vs. 4x10E7). In contrast, mice that received Myrcludex-B for 5 weeks showed no serological and intrahepatic markers of HDV infection. Moreover, 5 weeks of Myrcludex-B treatment and establishment of HDV superinfection reduced HBV viremia (46% and 62%, respectively). Of note, after treatment cessation, one mouse showed development of HDV viremia (1x10E4 and 1x10E6 HDV-RNA copies/ml after 3 and 6 weeks), indicating that Myrcludex-B strongly hindered but did not fully abrogate the establishment of HDV infection.

Discussion/Conclusion: In the presence of productive HBV infection, HDV entry was significantly inhibited by Myrcludex-B. However, continuous drug administration was necessary to prevent HDV spreading from the very few initially infected cells. Both the intracellular persistence and the high infection efficiency demonstrated by HDV in vivo underscore the great survival capacities of this virus and explain difficulties encountered by treating HBV/HDV infection in patients.
HCV-therapy reduced not only the liver-related, but the overall mortality too – results in the Leipzig anti-D cohort

Manfred Wiese on behalf of East German HCV Study Group
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Introduction: The long-term natural history of hepatitis C (1b) infection is still not fully understood. So far, there are few long-term studies with a known date of infection, which include all those infected without bias. According to the initial reports it was assumed, that after 20 years, 30% of patients would develop liver cirrhosis. A new issue is to what extent the prognosis after more than three decades was influenced by the anti-HCV therapy.

Methods: Between August 1978 and March 1979 had come to the administration of 14 hepatitis C-contaminated anti-D immunoglobulin batches at 2867 East German women to prevent Rh isoimmunization. These HCV cohort is of particular interest, because there are few cohorts with known HCV infection time that would allow precise statements about the spontaneous course and therapy results. Our data were collected in 15 study centers in East Germany since the beginning. The Leipzig anti D cohort as part of the total cohort includes 356 women, of whom now 181 were followed up after 32 years. 79 women were treated with IFN-based therapies, 48 of them successfully (SVR).

Results: After 32 years 85% of the 181 women in the HCV ELISA were positive. 33% were viremic (HCV PCR positive). Only 11 (8.3%) of viremic women had liver cirrhosis, 7 (5.3%) had advanced fibrosis sufferers. In the last 15 years a continuous, but slow rise of advanced fibrosis score was observed. HCC has not been diagnosed. Since 1978 six HCV RNA-positive women of the Leipzig cohort died; eight women died after viral clearance. The overall mortality in the therapeutic SVR group was lower than in the treatment-naïve group.

Discussion/Conclusion: Young women without comorbidity eliminate HCV (1b) infection spontaneously in approximately half of the infected cases. After 32 years, a continuous but relatively low progression with regard to final states such as cirrhosis, HCC or death could be confirmed in this cohort. Anti-HCV therapy should be recommended because the overall mortality after successful therapy (SVR) was lowest.
Increased hepatic expression level of novel microRNA in ABCB4⁻/⁻ mice

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Introduction: MicroRNA play a crucial role in maintenance of liver homeostasis as well as in the modulation of various pathological processes associated with liver damage. The aim of the present study was to assess microRNA expression pattern in the liver of mouse models for sclerosing cholangitis (ABCB4⁻/⁻), in HBV transgenic mice expressing hepatitis B surface proteins (HBV⁺/⁻) and in a murine hybrid model (HBV⁺/⁻ - ABCB4⁻/⁻).

Methods: At first, the expression of 92 microRNA in liver of 16-week-old HBV⁺/⁻ - ABCB4⁻/⁻ hybrid mice was analyzed using miFinder microarray (Qiagen®). Transgenic mice, knockout mice and corresponding wild type mice were used as control group. After evaluation of the array data, selected MicroRNA levels were verified by qPCR in liver of 8, 16, 26 and 52 weeks old mice.

Results: The array data analysis revealed a more than two-fold upregulation of the following microRNA: miR-34c-5p, 182-5p, miR-214-3p, miR-141-3p, miR-411-5p, miR-27a-3p, miR-125b-5p, miR-199a-5p, miR-199a-3p, miR-96-5p, miR-15b-5p and miR-16-5p. In the subsequent quantitative real-time PCR, these results were confirmed in ABCB4⁻/⁻ mice. However, HBV transgenic mice (Churin 2014) showed no significant change in microRNA expression level.

Discussion/Conclusion: The present study shows that, in addition to previously known miRNA associated with liver damage, several additional miRNA play a role in the context of specific liver injury (ABCB4⁻/⁻) and are altered in their expression. These new findings may help to understand the pathogenesis of cholestatic liver diseases and to develop new therapeutic options.

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