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Liver Diseases in 2013: Advances in Pathogenesis and Treatment

October 4 – 5, 2013
Park Plaza Westminster Bridge
London, Great Britain

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Poster Abstracts
Falk Symposium 191

LIVER DISEASES IN 2013: ADVANCES IN PATHOGENESIS AND TREATMENT

London (Great Britain)  
October 4 – 5, 2013

Scientific Organization:  
R.W. Chapman, Oxford (Great Britain)  
D.H. Adams, Birmingham (Great Britain)  
U. Beuers, Amsterdam (The Netherlands)  
C.P. Day, Newcastle-Upon-Tyne (Great Britain)  
M.P. Manns, Hannover (Germany)
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* = Posters of Distinction
Session I

From bench to bedside in hepatology
The therapeutic potential of stem cells in liver disease

Phil Newsome
Centre for Liver Research, Institute of Biomedical Research, University of Birmingham, UK

Due to the increasing burden of liver disease and the paucity of available treatments, there has been excitement about the possible role that stem, and other cell types, may have in liver disease. This talk will describe the varying contributions that cell/stem cells can have in the various types of liver damage. Demonstrations of efficacy in pre-clinical mouse models have prompted moves to introduce cell therapy into clinical studies, although consideration should be given to tailoring the cell used to the clinical indication. The talk will also describe some of the regulatory hurdles which need to be overcome before starting translational into clinical trials. Notwithstanding that the mechanism of action on many of these cells has not been fully delineated, there have been several clinical studies in a range of different clinical conditions. The talk will summarise the clinical studies to date, as well as covering some of the studies that are currently in progress.
Cellular therapy to modulate autoimmune liver disease

A.W. Lohse
I. Medizinische Klinik und Poliklinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany

Even though the pathogenesis of autoimmune liver diseases is poorly understood, all evidence points toward an immune dysregulation, triggered by unknown events in a genetically susceptible host. Immune dysregulation obviously is only partial, as disease is only very rarely fulminant, but mostly slowly progressive or relapsing and remitting. Cellular therapy should aim at either dampening pro-inflammatory actors, or strengthening anti-inflammatory regulators. One approach is in vitro propagation of regulatory T-cells and their passive transfer. Another approach is using autoreactive antigen-specific T-cell vaccine by prior alternation, for example by irradiation of fixation with glutaraldehyde. More simple solutions could lie in treatment with antibodies to inflammatory cytokines, co-stimulating signals or depleting antigen-presenting B-cells with rituximab (anti-CD20-antibody). As the immune system seems to retain some of its ability for healthy immune regulation even in affected patients, careful tapering of long-term immunosuppression may in itself be a cellular immune therapy for many patients while waiting for more specific interventions in the future.
The inflammasome in liver injury and NAFLD

W.Z. Mehal
Yale School of Medicine, New Haven, CT, USA

Tissue injury in the absence of pathogens has been recognized since ancient times to result in an inflammatory response. This is termed sterile inflammation, and is a response common to all organs. There is a weak adaptive immune response to antigens in the liver, but in contrast the liver possesses a strong inflammatory response. This is seen experimentally and clinically with liver inflammation due to toxic and metabolic stress, sepsis and ischemia [1]. The molecular and cellular mechanistic basis for this clinically important inflammation has been significantly clarified, and requires the interaction of two types of extracellular signals which collectively up-regulate and activate a cytosolic molecular complex termed the inflammasome. The two types of signals can be divided into i) activation of pattern recognition receptors, and ii) a second set which includes stimuli as diverse as particulates and ATP. The common end result of inflammasome activation is the activation of protease caspase-1 with release of active IL-1β.

The importance of the inflammasome machinery has been demonstrated in a wide range of liver inflammatory conditions including alcoholic and non-alcoholic steatohepatitis. The predominant cell type in which this is occurring is thought to be the Kupffer cell, but the consequences of inflammasome activation in other hepatic immune cells have not been characterized. In addition to inflammation the inflammasome pathway is also known to be required for a full fibrotic response, as demonstrated by reduced lung, skin and liver fibrosis in inflammasome deficient mice. Identification of the inflammasome machinery has opened up novel therapeutic avenues by the use of antagonists for TLRs, as well as the ATP receptor P2x7, and the IL-1 receptor. Many of these are fertile areas for clinical investigation.

With characterization of the major signals and pathways in initial inflammasome activation, there is now great interest in how these are regulated. The initial challenge is how an acute inflammatory response is sustained. This is a significant issue as all the known stimuli result in an acute response that is self-limited to under 24 hrs. Many conditions of great interest such as NASH have sustained inflammation over months and years. This suggests that there are other significant regulators that are yet to be identified.

Reference:

Immune signatures as biomarkers of tolerance and immune activation

Alberto Sanchez-Fueyo, MD, PhD
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Attempts to intentionally induce tolerance in clinical organ transplantation have been unsuccessful other than in highly selected groups of recipients. In contrast, human transplant recipients occasionally develop spontaneous operational tolerance, a phenomenon in which recipients receiving no immunosuppressive therapy exhibit stable graft function for remarkably long periods of time in the absence of harmful immune responses. This state of spontaneous operational tolerance is particularly prevalent in liver transplantation, where a sizable proportion of stable recipients could probably cease all immunosuppression without compromising the graft’s viability. In recent years, considerable efforts have been devoted to the identification of non-invasive biomarkers of operational tolerance in kidney and liver transplantation. Most of these studies have employed blood cell immunophenotyping and gene expression profiling to search for immune parameters associated with tolerance. Methodological drawbacks common to all studies have been the small number of tolerant recipients available for study and their cross-sectional retrospective design. We have investigated the immunological traits of operationally tolerant liver recipients by comparing blood and liver tissue immune-related parameters. Overall, our results indicate that tolerant liver recipients, but not kidney recipients, exhibit an over-enrichment in innate immune related transcripts in blood. This contrasts with the identification of a B-cell related transcriptional signature in the blood of tolerant kidney recipients, and is associated with an expansion of NK cells. In tolerant liver recipients these findings are stable over time, can be detected before immunosuppression is withdrawn, and could serve as a non-invasive means to identify tolerant patients before drug weaning is attempted. The mechanistic interpretation of these results, however, remains elusive. In contrast to these results, molecular profiling of liver tissue samples collected before immunosuppressive drugs are withdrawn reveals that tolerant and non-tolerant grafts mainly differ in genes involved in the regulation of iron metabolism. These findings correlate with differences in clinical iron parameters, and suggest that regulation of iron metabolism could constitute an unrecognized pathway involved in the control of intra-graft alloimmune responses.
Adenosine triphosphate (ATP) is essential for the myriad of metabolic processes upon which life is based and is known widely as the universal energy currency unit of intracellular biologic reactions. ATP, adenosine diphosphate (ADP), adenosine, as well as other purines and pyrimidines also serve as ubiquitous extracellular mediators, which function through the activation of specific receptors (viz. P2 receptors for nucleotides and purinergic P1 receptors for adenosine). Extracellular nucleotides are rapidly converted to nucleosides, such as adenosine, by highly regulated plasma membrane ectonucleotidases that modulate many of the normal biological and metabolic processes in the liver – such as gluconeogenesis and insulin signaling. Under inflammatory conditions, as with ischemia reperfusion, sepsis or metabolic stress, ATP and other nucleotides can also act as “damage-associated molecular patterns” causing inflammasome activation in innate immune cells and endothelium resulting in tissue damage. The phosphohydrolysis of ATP by ectonucleotidases, such as those of the CD39/ENTPD family, results in the generation of immune suppressive adenosine, which in turn markedly limits inflammatory processes. Experimental studies by others and my group have implicated purinergic signaling in experimental models of hepatic ischemia reperfusion and inflammation, transplant rejection, hepatic regeneration, steatohepatitis, fibrosis and cancer, amongst others. Expression of ectonucleotidases on sinusoidal endothelial, stellate or immune cells allows for homeostatic integration and linking of the control of vascular inflammatory and immune cell reactions in the liver. CD39 expression identifies hepatic myeloid dendritic cells and efficiently distinguishes T regulatory-type cells from other resting or activated T cells. Our evolving data strongly indicate that CD39 serves as a key ‘molecular switch’ and is an integral component of the suppressive machinery of both dendritic and T cells. Increased understanding into mechanisms of extracellular ATP scavenging and specifically conversion to nucleosides by ectonucleotidases of the CD39/ENTPD family have also led to novel insights into the exquisite balance of nucleotide P2-receptor and adenosinergic P1-receptor signalling in those inflammatory and hepatic diseases, characterized by steatosis, fibrosis, thrombophilia and malignancy. Importantly, CD39 and other ectonucleotidases exhibit genetic polymorphisms in humans, which alter levels of expression/function and are associated with predisposition to inflammatory and immune diseases, diabetes and vascular calcification, amongst other problems. Development of therapeutic strategies targeting purinergic signaling and ectonucleotidases offers promise for the management of disordered inflammation and aberrant immune reactivity. This presentation will focus the developing role of purinergic signalling in the pathophysiology of liver diseases and propose potential future clinical applications.
Platelets: Novel anti-inflammatory targets in immune-mediated liver disease

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Hepatitis B virus (HBV) causes a liver disease of variable duration and severity. HBV replicates noncytopathically in the hepatocyte and most of the liver injury associated with this infection reflects the immune response. Virus-specific effector CD8 T cells play a major role in the development of liver disease and the resolution of HBV infection during self-limited acute viral hepatitis. Viral persistence reflects the failure to induce CD8 T cells with full antiviral capacity, so that a T cell-dependent chronic necroinflammatory process failing to eradicate the infection is maintained over time, often resulting in cirrhosis and hepatocellular carcinoma (HCC). Through the use of unique mouse models of HBV pathogenesis and advanced in vivo imaging, we found that the hepatic recruitment of pathogenic effector CD8 T cells is independent of selectins, integrins, chemokines and MHC/TCR interactions but it involves platelets and their CD44-dependent capacity to interact with hyaluronic acid within liver sinusoids. Our results suggest a model in which platelets that initially adhere to liver sinusoidal endothelial cells provide a preferential surface onto which CD8 T cells arrest their run within the hepatic microcirculation. Following their initial adhesion to platelets, effector CD8 T cells exhibit an intrasinusoidal crawling behavior that is inhibited by the recognition of hepatocellular HBV antigens. This latter process occurs in a diapedesis-independent manner (i.e. effector CD8 T cells become IFN-γ positive and trigger hepatocellular apoptosis while still intravascular). That platelets represent key players in the pathogenesis of viral hepatitis is also indicated by experiments we recently performed in a mouse model of chronic HBV infection. There, the continuous administration of anti-platelet drugs inhibiting platelet-T cell interaction (i.e. aspirin and clopidogrel) constantly contains the hepatic accumulation of pathogenic effector CD8 T cells and the consequent liver disease, ultimately preventing the development of cirrhosis and HCC.
Role of the innate and adaptive immune system in viral hepatitis

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Orchestrated innate and adaptive immune responses are required to control hepatotropic viruses but are also responsible for mediating and regulating liver pathology. A detailed dissection of protective and pathogenic immune responses in viral hepatitis is therefore necessary to allow their safe manipulation to promote sustained antiviral responses whilst limiting liver disease.

NK cells are a key component of the innate immune system that are highly enriched in the liver. I will review recent data revealing that NK cells are capable of exerting antiviral and immunoregulatory functions, whilst also contributing to the pathogenesis of liver injury via death receptor pathways. I will show that pegylated IFN-alpha potently expands activated NK cells, underscoring their potential importance in the treatment of viral hepatitis.

CD8 T cells are pivotal in the control of HBV and are profoundly diminished in patients with persistent infection. They exhibit a hierarchical loss of effector function, culminating in deletion, that is termed “exhaustion”. I will describe recently identified mechanisms driving T cell exhaustion and highlight their relevance to HBV. I will illustrate how the cellular, cytokine and nutrient environment of the liver drives the functional impairment of the antiviral T cell response and discuss avenues for therapeutic manipulation of these factors.
The HCV life cycle: In vitro tissue culture systems and therapeutic targets

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Approximately 2% of the world population is chronically infected with hepatitis C virus (HCV). If untreated ca. 20% of these patients will develop liver cirrhosis and of these, approximately 15% will progress to liver cancer within ten years. Past standard of care was based on the combination of pegylated interferon-alpha (PEG-IFN-α) and the nucleoside analogue ribavirin. However, only a fraction of patients was cured, treatment was associated with severe side effects, and contraindicated in a substantial number of chronic HCV patients. The first directly acting antiviral drugs which target the viral NS3-4A protease have been licensed in 2011 thus considerably improving therapeutic options. However, resistance development and viral genotype-specific efficacy of these drugs demand more efforts to develop well tolerated treatments with a high barrier to resistance and efficacy across all viral genotypes.

HCV is a highly variable plus-strand RNA virus of the family Flaviviridae. Viral strains are grouped into six epidemiologically relevant genotypes that differ from each other by more than 30% at the nucleotide level. The variability of HCV allows immune evasion and facilitates persistence. It is also a substantial challenge for development of specific antiviral therapies effective across all HCV genotypes and for prevention of drug resistance. Novel HCV cell culture models were instrumental for identification and profiling of novel therapeutic strategies. Moreover, these models revealed numerous host factors critical for HCV propagation, some of which have emerged as alternative targets for novel therapies. It is generally assumed that use of host factors is conserved among HCV isolates and genotypes. Moreover, the barrier to viral resistance is usually thought to be high. Therefore, current drug development includes both viral factors but also host factors essential for virus replication. In fact, some of these host targeting strategies like for instance inhibitors of cyclophilin A have advanced to late stage clinical trials. Here, we discuss the most prominent host targeting strategies against hepatitis C and critically discuss opportunities and risks associated with host targeting antiviral strategies.
The role of molecular HCV diagnostics in the management of chronic hepatitis C

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The rapid evolution of therapy for chronic hepatitis C virus infection is putting an increasing demand on the performance of molecular diagnostics. Currently we need viral load assays with a broad dynamic range and yet requiring exquisite sensitivity. It is now clear that the difference between lower limit of quantification and lower limit of detection are indeed clinically significant as treatment success rates vary according to these parameters. Molecular based genotyping assays are currently taken for granted but as patterns of antiviral resistance associated variants become clearer there will be a demand for more specific, rapid turnaround genotyping. However, in the long term when the majority of patients will be cured with a simple, all oral 12 week regimen then molecular diagnostics may be reduced to a pre-treatment and a post-treatment qualitative assay.
Viral hepatitis and liver transplantation – Pathogenesis, prevention and therapy of recurrent disease

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Financial disclosure: Xavier Forns has received unrestricted grant support from Roche and MSD and has acted as advisor for Jansen and MSD.

Natural history of hepatitis C recurrence
Infection with hepatitis C virus (HCV) is the leading cause of chronic liver disease and the main indication for LT. Hepatitis C recurrence occurs in all patients who undergo LT with detectable serum levels of HCV-RNA. Compared with patients who are not immunosuppressed, fibrosis deposition is accelerated in LT recipients with recurrent HCV infection and a significant proportion of them (20–30%) will develop graft cirrhosis only 5 years after LT.

The natural history of hepatitis C recurrence is highly influenced by events occurring during the first months following transplantation. Indeed, the presence of portal hypertension (hepatic venous pressure gradient [HVPG] ≥ 6 mmHg), or significant fibrosis (fibrosis score ≥ 2) 1 year after transplantation accurately identifies patients at risk for clinical decompensation and graft loss (rapid fibrosers), whereas patients with normal portal pressure and mild fibrosis 1 year after LT have excellent outcomes (slow fibrosers). A significant number of studies have evaluated which variables are associated with an accelerated course of the disease: among the most important are donor age, recipient sex, post-transplant diabetes, infection with cytomegalovirus, and biliary complications.

Assessment of hepatitis C recurrence after LT
Liver biopsy has been the gold standard and the method most widely used to monitor progression of liver fibrosis in patients with hepatitis C recurrence. Diagnosis of significant fibrosis is relevant in this setting, since antiviral therapy is clearly indicated in patients in whom fibrosis progresses over time. In the last years, non-invasive methods have been developed and its accuracy validated in liver transplant recipients. The accuracy of transient elastography for liver fibrosis assessment in this setting has been validated in several studies and it is currently used in routine clinical practice in most transplant centers in Europe.

Antiviral treatment before LT to prevent hepatitis C recurrence
Eradicating hepatitis C before LT is one of the potential strategies to prevent liver damage due to hepatitis C recurrence. A few studies have examined the efficacy and safety of interferon and ribavirin therapy before LT, giving patients a complete course of therapy aimed at achieving sustained virologic response before LT, or a short course of therapy (3–4 months) to reach viral clearance at the time of transplantation. Both strategies had similar levels of efficacy, with around 20% of patients achieving long-term viral clearance after LT. Variables associated with viral clearance are infection with HCV genotypes 2 or 3, duration of antiviral therapy before LT and the CC polymorphism in IL28B.
The addition of the recently approved HCV protease inhibitors telaprevir or boceprevir increases the efficacy of peginterferon and ribavirin therapy. It is important to notice, however, that rates of sustained viral response (SVR) were below 15% in patients with early cirrhosis who were previous null responders (a common feature in the waiting list of LT). Data from the French Cupic Cohort indicate that the rate of severe side effects (SAE) is very high in patients with cirrhosis who undergo triple therapy. Of special concern is the relatively high incidence of infections. For this reasons, indication of triple therapy in this setting should be restricted to patients with compensated cirrhosis in whom the indication for LT is hepatocellular carcinoma, and who do not have clinically significant portal hypertension.

In the pre-transplant setting, only interferon-free regimens will be useful in patients with decompensated liver disease. Currently, most results from interferon-free regimens have been obtained in patients with mild liver disease, but they have clearly shown that SVR is possible when combining two or more DAAs. Some of these drug combinations will be soon explored in patients with advance liver disease; if proven safe, this will change the current view of hepatitis C in liver transplantation, since most infections will be prevented.

Treatment of hepatitis C recurrence after LT
Most transplant centers begin antiviral therapy once significant fibrosis has been documented, usually more than 1 year after LT. A systematic review that included 19 studies and more than 600 patients with established recurrent hepatitis treated with pegylated interferon and ribavirin revealed a pooled SVR of 30% (range 8–50%). The rate of discontinuation was around 30% and most patients required dose reduction (mostly because of anemia).

Despite the lack of studies evaluating the safety and efficacy of protease inhibitors in the transplant setting, small series of patients with hepatitis C recurrence treated with peginterferon, ribavirin and telaprevir or boceprevir have been presented in recent meetings. Overall, preliminary results seem to indicate that SVR will be high in patients treated with triple therapy (rates of undetectable HCV-RNA at week 12 of therapy are above 70%). Nevertheless, it is important to notice that exposure to CsA and particularly TAC increases significantly in patients treated with protease inhibitors (boceprevir and telaprevir are both substrates and inhibitors of the CYP3A4 system). This effect is more pronounced for telaprevir than for boceprevir. Since immunosuppressants have a very narrow therapeutic range, they must be carefully managed in this situation, to prevent life-threatening side effects (renal failure, neurological toxicity, hypertension, diabetes).

Currently, very little is known on the efficacy and safety of new DAAs in the post-transplant setting. Most companies are exploring the potential interactions of these new compounds with immunosuppressants and we will soon start to see the first studies in this population. As in the pre-transplant setting, the field will completely change a few years from now, with the use of DAAs combinations that will increase efficacy and tolerability.
Session III

Viral hepatitis II
Biomarkers for current and future HBV therapies

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Chronic hepatitis B (CHB) is a global health challenge with > 350 million people chronically infected indicated by HBsAg positivity. Clinical cure is only achieved if patients clear HBsAg\(^1\). Current direct antiviral therapies with nucleoside analogues (NA) only achieve HBsAg clearance in less than 5% and life-long treatment is required in almost all cases. Treatment with interferon alpha slightly increases HBsAg loss rates to 10%. There is an urgent unmet need for concepts that improve HBsAg clearance, especially in patients who are treatment experienced. So far the drug development pipeline for HBV is dry. Concepts to increase HBsAg loss will depend on the combination of NA and IFN in the next five to ten years. Toll-like receptor agonist and therapeutic vaccinations will be developed but are at an early stage. It will be essential to establish biomarkers indicating immune control of HBV and predicting HBsAg loss, which allows monitoring the success and personalization of therapies. HBV DNA will be not useful as biomarker because HBV DNA can be easily suppressed by NAs without affecting intrahepatic cccDNA. It will take decades to clear HBsAg with NA treatment\(^2,3\). Quantification of Hepatitis B surface antigen (HBsAg) may be a better marker to indicate immune control and can be a useful diagnostic tool in the management of HBV (reviewed by\(^4\)). The recent availability of commercial quantitative assays\(^5\) has restarted the interest in quantitative HBsAg as a biomarker for prognosis and treatment response in CHB. HBsAg levels in serum are affected by the amount of cccDNA, its transcriptional activity and the secretion behavior of the expressed HBsAg proteins (reviewed by 4). Serum HBsAg levels are associated with the phase of chronic HBV infection. In HBeAg-negative patients, combination of low hepatitis B virus (HBV) DNA and low HBsAg levels can predict inactive carrier status\(^6,7\). HBsAg decrease by interferon alfa (IFN) is more pronounced as by NA treatment, although NAs show a stronger inhibition of HBV DNA replication\(^8\). HBsAg kinetics has been successful to predict treatment response to IFN treatment\(^9,10\). Although HBsAg decline is absent or very slow with NA therapy in most patients, some patients show a significant HBsAg decrease which may predict future HBsAg loss and subsequent seroconversion\(^11\). This may be limited to patients with efficient protective immune responses (i.e. high IP-10 levels), as we have previously discussed (Figure 1)\(^11\). Despite the large amount of data that have been published over the last two years, there are many open questions regarding HBsAg. Data in co-infected patients are rare. In some HBV/HDV co-infected patients HBsAg levels are very high despite relatively low HBV DNA levels 12. As HBsAg is crucial for the virogenesis of HDV, further insights may help to optimize treatment for HBV/HDV. We have initial data, that HBsAg is relatively low in HBV/HCV co-infected patients with HCV dominance\(^13\). The mechanisms are unknown but may help to understand the viral interactions in co-infected patients. Cytokines or immune responses may explain the findings. There are also limited data in HBV/HIV co-infected patients. Our results suggest that HBsAg correlates with CD4 T cell count in this setting\(^14\) (Figure 2). All data emphasize the importance of HBsAg for immunomodulatory therapies for CHB. Besides differences in the quantity of HBsAg there may be also differences in the protein composition of HBsAg. The gene for HBsAg contains three start codons and a common stop codon, thus coding for 3 co-carboxyterminal proteins, called large (L), middle (M) and small (S).
protein (Figure 3). S protein is the main component of the viral envelope and the HBsAg subviral particles. M protein and its preS2 domain is dispensable for virogenesis and infectivity\textsuperscript{15}, but is conserved in the mammalian hepadnaviruses. The preS2 domain may have impact for HBsAg secretion and is a target of immune responses. The preS1 domain is essential for viral envelopment and for the high affinity binding to susceptible hepatocytes. It is a major component of the complete virions but a minor component of the small subviral particles. L and M protein have a variable, complex topology, which results in variable glycosylation patterns. L protein expression is governed by the liver specific preS1 promoter whereas M and S expression is initiated from the cell type-independent preS2/S promoter. The ratio of subviral to virus particles is highly variable in patients with CHB. Our initial data suggest that LHBs may decline faster in patients with acute hepatitis B compared to total HBsAg, which suggest that the ratio of HBsAg fractions may be a better marker for immune control. In addition to viral proteins and cytokines, further easy to apply biomarker should be evaluated. Genome wide association studies are underway to correlate SNPs with response to PEG-IFN. Some studies have shown a correlation of IL28B polymorphism with response to PEG-IFN\textsuperscript{16,17}, but larger studies have to confirm these results. Overall, biomarkers associated with HBsAg loss are important to optimize the management of current and future HBV therapies.

**Figure 1:** HBsAg levels decline in patients during NA therapy who have higher baseline serum IP-10 level.
Figure 2: HBsAg levels correlate with the immune status in HIV/HBV co-infected patients.

Figure 3: Total HBsAg consist of three different fractions: LHBs, MHBs, sHBs.

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Interferon free treatments for chronic hepatitis C

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The primary aims of antiviral therapy for chronic hepatitis C (CHC) are the prevention of progression to cirrhosis and the prevention of decompensated cirrhosis or hepatocellular carcinoma in patients with cirrhosis. New direct acting antiviral agents have been introduced. Several new direct acting antivirals (DAAs) in development portend improved response rates for both genotype 1 and other HCV genotypes. The future potential combination of all oral DAAs without IFN is an attractive objective and will become reality for many patients. Several important HCV replication targets have been identified. These include the HCV protease, NS5a region and the NS5b RNA-dependent RNA polymerase. Both nucleos(t)ide and non-nucleoside inhibitors of the NS5b polymerase have been characterized. Host targeting agents have been identified. The next wave of treatment for patients with genotype 1 will comprise newly developed DAAs to be used in combination with PEG-IFN and ribavirin for treatment periods of 12–24 weeks, with high rates of cure. Several regimens have been tested to date, albeit often in small numbers of patients. Examples include: An all-oral combination of daclatasvir plus sofosbuvir in 126 treatment-naive, noncirrhotic patients infected with subtypes 1a or 1b for 12 or 24 weeks with or without weight-based ribavirin; sofosbuvir and ribavirin in genotype 1 treatment-naive and prior null responders; sofosbuvir and ribavirin with ledipasvir for 12 weeks in treatment-naive patients and null responders. These DAA regimens resulted in encouraging response rates in both naive and null responders with both regimens, with SVR rates of 92–100% in small numbers of patients.

The AVIATOR study comprised the combination of ABT 450 (a protease inhibitor) with ritonavir, together with varying combinations of ABT 267 (an NS5a inhibitor) and ABT 333 (a non-nucleoside NS5b inhibitor) with or without ribavirin for varying durations ranging from 8–24 weeks in 571 genotype 1 treatment-naive and null responder patients without cirrhosis. Daclatasvir, asunaprevir and BMS 791325 (a non-nucleoside protease inhibitor) given for 12 or 24 weeks has been tested in 66 treatment-naive genotype 1 patients without cirrhosis. Faldaprevir 120 mg daily and 600 mg of the non-nucleoside BI 207127 (deleobuvir) given twice daily, for 28 weeks plus ribavirin is effective in treatment-naive patients with subtype 1b, as is the combination of daclatasvir and asunaprevir in null responders with cirrhosis infected with subtype 1b but not subtype 1a. Results from the COSMOS study have been reported, evaluating SVR rates at post-treatment week 8 in patients in the shorter 12-week arms; in this four-arm study simprevir 150 mg once daily was given with sofosbuvir 400 mg daily with or without ribavirin for 12 or 24 weeks to prior PEG-IFN genotype 1 null responders without cirrhosis.

Non-1 genotypes: Sofosbuvir and ribavirin has been tested in genotype 2 and 3. Response rates were the same in patients with genotype 2 irrespective of the presence or absence of cirrhosis, but were reduced from 61% to 21% in patients with genotype 3 and cirrhosis; Daclatasvir plus sofosbuvir in treatment-naive patients infected with genotypes 2 or 3 for 12 or 24 weeks with or without ribavirin reported SVR rates of 93–100%. Genotype 3 has (perhaps temporarily) become the ‘difficult to treat genotype’. PEG-IFN
and ribavirin might still be required together with a DAA, for example sofosbuvir or daclatasvir. Increased treatment duration with sofosbuvir and ribavirin, or the addition of a third DAA will be required to achieve high rates of cure for genotype 3.

**Special groups:** Successful retreatment following failure with boceprevir and telaprevir will be dependent on the development of agents that lack cross-resistance across the class. Importantly, almost 100% SVR rates have been reported with daclatasvir 60 mg plus sofosbuvir 400 mg with or without ribavirin in genotype 1 patients who previously failed telaprevir or boceprevir treatment. Patients with decompensated cirrhosis are poor candidates for treatment with IFN. The advent of IFN-free treatments with high rates of efficacy in patients with cirrhosis or decompensated cirrhosis will transform the management of patients with advanced liver disease due to CHC and are urgently awaited. Given this exiting landscape, fundamental consideration exists, that determine who should be treated now and who should wait.
Session IV

Hepatobiliary tumors
The pathogenesis of hepatocellular carcinoma

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Worldwide, hepatocellular carcinoma (HCC) is the fifth most common cancer in men and the seventh in women. In the year 2008, 748,300 new HCCs and 695,900 deaths have been registered. Almost 85% of all cases occur in the developing countries. Regions with a high incidence are Eastern and South-Eastern Asia and Middle and Western Africa. More than half of all new HCC are diagnosed in China.

In almost all populations, more men than women are affected. The fact that men are more often exposed to risk factors partly accounts for the higher incidence for men. However, as gender differences can be reproduced in mouse experiments, hormonal changes are likely to influence hepatocarcinogenesis as well. One possible mechanism is that androgens enhance DNA damage and oxidative stress during hepatocarcinogenesis. Recently, it was shown that the transcription factor Foxa1/2 protects female mice from HCC while promoting HCC in male mice. All these results indicate that there exist gender specific mechanisms in hepatocarcinogenesis and that the higher incidence of HCC in men is not restricted to the exposure to risk factors.

In more than 70% of cases, HCC develops in patients with advanced liver cirrhosis. In 90%, the responsible risk factor is known. The main risk factors for liver cirrhosis and HCC strongly depend on the geographic region. Worldwide, the most important risk factors are viral hepatitis, alcohol, non-alcoholic steatohepatitis und aflatoxin exposure. 54% of all HCC can be attributed to hepatitis B and 31% to hepatitis C. These classical risk factors promote the occurrence of gene alterations in hepatocytes, which subsequently lead to an accumulation of alterations in genes that control cell cycle and cell proliferation. Several oncogenic pathways have been identified among them WNT signaling, PI3K/AKT/mTOR and p53 pathway, which subsequently induce tumor formation and progression. These findings led to the identification of molecular HCC subclasses, which are classified according to the deregulated molecular pathway. A more comprehensive knowledge of the genetic alterations and activated pathways will allow us to further dissect the diversity of HCC and to develop a more refined molecular classification to not only prevent hepatocarcinogenesis at early steps, but also to identify more effective treatments for patients with advanced diseases.
Major advancements in the diagnosis and treatment of hepatocellular carcinoma (HCC) have been produced in the recent decades. Development of HCC is now recognized as the major cause of death in patients with cirrhosis, and clinical practice guidelines [1–4] recommend patients who would be treated if diagnosed with HCC to be included in screening programs based in abdominal ultrasonography every 6 months. Upon nodule detection, HCC may be diagnosed by non-invasive imaging criteria [1–4] or by biopsy. Treatment is decided after carefully assessing tumor burden, liver function and general health status. The strategy that has been endorsed for prognosis evaluation and initial treatment approach is the BCLC strategy [5] that is exposed below. It allows to raise the 1st line recommended therapy for each stratum of patients and its development is supported by evidence based data derived from randomized controlled trials, meta-analysis and prospective cohort studies. Current effective options for conventional practice are: surgical resection, liver transplantation, ablation, chemoembolization and sorafenib. Survival in patients diagnosed at early stage is 60–70% at 5 years, at intermediate stage is 50% at 4 years, and at advanced stage is 50% at 1 year [5]. Options beyond conventional ones should be evaluated in research trials designed according to current knowledge about predictive factors as derived from recent investigations [6, 7].

References:

The pathogenesis and biliary tract cancer

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Biliary tract cancer is tightly coupled to hepatobiliary inflammation. Indeed, of all the risk factors known to be associated with biliary tract cancer, the best understood are liver fluke infection of the biliary tract, stone disease, primary sclerosing cholangitis, and fibrocystic diseases associated with biliary tract inflammation. Moreover, in the genetic studies of cholangiocarcinoma, there is clearly a subset with a very strong inflammatory genetic signature driven by STAT3 signaling cascades. We have shown that interleukin-6 (IL-6), which activates STAT3, is a potent biliary tract mitogen, and renders cholangiocarcinoma cells resistant to apoptosis by increasing expression of the antiapoptotic protein Mcl-1. Moreover, studies of the stroma cells in biliary tract cancer have also identified a strong interleukin-6 signal. Socs 3 which is an inhibitor of IL-6 signaling is often silenced by epigenetic mechanisms in biliary tract cancer providing a mechanism for a sustained and unrelenting IL-6 signaling in these cells. The role of changes in bile composition are likely to be important in biliary tract carcinogenesis but have received scant attention. Oxysterols are oxidized metabolites of cholesterol, are abundant in bile and are bona fide endogenous ligands for the hedgehog signaling pathway. The hedgehog signaling pathway has also been strongly implicated in biliary tract cancer progression. We note that induction of inflammatory mediators such as inducible nitric oxide synthase (iNOS) have been associated with upregulation of NOTCH signaling, which has been strongly implicated in genetic mouse models as an inducer of cholangiocarcinoma. Finally, unpublished data suggest interleukin-33, another inflammatory cytokine may be important in the genesis of biliary tract carcinogenesis in the presence of oncogenic mutations (unpublished observations). As we look at ways to prevent the onset of cholangiocarcinoma and/or treat established carcinoma, interruption of these inflammatory signaling cascades will likely prove to be therapeutically useful.
Session V

Fatty liver diseases
Mechanisms of disease

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Nonalcoholic fatty liver disease (NAFLD) has evolved as a serious public health problem in the US and around the world. NAFLD ranges from simple steatosis to nonalcoholic steatohepatitis (NASH) to liver fibrosis and liver cirrhosis. Within this spectrum, NASH is characterized by hepatocellular lipid accumulation (steatosis) along with inflammation and varying degrees of fibrosis. NASH is a potentially serious condition, and about 10 to 25% of patients with NASH may progress to cirrhosis. Indeed, as NAFLD is an increasingly common in the developed world, NASH is projected to be the leading cause of liver transplantation in the United States by 2020. The pathogenesis of NAFLD and NASH, in particular the mechanisms responsible for liver injury and fibrosis, is the result of a complex interplay between host and environmental factors, and is the center of intense investigation. In this talk, I will review recent works in the field that brought new insights into this complex disorder and suggest novel therapeutic and diagnostic strategies that may allow for a personalized approach to the disease. I will make an effort to integrate the role and influence of dietary factors, in particular certain lipid and carbohydrate components, the interplay between host genetics and environment, the role of innate immune activation and inflammation, as well as the impact of hepatocellular injury and cell death in the progression of NAFLD.
Diagnosis and investigation

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The field of nonalcoholic fatty liver disease (NAFLD) is a paradox: both narrow when it comes to identifiable diseases and deeply intricated, with the liver only part of a complex phenotype. Primary nonalcoholic fatty liver disease is by far the most common diagnosis; secondary causes are either very rare or often overlooked. Yet, for each patient, other causes than primary NAFLD should be ruled out: Wilson’s disease, hypo or abetalipoproteinemia, drug-induced steatohepatitis, lipodystrophic syndromes, HCV infection (genotype 3 induces steatosis independent of coexisting overweight), exposure to petrochemical toxicants, hypothalamic or hypophyseal disorders, surgical interventions for weight-loss. If none of these causes are present, metabolic risk factors should be thoroughly assessed: most NAFLD patients are either overweight, have increased central adiposity, arterial hypertension dyslipidemia or altered glucose metabolism. Occasionally markers of insulin resistance (hyperinsulinemia, a high HOMA score or an oral glucose tolerance test) are required to demonstrate insulin resistance in some patients with bona-fide steatosis but a lean phenotype.

Further explorations should be performed only in patients in a stable metabolic condition. It is advisable to delay hepatological work-up in case of recent and continuing weight-loss. A careful assessment of concurrent comorbidities and of cardiovascular risk is critical. The condition called non alcoholic fatty liver (NAFL, which encompasses steatosis alone and steatosis with mild inflammation but without steatohepatitis) should be distinguished from steatohepatitis (NASH) as the prognosis of these entities is different. Despite considerable work with non-invasive serum markers or imaging methods, this distinction is still best made by liver biopsy, with a significant margin of error due to sampling variability. Additionally, the stage of fibrosis needs to be assessed both for prognostic purposes and for refining indications for therapy-introduction of pharmacological agents or reinforcement of non-pharmacological measures. There are quite a few serum markers that achieved good diagnostic value for advanced fibrosis (the NFS score, FibroTest, ELF score) and the same holds true for elastometry. More sophisticated imaging methods such as magnetic resonance elastometry and FibroMRI are not yet available on a large scale.

The follow-up of these patients should include at least an annual visit for metabolic comorbidities, cardiovascular events, neoplasia, fibrosis progression or cirrhosis occurrence as monitored per non-invasive methods. A repeat liver biopsy is advisable within 5 years especially if steatohepatitis was present on the index biopsy and if persistent or worsening metabolic risk factors. Importantly, best cost-effective strategies for hepatocellular carcinoma screening need to be determined by future studies, both in cirrhotic and non-cirrhotic patients.
NASH Natural history and HCC

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Non-alcoholic fatty liver disease (NAFLD) is best characterized as a broad prognostic spectrum ranging from long-term stability to chronic liver failure and in some cases to hepatocellular cancer. NAFLD can be viewed as a larger population of non-NASH fatty liver (NNFL) patients and a smaller population of NASH patients distinguished by more severe histological findings. NNFL and NASH are usually two different populations although it is recognized that NNFL can transition to NASH. In general, NNFL tends to be stable while NASH carries risk of progression from early stage fibrosis to bridging fibrosis and then to cirrhosis. It is estimated that patients with NASH have about a 10–20% chance of developing cirrhosis over 5–10 years and about 15–20% chance of dying from a liver-related condition over 10–15 years. The most significant predictor of liver-related mortality is the initial fibrosis stage whether determined by biopsy or more-recently by non-invasive markers. As pharmacological treatment evolves, it will be increasingly important to better identify predictors of fibrosis in order to weigh the relative risk and benefit of newer, potentially risky and more costly interventions. Currently, the grade of inflammation stands out as the major predictor of fibrosis although as diagnostic tools are refined it seems likely that other markers will emerge. Once cirrhosis is present, the rate of complicating events such as ascites or variceal bleeding is steady at a few percent per year at a rate somewhat slower than cirrhosis due to hepatitis C. Hepatocellular cancer can develop in non-cirrhotic fatty liver but by far the greater risk for supervening HCC is in patients who progress to cirrhosis. The risk can be estimated as up to about 2–3% of cirrhotic NASH patients over 5–10 years. Coronary artery disease is also a leading cause of death among patients with NAFLD and in some series it eclipses liver-related mortality even among patients identified non-invasively as having NASH. Certainly, these two conditions can be expected to co-exist in a significant number of patients adding to the complexity of managing a patient with advanced liver fibrosis or cirrhosis and coronary vascular disease. Whether this situation represents shared risks or shared pathogenic mechanisms remains unclear although the latter seems increasingly likely. The importance of better defining and understanding the natural history of NAFLD and NASH lies in better prognostication but also and especially in triaging patients to emerging therapeutic interventions.
Non-alcoholic fatty liver disease (NAFLD) is increasingly diagnosed worldwide and considered to be the commonest liver disorder in Western countries. It comprises a disease spectrum ranging from simple steatosis (fatty liver), through non-alcoholic steatohepatitis (NASH) to fat with fibrosis and ultimately cirrhosis. Simple steatosis is largely benign and non-progressive, whereas NASH, characterized by hepatocyte injury, inflammation and fibrosis can lead to cirrhosis, liver failure and hepatocellular carcinoma (HCC). NAFLD is strongly associated with obesity, insulin resistance, hypertension and dyslipidemia and is now regarded as the liver manifestation of the metabolic syndrome. Therapeutic strategies can be divided into those directed at components of the metabolic syndrome with potential beneficial liver effects and those directed specifically at the liver. The former group includes weight reduction therapies, insulin sensitisers, lipid lowering agents and anti-hypertensives. With respect to weight reduction, data from controlled trials suggest that diet and exercise improves NASH, particular in those achieving > 7% weight loss. Obesity surgery has been shown to improve steatosis in all studies and inflammation and fibrosis in some. Insulin sensitisers are the rational choice for patients with NASH and associated diabetes, however, results for metformin have not been convincing and concerns over the safety of glitazones has reduced the initial enthusiasm for their use based on encouraging pilot data. Metformin may have the added benefit of reducing the risk of HCC in patients with NAFLD. There has been no convincing evidence of any benefit of lipid lowering agents, however, importantly, statin therapy is safe in patients with NASH and should be given for the normal indications, and, as with metformin statins may lower the HCC risk. Given the role of the renin-angiotensin system in liver fibrosis, ACE inhibitors and angiotensin II receptor blockers hold most promise as anti-hypertensive agents for patients with NASH and hypertension. Again, pilot data have been encouraging. With respect to more specific liver-directed therapies, there have been promising studies of antioxidants, including betaine and probucol and Vitamin E may improve NASH in adults and children either alone or taken together with ursodeoxycholic acid. Ursodeoxycholic acid alone appears to have no beneficial effect. As in alcoholic hepatitis, the TNFα lowering agent, pentoxifylline may have beneficial effects on NASH. Liver transplant is successful but disease recurrence rate is high in the absence of treatment of the underlying metabolic syndrome. Promising therapies at present, based largely on animal studies, include probiotics, drugs directed at ER stress and inhibitors of the IKK/NF-KB system.
Session VI

What’s hot in hepatology
IgG4-associated cholangitis

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IgG4-associated cholangitis (IAC) is the hepatobiliary manifestation of Immunoglobulin G4-related disease (IgG4-RD), an increasingly recognized systemic fibroinflammatory disorder with a wide variety of clinical presentations and organ manifestations which predominantly affects the hepatobiliary tract (IAC) and pancreas (autoimmune pancreatitis, AIP)\(^1\)–\(^5\). IgG4-RD is characterized by fibrosing inflammation of the affected organs, tissue infiltration of IgG4-loaded plasma/B cells and often elevated serum levels of IgG4. A large number of medical conditions fall within the spectrum of IgG4-RD and the list of organ manifestations (pancreas (AIP); biliary tree (IAC), gallbladder and liver; salivary, parotid and lacrimal glands; retroperitoneum; kidney; lungs; lymphatic system (especially hilus); stomach, intestine including ileal pouch; vascular system (aortitis); nervous system, eye (uveitis); prostate, testis; thyroid; pseudotumor) is still expanding.

Diagnosis of IAC and IgG4-RD

In clinical practice, it is difficult to make a clear-cut diagnosis of IgG4-RD as an accurate diagnostic marker is lacking. Patients often present with painless obstructive jaundice and tumor-like swelling of involved organs that can be easily mistaken for pancreatic or bile duct cancer, as well as primary sclerosing cholangitis (PSC) or other forms of secondary sclerosing cholangitis. In up to one of three patients, extensive surgery for presumed malignant hepatobiliary or pancreatic malignancy has taken place prior to diagnosis of IAC and IgG4-RD\(^6\). For these reasons, consensus criteria have been developed first for AIP\(^7\) and subsequently for IAC\(^2\) to improve the accuracy of diagnosis of IgG4-RD including clinical, biochemical, radiological and histomorphological features. Serum levels of IgG4 are often elevated in IAC and IgG4-RD, but not diagnostic at moderately elevated levels (< 4 x ULN) as other conditions such as primary sclerosing cholangitis (PSC), cholangiocarcinoma or pancreatic carcinoma are also associated with elevated IgG4 serum levels\(^8\)–\(^10\). In addition, about 10–20% of patients with IAC and AIP do not show elevated serum IgG4 levels upon presentation\(^5,11\). The use of serum IgG4 as a biomarker is, therefore, limited due to its limited sensitivity and specificity when only moderately elevated.

Histopathological examination discloses infiltration with IgG4+ plasma cells and storiform fibrosis, regardless of the affected organ\(^2\). However, such infiltrates are also observed in other diseases.

Radiologic studies commonly show diffuse swelling of the affected organ\(^1,12\), e.g. pancreas, salivary glands, lymph nodes. However, swelling is a nonspecific feature of inflammation or neoplasia. Cholangiography discloses alterations compatible with sclerosing cholangitis or cholangiocarcinoma.

We recently identified dominant IgG4+ B cell receptor (BCR) clones in blood and tissue of all patients with active IAC under study, but in no single healthy or disease control including patients with PSC and hepatobiliary/pancreatic malignancy and elevated serum IgG4\(^13\). Many more studies are needed before identification of dominant IgG4+ BCR clones may become the golden standard for the diagnosis of IAC and AIP as well as other organ manifestations of IgG4-RD.
**Pathogenesis of IgG4-RD**

Pathophysiological mechanisms underlying IAC and IgG4-RD are poorly understood. Whether IgG4 antibodies behave as tissue-destructive immunoglobulins or as an anti-inflammatory antibody to dampen the immune system in reaction to an unknown stimulus remains to be clarified. IgG4 normally form the smallest fraction of total IgG in serum\(^{14}\) are upregulated in chronic immune stimulation\(^{14}\), are unable to bind C1q and have a low Fc affinity thereby barely initiating a complement response\(^{15,16}\) and exchange their Fab arms\(^{17}\). The recent finding of dominant IgG4+ BCR clones in blood and tissue of patients with active IAC (and other organ manifestations of IgG4-RD), but not in controls, shed a new light on development of IgG4-RD and may suggest that specific B-cell responses are pivotal to the pathogenesis of IAC possibly under chronic immune stimulation. These findings support the hypothesis that the abundant production of IgG4 antibodies is part of an antigen-driven immune response.

Remarkably, the majority of patients with IAC are over 60 years old and 80–85% are of male sex\(^{18}\). These findings contrast strongly with comparable autoimmune diseases such as primary biliary cirrhosis or Sjögren’s syndrome, which predominantly affect middle-aged female patients\(^{19}\), raising the question whether IgG4-RD is really an autoimmune disease. Thus far, no specific antigen has convincingly been identified in IgG4-RD patients.

**Treatment of IgG4-RD**

Most patients with IAC or AIP have an excellent response to initial high to moderate dose corticosteroid therapy, typically with diminution of symptoms, of organ enlargement and of serum levels of IgG4\(^{5,20}\). Notably, dominant IgG4+ clones in blood disappeared upon successful corticosteroid therapy within a month. Long-term low dose maintenance immunosuppressive therapy is needed in the majority of patients to prevent symptom recurrence.

**References:**

Developing vaccines against hepatitis C virus (HCV)

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HCV infects millions of people worldwide and is a leading cause of liver cirrhosis, and hepatocellular cancer. Although new oral antivirals are now available and represent a real advance in the field, these are unaffordable and unavailable to most people. Furthermore these drugs are least effective in patients with advanced liver disease who need them most, are associated with the development of viral resistance, and do not provide protection from re-infection. For these reasons a protective vaccine against hepatitis C virus remains a real unmet clinical need.

HCV may be particularly susceptible to a T cell vaccine strategy; a significant proportion of people acutely infected spontaneously clear infection and the assessment of host immunity, HLA genetic association studies, and chimpanzee challenge experiments highlight the critical role that effective T cell immunity plays in viral control. Our aim through vaccination is not to provide sterilising immunity, but rather to prevent the establishment of chronic infection.

After many years of defining the immune correlates of protection during natural HCV infection in the laboratory, we have recently conducted a series of phase-I experimental medicine studies to develop novel T cell vaccine candidates in healthy human volunteers and in patients with persistent HCV infection. We have employed both Adenoviral (Ad) and Modified Vaccinia Ankara vectors in prime-boost vaccination strategies. To overcome the issue of pre-existing anti-Ad vector immunity, we have used Ad vectors derived from rare human serotypes (Ad6) and chimpanzees (ChAd3), found at low sero-prevalence (28% and 3% respectively) in association with an SME partner (Okiaros). These vectors encode the HCV NS3-NS5B polyprotein (1,985 amino acids), with a genetically inactivated NS5B polymerase.

Using this approach, we are able to generate very high numbers of polyfunctional CD4+ and CD8+ HCV specific T cells that target multiple HCV antigens in healthy volunteers. Analysis employing single cell mass cytometry (CyTOF), and HLA class-I peptide tetramers showed that substantial memory T cell populations are primed, with overall improvement of quality (proliferation, polyfunctionality) following heterologous boost. Assessment of vaccine induced T cells in association with autologous circulating virus in patients with HCV infection highlights the challenges of developing effective vaccines against pathogens with diverse genomes in therapeutic vaccine strategies.

In summary, we have developed a novel HCV vaccine strategy paving the way for the first efficacy studies of a prophylactic HCV vaccine that are currently being assessed in intravenous drug using populations.
Coagulopathy in liver diseases: Complication or therapy?

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Keywords: Cirrhosis, Coagulopathy, Bleeding, PVT, Enoxaparin

Coagulopathy in cirrhosis is a composite condition where liver synthetic deficit re-balances coagulation to a parallel reduction of both procoagulant and anticoagulant factors [1]. Therefore cirrhosis is no longer considered a hypocoagulable state but a more unstable hemostatic balance with a lower threshold toward thrombosis or bleeding [2].

Bleeding inclination is due to the reduction in procoagulants synthesis and low platelets count as well as hyperfibrinolysis. Variceal haemorrhage is a frequent bleeding complication in decompensated cirrhosis. However the possible contribution of coagulopathy as a precipitant or an aggravating factor is poorly documented and further data are required to clarify its real burden at the point of presentation of haemorrage [3]. Moreover, apart from the gastrointestinal tract, the occurrence of spontaneous and procedure-related bleeding elsewhere, whilst not uncommon, is less than expected [4].

By contrast a large-scale population-based study has shown the propensity toward venous thrombosis in patients with liver diseases [5]. Portal vein thrombosis (PVT) is a critical but frequent event occurring up to 40% of cirrhotics. PVT causes deterioration of the clinical course, portal hypertensive complication and post-transplant mortality [6–7]. Pathogenesis of PVT includes both local alterations, like blood flow reduction and endothelial activation, and systemic derangements. Systemic prohemostatic alteration include high von Willebrand factor, low ADAMTS 13, low levels of anticoagulants (antithrombin, protein C–S) and increase in procoagulants like factor VIII [1].

Low Molecular Weight Heparin such as Enoxaparin has proved to be safe and effective both in treatment and prevention of PVT [8–9]. In addition patients in prophylaxis with enoxaparin showed a lower rate of decompensation and a better survival without bleeding complications. In such patients circulating bacterial DNA, endotoxemia and markers of inflammation were attenuated compared to controls, confirming the well known anti-inflammatory activity of enoxaparin [10]. These results therefore suggest a possible connection between enoxaparin, decrease of endotoxemia and reduce in portal hypertension [9].

The approach to the coagulopathy in patients with liver diseases is changing: while the main goal for clinicians so far has been to reduce the risk of bleeding, the results of these new studies highlight the importance of avoiding or treating thrombophilic disorders like PVT to protect microcirculatory damage and prevent liver decompensation [11].
References:

Cell based therapy for $\alpha_1$-antitrypsin deficiency and inherited liver diseases

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My research focuses on the use of human induced pluripotent stem cells (hiPSCs). This exciting new technology, still only a few years old, promises to bring great advancements to studies in developmental biology, modelling of human diseases and cell-based therapy. To this end, I recently developed disease specific human hepatocyte-like cells by reprogramming dermal fibroblasts taken from individuals with PiZ $\alpha_1$-antitrypsin deficiency. The resulting cells were found to possess key functional attributes common to adult hepatocytes and could also reproduce the protein misfolding and intracellular polymer formation characterizing this disease (Rashid et al., J Clin Invest. 2010). I believe that this new discovery, which uniquely allows generation of unlimited quantities of patient specific hepatocytes, has the potential to change our whole approach to modelling human liver disease in vitro. In addition to investigating the use of hiPSCs for disease modelling, I have also been interested by their potential use as a therapeutic agent. Using hiPSC-products for cell therapy of patients with inherited genetic disorders, such as PiZ $\alpha_1$-antitrypsin deficiency, requires correction of the underlying genetic abnormality in a manner fully compatible with clinical applications. To demonstrate this, the defect responsible for PiZ $\alpha_1$-antitrypsin deficiency (Glu342Lys) was corrected using a novel approach, combining engineered Zinc finger nucleases with a piggyBac vector in patient specific hiPSCs (Rashid & Yusa et al., Nature. 2011). Importantly, gene correction was found to restore normal structure, function and secretion of $\alpha_1$-antitrypsin in subsequently derived liver cells in vitro, with injection of corrected cells into a mouse model of liver injury confirming their functional capacity in vivo without tumour formation. Overall, these data provided the first proof of principle for the combined use of gene therapy with hiPSCs in autologous cell therapy of inherited liver disorders. But understanding how such cells may behave post transplantation within the varied extracellular milieu found at different stages of human liver disease is an outstanding and critical question prior to safe translation of this work. To try to answer this question I have started to develop high throughput “-omics” based approaches to profile the effects of relevant extracellular constituents on hepatocyte behaviour (Rashid & Humphries et al., J Prot Res. 2013). This knowledge will complement ongoing efforts to understand the mechanisms regulating the biology of adult and progenitor hepatocytes in development, health and disease. Such understanding is pivotal to our attempts to produce truly representative cell models in vitro as well as efforts to achieve therapeutic efficacy through cellular manipulation in vivo.
Session VII

Autoimmune liver diseases
Genetics of autoimmune liver disease

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Autoimmune liver diseases represent a group of chronic immune mediated liver injuries, wherein targeted inflammatory damage to hepatocytes and biliary epithelium results in liver fibrosis and the development of end stage liver disease. The diagnosis and treatment of primary biliary cirrhosis (PBC), autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC) remains hampered by a failure to fully ascertain all the pathogenic mechanisms that lead up to loss of immune tolerance, and chronic self-mediated liver injury.

Akin to all disease, autoimmune liver diseases are complex and reflect the interaction, dynamically of the host (e.g. genome, epigenome, microbiome) and their environment (e.g. xenobiotics and infection). Because of the long lag time likely between environmental exposure and development of overt disease, it remains challenging to make progress on defining fully environmental triggers. However genetic insights into autoimmune liver disease have been more fruitful, particularly of late. These have harnessed the willingness of international collaborators to share DNA collections, and the increasing confidence with which it has become possible to determine genetic association across whole genomes in well characterised cohorts.

To date association studies have focused on disease risk, rather than disease phenotype (clinical, laboratory, outcome). Association studies confirm clearly across PBC, AIH and PSC the strength and importance of HLA risk haplotypes in disease initiation. Translating HLA genetic signals into disease mechanisms continues however to prove hard, and remains an important area to focus future research towards. Non-HLA loci have with application of genome wide studies (and the Immunochip project) provided numerous robust non-HLA risk loci for follow up in larger genetic studies, as well as mechanistically. PBC risk studies have highlighted for example the role of the IL-12/STAT4 signalling cascade whilst in PSC, loci such as IL2RA, IL-2/IL-21, and MST1 are amongst many now recognised as associated with disease risk. AIH studies have lagged behind those of PBC and PSC, as cohorts are evolving at a slower pace, but it is expected that similar immunogenetic insights will arise.

The attempts to date to address the genetic risk factors for autoimmune liver disease, highlighting advances in knowledge, will be discussed, as well as the challenges ahead for translation of findings into new therapies for patients. The need to stratify association by clinical phenotype is highlighted, alongside the importance not only of further genetic studies using whole genome/epigenome technologies, but the alignment of genetic risk with microbiome data and environmental triggers.
Pathogenesis and ‘State-of-the-Art’ treatment of autoimmune hepatitis

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Autoimmune hepatitis (AIH) is a disease of unknown etiology. However, a loss of tolerance against the patients’ own liver is regarded as the main pathogenetic mechanism. One major pathogenetic hypothesis is that environmental agents trigger the self-perpetuating autoimmune process in a genetically susceptible individual. Genes of the MHC locus are responsible for approximately 50% of the genetic risk; other non-MHC genes include CTLA-4, the vitamin D receptor, but not the AIRE gene. Among the environmental candidates triggering AIH are drugs and infectious agents like hepatitis A, C and lately E virus.

Immunosuppressive therapy prolongs survival in patients with severe AIH. Two phases of therapy have to be distinguished. In newly diagnosed AIH induction of remission is the first main goal. In the past reduction of aminotransferase levels below two times the upper limit of normal was the aim of therapy. Nowadays, normalisation of aminotransferase levels should be achieved. The majority of patients usually respond to therapy within six to twelve months. A significant reduction of aminotransferase levels is achieved within a few weeks of therapy. Improvement in clinical symptoms is followed by improvement in biochemical parameters of disease activity and then by improvement in histology. Prednis(ol)one alone or in combination with azathioprine was shown to induce remission in the majority of patients. Nowadays, the topical steroid budesonide in combination with azathioprine is an alternative to prednis(ol)one. Budesonide was shown to induce disease remission in combination with azathioprine with less steroid specific side effects when compared to prednisone. In addition, budesonide can maintain a remission previously induced by prednisone in combination with azathioprine. Once remission is achieved patients should be kept in remission with the lowest dose of immunosuppression possible. Careful evaluation of the individual patient should lead to the decision whether prednis(ol)one, budesonide, azathioprine or a combination of one of the steroids with azathioprine is used to maintain remission. Patients treated with budesonide should not have liver cirrhosis since the benefit of budesonide with its 90% pass effect in the liver is lost if the patient has already developed porto-systemic shunting; in addition there are safety concerns for budesonide use in cirrhotic patients. Around 20–40% of patients do not achieve remission. In these patients alternative therapies should be evaluated for the individual patient. Prospective controlled trials with a larger number of patients are missing in this population. At the moment mofetil/mycophenolate (MMF) at a dose of 2 x 1 g daily is able to achieve remission in a significant proportion of patients, either given alone or in combination with prednis(ol)one as second line treatment. Based on recent restrospective observations MMF is beneficial in previous azathioprine intolerant rather than azathioprine failure patients. Again prospective trials are missing. Alternative drugs include cyclosporin A, tacrolimus, cyclophosphamide, and others. In particular women suffer from steroid specific side effects including weight gain, moon face, diabetes, glaucoma and bone disease. If the diagnosis is correct and the appropriate therapy is choosen liver transplantation should be avoidable in patients with AIH. AIH is still responsible for 4% of liver transplantations in Europe and the US.
Pathogenesis of PBC and PBC-associated fatigue

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The advent of large national and international patient consortia has dramatically changed the landscape regarding study of the pathogenesis of PBC. National cohorts are reaching 6,000 patients with international collaboration leading to the potential for cohorts of 10,000 or more patients. Initially work has focused on the genetic basis of PBC with a clear and consistent pattern of findings from GWAS and related studies, implicating immunoregulatory pathways in PBC pathogenesis. These are particularly focused around HLA and, in particular, the IL12 and IL12 signalling pathways. Other important genetic factors appear to regulate T cell trafficking into tissues. The remarkably consistent genetic findings all point to PBC being a disease in which dysregulation of the immune response plays a critical role. Studies looking at potential triggers of immune abnormality have been more limited and will represent a major area of opportunity over the next few years. Data on epidemiology of the disease does all, however, point to environmental factors contributing to disease pathogenesis with some evidence to suggest that temporal as well as physical associations are important. Whether knowledge with regard to disease triggers leads to changes in disease management through, for example, risk factor avoidance, is far from clear. Work on mechanisms of disease expression have focused on the pathway of abnormality of bile duct biology and in particular the potential role played by hydrophobic bile acids in epithelial cell physiology in the bile duct. This is likely to be an area of major therapeutic progress in the next few years. National patient cohorts have also given adequate power to the studies of symptoms of PBC. It is now clear that fatigue is indeed a major factor in the lives of patients and is associated with poor quality of life, particularly when associated with significant social dysfunction. Fatigue appears quite resistant in PBC and shows little if any improvement with transplantation. Autonomic dysfunction and sleep disturbance, together with abnormalities in muscle biogenetic function, appear to be associated with fatigue and studies of the integrated biology of fatigue, and its treatment are now underway. Ultimately national and international patient platforms will represent the context in which to do innovative large scale clinical trials of agents both able to treat the disease itself and its important manifestations such as fatigue.
Evidence-based treatment of primary biliary cirrhosis (PBC)

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The natural history of PBC has improved greatly during the past two decades because of its diagnosis at earlier stages and the widespread use of ursodeoxycholic acid as treatment. As a result, far fewer patients require liver transplantation, and patients with stages I and II PBC appear to have a normal life expectancy. The treatment of PBC will be reviewed here. These issues are also discussed in 2009 guidelines from the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver.

First-line therapy

All PBC patients with abnormal liver biochemistries should be considered for specific therapy.

Ursodeoxycholic acid (UDCA)
UDCA, at a dose of 13–15 mg/kg/day is currently considered the mainstay of therapy for PBC. This is based on a series of evidence which are summarized below.

Effect on survival
Because the number of patients randomized in almost all individual trials was too small to detect a difference in survival in the short term, a combined analysis of three randomized trials (with a total of 548 patients) [1–3] was subsequently performed [4]. UDCA was associated with a significant reduction in the likelihood of liver transplantation or death (47 versus 66 patients at four years) [4]. This benefit was seen in patients with moderate and severe disease. In several separate reports, the benefit of UDCA persists for up to 20 years.

Four other meta-analyses have been reported, which have reached variable conclusions [5–8]. Two meta-analysis [5, 6] found, that UDCA had no effect on overall or transplant-free mortality. Two other meta-analyses (excluding trials of short duration < 2 years and suboptimal dose of UDCA, < 10 mg/kg/day, concluded that long-term UDCA significantly improved transplant-free survival and delayed histologic progression in early-stage patients.

Long-term follow-up reports consistently found that patients with a good biochemical response to UDCA have an excellent prognosis. The annual rate of liver transplantations is decreasing in several countries despite an overall increasing incidence of the disease.

Other effects
UDCA improves liver biochemistries, immune parameters (IgM, IgG, AMA titers), delays fibrosis progression and development of oesophageal varices. UDCA is extremely safe and well tolerated [9, 10].

UDCA is thus advocated as first-line therapy in PBC.
The extent of the biochemical response to UDCA during the first year of therapy is a very simple and useful marker of long-term prognosis. About 35% of patients have a suboptimal response to UDCA. These patients need adjuvant therapy.

**Drugs that are ineffective and/or toxic**
Several drugs are ineffective or toxic in PBC. These include penicillamine, colchicine, azathioprine, chlorambucil, prednisolone, cyclosporine, thalidomide and silymarin.

**Adjuvant therapies**

**Budesonide**
Budesonide is a nonhalogenated glucocorticoid absorbed in the small intestine. Of an oral dose, 90% is metabolized during the first liver pass in healthy individuals. Compared with prednisolone, glucocorticoid receptor binding activity of budesonide is 15–20 times higher, so its effect on liver inflammation and biliary alkaline secretion may be greater. In patients with inflammatory bowel disease and autoimmune hepatitis, oral budesonide has been shown to exert fewer systemic side effects than do conventional corticosteroids. Two randomized studies showed budesonide (6–9 mg/d) combined with UDCA to be more effective in improving liver biochemistries and histology than did UDCA alone in patients with stage I to III PBC [11, 12]. Combination therapy (UDCA-budesonide) is currently evaluated in a randomized control trial in Europe.

**Controversial approaches**
Two drugs, methotrexate and colchicine, have a long-history in the care of patients with PBC, but their role remains uncertain. Data supporting the benefit have been derived mostly from case series and small controlled trials, not all of which have suggested a benefit. While they continue to be used in some centers, their role is generally considered unproven.

**Investigational approaches**
Investigational approaches (Phase 2–3 trials) include:
- fibrates (fenofibrate, a specific PPAR alpha agonist and bezafibrate, a pan PPAR agonist)
- FXR agonists
- triple therapy wit UDCA-budesonide and mycophenolate mofetil
- antiretrovirals
- molecular therapies (anti-CD20, CD80, CXCL10, anti-interleukin 12/interleukin 23)

**Liver transplantation**
PBC is a common, albeit decreasing, indication for liver transplantation. The prevalence of recurrent PBC post transplant ranges between 9% and 35% with a mean time that ranges between 1.6–6.5 years. Among factors proposed to affect the rate of recurrence include is the use of tacrolimus as the mainstay for immunosuppression. Despite the possibility of recurrence, OLT has greatly improved survival in patients with PBC with a reported survival rate of 92% and 85% at 1 and 5 years, respectively.
References:


Immunopathogenesis of PSC

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Autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are all associated with gut inflammation; PSC and AIH being strongly linked to inflammatory bowel disease (IBD) and PBC to coeliac disease. This clinical observation has stimulated several intriguing hypotheses to explain how gut commensals, pathogens and intestinal antigens can trigger liver injury. Th17 cells have been linked to the pathogenesis of AIH, PBC and more recently PSC. Given the key role of the intestine in regulating immunopathogenic Th17 responses this may provide one shared disease mechanism. The discovery that long-lived mucosal memory T-cells can be recruited to the liver in response to aberrantly expressed endothelial-cell adhesion molecules and chemokines which are normally ‘gut-restricted’ provides a mechanism to explain why these diseases are associated with site-restricted tissue distributions. It also opens up new therapeutic strategies based on modulating tissue specific lymphocyte homing. The recent findings that particular gene-polyorphisms confer combined PSC/IBD susceptibility underscores the fundamental role of mucosal immunity in disease pathogenesis. Thus understanding how immune mechanisms regulate immunity in the liver and gut could open up new therapeutic avenues and encourage clinical trials to test the efficacy of biologics shown to work in IBD in IBD associated liver disease.
Therapeutic role of bile salts and nuclear receptor agonists in fibrosing cholangiopathies

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Improved understanding of the molecular mechanisms of bile formation and cholestasis has opened new perspectives for targeted therapies. The etiology and pathogenesis of chronic cholestatic disorders/fibrosing cholangiopathies such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are still poorly understood. Cholestatic liver diseases are generally characterized by impaired hepatobiliary excretory function ultimately resulting in accumulation of bile salts (BS) and other cholephils. The accumulation of potentially toxic BS leads to hepatocellular damage followed by inflammation, fibrosis, and finally – depending on the disease etiology – may culminate in liver cirrhosis and hepatocellular or cholangiocellular cancer. To handle potentially toxic cholephils under physiological and pathological conditions, the liver possesses a complex network of BS-activated nuclear receptor (NR)-regulated pathways that coordinate BS homeostasis and bile secretion to limit their concentrations and prevent hepatic as well as systemic accumulation. The most relevant BS-activated NRs for regulation of hepatobiliary homeostasis, bile secretion and thereby understanding and treating cholestasis, include the farnesoid X receptor (FXR, NR1H4), pregnane X receptor (PXR, NR1I2) and vitamin D receptor (VDR, NR1I1). Apart from BS other biliary constituents such as bilirubin can also activate NRs such as the constitutive androstane receptor (CAR, NR1I3). Furthermore other NRs such as glucocorticoid receptor (GR, NR3C1) and fatty acid-activated peroxisome proliferator-activated receptors PPARs, in particular PPARα (NR1C1) and PPARγ (NR1C3) as regulators of inflammation, fibrosis and energy homeostasis, may also impact on BS homeostasis and cholestatic liver injury. Due to their capability to control hepatic BS metabolism, hepatic inflammation and fibrosis, NRs in general and BS-activated NRs in particular have emerged as promising therapeutic targets in cholestatic disorders and fibrosing cholangiopathies. Many drugs used as treatment for cholestasis act via NRs. Ursodeoxycholic acid (UDCA) is the paradigm therapeutic BS which is effective in PBC and many other cholestatic disorders. UDCA has multiple beneficial mechanisms which may be mediated to at least in part by NRs (e.g., GR, PXR). Apart from UDCA, other already available drugs may exert their beneficial effects in cholestasis via NR activation (e.g., rifampicin via PXR; fibrates via PPARα, budesonide via GR). The most promising future BS-based therapeutic options for targeted therapy of cholestatic liver diseases include 24-norursodeoxycholic acid (norUDCA) and bile acid receptor/farnesoid X receptor (FXR) agonists (e.g., obeticholic acid (OCA)). Such new therapeutic approaches may be particularly relevant for PSC (with currently limited therapeutic options) and non-respondes to UDCA standard therapy in PBC.

norUDCA is a side chain-shortened C23 homologue of UDCA which possesses one less methylene group in its side chain and is more resistant to conjugation with taurine or glycine than UDCA, but instead is secreted into bile mostly in unchanged form. The secreted norUDCA undergoes absorption by cholangiocytes, returns to the liver and is resecreted into bile. Such cholehepatic shunting leads to a bicarbonate-rich hyper choleresis and may also result in improved targeting to the liver and disease bile ducts (‘ductular targeting’). norUDCA (but not “conventional” UDCA) reversed sclerosing
cholangitis in the Mdr2 (Abcb4)^−/− cholangiopathy model within 4 weeks of treatment. Its possible therapeutic mechanisms include (i) amelioration of bile hydrophobicity by biliary enrichment with hydrophilic norUDCA and its metabolites, (ii) flushing of injured bile ducts by stimulation of bile flow and bicarbonate-rich choleresis, which dilutes toxic biliary content and reinforces the bicarbonate umbrella protecting against potentially toxic bile acids, (iii) induction of alternative bile acid detoxification (phase I and II enzymes) and elimination routes for bile acids, and (iv) direct anti-inflammatory and antifibrotic properties. Notably, tauro-norUDCA which lacks cholehepatic hepatic shunting with stimulation of bicarbonate secretion also looses the therapeutics effects. Gene expression and metabolomic profiling revealed profound alterations in fatty acid and triglyceride metabolism, including a restoration of elevated short-chain and medium-chain fatty acids and reduced long-chain fatty acids resulted in a less lipotoxic lipid profile in the Mdr2^−/− cholangiopathy model by norUDCA. norUDCA also targets the inflammatory cross talk between cells involved in inflammation and fibrogenesis in sclerosing cholangitis. As such, norUDCA represents a multi-targeted therapeutic approach, targeting hepatocytes, cholangiocytes and Kupffer cells. Such a multi-targeted therapeutic approach may be essential for the treatment of a complex multifactorial disease such as PSC, as well as other cholangiopathies such as PBC. Following the very encouraging experimental data in preclinical (P)SC models, Phase I clinical trials have been completed and a multicenter Phase II dose-finding study testing norUDCA in PSC is well on its way.

A non-BS synthetic FXR agonist GW4064 and a BS-derived 6α-ethyl derivative of CDCA (also known as 6-ECDCA or INT-747 or obeticholic acid [OCA]) have shown beneficial effects in mouse models of chemically-induced liver injury (ANIT and estradiol-induced) or in bile duct-ligation (BDL). A dual ligand with high affinity to FXR (INT-767, but not the clincial lead compound INT-747/OCA) was able to cure bile duct injury in the Mdr2 (Abcb4)^−/− cholangiopathy model. Subsequent studies in FXR knock-out mice revealed that these effects were mediated exclusively by FXR and not by TGR5. The therapeutic mechanisms involved suppression of BS synthesis and indirect anti-inflammatory and antifibrotic effects and silencing of the reactive cholangiocyte phenotype. Notably, similar to norUDCA this therapeutic effect was also linked to generation of a bicarbonate-rich choleresis which appears to be a common denominator for successful treatment of cholangiopathies in general.

Apart from targeting the underlying cholestatic liver disease, additional challenges in the treatment of PSC may come from associated IBD and the high risk for malignancy in the hepatobiliary tract and colorectum. FXR ligands may target both PSC and associated IBD. Moreover, FXR is linked to the control of the gut flora and maintenance of gut integrity, making it a valid target in IBD. FXR also protects mice against intestinal tumorigenesis and loss of FXR has been linked to colorectal in mice and men. In a phase II clinical trial in PBC patients not responding to UDCA, addition of OCA showed substantial reduction of biochemical cholestasis parameters. In line, OCA monotherapy also improved biochemical cholestasis parameters. Dose dependent itching was the most common adverse event in patients receiving higher doses of OCA. A multicenter, placebo-controlled, randomized phase III clinical trial, testing OCA in PBC patients who have not non-responded to standard UDCA is about to be completed and the results are eagerly awaited.

Another interesting target for therapy in cholestasis may be TGR5, a G-protein coupled BS receptor at a plasma membrane. Notably, TGR5 polymorphisms have been associated with pathogenesis of PSC and ulcerative colitis. A selective TGR5 agonist was not able to reverse sclerosing cholangitis in the Mdr2/Abcb4^−/− model but may have
anti-inflammatory effects in the gut. Of great concern may be, that TGR5 has been shown to be up-regulated in CCC where it may confer resistance to apoptosis. It is important to emphasize, that neither UDCA nor norUDCA are FXR or TGR5 ligands.

In conclusion, we witness a revolution of expanding use of BS-derived and/or BS-targeted therapies in cholestatic liver diseases and fibrosing cholangiopathies. The translation of expanding knowledge on NRs and novel insights into BS (patho)biology should result in optimization of the currently available therapies with careful selection of patients’ subgroups benefiting from such novel targeted therapeutic approaches.

References (further reading):

Pathogenesis and treatment of pruritus in cholestasis

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Chronic pruritus is a burdensome feature of numerous hepatobiliary disorders such as primary biliary cirrhosis, primary sclerosing cholangitis, cholangiocarcinoma, inherited forms of cholestasis and intrahepatic cholestasis of pregnancy. Pruritus may be mild, but can also become the most agonizing symptom for patients with cholestatic disorders resulting in sleep deprivation, depressive mood and even suicidal ideations in severe cases. Although primary skin lesions are not observed in these patients, secondary skin alterations may result as sequelae of intense scratching.

Bile salts, μ-opioids, histamine and steroids have been controversially discussed in the pathogenesis of cholestatic pruritus. However, for these substances neither a correlation with itch severity nor a causative link has ever been established[^1,4]. Clinical and experimental observations indicate that the itch-causing molecules (i) accumulate in the systemic circulation as suggested by attenuation of severe pruritus after treatment with plasmapheresis or albumin dialysis; (ii) are secreted into bile as indicated by rapid relief of severe, treatment-refractory pruritus after nasobiliary drainage; (iii) are (biotrans-)formed in liver and/or gut as indicated by effective treatment with the potent pregnane X receptor (PXR) agonist, rifampicin; (iv) affect the endogenous opioidergic and serotoninergic system as suggested by moderate anti-pruritic activity of μ-opioid antagonists and serotonin reuptake inhibitors.

The anion exchange resin colestyramine, the PXR-agonist rifampicin, the μ-opioid antagonist naltrexone and the serotonin reuptake inhibitor sertraline are recommended by evidence-based guidelines as a stepwise therapeutic approach to treat cholestatic pruritus[^5,6]. Patients unresponsive to these drugs should be referred to specialized centres to receive experimental approaches such as UVB phototherapy, albumin dialysis, plasmapheresis or nasobiliary drainage.

Screening sera of pruritic cholestatic patients for the capacity of neuronal activation we could recently unravel lysophosphatidic acid (LPA) as main mediator[^7]. Intradermally applied LPA caused scratching behaviour in mice. The serum activity of lysophospholipase autotaxin (ATX), which largely determines serum LPA levels, correlated with itch intensity and response to treatment in patients with cholestatic pruritus, but not other forms of pruritus. Autotaxin activity had a positive predictive value of 70% in differentiating cholestatic pruritus from pruritus associated with atopic dermatitis, uremia and Hodgkin lymphoma. The beneficial effect of rifampicin may be due to inhibition of ATX expression[^8]. As ATX and LPA are not present in bile an additional factor potentially exists in bile which may interact with the ATX-LPA-axis[^8,9]. The causal relationship, expression pattern and exact mode of action of ATX and LPA during cholestasis remain to be further elucidated.
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POSTER ABSTRACTS

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Evaluation of serum nitric oxide before and after local radiofrequency thermal ablation for hepatocellular carcinoma

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HCC is one of the leading causes of worldwide cancer mortality due to late diagnosis. Chronic hepatitis C virus is one of the main risk factor for the development of hepatocellular carcinoma (HCC), which is a multi-step process involving different genetic alteration that leads to malignant transformation of hepatocyte. Genetic and molecular abnormalities associated with viral infection or due to inflammatory condition represent an early step in hepatocarcinogenesis. (HCC) is a hypervascular solid cancer. Tumor growth depends on angiogenesis, and the “angiogenic switch” of preexisting vessels is required to allow tumor progression, growth, and propagation to supply nutrients and oxygen. Inducible nitric oxide synthase (iNOS) also play an important role in angiogenesis, regulating several biological processes crucial for tumor growth.

Objective: Evaluation of serum nitric oxide before and after local radiofrequency thermal ablation for hepatocellular carcinoma.

Subjects: 20 patients with proven hepatocellular carcinoma and 15 healthy as controls were enrolled in the study.

Methods: History taking, clinical examination, laboratory testing (ALT, AST, Bil, γGT, ALP, albumin, AFP, NO), ultrasound and Spiral-CT. Evaluation was done initially and repeated after 2 weeks of tumor ablation by local radiofrequency thermal ablation.

Results: Median of serum nitric oxide was statistically significantly higher among HCC patients before radiofrequency thermal ablation (1200 µmol/l) compared to controls (22 µmol/l) were p < 0.001, also the median of NO was statistically significantly declined after radiofrequency thermal ablation compared to before (160, 1200 µmol/l) respectively were p < 0.001.

Conclusion: The data suggest that there is elevation in serum nitric oxide in HCC patients and that is locally produced from the tumor and hence its level significantly drops after local radiofrequency thermal ablation.

Keywords: nitric oxide, hepatocellular carcinoma, radiofrequency
Adiponectin a differential marker between steatosis and steatohepatitis

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Nonalcoholic fatty liver disease (NAFLD) becoming a worldwide public health problem. It represents a spectrum of disease ranging from simple steatosis to steatohepatitis (NASH). Adipocytokines refer to adipocyte-derived biologically active molecules TNF-\(\alpha\), leptin and adiponectin, all been implicated in development of hepatic inflammation and fibrosis in NAFLD patients. This new hormone differs from its predecessors in important feature, production and concentration acutally decrease in obesity, and all adipose-derived hormones are increased. It is possible that adiponectin expression is activated during adipogenesis, a feedback inhibition on its production may occur during the development of obesity. Adiponectin may exert a hepatic protective effect.

**Aim:** Evaluation of adiponectin level as a differential marker between steatosis and steatohepatitis.

**Subjects and Methods:** 20 NAFLD patients, 20 biopsies proved NASH and 20 control subjects, matched for age, sex and BMI. All the subjects were subjected to an abdominal ultrasonography, routine biochemical evaluation: liver function ALT & AST, lipid profile (cholesterol, triglycerides, HDL-C, LDL-C), CRP & adipocytokines (TNF-\(\alpha\), IL-6, leptin & adiponectin).

**Results:**
1. Plasma adiponectin levels were significantly lower in NAFLD patients than control gp (6.15 ± 1.39 ng/ml vs. 12.03 ± 3.46 ng/ml).
2. Adiponectin was significantly lower in NASH than NAFLD (1.800 ± 0.96 ng/ml vs. 6.15 ± 1.39 ng/ml).
3. Leptin level was significantly higher in NAFLD than NASH gp (69.50 ± 18.70 ng/ml vs. 43.20 ± 6.93 ng/ml).
4. Adiponectin ROC curve showed an AUROC curve in NAFLD gp (0.945 p = 0.049) while in NASH was (0.995 p = 0.007).
5. TNF-\(\alpha\) & IL-6 was significantly higher in NASH than NAFLD gp (79.25 ± 13.89 pg/ml vs. 41.25 ± 17.53 pg/ml) and (110.20 ± 55.34 pg/ml vs. 43.85 ± 16.13).
6. Plasma adiponectin level in NAFLD gp was inversely correlated with T.G (r = -0.368 p = 0.111), GOT (r = -0.037 p = 0.878) & GPT (r = -0.022 p = 0.926) while it was +ve correlated in NASH gp with cholesterol (r = 0.317 p = 0.174) & T.G (r = 0.042 p = 0.861).

**Conclusion:**
1. This data support a role for low circulating adiponectin in the pathogenesis of NAFLD and hypoadiponectinemia found to be a feature of NASH.
2. Adiponectin found to be a non-invasive differential marker between NAFLD & NASH.
Evaluation of nitric oxide as a novel diagnostic marker for hepatocellular carcinoma

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Background: Liver cancer is the sixth most common cancer worldwide. HCC is the most common primary tumour of the liver. The National Comprehensive Cancer Network (NCCN) clinical practice guidelines for treatment of hepatobiliary cancers propose surveillance for the early detection of HCC by liver ultrasonography every 3 to 6 months and evaluation of AFP. AFP > 200 ng/ml is considered diagnostic for HCC, although fewer than half of patients of HCC may generate levels that are high, so that the specificity of AFP is close to 100% but the sensitivity is 45%. Nitrite/Nitrate is a stable end product of nitric oxide increase in patients with HCC.


Material and Methods:
80 patients and 15 normal individuals enrolled in the study:
- Group 1: 15 normal individuals
- Group 2: 30 patients with chronic liver disease without HCC
- Group 3: 50 patients with HCC

History taking, clinical examination (detection of liver masses, ascites, spleen size, grade of encephalopathy) Child-Pugh scoring. Laboratory investigation: (ALT, AST, bilirubin, albumin, prothrombin, GGT, platelet count, AFP, nitric oxide, HBs-Ag, HCV-Ab). Abdominal ultrasonography and spiral CT.

Results: The median level of nitric oxide was significantly higher in Group 3 (170 µmol/l) than in Group 2 (56 µmol/l) than in Group 1 (22 µmol/l), with a sensitivity of 68% and a specificity of 90% at a cut-off level of 110 µmol/l and area under the curve of 0.810. While AFP; at a cut-off level of 200 ng/ml had a sensitivity of 52%, a specificity of 100% & an area under the curve of 0.855. Indeed nitric oxide was high in 42% of AFP-negative HCC patients.

Conclusion: Nitric oxide is a novel diagnostic marker for hepatocellular carcinoma, the simultaneous determination of serum nitric oxide & AFP gave significant improvement in detection of HCC patients compared to that of AFP alone.
Decompensated alcoholic liver disease (ALD): High long-term mortality despite initial survival

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Introduction: Early management and outcome of decompensated ALD has been extensively studied, there are few published data on long-term outcome. We studied 249 patients (163 men, age [mean 50 yr] admitted consecutively to our unit (1/4/1998–31/12/2005) with first presentation of decompensated ALD (Child grade B or C). We aimed to assess long-term mortality and its associations.

Methods: We reviewed hospital records and death certificates to assess who had died, causes of death and overall alcohol drinking behaviour (classified as: abstinent, continued drinking but reduced, did not reduce).

Results: 37 patients died at index hospital episode because of liver disease. The other 212 patients were followed for 4.3 (0.03–13.0) years. 154 patients have subsequently died. Cause of death is known in 152 (98%) and was due to liver disease in 102 (67%). Overall 5, 10 year total mortality rates 52 ± (SEM)4% and 75 ± 3% respectively; corresponding rates from causes known to be liver related 41 ± 5% and 51 ± 4%. Patients were abstinent (n = 52) had lower total and known liver-related mortality (61 ± 9% and 20 ± 6% after 10 yr) compared to those who continued but reduced (n = 105; 73 ± 5% p = 0.122 and 53 ± 6% p = 0.013) and to those who did not reduce (n = 53; 91 ± 4% p < 0.001 and 71 ± 7% p < 0.001). In Cox regression analysis, total and known liver-related mortality were independent of age, gender and severity of liver dysfunction at presentation but were strongly associated with subsequent drinking behaviour (both p < 0.001) and inversely associated with serum albumin at discharge (p = 0.001 and 0.019).

Discussion/Conclusion: Patients with decompensated ALD who survive their first hospital episode have high long-term mortality, mainly due to liver disease, which is reduced but not prevented by abstinence.
Glucagon-like peptide-1 analogue, Liraglutide, reduces adipose insulin resistance and hepatic de novo lipogenesis in nonalcoholic steatohepatitis: Sub-study results of a phase II randomised-control clinical trial

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Introduction: Adipose tissue insulin resistance and lipotoxicity are key pathognomonic features in nonalcoholic steatohepatitis (NASH). Liraglutide is a once-daily, glucagon-like peptide-1 (GLP-1) analogue that significantly improves glycaemic control, weight and hepatic steatosis. The aim of this phase II study was to determine the effect of Liraglutide on insulin sensitivity (hepatic, muscle, adipose), hepatic lipogenesis and markers of adipose inflammation.

Methods: 14 patients with biopsy-proven NASH were randomly assigned to 1.8 mg Liraglutide or placebo (once-daily subcutaneous) for 12-weeks as part of the metabolic sub-study of the double-blind, randomised, placebo-controlled LEAN trial (clinicaltrials.gov. NCT01237119). At baseline and 12-weeks, patients underwent paired 2-step hyperinsulinaemic euglycaemic clamps incorporating stable isotopes with concomitant adipose tissue microdialysis. Serum adipocytokines were quantified with Fluorokine® MAP multiplex kits. In-vitro isotope experiments were performed with Huh-7 and primary human hepatocytes.

Results: 1.8 mg Liraglutide significantly decreased weight, waist circumference, HbA1c, fasting glucose, LDL and liver enzymes versus placebo. Liraglutide significantly increased the suppression of hepatic glucose production with low-dose insulin. Liraglutide significantly decreased circulating NEFA in the fasting, low-dose and high-dose insulin states. Liraglutide significantly reduced the insulin concentration required to ½-maximally suppress circulating NEFA. Liraglutide significantly decreased adipose tissue lipolysis, as demonstrated by a reduction in interstitial fluid glycerol concentrations. Furthermore, Liraglutide significantly improved serum markers of adipose inflammation, namely leptin, adiponectin, and CCL-2. In addition, Liraglutide significantly decreased de novo lipogenesis in-vivo, as measured by incorporation of deuterated ²H₂O into palmitate, versus placebo. Endorsing our clinical observations, in-vitro experiments using both the Huh-7 cell line and primary cultures of human hepatocytes showed decreased de novo lipogenesis, measured by ¹⁴C-acetate incorporation into cellular lipid following treatment with GLP-1 (exendin-4).
Discussion/Conclusion: Liraglutide significantly reduces metabolic dysfunction, hepatic lipogenesis, hepatic/adipose insulin resistance and adipose inflammation in patients with NASH. GLP-1 analogue therapy may represent a novel treatment for patients with NASH.
Comparative study of the efficacy of rifaximin in comparison with lactulose for the treatment of hepatic encephalopathy

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Introduction: The most widely accepted theory of the pathogenesis of HE is, that nitrogenous substances derived from the gut adversely affect the cerebral function. The main substance implicated is ammonia. Rifaximin is a derivative of rifamycin, which acts by inhibiting bacterial ribonucleic acid (RNA) synthesis. Rifaximin is virtually unabsorbed after oral administration and exhibits broad spectrum antimicrobial activity against both aerobic and anaerobic gram-positive and gram-negative microorganisms within the gastrointestinal tract.

Methods: The study population included 50 patients were diagnosed to have signs of the first to third degree HE and classified into two groups: Group I: included 25 patients who had hepatic encephalopathy and were treated with rifaximin (1200 mg daily divided into 3 doses for 7 days). Group II: included 25 patients who had hepatic encephalopathy and were treated with lactulose (90 ml daily divided into 3 doses for 7 days).

Results: The results showed that the both rifaximin & lactulose can decrease ammonia level by different mechanisms, but the response of patients regarding improvement of symptoms of HE was more & rapid in rifaximin group than lactulose group.

<table>
<thead>
<tr>
<th></th>
<th>Pre treatment</th>
<th>Post treatment</th>
<th>p value³</th>
</tr>
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<tbody>
<tr>
<td>Group I Rifaximin (n = 25)</td>
<td>182.4 ± 36.436</td>
<td>120.8 ± 44.8</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Group II Lactulose (n = 25)</td>
<td>162.2 ± 60.4</td>
<td>118.7 ± 50.7</td>
<td>&lt; 0.001*</td>
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Discussion/Conclusion: Patients treated with rifaximin required shorter duration of hospitalization compared to lactulose, also rifaximin was better tolerated than other pharmacologic treatments.
**Frequency and predictive factors of granulomatous hepatitis in patients with peritoneal tuberculosis**

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**Introduction:** The aims of our study were to determine the frequency of granulomatous hepatitis in patients with peritoneal tuberculosis, to identify factors for high risk and whether it is associated with higher frequency of antituberculous treatment side effects.

**Methods:** We carried out a prospective study on patients with histologically proven peritoneal tuberculosis. We performed a liver biopsy in all the patients before starting the antituberculous treatment. Granulomatous hepatitis was systematically searched in all patients. Statistical analysis was performed with SPSS software version 19.0.

**Results:** The study was conducted in 52 patients, 9 men and 43 women of median age of 35.5 years. A granulomatous hepatitis was seen in 24 patients (46%). In univariate analysis the factors associated with a high risk of liver involvement were a higher level of gamma-glutamyl transpeptidase (44.5 ± 36.8 IU/l vs. 23.3 ± 9.28 IU/l p = 0.005), a higher level of phosphatases alkalines (233.9 ± 96.6 IU/l vs. 189.4 ± 49.9 IU/l p = 0.03) and a lower level of cholesterol (1.22 ± 0.2 g/l vs. 1.56 ± 0.3 g/l p < 0.0001). In multivariate analysis, only a cholesterol level lower than 1.31 g/l was significantly associated with a granulomatous hepatitis (p = 0.006 OR [95% CI]: 0.10 [0.02–0.52]).

**Discussion/Conclusion:** We have found a frequent liver involvement in the case of peritoneal tuberculosis (46%). Cholesterol level lower than 1.31 gr/l was an independent predictor of granulomatous hepatitis in patients with peritoneal tuberculosis. We suggest, in this case, that percutaneous liver biopsy can be considered as an alternative to laparoscopy.
Analysis of polymorphisms of BER genes (hOGG1, XRCC1) in HCC patients

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Introduction: It is known that increased oxidative DNA damage, as well as mutations and deletions in genes encoding antioxidant and DNA repair proteins are associated with the development of primary hepatocellular carcinoma. DNA base-excision repair genes hOGG1 and XRCC1 play an important role in preserving genetic stability in mammalian cells against any damage caused by different factors. However, it is unclear whether altered expression and function of these DNA repair genes could lead to hepatocellular carcinoma (HCC) susceptibility. In our study determined the association between polymorphisms of the genes encoding two key proteins of DNA base excision repair (hOGG1 ser326Cys and XRCC1 Arg 280His) and HCC risk. The data from the current study demonstrated the association of these two DNA repair gene polymorphisms with HCC risk.

Methods: For genotyping, DNA will be isolated from 40 HCC patients paraffin blocks and 40 histologically unchanged liver paraffin blocks. Polymorphism of DNA base-excision repair genes hOGG1 and XRCC1 was identified by High Resolution Melting (HRM) in the presence of LCGreen Plus dye (Idaho) or sequencing method.

Results: To evaluate the role of the hOGG1 Ser326Cys polymorphism in HCC patients, we screened 40 HCC patients paraffin blocks and 40 histologically unchanged liver paraffin blocks as controls. We observed association between both the Cys326 allele ($p = 0.02$) and the combined Ser326Cys + Cys326Cys genotype (OR = 1.65, 95% CI = 1.06–2.88) and increased risk of disease. Combining the hOGG1 326 Cys/Cys genotype with the XRCC1 280 Arg/Arg genotypes (which may enhance BER) resulted with induction of disease risk (OR = 0.09; 95% CI = 0.01–0.56).

Discussion: The data showed that the hOGG1 Cys326Cys and Ser326Cys genotypes were associated with increase in HCC risk. In contrast, there was no association between HCC susceptibility and the distribution of XRCC1 His 280 His and Arg280His. However, combination of these two gene polymorphisms (XRCC1-280 Arg and hOGG1-326Cys) is associated with significant induction of HCC risk. In addition, the data also showed that XRCC1 280His polymorphism was associated with HBV infection. Polymorphism was associated with HBV infection and HCC family history to increase HCC risk. The hOGG1 326cys genotype was associated with HBV infection to increase HCC risk.

Conclusion: The data from the current study demonstrated the association of these two DNA repair gene polymorphisms with HCC risk.
Rat hepatocytes isolated from fatty liver are more sensitive to tert-butyl hydroperoxide-induced oxidative stress

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver disease. Oxidative stress was approved as an important pathogenetic factor. Aim of our study was to evaluate whether tert-butyl hydroperoxide (tBHP) causes more severe damage in hepatocytes isolated from rat fatty liver compared to non-steatotic liver.

Methods: Male Wistar rats were fed by standard diet (10% energy from fats) or high-fat diet (HFD, 70% energy from fats) for 6 weeks; hepatocytes were isolated by two-step collagenase perfusion, cultured in William’s E medium on collagen-coated dishes. tBHP was added in medium (0.1–1 mmol/l) for up to 60 min. To investigate role of mitochondrial permeability transition (MPT) cells were preincubated with MPT pore inhibitor trifluoperazine (TFP) for 30 minutes. Viability, integrity and functional status of hepatocytes were evaluated by measurement of lactatedehydrogenase (LDH) leakage, activity of cellular dehydrogenases (WST-1), content of glutathione (GSH and GSSG), reactive oxygen species (DCFDA), mitochondrial membrane potential (JC-1) and mitochondrial respiration (high-resolution oxygraphy – hepatocytes in suspension).

Results: Increased markers of cell injury were found even in non-treated steatotic hepatocytes in comparison with control cells (p < 0.001). tBHP causes earlier and more severe toxic effect on fatty hepatocytes, this effect is dose dependent. Activity of complex I (glutamate+malate) was significantly lower in intact steatotic hepatocytes, instead of activity of complex II was not affected. Higher sensitivity of complex I to oxidative stress was found both in lean and steatotic hepatocytes, nevertheless significantly more pronounced in fatty hepatocytes. TFP reduced cell damage caused by tBHP documented by LDH, WST-1 and especially JC-1 (p < 0.001).

Discussion/Conclusion: Steatotic hepatocytes exert higher sensitivity to oxidative stress compared to lean hepatocytes. This susceptibility may be caused by higher sensitivity to oxidative stress-induced reduction of complex I activity and opening of MPT pore in steatotic hepatocytes.

Supported by PRVOUK P37/02.
Intraoperative platelet transfusion is associated with poor graft and overall survival in liver transplantation

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Introduction: Hypersplenism causes significant thrombocytopaenia in patients with advanced liver cirrhosis awaiting liver transplantation. Recent studies have shown that intraoperative platelet transfusion is an independent risk factor for survival after orthotopic liver transplantation (OLT). We examined if there is a difference in graft and overall survival at 90 days and 1 year in patients receiving platelets intraoperatively during OLT.

Methods: A retrospective analysis was performed on 399 patients who had undergone first OLT from 2000 to 2009. Data on intraoperative platelet transfusion were retrieved from the UK and Ireland Transplant Registry and the haematology laboratory database. Graft and overall survival were analysed by Kaplan Meier and Mantel-Cox log rank test. Graft survival was defined as time from OLT to death or re-transplant.

Results: Between 2000–2009 258 patients (65%) received platelet transfusion during OLT. Graft survival was reduced significantly in patients receiving platelets at one year follow up (81.5% vs. 89.9%; p = 0.026) but not at 90 days (88.8% vs. 93.5%; p = 0.112). Overall survival was significantly reduced at both 90 days (91.9% vs. 97.1%; p = 0.039) and one year (87.6% vs. 94.2%; p = 0.036) in patients with platelet transfusion. The hazard ratio of platelet transfusion on 90 days survival was 2.37 (95% CI of 1.05 to 5.37) and at one-year was 2.02 (1.05 to 3.88).

Discussion/Conclusion: Platelet transfusion is associated with reduced survival after liver transplantation.
Can spleen stiffness reflect changes in portal pressure after liver transplantation?

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**Introduction:** Spleen stiffness measured by transient elastography has been shown to correlate with hepatic venous pressure gradient and can predict oesophageal varices. Elevated spleen stiffness in cirrhosis has been attributed to splenic tissue hyperplasia and fibrosis, portal hypertension and its consequent hyperdynamic circulation. The aim of this study was to evaluate changes in spleen stiffness after orthotopic liver transplantation (OLT) when portal hypertension resolves.

**Methods:** Twenty patients on the OLT waiting list were recruited prospectively. Spleen and liver stiffness were measured with Fibroscan before and at 2 to 4 weeks after OLT. Criteria applied for spleen stiffness measurement were similar to liver stiffness (≥ 10 measurements; ≥ 60% success rate; interquartile range, IQR < 30% of median). For patients with mild to moderate ascites, spleen stiffness was measured in the right lateral decubitus position before and after OLT.

**Results:** 90% (18/20) of patients have oesophageal varices on endoscopy or oesophageal and/or splenic varices on CT imaging. Spleen stiffness decreased significantly after OLT, from a median of 75.0 (IQR 63.9–75.0) kPa before transplant to 34.6 (28.1–43.7) kPa at weeks 2 to 4 after OLT (n = 16, p = 0.0005). As expected, liver stiffness measurements were higher before OLT at 44.3 (27.4–75.0) kPa (n = 7) and falls to 8.1 (6.2–9.9) kPa (n = 18) after transplant (p = 0.0002).

**Discussion/Conclusion:** Spleen stiffness can measure changes in portal pressure after liver transplantation and decreases significantly when portal hypertension resolves.
Large volume paracentesis (LVP) can be safely performed by junior doctors without ultrasound guidance

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**Introduction:** Introduction of the European Working Time Directive has lead to concerns about reduced exposure of junior doctors to practical procedures\(^1\). Use of ultrasound guidance for LVP has been suggested\(^2\). Our aim was to assess the safety of LVP performed at our centre according to the clinical grade of the operator.

**Methods:** Patients undergoing LVP in a 12 month period from October 2010 were identified by ascitic samples received by our microbiology department and also the departmental planned admission book. Case notes were reviewed and data was collected on patient demographics, method of insertion (blind vs. ultrasound guided), grade of operator, and occurrence of complications.

**Results:** 56 LVP were performed on 28 patients. 53 drains were successfully inserted blindly, 3 required ultrasound guidance. 2 drains were inserted by consultants (both ultrasound-guided) and 9 by registrars. 15 were inserted by core training doctors (1 procedure was supervised) and 28 by foundation doctors (19 supervised). Ascites was sent for white cell count after 53 (95%) procedures. No major procedure related complications occurred, 1 patient required a stitch for a minor cutaneous bleed following drain removal. 2 patients on surgical wards had the drain left in for more than 6 hours (10 hours and 3 days).

**Discussion/Conclusion:** LVP can be safely performed without ultrasound guidance by adequately trained or supervised junior doctors. Some failings occurred with timely drain removal and request for ascitic white cell count. However these would not have been prevented by using ultrasound guidance. Patients receiving ultrasound guided drains were actually more likely to receive sub-optimal post-procedure care. Protocols are required for the management of ascitic drains and clear communication with nursing staff is essential.

**References:**

Renal impairment occurs after large volume paracentesis (LVP) in patients with cirrhosis despite adequate albumin administration

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Introduction: Although generally a safe procedure when performed with albumin replacement, Large Volume Paracentesis (LVP) can precipitate post paracentesis circulatory dysfunction (PPCD) and acute kidney injury (AKI). We retrospectively reviewed all LVP performed at our hospital and assessed the adequacy of albumin replacement and the incidence of renal dysfunction.

Methods: We identified patients undergoing LVP during 12 months from October 2010 using ascitic samples received by the microbiology laboratory and the departments planned admission book. Patient case notes were reviewed and data on patient demographics, blood results pre and post procedure, use of blood products and human albumin solution (HAS), volume of ascites drained and the occurrence of complications was collected. AKI was defined as a 50% increase in creatinine or creatinine rise of > 26.4 µmol/L. We also identified occurrences of milder renal dysfunction defined as an increase in creatinine of > 20%.

Results: 56 LVP were performed on 28 patients. 24 were male, age range 30–84 years (median 59). 5 patients developed 6 episodes of AKI after LVP. 2 followed lack of HAS replacement and failure to remove the drain within 6 hours. 2 episodes in the same patient occurred in the context of SBP. 2 episodes had no obvious precipitating factors and renal function subsequently returned to baseline, which was abnormal in 1 case. 3 procedures (all with normal baseline renal function) resulted in a lesser degree of renal impairment. 2 of these had no obvious precipitant. Renal function normalised in all cases. Thus in a total of 4 patients (14%) LVP lead to either AKI or minor but clinically significant renal impairment despite adequate HAS replacement, without identifiable precipitating factors.

Discussion/Conclusion: Although administration of HAS is known to prevent PPCD and reduce the incidence of renal failure, 14% of our patients developed renal impairment despite optimal management.

The development of AKI negatively impacts on patient survival. It is therefore important to monitor renal function after LVP in order to identify and treat AKI aggressively.

Reference:

Can weight loss and exercise improve NAFLD?

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Introduction: There appears to be a benefit from lifestyle modification involving increased physical activity and weight loss to reverse fatty liver disease, although the results have been variable.

Methods: In a prospective observational study, 86 patients (51 men, 35 women), with a BMI between 25–40 kg/m² and liver steatosis (steatotest, echo and CT) were recruited after excluding individuals with other liver diseases. All patients were assigned a caloric goal based on their starting weight (1000–1500 kcal/day) and were instructed to gradually progress to a goal of 200 min/week of moderate-intensity physical activity. Blood was obtained at entry and months 6 and 12 and was tested for biochemical analysis, HOMA, cytokine levels (leptin, adiponectin), and steatotest. All subjects underwent abdominal CT scan at the beginning and after 12 months for grading steatosis and changes in visceral and subcutaneous tissue (VAT/SAT).

Results: After 12 months all baseline descriptive characteristics for the 86 subjects decreased significantly: body weight by 14.8 ± 6.4 kg, BMI by 5.2 ± 2.5, waist circumference by 10.6 ± 5.1 cm. Steatotest decreased from 0.58 ± 16 at baseline to 0.28 ± 0.14 (p = 0.02), reflecting reduction of intrahepatic fat. The decreases of adipocytokines was significant for leptin (15.3 ± 4.8 ng/mL vs. 7.8 ± 3.1 ng/mL, p = 0.018) and adiponectin (8.1 ± 3.3 µg/mL vs. 9.8 ± 3.8 µg/mL, p = 0.003). Steatosis regression at the end of the study (> 5 HU) assessed by hepatic-splenic attenuation difference was significant. In multivariate logistic regression analysis the following factors were associated to improved steatosis: BMI < 25 kg/m² (p = 0.020), ALT < 42 UI/L (p = 0.027), leptin (p = 0.017) and adiponectin (p = 0.010). There was also a difference in the reduction of VAT and SAT (p = 0.01).

Discussion/Conclusion: Obese persons compliant with 12 months of physical activity and low diet can safely and effectively achieve significant reduction in body weight, liver fat, VAT and SAT. Changes in BMI, ALT, leptin and adiponectin could predict improvement in liver steatosis.
Alternative treatments for hepato-renal syndrome in patients with cirrhosis

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Introduction: Terlipressin plus albumin and octreotide plus albumin are the two therapeutic options most commonly used in hepato-renal syndrome (HRS). We compared terlipressin and albumin vs. octreotide and albumin in HRS treatment.

Methods: 40 patients with cirrhosis and HRS (36 with type I and 4 with type 2 and serum creatinine > 2.5 mg/dl) were randomized to receive terlipressin plus albumin (group A = 20 pts) or octreotide and albumin (group B = 20 pts). Group A patients received terlipressin by continuous intravenous infusion at initial dose of 4 mg/24 hrs, which in case of non-response was progressively increased to 12 mg/24 hrs. Group B patients received octreotide at initial dose of 100 µg subcutaneously TID, which in case of non-response was increased to 200 µg TID. Patients in both groups received albumin 1 g/kg body weight on first day, followed by 20–40 g/day. Full response to treatment was defined by decrease in serum creatinine to < 1.5 mg/dl. Partial response was defined by decrease in pretreatment peak serum creatinine > 50% to a final value > 1.5 mg/dl.

Results: Improvement of renal function was significantly more frequent in patients of group A (80%), than in group B (45%), p < 0.01. A full response to treatment was observed in 11 of 20 (55%) patients of group A and in 4 of 20 (20%) in group B, p < 0.01. The 30 day survival was not different between the two groups (70% in group A and 60% in group B, p = NS), but percent of responders was significantly higher among survivors in group A (90%) than among survivors in group B (55%), p < 0.05.

Discussion/Conclusion: Terlipressin plus albumin is more effective in the treatment of HRS in patients with cirrhosis. The efficacy of terlipressin is strongly associated with 30 day survival in these patients.
Long term entecavir treatment, for nucleotide naïve, non-cirrhotic, hepatitis B HBsAg(+) and eAg(+) field patients in the northwest of Greece

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Introduction: Hepatitis B is a significant health care issue in Greece. Although most native patients are infected with an eAg(-) virus, in the Northwest of Greece most patients are eAg(+). The aim of this study was to look at effectiveness and safety of long term entecavir treatment in a cohort of patients who were not cirrhotic and were eAg(+). All patients were nucleotide naïve.

Methods: Thirty five patients with chronic hepatitis B were followed up at the clinic. They were all prescribed entecavir 0.5 mg a day. At baseline mean age was 46 years, 86% males, HBV DNA 3.85 log IU/ml, 94% had elevated ALT. No patient had clinical evidence of cirrhosis. Liver function test and HBV DNA were assessed every 3 months.

Results: Mean follow up was 63 months. The rates of undetectable HBV DNA progressively increased overtime reaching 100%. A partial response on week 12 occurred in 21% of patients and a virological breakthrough in 9% of patients. There was one case of primary non response. ALT levels normalised in 93% of cases. HBeAg seroconversion occurred at a medium of 46 months at 60% of patients. At 5 years 16% of patients achieved HBsAg seroconversion. Subsequently 5 patients were successfully taken off therapy. No major safety issues were observed. No patient developed new onset renal failure. In 9 patients with a degree of renal failure at the beginning of the study no progression was observed.

Discussion/Conclusion: Entecavir is a safe and effective treatment option long term in eAg(+) hepatitis B patients.
Vitamin D status in Mongolian patients with HCV

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There is well established that viral hepatitis infections are endemic in Mongolia. But non clear the basic cause of endemicity. Nowadays there is much interested and discussing the role of vitamin D in pathogenesis for hepatitis, in particularly HCV.

Aim of the study: To estimate the vitamin D status in Mongolian patients with HCV infection.

Patients and Methods: We have observed 30 patients with chronic HCV infection (age: 36–77 years old, man female) According to the level of ALAT divided into 3 groups: normal range, slightly elevated and significantly elevated. In serum of all patients detected 25-hydroxyvitamin D (25-OH D) by quantitative EIA (Immunodiagnostic Systems, Denmark). By the instruction of used kits, there was estimated as deficient < 25 < 10 nmol/L; as insufficient 25–74 nmol/L and sufficient 75–250 nmol/L.

Results: There was detected level of 25-OH D in range 15–45 nmol/L (table).

<table>
<thead>
<tr>
<th>Range of ALAT</th>
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<th>deficient</th>
<th>insufficient</th>
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<tbody>
<tr>
<td>ALAT in normal range</td>
<td>12</td>
<td>4 (33.3%)</td>
<td>8 (66.7%)</td>
</tr>
<tr>
<td>ALAT slightly elevated than normal</td>
<td>9</td>
<td>3 (33.3%)</td>
<td>6 (66.7%)</td>
</tr>
<tr>
<td>ALAT up to 2 fold and more of normal</td>
<td>9</td>
<td>4 (44.4%)</td>
<td>5 (55.6%)</td>
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</table>

Discussion and Conclusion: Recently, some study on vitamin D in deference groups of Mongolian population. By these, there was concluded that the level of vitamin D is comparatively low.

1. In Mongolian HCV patients, the level of vitamin D is low and deficient case is wide spread. The activity of HCV infection and vitamin D deficiency have a slight correlation.

2. May be some relationship deficiency of vitamin D and high prevalence of HCV infection in Mongolia.
The effect of HBV infection on liver metastases of colorectal cancer

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The liver is the most common site of metastases in colorectal cancer but metastases seem to be less common in patients with a chronically liver damage.

Introduction: The objectives of our study are to evaluate the effect of hepatitis B virus (HBV) infection on liver metastases of colorectal cancer.

Methods: A total of 542 colorectal cancer patients were recruited from January 2008 to December 2011 in this study. Enzyme-linked immunosorbent assay was used to test serum HBV markers for colorectal cancer. Patients were divided into study (infection) group and control (non-infection) group. The two groups were compared regarding the incidence of colorectal liver metastases and survival. Clinical features of patients in two groups were compared. The criteria of colorectal liver metastases were based on liver CT examination.

Results: Liver metastases were found in 147 out of the 542 colorectal cancer patients. The incidence of liver metastases was significantly lower in study group than control group (16.3% vs. 27.1%, p < 0.01). HBV infection significantly decreased the risk of liver metastases, but the incidence of extrahepatic metastases was significantly higher in study group than in control group (32.5% vs. 16.1%). Five-year survival rates were 62% and 42.4% in the chronic hepatitis infection group and the non-hepatitis virus infection group, respectively (p < 0.05). The degree of progress in the two groups of patients showed no significant difference. The number of liver metastatic lesions was significantly less in study group than in control group with a higher surgical resection rate. However, no significant difference was found in survival rate between the two groups (p = 0.93).

Discussion/Conclusion: HBV infection decreases the risk of liver metastases in patients with colorectal cancer and elevates the surgical resection rate of liver metastatic lesions and also the patients in our study had good prognoses.
Chronic hepatitis B virus infection – Clinical, laboratory, serological and histologic characteristic

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Hepatitis B infection remains the most common form of chronic hepatitis.

Introduction: The objectives of this study are to determine the laboratory, clinical, histologic and serological characteristic of chronic hepatitis B virus carriers in our environment.

Methods: A retrospective study was performed that included chronic AgHBs carriers obtained from January 2011 to December 2011.

Results: A total of 248 patients were included. At diagnosis 61.4% were men, with a mean age of 39.6 ± 11.4 years and 38.6% were women with a mean age of 40.9 ± 12.3 years. Alanine aminotransferase (ALT) levels were within the normal range in 58.6% of the patients and 85.7% were AgHBe(-). Liver biopsy was performed in 29.3%; varying grades of inflammation-fibrosis were found in 64.5% and cirrhosis was found in 14.9%. Hepatitis C and D virus coinfection was found in 4.1% and 2.4% respectively. A familial history of chronic HBV was found in 19.6%. Compared with AgHbe(-) patients, those who were AgHBe(+) were younger and had greater disease activity; the difference was statistically significant. Patients in the immunotolerant phase were the least numerous (6.6%), while AgHBe(-) patients with chronic HBV infection were the most numerous (47.8%).

Discussion/Conclusion: Chronic HBV infection in our environment occurs mainly in middle-aged persons. GPT values are normal in more 50%, most are AgHBe(-) and approximately half are inactive carriers.
**Combined treatment of a carcinoma of the stomach with metastases in a liver**

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In 48 patients the stomach cancer with metastases in liver was performed palliative gastrectomy (GE) and distal subtotal resection (DSR). Histological in 34 patients was established intestinal form and in 34 – diffuse form of cancer. In 32 (66.6%) patients tumor located in the antral part of stomach, which one was accompanied by a different degree of stenosis and in 16 (33.3%) – process was located in the body and proximal part of stomach. Quantity of metastases from 4 up to 11 clusters, diameter from 0.6 up to 4.5 cm.

In 32 (66.6%) patients was performed DSR. From them for 14 (43.7%) patients DSR was combined with a planar resection of the pancreatic head. In 16 (33.6%) patients performed GE, the operation till necessity was combined with splenectomy in 6 (37.5%) patients. Depending on adopted tactics in treatment of liver metastases distributed on main (25 patients) and control (23 patients) groups. Distribution by the form and volume of the transaction in both groups were identical.

In a main group after 3–4 weeks after operation with the purpose of liquidation and depressing the growth of metastatic clusters was conducted long term endoarterial chemotherapy by installation the catheter into *A. hepatica communis*. Fluouracil 5 g and Doxorubicinum 60–80 mg injected relation to weight of a body, slowly with the special metering device during 120 hours. The treatment was repeated 2–3 times with an interval 1.5–2 months. And in control group the same drugs in the same doses were entered system intravenously during 7–8 day. The treatment to 11 patients was repeated 2 times and 9 patients – 3 times.

**Outcomes:** The postoperative complications were advanced in 13 (27%) patients, died – 4, the letality has compounded – 8.3%. The full regressions of metastatic loci, in both groups was not observed. The partial regression of metastases in main group has compounded – 68%, the stabilization – 32%, development is not marked. In control group the partial effect is marked – in 34.8% patients, stabilization – 39.1% and development – 26.1 %.

The median lifetime of patients in main group has compounded 16.2 ± 0.4 months and in control group 11.4 ± 0.7 months ($p < 0.05$).
Diagnostic difficulties, therapeutic strategies and performance of scoring systems in patients with autoimmune hepatitis and concurrent hepatitis B/C

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Background: The diagnosis of autoimmune hepatitis (AIH) is already difficult, and that of AIH with chronic viral hepatitis including hepatitis B (HBV) or hepatitis C (HCV) is even more challenging. To date, only a few case based studies have described this association.

Aims: To retrospectively assess diagnostic difficulties, therapeutic approaches and performance of the scoring systems in AIH patients with concurrent HBV and HCV.

Methods: Twenty-five patients from USA, Sweden, Italy and Turkey were retrospectively evaluated. Both revised and simplified criteria suggested by the International Autoimmune Hepatitis Group were applied for each patient. All study data were obtained from medical records.

Results: Of the 25 patients, 20 (80%) had concomitant HCV and five (20%) HBV. Based on the revised scoring system and simplified criteria, 18 (72%) and 12 (48%) of patients were diagnosed as ‘probable’ AIH. None of the patients were diagnosed as ‘definite’ AIH according to both scoring systems. Patients with HCV initially treated by immunosuppressive and anti-viral (interferon alone or combination with ribavirin) therapy was commenced when biochemical remission occurred. Anti-viral therapy was administered in 13 (65%) of the 20 HCV patients while seven patients (four due to advanced age and three refused) did not receive therapy. Among these 13 patients, seven had sustained response after anti-viral therapy whereas the remaining six patients were non-responders. Patients who received anti-viral were followed closely and relapse of AIH was not observed in any of the 13 patients during therapy. AIH patients with HBV were treated by anti-viral first and thereafter, immunosuppressive therapy was started.

Conclusions: This is the largest population based study that describes concurrent AIH and chronic viral hepatitis. The revised scoring system had a better performance than the simplified scoring system. However, performance of both scoring systems is not optimal enough to reach the diagnosis of AIH alone. In these patients, a more definitive diagnosis of AIH requires further evaluation and should be made based on combination of serological profiles, histological findings, performance of scoring systems as well appropriate treatment regimens and outcomes.
Novel redox nanoparticles reduce oxidative stress and improve fibrosis in NASH mouse model

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Introduction: Oxidative stress (OS) is largely thought to be a core abnormality responsible for liver damage and disease progression in nonalcoholic steatohepatitis, or NASH. Moreover, OS, caused by an increase in reactive oxygen species (ROS), has been closely associated with fibrosis. The aim of our study was to determine the therapeutic efficacy and safety for OS suppression using novel redox nanoparticles (RNP).

Methods and Results: RNPss were prepared by self-assembling amphipilic block copolymers composed of a hydrophilic polyethylene glycol (PEG) segment and hydrophobic poly (4-methylstyrene) segment possessing nitroxide radicals via amine linkage. C57BL/6 mice were placed on a choline-deficient L-amino acid defined (CDAA) diet to generate a NASH mouse model. To investigate whether the polymer derived from RNP is delivered into the liver, mice were treated with RNP-Rhodamin (300 mg/kg), or vehicle, and the liver was collected for measurement of Electron Spin Resonance (ESR) and fluorescence 30–60 min post-treatment. The strong ESR signal and fluorescence were observed in the liver resulting from polymer delivery after disintegration of nanoparticle in the stomach and absorption into the bloodstream through the mesentery. For NASH treatment, after 16 wks of CDAA diet with severe fibrotic-NASH, mice were treated with RNP, control NP (300 mg/kg/day), or vehicle for 4 weeks via gavage. The ROS in the liver were reduced in mice treated with RNP, as assessed by Dihydroethidium (DHE) staining. Liver fibrosis was significantly improved in RNP-treated group as compared to control NP, or vehicle, as assessed by Sirius-red quantitation, as well as mRNA expression of fibrosis genes such as COL1, a-SMA and TIMP-1 (p < 0.05). The mRNA expressions of inflammatory cytokines, including IL-6, were slightly decreased with RNP treatment, whereas no significant changes were detected in hepatic steatosis and inflammatory foci in the CDAA-treated groups.

Discussion/Conclusion: This study demonstrates that oral administration of RNP reduces HSC activation and improves liver fibrosis associated with experimental NASH. These findings uncover RNP as a potentially novel anti-fibrotic therapy for NASH.
The balance between T helper 17 and FoxP3⁺ T regulatory cells in patients with chronic hepatitis C: Relation to disease activity and hepatic fibrosis

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Introduction: T helper (Th) 17 cells are major mediators of inflammation and have a reciprocal developmental relationship with the immunosuppressive T regulatory (Treg) cells. The present work was designed to study the balance between Th17 and Treg cells in patients with chronic hepatitis C (CHC) in relation to disease activity and hepatic fibrosis.

Methods: Twenty patients with treatment-naïve CHC and 20 healthy subjects were included in the study. The Th cells, Th17 cells and Treg cells in blood samples were identified as CD4⁺, CD4⁺IL17A⁺ and CD4⁺CD25⁺FoxP3⁺ cells respectively using flow cytometry. Serum IL17 levels were measured using enzyme linked immunosorbant assay kit. Liver biopsies were examined to assess METAVIR histological activity grade and fibrosis stage. Liver-infiltrating CD4⁺ Th cells, IL17A⁺ cells (Th17 cells) and FoxP3⁺ cells (Treg cells) were counted using immunohistochemical staining.

Results: Patients with CHC showed significant increases in the percentages of Th17 cells, Th17/FoxP3⁺Treg ratio in peripheral blood and serum IL17 levels and a significant decrease in the percentage of circulating FoxP3⁺Treg cells compared with healthy subjects (P < 0.01). The percentages of peripheral blood CD4⁺ Th cells were not different between the two groups (P = 0.284). The proportions of liver-infiltrating IL17A⁺ cells and FoxP3⁺ cells were inversely correlated and showed positive correlations with the percentages of circulating Th17 cells and FoxP3⁺ Treg cells respectively in patients with CHC (P < 0.05). The METAVIR histological activity grade and fibrosis stage were directly correlated with the proportion of intrahepatic IL17A⁺ cells and IL17A⁺ cells/FoxP3⁺ cells ratio and serum IL17 levels and were inversely correlated with the proportion of liver-infiltrating FoxP3⁺ cells (P < 0.05).

Discussion/Conclusion: The CD4⁺ Th cell phenotype is skewed toward Th17 phenotype in CHC. The imbalance between Th17 and Foxp3⁺ Treg cells plays an important role in disease progression and hepatic fibrosis in chronic HCV infection.
Can rapid virological response predict sustained virological response in naïve Egyptian patients with HCV genotype 4 treated by peginterferon plus ribavirin?

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Introduction: The effect of peginterferon and ribavirin treatment on chronic hepatitis C virus infection has early been established. However, predictors of treatment success need more elucidation.

Objective: to estimate the importance of rapid virological response as well as other host and viral factors as predictors of sustained virological response (negative HCV RNA at the fourth week) in genotype 4 hepatitis C virus naïve Egyptian patients treated with pegylated interferon and ribavirin.

Methods: A total of 111 naïve patients with chronic hepatitis C genotype 4 were randomly completed 48 weeks of either peginterferon-alpha-2a (180 μg/week) or peginterferon-alpha-2b (1.5 μg/kg/week) plus weight based oral ribavirin with a 24 weeks follow up. The end point was the sustained virological response (negative HCV RNA after 72 weeks).

Results: Overall, sustained virological response was achieved by 85 patients (76.6%), while 26 patients relapsed (23.4%). Rapid virological response occurred in 95 patients where 77 of them achieved SVR (84.6%) and 14 of them relapsed (15.4%). According to Metavir score, F3 stage significantly affect SVR compared to F1 stage.

Discussion/Conclusion: Rapid virological response is an independent factor affecting sustained virological response. Also, low pre-treatment fibrosis stage is a predictor of sustained virological response.
Transient elastography is a useful clinical tool to predict the presence of minimal hepatic encephalopathy in a cohort of compensated cirrhotic patients

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Introduction: Minimal hepatic encephalopathy (mHE) is a common cause of neurocognitive dysfunction in patients with cirrhosis. Treatment has been shown to improve psychometric performance and enhance quality-of-life parameters. The diagnosis is based on psychometric and/or neuro-physiological tests but a consensus on a gold standard test has yet to be reached. These tests are time consuming and currently not widely used outside of the research setting. Transient elastography (TE) is an established non-invasive tool to determine the severity of hepatic fibrosis. The aim of this study was to investigate if TE could be used in a population with compensated cirrhosis to identify patients most likely to have mHE.

Methods: Consecutive compensated cirrhotic patients attending the outpatient department over a six-month period completed the Psychometric Hepatic Encephalopathy Score (PHES) and had TE performed on the same day. PHES raw data was compared to UK normative data and a score of two or more standard deviations below the mean was considered consistent with a diagnosis of mHE. TE was performed using Fibroscan (Echosens) and the median value of ten valid acquisitions gave the liver stiffness measurement (LSM) in kPa. The diagnostic performance of LSM was assessed by using receiver operating characteristics (ROC) curves. The optimal cut-off value for LSM was chosen to maximise the sum of sensitivity and specificity.

Results: 22/70 patients (31.4%) had mHE on PHES. LSM was significantly higher in those with mHE than in those without mHE (median 40.4 kPa v 17.2 kPa; p = 0.001). Based on the ROC curve a cut-off of 20.9 kPa had a sensitivity of 73% and a specificity of 63% to predict presence of mHE.

Discussion/Conclusion: TE can be used to risk stratify patients for the presence of mHE. All patients with a LSM > 20.9 kPa should either be tested for mHE or empirically treated.
Review of hospital patients with ALT > 1000 IU/L

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Introduction: There are many potential causes for an ALT > 1000 IU/L, the commonest being ischaemia, drug induced liver injury (DILI) and viral hepatitis. There are, however, many other potential causes and sometimes the underlying aetiology is cryptogenic. Our aims were:
– To establish the causes of ALT > 1000 IU/L in patients in tertiary referral university hospital and to determine the admission and mortality rates.
– To determine the frequency of cryptogenic hepatitis and in how many of these cases had HEV been checked.

Methods: All ALT values over a two-year period (2010/11) were examined. Those with an ALT > 1000 IU/L were identified and their investigations/notes reviewed. All data was anonymised and recorded in a dedicated electronic database.

Results: 182 patients (57% male and 43% female) with an ALT > 1000 IU/L were identified. The most common causes of an ALT > 1000 IU/L were ischaemic hepatitis, (n = 111, 61%), DILI, (n = 30, 16.5%) and viral hepatitis, (n = 22, 12.1%). The remaining causes included choledocholithiasis (n = 8) and autoimmune hepatitis (n = 3). No cause was identified for 8 patients. Of these, none had HEV IgM checked. 35.7% (n = 65) of those with an ALT > 1000 IU/L died during their admission. Of these, 94% (n = 61) had ischaemic hepatitis, 3% (n = 2) had DILI (1 = paracetamol, 2 = cocaine) and 3% (n = 2) had a cryptogenic cause.

Discussion/Conclusion: This review confirms that ischaemia, DILI and viral hepatitis account for almost 90% of ALT > 1000 IU/L. Ischaemic liver injury is the commonest cause of ALT > 1000 IU/L and is associated with a high mortality rate. DILI and viral hepatitis are associated with low mortality rates and in many cases may not require admission to hospital. It is important to recognise that choledocholithiasis and AIH, while a significant minority, can cause an ALT > 1000 IU/L. Only 5% of patients had no aetiology identified. This number may have been significantly lower if all had had HEV IgM checked.
Diagnostic approach in Wilson disease using ceruloplasmin and urinary copper excretion according to Leipzig scoring system

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Introduction: Wilson disease (WD) is an autosomal recessively inherited disorder of the copper accumulation and toxicity. It’s treatable, if diagnosed early. The recognition is clear in typical clinical presentations. Unexplained liver diseases are a diagnostic challenge and require more examinations.

Methods:
Aim: To investigate the clinical features and diagnostic possibilities in WD.
Sixty five patients with WD were analysed (22 female, 43 male), from June 2010 to May 2013, aged at the end of evaluation between 18 and 65 years and a control group of 17 patients with other liver diseases. Leipzig scoring system was used for diagnosis.

Results: The patients were followed up from 3 months to 21 years from diagnosing. The diagnosis was based on clinical findings, parameters of copper metabolism, instrumental, ophthalmological, DNA examination, liver biopsy and assessment of the Leipzig scoring system at the final evaluation. Twenty eight patients (43.08%) were with liver disease alone, thirty two (49.23%) – with hepatic and neurological presentation, three with neurological features without signs of a liver injury and two asymptomatic patients. Cirrhosis was found in 46.15% (30/65), acute liver failure after a discontinuation of the treatment in 2, hepatocellular carcinoma in one cirrhotic patient. The average level of the ceruloplasmin was 0.149 g/l (± 0.067) vs. 0.224 g/l (± 0.062) in control group. 24-hour urine copper was increased. D-penicillamine challenge test showed mean value 14.4 µmol/24 h (± 13.13) of urine copper excretion vs. 5.46 µmol/24 h (± 2.99) in controls. According to the Leipzig diagnostic criteria 58 (89.23%) patients had a score ≥ 4 (max. 12). Seven patients had score 3, but the exclusion of another etiology and the clinical course of the disease confirmed the diagnosis. 76.5% of controls had score ≤ 2. Liver biopsy was performed in 24 patients, with rodanine positive staining in 10.

Discussion/Conclusion: WD has to be considered in the liver diseases to prevent the delay of the recognition and the progression of the copper metabolic disorder. Our experience confirms that the Leipzig scoring system with a combination of clinical symptoms, laboratory parameters of copper metabolism, genetic testing and liver biopsy is reliable method for diagnosing of WD in the clinical practice.
TGFβ1 stimulation of human bone marrow mesenchymal stem cells (MSC) enhances their hepatic engraftment and therapeutic effect in injured liver via upregulation of CXCR3 function

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Introduction: Bone marrow mesenchymal stem cells (MSC) have been proposed as therapies for liver injury. However, MSC migration and engraftment within the liver is poor, potentially limiting their therapeutic action. The aim of this study was to enhance the hepatic migration of MSC by cytokine stimulation to determine if this improved their biological efficacy.

Methods: MSC (Lonza®) were cultured with a range of cytokines for 24hrs and effects on chemokine receptor (CCR) expression were determined with qPCR and flow cytometry. Furthermore, the ability of TGFβ1-MSC to engraft and modulate liver injury in carbon-tetrachloride (CCl4) injured C57BL/6 mice were tested following portal and systemic (tail-vein) infusion. Fluorescent MSC in livers were quantified per field of view (c/fov) or by in vivo imaging. Livers sections were tested for necrosis by H&E and digital-morphometric analysis.

Results: TGFβ1 treatment demonstrated up-regulation of CCR expression; CCR4 (24.82 ± 3.29% to 40.32 ± 4.41%, p < 0.05), CCR5 (20.8 ± 3.4% to 33.04 ± 4.86%, p < 0.05) & CXCR3 (18.33 ± 3.4% to 32.51 ± 4.51%, p < 0.05) without any change in gene expression. TGFβ1-MSC had increased hepatic engraftment in CCl4 injured mice (2.02 ± 0.1 c/fov vs. 10.56 ± 0.39 c/fov, p < 0.001) which was only significantly reduced by blockade with anti-CXCR3 antibodies. TGFβ1-MSC reduced levels of liver damage and inflammation, as assessed by (i) necrosis; 38.98 ± 1.22% vs. 23.52 ± 1.17% vs. 3.09 ± 0.55, p < 0.01; (ii) reduced infiltrating CD45+ c/fov; 42.27 ± 3.06 vs. 33.18 ± 1.68, p < 0.05; vs. 20.02 ± 1.80, p < 0.001; all for no MSC vs. MSC vs. TGFβ1-MSC respectively, (iii) serum ALT; TGFβ1-MSC reduced ALT compared to no MSC (228.1 ± 26.5 vs. 404.7 ± 53, p < 0.001). Furthermore, MSC treated mice had greater hepatic macrophage numbers in the portal regions (c/fov); 40.4 ± 3.36 vs. 51.2 ± 4.5, p < 0.001. This was greater still with TGFβ1-MSC (88.9 ± 5.2, p < 0.001).

Discussion/Conclusion: Stimulation of MSC with TGFβ1 significantly increases their hepatic homing and ability to reduce CCl4 induced liver damage. These data suggest a powerful new protocol for pre-therapeutic manipulation of MSC to improve clinical potency.
Five year clinical review of 78 cases of hepatocellular carcinoma

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Background/Aims: Hepatocellular carcinoma is an important disease worldwide, with an increasing incidence in the world. Chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) are well-recognized risk factors for cirrhosis and liver cancer.

Methods: The study population included 78 patients (58 males and 20 females). The prevalence of serologic markers of HBV and HCV infections among patients diagnosed with cirrhosis or hepatocellular carcinoma (HCC) and detection of alphafetoproteins, was obtained from representative samples of published reports. Each case was verified with biopsy. We have followed the most common symptoms, the most frequent clinical stage and the modality of treatments.

Results: The mean age was 65 years (range 30–85). Majority of cases (80%) had preexisting liver diseases: hepatitis B (50%), hepatitis C (20%) and alcoholic liver disease (10%). In 67% of cases the hepatoma was symptomatic at the time of diagnosis. The most common symptoms were abdominal pain (49%), weight loss (40%) and anorexia (29%). The most frequent clinical stages were T3 (30%) and T4 (48%). The percentage of the positive alphafetoprotein level in serum was 80%. Therapy was undertaken in 45% of patients: surgical treatment (15%), alcohol injection (9%) and systemic chemotherapy (21%).

Conclusions: HBV and HCV infections account for the majority of cirrhosis and primary liver cancer throughout most of the world, highlighting the need for programs to prevent new infections and provide medical management and treatment for those already infected. Early screening for HCC in the target population of patients with established liver disease due to viral hepatitis B, C or alcoholic liver disease may be indicated.

Key words: hepatitis B, hepatitis C, risk factors, hepatocellular carcinoma, alphafetoprotein
The safety and tolerability of the budesonide in treatment of autoimmune hepatitis

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Introduction: The aim of this comparative study was the retrospective assessment of the safety and tolerability of the budesonide-azathioprine combined therapy versus prednisone in association with azathioprine in patients with autoimmune hepatitis (AIH).

Methods: We studied 42 patients (28 females/14 male, mean ages 43.2 years) with AIH. A comparative study was performed on two groups of patients: A group composed of 25 patients who received a combined therapy with prednisone (40 mg/day and tapered to 10 mg/day) and azathioprine (1–2 mg/kg/day) and B group treated with Budenofalk® (3 mg, oral doses three times daily) in association with azathioprine (1–2 mg/kg/day). After the liver enzymes level was normalized, the dose of budesonide was reduced at 6 mg daily. The tolerability of therapies, the incidences and severity of adverse events was monitored for a 12 months period.

Results: At 6 months, complete biochemical remission occurred in 9 cases (36%) of the A group and in 11 cases (64.7%) in B group. In A group the side effects were: mild anemia (4 cases), osteoporosis (5 cases), severe leukopenia (2 cases), steroid diabetes (2 cases) and Cushing’s syndrome (3 cases). Multiple side effects were observed in 6 patients (24%). Comparative, the rate of side effects in B group was significantly reduced (27.77%) and 15 patients (83.3%) did not develop steroid-specific side effects. After 6 months, disappearance of clinical symptoms, normal liver biochemistry and histological remission was observed in 18 cases: 7 patients in A group and 11 in B group.

The incidences of the side-effects which appeared in a period between 6 and 12 months after debut of therapy were significantly reduced in B group: only one case with leukopenia due to azathioprine maintenance therapy and one case with trombopenia. Comparatively, in group A were appeared most side effects: osteoporosis (2 cases), gastrointestinal bleeding (3 cases) diabetes (one case) and trombocytopenia (one case). For whole 12 months period, the rate of the discontinuation of the therapy due to adverse events was: 36.0% in group A and 17.6% in B group.

Discussion/Conclusion: The combined therapy with budesonide and azathioprine assure a high efficacy in patients with AIH and determined low rate of steroid specific side effects. In association with azathioprine, budesonide is more tolerable than prednisone.
Therapeutic options in the treatment of nonalcoholic steatohepatitis in obese patients

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Introduction: Aim was to evaluate and compare the effectiveness of ursodeoxycholic acid (UDCA) monotherapy, simvastatinum and combination of UDCA and vitamin E in the treatment of NASH.

Methods: We studied 53 patients with NASH and obesity. We excluded patients with viral or autoimmune hepatitis, diabetes mellitus or drug abuse. The diagnosis was based on the correlation of histologic and clinical findings. Liver biopsy was performed before and after therapy. Group A composed of 18 normolipidemic cases, treated with UDCA 13–15 mg/kg/day, B group consist of 15 hyperlipidemic cases which received simvastatinum 20 mg/day and C group (20 patients) with UDCA and vitamin E (400 IU twice a day) therapy. We evaluated liver function tests, serum lipids and BMI at the beginning of therapy, after 6 and 12 months.

Results: A number of 39 patients had elevated serum aminotransferase level, but 14 had normal values. In B group, lipide profile was: 7 cases with hypercholesterolemia, 4 cases with hypertriglyceridemia and 4 with both. In group A, mean value of serum ALT-level was decreased from 88.3 ± 21.7 U/l at baseline, to 52.12 ± 17.5 U/l at 6 months. In B group, serum ALT was reduced (in mean with 19.3 ± 7.2 U/l) after 6 months and cholesterol was significantly improvement in 8 cases (72.7%). In 2 cases we increased simvastatinum dose at 40 mg/day. In C group mean ALT and AST levels was more decreased: in mean with 49.3 ± 5.2 U/l. After one year, aminotransferase levels reach normal range only in C group. Comparatively, in A and B groups the normalisation rates of ALT was lower (89.7% and 73.33%). Histopathologic examination was revealed improvement the steatosis grade: 83.3% in A group, 73.3% in B group and 90.0% in C group.

We could not establish a correlation between the values of serum aminotransferases and others parameters, but multivariate analysis showed that the BMI > 28 kg/m² and elevation of serum ALT were associated with steatosis grade. Patients which associated combined therapy with low caloric diet, had a good and rapid response.

Discussion/Conclusion: Combination of UDCA and vitamin E significantly improves aminotransferase levels and steatosis grade. The combined therapy and low caloric diet still remains first line therapy in patients with NASH and obesity.
Prevalence, virological and clinical characteristics of chronic hepatitis Delta (CHD) in Romania

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Background and Aims: Hepatitis D is considered to be associated with accelerated fibrosis progression, earlier hepatic decompensation and increased risk for hepatocellular carcinoma. In Romania, the real prevalence of HDV infection is not known at this time. Published data from Romania since 1986 have indicated a high prevalence of hepatitis D with up to 10% of the general population in the south of Romania testing seropositive. The aim of our study was to assess the prevalence, virological and clinical characteristics of HDV infection in chronic hepatitis B Romanian patients.

Patients and Methods: We conducted a national, prospective study which included 10 centres in Romania. Virological markers of HBV and HDV infection, biochemical and clinical features of liver disease were evaluated in all patients included in the study. Qualitative or quantitative variables were analysed using nonparametric tests, the Chi-square, Fisher’s exact, Kruskall-Wallis or the Mann-Whitney test, as appropriate, and data have been analysed using STATA/SE 11 package. There were 2761 HBs antigen positive patients, 84.78% of the patients were negative for HBe antigen. The male gender predominated with 55.7% of the patients being males. The mean age in the study population was 43.8 ± 13.8 years with a wide range from 18 to 81 years. The most prevalent type of liver disease was chronic hepatitis 68.5%, followed by cirrhosis in 22.4% of the patients. More females were positive for anti-HDV antibodies than males (24.63% vs. 21.96% p = 0.243). A positive HDV RNA was found more frequently in females than in males (17.02% vs. 15.98%, p = 0.345).

Conclusion: HBs antigen positive population in Romania is characterized by increased prevalence of HBe antigen negative HBV infection and a high prevalence of HDV coinfection (23.14%). Such as in HCV and HBV monoinfections, a cohort phenomenon can be observed, with the highest prevalence in age groups 50–59 and 60–69 with many of these patients having advanced liver disease (22.4% cirrhotics) and a low HBV viral load.
Relationship between selectins and histologic changes in chronic viral liver diseases

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The aim of study is to assess the relationship between E-selectin, P-selectin and L-selectin plasma levels and histological changes in chronic viral hepatitis (CVH) and liver cirrhosis (LC).

Methods: 80 patients with CVH and 21 patients with viral LC were examined. The control group included 54 healthy volunteers. Blood concentration of E-selectin, P-selectin and L-selectin were carried out by means of ELISA. Data were analyzed using one-way ANOVA followed by Newman-Keuls test. Diagnostic value of parameters defined their sensitivity (Se), specificity (Sp), positive (PPV) and negative predictive value (NPV), accuracy (Ac) which expressed in percentage. Statistically significant values were considered for p < 0.05.

Results: Plasma levels of all selectins were higher in moderate and severe histological activity than in minimal hepatic morphologic changes. Parameters of E-selectin ≥ 90 ng/ml, L-selectin ≥ 5.52 pg/ml and P-selectin ≥ 33 ng/ml were associated with increased risk of histological activity index (HAI) > 8 in chronic viral liver diseases. Se, Sp, PPV, NPV and Ac of E-selectin ≥ 90 ng/ml, L-selectin ≥ 5.52 pg/ml and P-selectin ≥ 33 ng/ml for detection of HAI > 8 were 40.4, 94.4, 86.4, 64.6, 69.3; 97.9, 46.3, 61.3, 96.2, 70.3 and 100.0, 46.3, 61.8, 100.0, 71.3 accordingly. Levels of E-selectin, P-selectin and L-selectin increased with intensifying of hepatic fibrotic changes and were maximal in fibrosis 3-4. Patients with E-selectin ≥ 79 ng/ml and P-selectin ≥ 95 ng/ml characterized by higher risk of severe fibrosis/cirrhosis. Se, Sp, PPV, NPV and Ac of E-selectin ≥ 79 ng/ml and P-selectin ≥ 95 ng/ml for detection of severe fibrosis/cirrhosis were 70.6, 82.1, 66.7, 84.6, 78.2 and 58.8, 77.6, 57.1, 78.8, 71.3 accordingly. There was positive correlation between E-selectin, P-selectin, L-selectin and values of HAI and fibrosis index.

Conclusion: The relationship of selectins and histologic changes in liver testifies to their involvement into processes of inflammation and fibrogenesis in chronic viral liver diseases.
Decompensated alcoholic liver disease (ALD) is associated with starting heavy drinking at an older age: A case-control study

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Background and Aims: The relationship between development of ALD (which affects only 10–15% of heavy drinkers) and rate, duration and age of onset of alcohol consumption is incompletely understood. We have previously (J Hepatol 2012; 56: S530) reported on total lifetime alcohol consumption in two cohorts of heavy drinkers (> 60 Units/wk (M) or > 40 Units/wk (F) for ≥ 5 years): one (patients) with decompensated ALD (Child Grade B or C, negative tests for other liver diseases) and one (controls) without serious liver disease on clinical, laboratory and ultrasound examination. Here, we aimed to compare alcohol consumption patterns in these cohorts in more detail.

Methods: Subjects (330 patients, 234 male, mean age 48 yr and 238 heavy-drinking controls, 187 male, mean age 48 yr) completed a lifetime alcohol questionnaire. Alcohol consumption was calculated at home and outside home, and during Monday–Thursday and Friday–Sunday. Data were summed over each stable drinking period during the subject’s lifetime. We calculated total duration, and age at start and at cessation of all periods during which the subject drank > 0, > 40, > 80, > 120 and > 160 units (U)/wk.

Results: Neither total duration of periods consuming > 0, > 40, > 80, > 120, and > 160 U alcohol/wk (table) nor mean weekly consumption during those periods (not shown) differed between patients and controls. However, patients first started drinking over each level at an older age than did controls (table). The relationships between ALD and age of starting drinking > 0, > 40, > 80, > 120 and > 160 U/week persisted in multivariate analysis (p = 0.00–0.013).

Conclusions: Development of decompensated ALD in heavy drinkers is associated with starting heavy drinking at an older age.

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<tr>
<td>&gt; 120</td>
<td>3 (0–12)</td>
<td>32 (24–41)++</td>
<td>4 (0–10)</td>
<td>28 (21–37)</td>
</tr>
<tr>
<td>&gt; 160</td>
<td>0 (0–7)</td>
<td>33 (27–41)+++</td>
<td>0 (0–7)</td>
<td>30 (24–39)</td>
</tr>
</tbody>
</table>

*: median (interquartile range); +: p < 0.001; ++: p = 0.02; +++: p = 0.017 by Mann-Whitney test for patients versus controls
Pregnancy in biliary atresia; Transient post-delivery hypoalbuminemia with massive ascites

1,4E. Granot, 2Z. Cohen, 1,4A. Ben-Arie, 3,4S. Yagel
1Kaplan Medical Center, 2Soroka Medical Center, 3Hadassah Medical Center and 4Hebrew University-Hadassah Medical School, Israel

Introduction: Biliary atresia (BA) patients have improved prognosis and increased long term survival. In BA patients, deterioration of liver function and even liver failure requiring liver transplantation have been observed during pregnancy or after delivery.

Methods: A report of BA patients with portal hypertension who following an uneventful pregnancy and delivery developed transient massive ascites and hypoalbuminemia.

Results: Case 1: 21 y old, post-Kasai course-splenomegaly, hypersplenism, esophageal varices. Liver function tests – normal. Prior to C/S: WBC 3600, hem 11 g%, PLT 30000, transaminases, bilirubin & PT–INR normal, albumin 3.7 g%. 2nd day post partum – massive ascites. Albumin 2.5 g%, other liver function tests – normal. Ascitic fluid: albumin 0.7 g%, triglycerides 120 mg%. Discharge (day 15) – no ascites, albumin 3.4 g%. 1 year later – liver function tests normal, ascites has not recurred. Case 2: 22 y old, post-Kasai course-splenomegaly, liver function tests – normal. On day prior to delivery: WBC 11280, hem 12.9g%, PLT 96000, transaminases & bilirubin – normal, albumin – 3.7 g%. C/S – uneventful. 8th post-partum day – distended abdomen. CBC – normal, transaminases, bilirubin & PT–INR normal, albumin 2.3 g%. U/S – massive ascites. Ascitic fluid – albumin 1.9 g%. Discharge (day 10) – ascites resolved, albumin 2.8 g%. At 1 month: albumin 3.5 g%, transaminases – normal. During 1 year follow-up liver function remains normal, ascites has not recurred.

Discussion/Conclusion: The sharp decrease in albumin and massive ascites, without deterioration of liver function are likely attributed to abrupt hemodynamic changes, following delivery, in a setting of severe portal hypertension. Shifting of blood into the splanchnic bed leads to venous and lymph engorgement with resultant leakage. Rapid return of albumin to normal with no recurrence of ascites support a hemodynamic mechanism for this previously not described complication.
Prevalence of falls in the UK primary biliary cirrhosis population

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Prof. David Jones, Institute of Cellular Medicine, Newcastle University, UK
Prof. Julia Newton, Institute of Health and Ageing, Newcastle University, UK

Introduction: Previous studies have documented the prevalence of falls in PBC in a geographical area but there is no published data on the prevalence of falls in the national cohort. Risk factors for falls are prevalent in PBC, particularly autonomic dysfunction and muscle weakness and in conjunction with osteoporosis carry a significant risk of falls. This study will assess the prevalence of falls, injuries and admissions in the National PBC cohort and explore the relationship between falls and autonomic symptoms.

Methods: Symptom assessment tools were completed by patients in the UKPBC genetics study. Information about falls was collected using a standardised data capture tool and autonomic symptoms were quantified using the Orthostatic Grading Score.

Results: Data was collected on 2328 patients with PBC. 862 (37%) of patients had fallen, 188 (8%) were current fallers (one fall within the past year) and 414 (17.7%) were recurrent fallers (more than one fall in the past year). 35% of patients attended A&E, 9.7% required admission and 24% sustained a fracture. Fallers were significantly more likely to be diabetic (5.7% of non-fallers and 12.2% of fallers, p < 0.0001) and more likely to be taking cardioactive medication (29% in non-fallers and 71% in fallers, p < 0.0001). Autonomic symptoms were significantly more prevalent in recurrent fallers (mean OGS 5.44, SD 4.15) compared to non-fallers (mean OGS 2.38, SD 2.13) and infrequent fallers (mean OGS 3.2, SD 3.36) p < 0.0001.

Discussion/Conclusion: Many patients with PBC are falling, sustaining fractures and being admitted to hospital. Autonomic symptoms, diabetes and the presence of cardioactive medications were all more common in fallers. The prevalence of falls has huge implications for patients in terms of morbidity, mortality and quality of life. All patients with PBC need a thorough assessment of falls risk factors and a multidisciplinary approach to reduce the risk of falls.
Expression analysis of zinc transporters in human peripheral blood immune cells

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Introduction: Dysregulation of zinc levels have been reported from various malignant diseases such as hepatocellular carcinoma (HCC). Stromal cells including infiltrating immune cells play a significant role in the paracrine regulation of tumours. Here, the expression pattern of all known 24 zinc transporters in resting and stimulated human peripheral blood immune cells was assessed.

Methods: Peripheral blood mononuclear cells (PBMC) were isolated from healthy probands and subsequently stimulated with Phytohaemagglutinin (PHA) for three days. Expression levels of 24 zinc transporters (ZIP and CDF/Znt family) were analyzed by q-RT-PCR using β-actin for normalization.

Results: Nineteen out of the 24 Zn²⁺ transporters were found to be ubiquitously expressed in all five PBMC samples. Median transcript levels of these 19 genes varied by less than two orders of magnitude. Individual expression levels among all 19 genes varied by 4 orders of magnitude. ZIP5, ZnT10 (5x negative); ZIP12, ZnT3 (4x negative) and ZIP2 (2x negative) were not expressed or only detected in limited numbers of samples. The stimulation of PBMC by PHA was associated with reduced or unchanged expression of the majority of all 24 Zinc transporters. Three genes only revealed a consistent pattern among the five samples. ZIP14 was found to be induced (1.2–27-fold), while expression of ZIP3 and ZIP4 was decreased in all five samples. Aside ZIP14, ZIP5 and ZnT3 were found to be induced by 12- and 15-fold. The analysis concerning the variability among the five samples stimulated showed a similar expression pattern for the 24 genes in 4 out of the five PBMC samples.

Discussion/Conclusion: The acquired knowledge about the general expression pattern of Zinc transporters in peripheral immune cells will facilitate better selection of potential deregulated Zinc transporters in chronic diseases, in which both epithelial and immune cells contribute to the expression.
Primary sclerosing cholangitis in the course of inflammatory bowel disease in children

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Hepatic manifestations are one of the most common parenteral symptoms of IBD. Diseases of the liver and bile ducts may have the pathomechanism that is common with IBD; such diseases include: primary sclerosing cholangitis (PSC), small duct PSC, and PSC/AIH overlap syndrome. The aim of this study was to evaluate the incidence of PSC and AIH/PSC in course of IBD in children, and to assess the clinical course of IBD in children with and without coexisting cholangitis.

Patients and Methods: The study involved 172 children: 92 with UC and 80 with CD, at the ages of 4–18 years. In all the study children disease activity was evaluated according to PUCAI and PCDAI scales; the following immunoassays were performed: p-ANCA, IgA ASCA, IgG ASCA. PSC was diagnosed on the basis of results of laboratory liver function tests, ultrasound imaging, cholangio-MR and histopathological examination of liver biopsies.

Results: In course of UC in examined children we found PSC in 12 (13%) – in 3 cases it was overlap syndrome PSC/AIH. PSC was significantly more frequent in boys. In the group of children with PSC/UC, p-ANCA were twice more frequent. The analysis of the location of inflammatory lesions demonstrated that in the group of children with PSC/UC, inflammatory lesions in the rectum were absent in half of these patients (p < 0.01) Moreover, insignificantly more frequent pancolitis was observed in this group, when compared with children affected by UC.

Conclusion:
1. Bile duct diseases in the course of IBD occur more frequently in children than in adult patients.
2. Differences in the clinical course of IBD with or without accompanying cholangitis re related to sex and extent of inflammatory lesions in the large intestine.
The seroprevalence of both hepatitis B and hepatitis C at the first step health organizations and the difference between the urban and rural areas

Fatma Kalem¹, Şerife Yüksekkaya² and Metin Başaranoğlu³
¹Konya Numune Hastanesi, Infectious Diseases

Background and Aim: Hepatitis B virus (HBV) and hepatitis C virus (HCV) are very important infectious agents for public health. The aim of this retrospective study was to assess the seroprevalence of HBsAg, anti-HBs and anti-HCV test results of patients who admitted to first step health organizations in central and peripheral districts of Konya, the middle region of Turkey during the period 2005–2010.

Material and Methods: In this study; HBsAg, anti-HBs and anti-HCV screening test results of patients who admitted to first step health organizations in Konya during the period of 2005–2010 were retrospectively investigated from the laboratory records. This study was approved by Konya Health Directorate. All of screening tests were performed on the automatic third-generation enzyme-linked immunosorbent assay (MEIA). This immunoassay method was carried out according to the instructions of the manufacturer (Architect, Abbott Laboratories, ABD). Borderline and positive results were retested.

Results: Konya is the largest city of Turkey in terms of surface area and one of the economically developed cities. For HBsAg, anti-HBs and anti-HCV screening whole test results of five years are given at table 1. The difference between the urban and rural for HBsAg (p = 0.062 > 0.05) and anti-HCV (p = 0.874 > 0.05) were not statistically significant. Among the markers only for anti-HBs; the difference between the urban and rural was statistically significant (p = 0.042 < 0.05). Of them 4.15% were positive for HBsAg, 36.46% were positive for anti-HBs and 1.16% were positive for anti-HCV.

Conclusion: In this study, Konya has been evaluated as two region; center and perifer. Our study showed us that distribution of the diseases varies from one region to another. We consider that difference in social diversity is one of the factors. These infections are major health problems. So the results of immunodiagnostic tests for HBsAg, anti-HBs and anti-HCV will be useful for guiding control actions and for new preventive strategies.

Table 1: HBsAg, anti-HBs, anti-HCV screening whole test results

<table>
<thead>
<tr>
<th>Tests</th>
<th>Positivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>4.15</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>36.46</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>1.16</td>
</tr>
</tbody>
</table>
Table 2: Anti-HBs test results according to years and region. For Anti-HBs the population was 105,546.

<table>
<thead>
<tr>
<th>Year</th>
<th>Urban (%)</th>
<th>Rural (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>29</td>
<td>34</td>
</tr>
<tr>
<td>2006</td>
<td>37</td>
<td>32</td>
</tr>
<tr>
<td>2007</td>
<td>37.3</td>
<td>30</td>
</tr>
<tr>
<td>2008</td>
<td>40.7</td>
<td>29.4</td>
</tr>
<tr>
<td>2009</td>
<td>49</td>
<td>31.5</td>
</tr>
<tr>
<td>2010</td>
<td>57</td>
<td>35</td>
</tr>
</tbody>
</table>
The increase is seen after 2007.

Table 3: Anti-HCV test results according to years and region. For anti-HCV the population was 102,680.

<table>
<thead>
<tr>
<th>Year</th>
<th>Urban (%)</th>
<th>Rural (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>2006</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>2007</td>
<td>1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>2008</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>2009</td>
<td>1.1</td>
<td>0.5</td>
</tr>
<tr>
<td>2010</td>
<td>0.9</td>
<td>2.2</td>
</tr>
</tbody>
</table>
Alkaline phosphatase – The next independent predictor of the poor 90-day outcome in alcoholic hepatitis

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Introduction: Up to 40% of patients (pts) with severe alcoholic hepatitis (AH) die within 6 months after the onset of the disease. Appropriate diagnosis and early risk stratification are essential for therapeutic decisions. The objective of our study was determination of risk factors relevant for 90-day prognosis in AH. We analysed and compared the conventional prognostic models such as the Maddrey’s modified discriminant function (mDF) and Child-Pugh-Turcotte (CPT) score with newer ones: The Glasgow Alcoholic Hepatitis Score (GAHS); Age, Bilirubin, INR, Creatinine (ABIC) score, Model for End-Stage Liver Disease (MELD), and MELD-Na in the death prediction.

Methods: The clinical and laboratory variables obtained at admission were assessed. The receiver operating curves were constructed in order to evaluate and compare the mDF, CPT, GAHS, ABIC, MELD, and MELD-Na scores’ different areas under the curve and the best threshold values. Logistic regression was used to assess predictors of the 90-day outcome.

Results: One hundred sixteen pts fulfilled the inclusion criteria. Twenty (17.4%) pts died and one underwent orthotopic liver transplantation (OLT) within 90-day of follow up. No statistically significant differences in the models’ performances were found. Multivariate logistic regression identified CPT score, alkaline phosphatase (AP) level higher than 1.5 times the upper limit of normal (ULN) and corticosteroids (CS) non-response as independent predictors of mortality.

Discussion/Conclusion: The CPT score, AP > 1.5 ULN and the CS non-response had an independent impact on the 90-day survival in AH. Accuracy of all studied scoring systems was comparable.
Gender-related alterations of inflammatory response in patients with alcoholic liver disease

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¹Department of Gastroenterology with Endoscopy Unit, Medical University of Lublin, Poland
²Department of Clinical Immunology, Medical University of Lublin, Poland

Introduction: Clinical observations indicate that women develop more severe alcoholic liver disease (ALD) at lesser intake of ethanol and fewer years of exposure. Exact mechanisms that underlie the sex difference in alcohol-induced liver damage remain obscure. The aim of our study was to explore the possible interaction between gender and different markers of inflammation and immune response.

Methods: 147 inpatients (pts) (40 females, 107 males) with ALD were prospectively recruited and compared with 30 healthy controls (HC) (13 females, 17 males). Pts were divided into two subgroups based on their gender. The plasma levels of IL17, IL1beta, IL6, TGFbeta1, C-reactive protein (CRP), neutrophils count, frequency of Th17 and Treg cells were assessed. Serum cytokine concentrations were evaluated using immunoenzymatic ELISA tests. A FACSCalibur flow cytometer (Becton Dickinson, USA) with CellQuest software was used to identify T cell phenotype. CD3+CD4+IL17+ cells were considered Th17 and CD4+CD25+FOXP3+ Tregs. They were expressed as the percentage of all CD3+CD4+ and CD4+CD25+ lymphocytes, respectively.

Results: The serum profile of studied cytokines indicated systemic inflammatory activation. We found increased IL17, IL1beta, IL6, but decreased TGFbeta1 levels in ALD group in comparison with HC. Significantly higher plasma concentrations of IL6 and lower concentrations of TGFbeta1 in females in comparison with males with ALD were found (median, 25–75 percentiles: 27.32, 26.45–36.15 versus 19.88, 14.77–24.91; p = 0.02, and 507.16, 305.70–701.53 versus 617.17, 388.10–711.21, p = 0.05; respectively). No differences in serum IL17, IL1beta and CRP levels between both sexes with ALD were detected. A trend to higher Tregs frequency and neutrophils count was observed in women compared to men in ALD group (3.49, 2.99–4.70 versus 3.03, 2.70–3.59, p = 0.07, and 8.44, 2.57–13.51 versus 5.02, 2.91–7.92, p = 0.053; respectively).

Discussion/Conclusion: We hypothesize that immune response may influence the sex susceptibility to the toxic effects of ethanol.
The Treg/Th17 lymphocyte imbalance in patients with alcoholic liver disease

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²Department of Clinical Immunology, Medical University of Lublin, Poland

Introduction: Two new subsets of T helper cells: Th17 and regulatory T cells (Tregs) are critically linked to inflammatory innate immune response. While Th17 exert pro-inflammatory effects, Treg are potent immune suppressors, but they may reciprocally regulate mutual function. The aim of our study was to determine, whether Th17/Treg balance has an impact on the course of alcoholic liver disease (ALD).

Methods: We studied the frequencies of Th17 and Tregs in peripheral blood of 147 inpatients (pts) (40 females, 107 males) with ALD in comparison to 30 healthy controls (HC). In order to confirm alcohol misuse the AUDIT-C (Alcohol Use Disorders Identification Test-Consumption) questionnaire was used. Pts were divided into subgroups according to the presence of liver disease complications at the time of hospital admission. A FACSCalibur flow cytometer (Becton Dickinson, USA) with CellQuest software was used to identify T cell phenotype. CD3+CD4+IL17+ cells were considered Th17 and CD4+CD25+FOXP3+ Tregs. They were expressed as the percentage of all CD3+CD4+ and CD4+CD25+ lymphocytes, respectively.

Results: The alteration of the Th17/Treg balance was observed in the most severely ill patients. Twelve of 147 pts died within the 90-day follow up. Significantly higher frequency of Th17 (median; 25–75 percentiles: 1.33; 0.98–1.73; p = 0.009) and a tendency to significantly lower percentage of Tregs (2.64; 2.03–2.93, p = 0.05) in the peripheral blood was found in the subgroup of patients who died compared to survivors (0.90; 0.59–1.24 and 3.40; 2.45–4.89, respectively). Multivariable logistic regression analysis showed that the frequency of Th17 in the blood was an independent predictor of mortality in the study group. Apart from mortality, no other liver disease complication was associated with Th17/Treg imbalance.

Discussion/Conclusion: Our results suggest that Th17/Treg serum imbalance, and particularly increased frequency of Th17, may serve as a indicator of poor prognosis for patients with ALD.
Association of serum adiponectin, leptin and resistin concentrations with the severity of liver dysfunction and the disease complications in alcoholic liver disease

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²Department of Clinical Immunology, Medical University of Lublin, 4A Chodzki street, 20-093 Lublin, Poland

Introduction: Excessive alcohol consumption leads to inflammation in adipose tissue, insulin resistance and hepatic steatosis. Moreover, alcohol abuse is associated with impaired energy intake and expenditure, as well as increased catabolism, processes modulated by adipokines. So, we investigated the association of serum adiponectin, leptin, and resistin concentrations with the severity of liver dysfunction in alcoholic liver disease (ALD) and its complications.

Methods: One hundred forty seven inpatients (pts) with ALD were prospectively recruited. They were divided into subgroups according to: 1. gender, 2. the severity of liver dysfunction according to the Child-Turcotte-Pugh and MELD scores; 3. the presence of ALD complications at the time of hospital admission i.e. ascites, hepatic encephalopathy, oesophageal varices, cholestasis, and renal dysfunction. The evaluation of plasma levels of adipokines was performed using immunoenzymatic ELISA test. Multivariable logistic regression was applied in order to select independent predictors of advanced liver dysfunction and the disease complications.

Results: Adiponectin and resistin levels were significantly higher in patients with ALD than in controls. Lower leptin levels in females with ALD compared to controls, but no significant differences in leptin concentrations in men, were observed. We found a significant positive correlation of serum resistin level with both the white blood cell count and CRP level. Serum resistin level was increased in pts with kidney dysfunction, but it lost their significance when adjusted for other variables. Moreover, adiponectin concentrations revealed an independent association with the severity of liver dysfunction and the development of ascites and hepatic encephalopathy.

Discussion/Conclusion: We hypothesize that gender related differences in serum leptin concentrations may influence the ALD course, which is different in males compared to females. Serum adiponectin level may serve as a prognostic indicator of the severity of liver dysfunction, as well as the development of ascites and hepatic encephalopathy in ALD.
Treatment peculiarities of nonalcoholic steatohepatitis in patients with diabetes mellitus

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National Medical Academy of Postgraduate Education, named after P.L. Shupyk, Kiev, Ukraine

The development of non-alcoholic steatohepatitis (NASH) in patients with diabetes mellitus (DM) type 2 (NASH/DM) deepens violation of lipid, carbohydrate and energy metabolism.

Objective: to study the biochemical indicators of liver functional state, lipid, carbohydrate metabolism, homocysteine content in the blood and correct disorders in patients with NASH/DM type 2.

Methods: The study involved 60 patients with NASH/DM type 2. Body mass index (BMI) was average (31.34 ± 1.16) kg/m². There were 2 groups of patients: primary group – 30 patients who received diet with the calculation of energy value. They were appointed carnitine orotate 300 mg per day. 30 patients were included in the comparison group who received the traditional antidiabetic diet. The course of treatment extended 2 months.

Results:

Table 1: Dynamic of carbohydrate metabolism and liver function biochemical indicators, homocysteine and lipids level in the blood of the patients with NASH/DM type 2 before and after treatment

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Healthy (n = 17)</th>
<th>Primary group (n = 30)</th>
<th>Comparison group (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/l</td>
<td>4.18 ± 0.20</td>
<td>7.15 ± 0.13*</td>
<td>5.59 ± 0.14*</td>
</tr>
<tr>
<td>Plasma glucose after eating, mmol/l</td>
<td>6.09 ± 0.17</td>
<td>14.4 ± 0.24*</td>
<td>8.3 ± 0.25*</td>
</tr>
<tr>
<td>Homocysteine, mmol/l</td>
<td>10.3 ± 0.35</td>
<td>39.6 ± 3.2*</td>
<td>18.6 ± 1.8*</td>
</tr>
<tr>
<td>Bilirubin, mmol/l</td>
<td>17.3 ± 1.1</td>
<td>23.9 ± 0.7*</td>
<td>18.1 ± 0.5*</td>
</tr>
<tr>
<td>ALT, mmol/g/l</td>
<td>042 ± 0.03</td>
<td>1.29 ± 0.05*</td>
<td>0.65 ± 0.04*</td>
</tr>
<tr>
<td>AST, mmol/g/l</td>
<td>0.38 ± 0.04</td>
<td>1.19 ± 0.04*</td>
<td>0.53 ± 0.03*</td>
</tr>
<tr>
<td>GGTP, mmol/g/l</td>
<td>38.7 ± 3.5</td>
<td>81.4 ± 2.9*</td>
<td>47.1 ± 1.8*</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>4.17 ± 0.09</td>
<td>7.35 ± 0.4*</td>
<td>5.8 ± 0.2*</td>
</tr>
<tr>
<td>LDL, mmol/l</td>
<td>2.35 ± 0.09</td>
<td>5.8 ± 0.3*</td>
<td>4.1 ± 0.4*</td>
</tr>
<tr>
<td>HDL, mmol/l</td>
<td>1.65 ± 0.04</td>
<td>0.9 ± 0.02*</td>
<td>1.14 ± 0.03*</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.62 ± 0.05</td>
<td>5.4 ± 0.6*</td>
<td>2.16 ± 0.2*</td>
</tr>
</tbody>
</table>

Difference between parameters is reliable (p < 0.05): *compared with healthy people, #compared with indicators before treatment
Conclusion: Carnitine orotate administrations in patients with NASH/DM type 2 improved clinical-biochemical parameters of liver function, fasting and after loading plasma glucose, lipid profile and homocysteine level in the blood.
New approaches to the correction of lipid exchange disorders in patients with metabolic syndrome

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National Medical Academy of Postgraduate Education, named after P.L. Shupyk, Kiev, Ukraine

Introduction: Prevalence of metabolic syndrome (MS) in the general population of different countries ranges from 14 to 24%. The main stages of MS pathogenesis are insulin-resistance and compensatory hyperinsulinemia. Development of hepatic steatosis is the initial starting point of the MS. We have developed a treatment complex, providing a diet with restriction of carbohydrates and fat, normal protein content, enriched with vitamin-mineral complexes and lecithin. Appointed also "Ursofalk®" in a dose of 500 mg per day, and recommended to increase physical activity.

Methods: Examined 136 patients with MS, aged from 21 to 69 years. All patients noted increased body weight and average BMI was (36.8 ± 2.7) kg/m². Patients were divided into two groups: primary group – 70 patients, the control group – 66. Patients of both groups were prescribed a developed diet. Patients of the primary group were appointed "Ursofalk®". The course of treatment extended 6 months.

Results:
Table 1: Indicators of blood lipids in patients with MS, mmol/l

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Healthy (n = 21)</th>
<th>Primary group (n = 70)</th>
<th>Control group (n = 66)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td></td>
<td>Pe &lt; 0.001</td>
<td>Pe &lt; 0.001</td>
<td>Pe &lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>4.34 ± 0.25</td>
<td>7.87 ± 0.31</td>
<td>5.83 ± 0.16</td>
</tr>
<tr>
<td>LDL</td>
<td>2.82 ± 0.11</td>
<td>5.71 ± 0.23</td>
<td>3.11 ± 0.12</td>
</tr>
<tr>
<td>HDL</td>
<td>1.56 ± 0.05</td>
<td>0.98 ± 0.03</td>
<td>1.43 ± 0.03</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.53 ± 0.09</td>
<td>3.91 ± 0.24</td>
<td>1.84 ± 0.12</td>
</tr>
</tbody>
</table>

Pe – reliability compared with indicators of healthy people,
Pt – reliability compared with rates before and after treatment

Conclusion: Applying "Ursofalk®" in the treatment of patients with MS reduces blood total cholesterol, LDL, triglycerides and increases HDL level.
Pioglitazone improves virological response to peginterferon alpha-2b/ribavirin combination therapy in hepatitis C genotype 4 patients with insulin resistance

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Introduction: Insulin resistance (IR) affects sustained virological response (SVR). The use of insulin-sensitizing agents has been proposed to improve therapy outcome. The safety and efficacy of pioglitazone on insulin sensitivity and SVR in treatment-naïve patients with chronic hepatitis C (CHC) genotype 4 with IR receiving standard antiviral therapy were evaluated in a randomized-controlled study.

Methods: Ninety-seven previously untreated patients with CHC and IR (homeostasis model assessment [HOMA > 2]) were randomly assigned into two arms; (arm A; n = 48) were given pioglitazone 30 mg/day combined with peginterferon (Peg-IFN)-alpha-2b/ribavirin (RBV) for 48 weeks, and (arm B; n = 49) were given standard of care (Peg-IFN-alpha-2b/RBV for 48 weeks); HOMA index and hepatitis C virus RNA (HCV RNA) levels were measured at baseline, during therapy and follow-up. Treatment was stopped in patients without an early virological response or those who were HCV RNA positive at 24 weeks.

Results: Baseline data of both groups were comparable, with no significant statistical differences. The percentages of rapid virological response (RVR) and SVR were significantly higher in patients given triple therapy compared with standard of care (27.08 vs. 6.1%; P = 0.006 and 60.4 vs. 38.7%; P = 0.04 respectively); patients in arm A showed a greater decrease in the HOMA index than those in arm B (-1.8 ± 0.3, -2.1 ± 0.3 vs. -1.1 ± 0.6, -1.3 ± 0.7) at week 24 and at the end of follow-up (P = 0.001 at both time points). The triple therapy was well tolerated.

Discussion/Conclusion: A combination of pioglitazone, Peg-IFN-alpha-2b and ribavirin increased RVR, SVR and decreased IR, compared with patients given Peg-IFN plus ribavirin without an increase in adverse events.
FTY720 increases the hepatic retention of purified haematopoietic stem cells in chronic liver injury resulting in an enhanced anti-fibrotic action

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Introduction: There is considerable interest in the use of bone marrow (BM) cells in liver cirrhosis, however the role of purified haematopoietic stem cells (HSC) and use of repeated infusions have not been studied. We also set out to determine whether increased retention of HSC within the injured liver by modulating their response to Sphingosine 1-phosphate (S1P) would augment their anti-fibrotic effect.

Methods: Liver injury was induced in BoyJ (CD45.1) mice by twice weekly ip injection of carbon tetrachloride (1 mg/kg) for ten weeks, whilst purified FACS-sorted HSC (c-kit+ sca1+ lineage-) isolated from BM of C57BL6 (CD45.2) mice were infused via tail vein at weeks 7, 8, 9. Tracking of DiR labelled HSC was performed using the IVIS Spectrum imaging system and cell numbers quantified by flow cytometry. The partial S1P receptor agonist FTY720 (1 mg/kg) was administered by ip injection.

Results: Repeated infusions of HSC resulted in a significant reduction in liver scarring as assessed by: picrosirius red (PSR) staining (48.8% reduction vs. control, p < 0.001), hepatic hydroxyproline content (436 vs. 313 mg/g liver, p < 0.01), αSMA immunostaining (7.0 vs. 2.4% staining, p < 0.001), as well as increased serum albumin (3.1 vs. 4.0 g/dl, p < 0.001). In separate BM transplantation studies liver injury was seen to result in a 4.4 fold increase in the number of BM-derived HSC in the liver (vs. controls, p < 0.001). Increased hepatic S1P levels in liver injury resulted in a reduced S1P gradient between liver and lymph, and were a result of increased hepatic sphingosine kinase 1 expression. FTY720 reduced HSC migration to S1P and resulted in a 1.7 fold increase in BM-derived HSC accumulating in the liver (vs. no FTY720, p < 0.01) and a 1.9 fold increase in the number of infused HSC in the liver 4 days after infusion (vs. no FTY720, p < 0.01). Intravital microscopy demonstrated this was not due to increased hepatic recruitment of HSC. Repeated administration of FTY720 during infusions of HSC resulted in a further reduction in hepatic fibrosis compared with HSC infusions alone (PSR 21.7% reduction, p < 0.05; αSMA 25% reduction, p < 0.05). The antifibrotic effect of HSC was also seen with infusions of lymphoid progenitors lacking myeloid potential. Infused cells (CD45.2+) were not detected in livers 7 days after infusion, although there were increased numbers of recipient (CD45.1+) neutrophils and macrophages (2.2 and 1.7 fold increase vs. control, p < 0.01) in the liver following HSC infusion.
Discussion/Conclusion: Our data demonstrate the potent anti-fibrotic action of repeated infusions of purified HSC, which is mediated by recruitment of endogenous cells. Moreover, we demonstrate that increasing hepatic retention of HSC with FTY720 augments their anti-fibrotic action.
The ratios of pro to anticoagulant factors: Index of hemostatic imbalance in cirrhotic patients

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Introduction/Aim: Patients with cirrhosis are characterized by decreased levels of most of pro- and anti-coagulant factors. This state results in an unstable balance. Therefore, patients are prone to hemorrhagic or thromboembolic events particularly in advanced stages. The aim of this study was to evaluate the ratios of pro coagulant to inhibitor coagulation factors in cirrhotic patients according to disease severity.

Methods: A case control study including cirrhotic patients and healthy subjects matched by age and sex was conducted. Patients were stratified according to Child Pugh classification. Pro coagulant factor activity (factor VII, II, V, VIII, XII) and inhibitor factor activity were determined (Protein C, protein S and antithrombin). Mean value of pro coagulant to inhibitor coagulation factor ratios in patients were compared to those in controls and investigated in patients according to Child Pugh classification.

Results: 51 cirrhotic patients and 51 controls were included. Their mean age was 56.8 years. Sex ratio (male to female) was 0.9. Patients were classified in Child Pugh A in 13 patients (25.5%), B in 23 patients (45.1%), C in 15 patients (29.4%). Among ratios, II/PC, V/PC, VII/PC, XII/PC were significantly higher in cirrhotic patients than in controls (respectively, p = 0.001, p = 0.002, p = 0.001, p = 0.001) but there wasn’t any difference between Child Pugh classes. Likewise, VIII/PC, VIII/PS and VIII/AT were significantly higher in cirrhotic patients than in controls (p < 0.001) and increased significantly from class A to C (p < 0.001), reaching a value of 5. On the other hand, II/PS was lower in cirrhotic patients than in controls showing marginal significance (p = 0.04). However, II/PS, V/PS, VII/PS decreased significantly from class A to C (p = 0.006, p = 0.013, p = 0.002, p = 0.024).

Conclusion: The ratios of pro- to anti-coagulant factors showed a coagulation imbalance in our patients with trend to hypercoagulability state. These hemostatic changes were significantly correlated with severity of cirrhosis.
Thrombotic risk factors in cirrhotic patients

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Background: Cirrhosis results in a complex pattern of defects in haemostatic functions with reduced synthesis of pro- and anticoagulant factors. As possible complication of coagulation disorders in cirrhosis, could be the development of arterial or venous thromboembolism (AVTE). The purpose of our study was to determine thrombotic risk factors in cirrhotic patients.

Methods: Cirrhotic patients were enrolled. The presence of personal and familial history of AVTE was investigated. Patients were divided into 2 groups. Group 1 included patients who developed arterial or venous thromboembolism after cirrhosis diagnosis and group 2 cirrhotic patients without thrombotic event. White blood cells, platelet count, prothrombine time, INR, albumin, urea, pro coagulant factors (VIII, XII, VII, II, V) were determined. Level of antithrombin, protein C and protein S were measured. Search for factor V Leiden and prothrombin gene mutation (G20210A) were performed with PCR-RFLP. Anticardiolipin and anti-B2-glycoprotein antibodies were also investigated. Both groups of patients were compared with regard of clinical and biological findings.

Results: Fifty one cirrhotic patients were included. Their mean age was 56.8 years. They were men and women. Among the 51 cirrhotic patients, 7 (13.7%) had experienced AVTE after cirrhosis diagnosis: deep venous thrombosis (n = 2), pulmonary embolism (n = 1), Budd Chiari syndrome (n = 1), portal thrombosis (n = 3). They were compared to 46 cirrhotic patients without thrombosis. No patient with AVTE had neither personal nor familial history of thrombosis. In a univariate analysis, white blood cell count and platelet count were significantly higher in patients with AVTE than other cirrhotic patients (respectively 8795 vs. 5032/mm$^3$, p < 0.018 and 91133 vs. 154375/mm$^3$, p = 0.03). However, in a multivariate analysis only the platelet count was independently predictive of VTE in cirrhotic patients (p = 0.05). Moreover, prothrombin time, INR, albumin, urea, level of pro and anticoagulant factors were not statistically different in both groups. There was no link between the presence of Factor V Leiden, prothrombin gene mutation (G20210A), anticardiolipin and anti-B2-glycoprotein antibodies to thrombosis.

Conclusions: Approximately 13.7% of cirrhotic patients resulted in a thromboembolic event. Platelet count was predictive of increased risk of AVTE as it was supported by other studies. Understanding the factors predisposing to thrombosis in cirrhotic patients could play a role in identifying a subgroup of patients at high risk of thrombosis and making decisions regarding the utility of anticoagulation therapy.
Prolonged entecavir therapy is not effective for HBeAg seroconversion in treatment-naïve chronic hepatitis B patients with a partial virological response

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Introduction: Entecavir (ETV) is a potent antiviral agent in treatemnt-naïve chronic hepatitis B (CHB) patients. The aim of this study was to investigate the long term efficacy of ETV in treatment-naïve CHB patients, particularly in those with detectable hepatitis B virus (HBV) DNA after 1 year, in whom treatment adaptation is suggested by current guidelines.

Methods: 133 treatment-naïve patients with CHB, who visited Chung-Ang University Hospital between January 2007 and January 2010, were enrolled. The mean duration of treatment was 57 months (range, 36–76). At baseline, the mean age was 49 years (range, 23–74), and 81 patients (61%) were HBeAg-positive. Partial virological response (PVR) was defined as detectable HBV DNA (> 116 copies/mL) at year 1.

Results: We investigated 133 treatment-naïve CHB patients treated with entecavir monotherapy (male, 101). virological response (VR) (HBV DNA < 116 copies/mL) was achieved in 37%, 64%, 88% and 95% of 81 patients with HBeAg-positive and in 67%, 94%, 95% and 100% of 52 patients with HBeAg-negative at year 1, 3, 5 and 6, respectively. Sixty-eight of 133 (51%) treatment-naïve patients had a detectable load at year 1 (PVR). 45 (66%) patients with PVR reached VR at year 3. 18 patients with PVR were treated with continuous ETV monotherapy for 6 years. Among them, 17 patients reached VR at year 6. ETV did not show limited efficacy in patients with PVR during prolonged therapy. However, the rate of HBeAg seroconversion at year 6 was significantly higher in VR group compared with PVR group (61.1% vs. 30.5%).

Discussion/Conclusion: ETV monotherapy can be continued in treatment-naïve patients with detectable HBV DNA at year 1, because long-term ETV leads to a virological response in the most of patients. However, long-term ETV monotherapy may be not effective for HBeAg seroconversion in NA-naive CHB patients with a PVR to ETV.
Preoperative TACE improves the survival in the resectable HCC with highly elevated AFP

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Background: To evaluate the effect of preoperative TACE for resectable hepatocellular carcinoma (HCC) with highly elevated alpha-fetoprotein (AFP) (≥ 200 ng/mL).

Methods: From August 2004 to March 2012, 297 consecutive patients with HCC underwent liver resection at Seoul St. Mary Hospital and St. Vincent Hospital. Among these patients, 39 patients had elevated AFP at diagnosis (≥ 200 ng/mL). We retrospectively compared recurrence rate and overall survival between patients who underwent preoperative TACE (TACE-LR group, n = 19) and those who did not (LR group, n = 20). The patients who received other treatments besides TACE and whose follow up duration is less than a year were excluded. Time to recurrence and overall survival were analyzed by Kaplan Meier method and log-rank test.

Results: The median age of the patients was 52 years (range, 16–67) and median follow-up duration was 25 months (range, 6–107). Most patients (87.1%) were related to chronic hepatitis B, and 26 patients (66.7%) were male. All the patients were Child-Pugh class A. Three patients had portal vein invasion. Baseline AFP level was median 739.7 ng/mL (range, 231.68–11781.4). Tumor size at diagnosis was median 4 cm (range, 1.3–17). The recurrence rates were 63.2% for TACE-LR group and 50% for LR group (P = 0.523). Mean time to recurrence were 585.9 ± 238.4 days for TACE-LR group and 265.5 ± 94.7 days for LR group (P = 0.206). The TACE-LR group had longer mean survival time than LR group (101.6 ± 5.1 months vs. 80.9 ± 9.3 months, P = 0.031).

Conclusions: Preoperative TACE improves the survival in the resectable HCC with highly elevated AFP (≥ 200 ng/mL).
Outcomes of surgical resection following downstaging the unresectable hepatocellular carcinoma with combination treatments

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Background: Therapeutic option for the patients with unresectable, large (> 10 cm) extensive hepatocellular carcinoma (HCC) is limited. We assessed the efficacy and safety of transcatheter arterial chemoembolization (TACE)-based multimodal treatment in patients with advanced HCC tumors ≥ 10 cm and/or portal vein thrombosis (PVT).

Methods: HCC patients who had a large HCC over 10 centimeters without distant metastases were assessed. A total of 146 consequent patients were included in the analysis, and medical records and radiological data of them were reviewed retrospectively. Among 146 patients, 119 patients (81.5%, treatment group) received TACE and during or after the TACE, additional therapeutic modalities (surgery, locoregional treatment, systemic chemotherapy or radiotherapy) were performed as necessary. The remaining 27 patients received conservative management (conservative group).

Results: There were no significant differences of basal characteristics including tumor type, stage, and PVT between the two groups. Overall survival (median 10.3 vs. 4.0 months, \( P < 0.001 \)) and objective tumor response (28.4 vs. 0%, \( P = 0.003 \)) were significantly better in treatment group than in conservative group. After subgroup analysis, survival benefits were observed not only in curative treatment group (TACE + resection or transplantation) than in localized treatment group (TACE + other modalities) (median 31.6 vs. 9.1 months, \( P < 0.001 \)), but also in combination treatment group (TACE + other modalities) than in TACE-only group (median 12.8 vs. 8.1 months, \( P = 0.002 \)). Multivariate analysis identified tumor stage (odds ratio = 1.869; 95% confidence interval (CI) 1.082–3.230, \( P = 0.025 \)) and objective tumor response (odds ratio = 3.114; 95% CI 1.778–5.455, \( P < 0.001 \)) as the two independent factors for survival. Aforementioned survival benefits were consistent after stratification of other known poor prognostic factors. There were no significant life-threatening adverse effects related to the treatment.

Conclusion: TACE-based therapeutic strategy with combination of other modalities was safe and beneficial than conservative management for advanced massive HCC without distant metastasis.
Carriage of PNPLA3 I148M is associated with an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma

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Introduction: Inter-patient genetic variation and environment determine NAFLD progression. There is a significant increase in prevalence of NAFLD-related hepatocellular carcinoma (HCC) but causative factors remain obscure. The PNPLA3 I148M polymorphism is associated with steatohepatitis and fibrosis and has been linked to HCC-risk in hepatitis C and alcoholic liver disease. We sought to determine whether carriage of PNPLA3 variants was associated with HCC risk in NAFLD.

Methods: Genotype for the PNPLA3 I148M (rs738409) SNP was determined by allelic discrimination (Taqman) in 84 European Caucasians with histologically confirmed NAFLD-related HCC and 285 controls with histologically characterised NAFLD but no evidence of HCC.

Results: PNPLA3 I148M genotype frequencies were significantly different between NAFLD-HCC cases (CC = 27, CG = 34, GG = 23) and NAFLD-controls (CC = 125, CG = 125, GG = 35) (ChiSq for Trend p = 0.0024). In a multivariate model adjusted for age, diabetes and gender, I148M genotype was significant (OR 2.652, 95% CI 1.46–4.81, p = 0.0013) and remained so even after inclusion of fibrosis stage (OR 2.128, 95% CI 1.11–4.08, p = 0.0231). Adopting a recessive model, homozygote minor (G) allele carriage was associated with increased HCC-risk (GG vs. CC/CG: OR 2.7, 95% CI 1.48–4.89, p = 0.0011), which was greater when compared to CC homozygotes (GG vs. CC: OR 3.04, 95% CI 1.56–5.95, p = 0.0011). Compared to the UK general population (1958 British Birth Cohort DNA Collection) rather than a NAFLD cohort, the risk-effect was even more pronounced (GG vs. CC/C: OR 7.16, 95% CI 2.59–19.85, p = 0.0002; GG vs. CC: OR 10.05, 95% CI 3.45–29.28, p < 0.0001).

Conclusions: This gene association study demonstrates that, within a population with histologically confirmed NAFLD, carriage of the PNPLA3 I148M single nucleotide polymorphism is significantly associated with HCC. These data therefore suggest that affected individuals are not only at greater risk of progressive steatohepatitis and fibrosis but also of development of HCC. If validated, PNPLA3 genotyping may offer a tool for patient-risk stratification.
Role of SLC22A1 gene in the response of hepatocellular carcinoma and cholangiocarcinoma to sorafenib

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Background and Aims: Reduced efficacy of pharmacological treatment of cancer is due in part by decreased intracellular content of active drugs. Thus, lowering uptake is an important mechanism of tumour chemoresistance. In this respect, impaired organic cation transporter-1 (OCT1, gene symbol SLC22A1) function may affect the response of hepatocellular carcinoma (HCC) and cholangiocarcinoma (CGC) to sorafenib in, which is taken up in part by OCT1. Here we have investigated whether changes in SLC22A1 expression and the appearance of genetic variants may contribute to liver cancer chemoresistance.

Methods: SLC22A1 mRNA was determined by RT-QPCR. Gel-electrophoresis-based complete sequencing and selective variant identification by RT-PCR was performed to detect SNPs in SLC22A1 cDNA. Modifications in OCT1 ORF sequence were mimicked by directed mutagenesis and used for cell transfection, immunofluorescence, transport assays and determination of sorafenib anti-tumour activity “in vitro”.

Results: In HCC and CGC, in addition to a marked decrease in OCT1 expression, 3 previously described plus 2 novel alternative spliced variants, and 11 previously described plus 3 novel SNPs were found. To study their functional consequences, SLC22A1 cDNA containing one of the identified SNPs was expressed in human hepatoma Alexander cells. Both c.181delCGinsT and c.262delT resulted in a reduction of tetraethylammonium uptake, which was due to the absence of OCT1 targeting to the plasma membrane. This was consistent with a reduced sensitivity to sorafenib. Screening of these SNPs in 23 HCC and 17 CGC revealed that c.181delCGinsT was present in both HCC (17%) and CGC (13%), whereas c.262delT was only found in HCC (17%). Considering all SLC22A1 variants, at least one inactivating SNP was found in > 40% HCC and > 30% CGC.

Conclusions: Liver carcinogenesis is accompanied by the appearance of aberrant variants of OCT1 that may dramatically affect the ability of HCC and CGC to take up and hence respond to sorafenib.
Do endosonography findings predict variceal recurrence after endoscopic band ligation?

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Introduction: Endoscopic band ligation (EBL) has a considerable tendency of variceal recurrence (VR). The aim was to analyse the value of esophageal collateral veins (ECV) as predictors for VR after EBL.

Methods: From September 2011 to January 2013 31 patients (67.7% men; average age 53.6 ± 8.9 years) with large esophageal varices and EBL indicated was enrolled in prospective study. Endosonography was performed before EBL and ECV were classified into 3 types (peri-ECV, para-ECV and perforator) and 2 grades: mild (peri-ECV < 2 mm; para-ECV < 5 mm) and severe (peri-ECV ≥ 2 mm and para-ECV ≥ 5 mm). Varices were ligated every 2 weeks until obliteration and endoscopy was performed every 3 month to detect VR. Patients, followed for 1 year (n = 16), were divided into recurrent (n = 12) and non-recurrent (n = 4) groups. Further, the recurrent group was divided into early (≤ 6 months (n = 8)) and late recurrent (> 6 months [n = 4]) groups. Relationship between endosonography findings and the VR rate was analysed (p value < 0.05 was considered significant).

Results: To this day 31 patients was being followed for 3 months, 22 – for 6 months, 21 – for 9 months and 16 – for 1 year. VR was detected in 16.1% of patients within 3 months, in 36.6 % of patients within 6 months and in 75% of patients within 1 year. No significant difference (p > 0.05) regarding ECV type and grade was found between recurrent/non-recurrent and early/late recurrent groups. Mathematical Cox proportional hazards model of data found that severe peri-ECV are associated with higher and earlier recurrence risk after EBL (hazard ratio 1.57).

Discussion/Conclusion: Endosonography may be a promising tool for predicting VR after EBL, but in our small sample size we do not reached statistical significance.
Liver tumors – The vascularisation pattern assessed by contrast-enhanced ultrasound

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Introduction: The development of contrast-enhanced ultrasound (CEUS) has considerably improved the possibilities of ultrasound in the assessment of liver tumors, based on tumor-specific vascularization pattern.

Aim: to investigate the ability of CEUS to differentiate between benign and malignant focal liver tumors in clinical practice.

Methods: In a prospective study between 1st September 2011 and 1st September 2012 we examined 120 patients with focal liver lesions diagnosed by conventional ultrasound. All patients were examined by CEUS with the contrast agent Sonovue, 5 minutes, using low – MI ultrasound with contrast – specific software (Hitachi). The final diagnostic was made by computed tomography, magnetic resonance imaging and in some cases histological exam.

Results: There were 64 men and 56 women, aged between 27 and 79 years. We found 76 malignant lesions (38 hepatocarcinoma, 34 metastases, 4 colangiocarcinoma) (group A) and 44 benign tumors (18 hemangiomas, 12 regenerative nodules, 6 focal steatoses, 4 focal nodular hyperplasia, 2 adenoma, 2 abscess) (group B). Hyperenhancement in the arterial phase with diffuse complete enhancement was found in 50 patients in group A and only in 4 patients in group B (p < 0.01). Hyperenhancement in the late phase was found in 2 patients group A and 16 patients group B. Hypoenhancement or no enhancement in the late phase was found in 72 patients group A and 12 patients group B (p < 0.01).

Discussion/Conclusion: Hypoenhancement in the late phase is the best CEUS parameter to differentiate benign from malignant liver tumors.
PNPLA3 rs738409 polymorphism and the possible involvement in development of NAFLD

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Introduction: Aim of our study was to identify the possible involvement of genetic factor in the development of NAFLD.

Methods: We included 138 subjects with NAFLD and 125 age and sex matched healthy controls. In both groups we evaluated anthropometric measures, aminotransferases level, presence of diabetes mellitus or metabolic syndrome, insulin resistance and the PNPLA3 gene polymorphism. The genotyping assays were performed using predesigned TaqMan SNP Genotyping Assays.

Results: The genotype frequencies for PNPLA3 rs738409 polymorphism in the study group was [CC] (59.42%) > [CG] (32.41%) > [GG] (7.97%). The [CG] genotype carriers had a 1.7 times higher risk of developing hepatic steatosis, compared with the [CC] genotype OR 1.768 (95% CI; 1.006–3.110) (p = 0.046). The PNPLA3 polymorphism was associated with an increased risk of hepatic steatosis in patients with BMI < 30 kg/m², compared with the control population, when the risk allele [G] carriers were compared with the [C] allele carriers (p = 0.038). By comparing the subgroup with steatosis without obesity with the subgroup with steatosis and BMI ≥ 30 kg/m², we have noticed that the [G] allele carriers compared to the [CC] homozygotes in the dominant model, have a 2.5 times higher risk of developing hepatic steatosis (p = 0.025) OR 2.514 (1.112–5.685). [G] risk allele was significantly associated with the risk of hepatic steatosis in patients without metabolic syndrome (p = 0.005) and without insulin resistance (p = 0.033). Also, we found no difference in cholesterol, triglycerides, aminotransferases and gamma-GT levels in [G] allele carrier versus [CC] homozygotes.

Discussion/Conclusion: The [G] allele carriers have a 3 times higher risk of developing hepatic steatosis in the absence of obesity, insulin resistance, or metabolic syndrome. Patients with similar metabolic risk factors (diet, obesity, insulin resistance) differ largely in terms of disease phenotype and progression of disease.
The relationship between non-invasive evaluation of fibrosis (by transient elastography) and genes polymorphism in NAFLD

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Introduction: Although, liver biopsy is the gold standard in evolution of liver damage in NAFLD, transient elastography (Fibro-Scan) is a technique that allows rapid and non-invasive measurement of tissue stiffness and estimate liver fibrosis in patient with NAFLD. Aim of our study was to correlate the degree of fibrosis estimated by Fibro-Scan and gene polymorphism for PNPLA3, MTTP and Mn SOD.

Methods: The study was prospective between November 2009–June 2012 and included 138 subjects with NAFLD and 125 age and sex matched healthy controls. Real Time PCR technique, using predesigned TaqMan SNP Genotyping Assays were performed for the following gene polymorphism PNPLP3 I148M- rs738409, MTTP 4936/T rs1800591 and Mn SOD 1183T>C. We performed transient elastography in both groups and we had 8 false results in patients with BMI > 50.1 kg/m².

Results: For the PNPLA3 gene polymorphism, [G] allele was more frequent in patients than in control group and was associated with high degree of fibrosis (p = 0.038). For MTTP 493GTrs1800591 polymorphism none of patients with LS ≥ 8.7 KPa was carrier of TT genotype. GT genotype carrier had 2 time higher risk for developing fibrosis than in controls (p = 0.022). For MnSOD polymorphism we did not notice any relationship with liver fibrosis.

Discussion/Conclusion:
1. For PNPLA3 the risk [G] allele carrier have a 2.6 time higher risk for developing severe degree of fibrosis (p = 0.024).
2. Although, Mn SOD polymorphism did not correlate with liver fibrosis, but [TT] homocygotes had correlated with high levels of aminotransferases.
Developing ImageStream™ as a sensitive and clinically relevant tool for detection of circulating tumour cells (CTCs) in patients with hepatocellular cancer (HCC)

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Introduction: Surveillance has little impact on HCC mortality as serum tests have a poor sensitivity and specificity. Novel biomarkers to aid diagnosis, predict prognosis, guide treatment and monitor response are needed. It is hoped CTC detection, number and characterisation will provide clinically relevant information. The commonly used CellSearch system is reliant on EpCAM expression on CTCs, which limits its usefulness. We have explored ImageStream™, an innovative multichannel flow cytometer with the added advantage of producing high-resolution fluorescent images of every cell analysed, for patients with HCC.

Methods: The numbers of cells positive, as well as level of expression (pixel intensity), has been assessed for candidate biomarkers (EpCAM, cytokeratin, AFP, GPC3, ZEB1, ARG1, vimentin) in a panel of HCC cell lines (HepG2, Hep3B, Huh-7, PLC/PRF5, SNU182, SNU475). Individual cell images as well as EpCAM, cytokeratin and AFP expression have been explored in blood (4 ml) of patients with HCC. Red cell lysis has been implemented and CD45 depletion of white cells optimised to enrich samples for CTCs.

Results: Between 1 and 35 nucleated CTC-like objects (CD45-ve) were detected in 12/17 HCC patient samples. Cells were EpCAM/cytokeratin/AFP-ve in 7 cases. CTC-like objects were detected in early (single ≤ 2cm) and advanced cases, occasionally travelling as aggregates attached to CD45+ve white cells. Cell line analyses identified significant correlations between biomarker positivities and intensities, in keeping with two distinct phenotypes defined by expression of either epithelial (EpCAM/CK/AFP/GPC3) or mesenchymal (vimentin/ZEB1) markers. Expression (pixel intensity) of ARG1 correlated closely with that of vimentin (SpearmanRho 0.886, P = 0.019).

Discussion/Conclusion: CTC-like objects were present in 70% of HCC patients samples, although in 50% of cases these were undetected by classical ‘epithelial’ biomarkers. HCC cell lines expressing higher levels of epithelial-mesenchyme transition (EMT) markers also expressed the candidate HCC biomarker ARG1. The use of a panel of biomarkers, including those associated with EMT, may increase the sensitivity of CTC detection.
Differential STAT3 expression in PBMCs from HCV genotype 1 & 3 infected patients may explain lack of responsiveness to IFN-α therapy

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Introduction: Interferon-α (IFN-α) is a type I IFN which upregulates over 500 IFN-stimulated genes playing a critical role in anti-viral immunity. The mainstay of treatment for chronic Hepatitis C (HCV) infection includes pegylated IFN-α and ribavirin. SVR is achieved in up to 82% of genotype 3 patients while those infected with genotype 1 achieve SVR in only 40–54% of cases. We have shown that STAT3 protein levels are depleted in peripheral blood mononuclear cells (PBMCs) from genotype 1 HCV-infected patients. Loss of STAT3 resulted in reduced signalling via the JAK/STAT pathway and reduction in IFN-stimulated gene induction. Our proposed mechanism is that HCV acts as an E3 ligase, causing ubiquitination and degradation of STAT3 by the proteosome. Since genotype 3 patients respond well to treatment we hypothesised that they have normal STAT3 levels, permitting optimal IFN-α signalling and viral clearance. Our aim was to compare the levels of STAT3 protein in genotype 1 & 3 HCV-infected PBMCs.

Methods: Blood samples were collected from 12 genotype 1 and 12 genotype 3 HCV-infected patients and 6 healthy controls. PBMCs were isolated and STAT3 protein levels were measured by Western blotting. Each blot compared STAT3 levels in both genotypes to 2 healthy controls. Flow cytometry was done to quantify STAT3 levels in immune cell subpopulations.

Results: All patients were treatment naïve. Over two-thirds of both genotype 1 & 3 patients had mild fibrosis (F0–F2). 11/12 (91.7%) of genotype 1 patients had significantly reduced STAT3 levels compared to 4/12 (33.3%) of genotype 3 patients (p = 0.009 Fisher's exact test). Flow cytometry showed a reduction of STAT3 in all immune cell subpopulations in genotype 1 HCV compared to healthy controls.

Discussion/Conclusion: HCV genotype 1 promotes STAT3 degradation in over 90% of patients. In contrast HCV genotype 3 is far less efficient at degrading STAT3. This may contribute to the reduced responsiveness of genotype 1 infected patients to interferon-α therapy and provide an opportunity to identify differential viral targets for therapeutic restoration of interferon responsiveness.
Expansion of arginase-expressing myeloid-derived suppressor cells in chronic hepatitis B virus infection

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Previous work from our group has pointed to a role for nutrient deprivation in driving a global T cell defect in chronic hepatitis B infection (CHB). We have shown that the conditionally essential amino acid L-arginine is depleted and arginase activity increased in the HBV-infected liver (Das, JEM 2008). In this study we postulated that myeloid-derived suppressor cells (MDSC), a population known to expand at sites of chronic antigenic stimulation and inflammation, may contribute to the arginase-rich suppressive milieu in CHB.

Using 11-colour flow cytometry, we analysed the circulating frequency of granulocytic MDSC (gMDSC) and their expression of putative regulatory mediators in patients with CHB (n = 56) and healthy controls (n = 49). Circulating gMDSC were expanded approximately 10-fold in CHB compared to healthy controls (p < 0.001). This expansion was also evident in HCV and correlated with age, suggesting that this suppressive population progressively accumulates during persistent viral hepatitis. The expansion of MDSC is known to be driven by inflammatory mediators such as TNF-α; in line with this, gMDSC decreased in those patients in whom liver inflammation was abolished by antiviral therapy (r 0.9, p < 0.01). A striking further enrichment of gMDSC was found in the intrahepatic compartment, the site of viral infection. One key function of gMDSC is their production of suppressive regulators, including arginase-1; we were able to demonstrate strong expression of this enzyme by the majority of gMDSC by flow cytometry and image stream. Preliminary sorting experiments have confirmed the capacity of MDSC to suppress T cell function and down-regulate the CD3ζ chain, a hallmark of arginine deprivation. Our data therefore show that gMDSC accumulate in CHB and have the potential to suppress T cell function through nutrient deprivation.
Presence of IgA class anti-neutrophil cytoplasmic antibodies (ANCA) in cirrhosis – Possible hint towards the involvement of gut mucosal immune system?


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**Introduction:** Anti-neutrophil cytoplasmic antibodies (ANCA) are a non-uniform family of antibodies recognizing diverse components of neutrophil granulocytes. ANCA formation might be induced by protracted bacterial infections or probably reflect an abnormal immune response to commensal microorganisms. Bacterial infections are common complications in cirrhosis with high incidence of episodes caused by enteric organisms, therefore, we sought to study the presence and clinical importance of ANCA in cirrhosis.

**Methods:** Sera of 385 patients with cirrhosis of different etiologies were assayed for ANCA of IgG, IgA, IgA1, IgA2 and secretory IgA subtypes by indirect immunofluorescence and ELISAs. Control group comprised of 202 patients with chronic liver diseases without cirrhosis and 100 healthy subjects. In cirrhosis, a 2-year follow-up, observational study was conducted to assess possible association between presence of ANCA and clinically significant bacterial infections.

**Results:** Prevalence of ANCA IgA was significantly higher in cirrhosis (52.2%) compared to chronic liver diseases (18.6%) or healthy controls (0%, p < 0.001 for both). ANCA IgA subtyping assays revealed marked increase in the proportion of IgA2 subtype (46% of total ANCA IgA) and presence of the secretory component concurrently. Presence of ANCA IgA was associated to disease-specific clinical characteristics (Child-Pugh stage and presence of ascites, p < 0.001). During a 2-year follow-up period, risk of infections was higher among patients with ANCA IgA compared to those without (41.8% vs. 23.4%, p < 0.001). ANCA IgA positivity was associated with a shorter time to the first infectious complication (pLogRank < 0.001) in Kaplan–Meier analysis and was identified as an independent predictor in multivariate Cox-regression analysis (HR: 1.74, 95% CI: 1.18–2.56, p = 0.006).
Discussion/Conclusion: Presence of IgA type ANCA is common in cirrhosis. Involvement of gut mucosal immune system is in center of the formation and probably reflects sustained exposure to bacterial constituents.
IL28B CC and IL10R -1087 GG genotypes are protective for chronic genotype 1 HCV infection in an East-Central European country

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Aim: We assessed the effect of IL28B and IL10R -1087 polymorphisms on the outcome of chronic genotype 1 HCV infection in an East-Central European country, Hungary.

Methods: A total of 748 chronic HCV positive patients have been enrolled; 420 of them were treated with PEG-IFN plus ribavirin (P/R) for 24–72 weeks, and 195 patients (46.4%) achieved sustained virological response (SVR). For genotyping studies DNA was isolated from peripheral blood by a standard desalting method. IL28 rs12979860 SNP was determined using Custom Taqman SNP Genotyping Assays (Applied Biosystems, Life Technologies, Foster, CA, USA). The IL10R -1087 (also known as IL10R- 1082) promoter region was required for the formation of the EcoNI recognition sequence 5'-AAGGCAACACTACTAAGGCTTCCTT-3'; the lower primer was 5’-TAAATATCCTCAAAGTCC-3’. After cutting the amplified product (590 bp) by EcoNI, homozygote GG was identified by two fragments 280 and 310 bp, while heterozygote AG had 310, 280, 252 and 28 bp fragments and homozygote AA had 310, 252 and 28 bp fragments.

Results: The IL28B rs12979860 CC genotype occurred with lower frequency in patients than in healthy controls (26.1% vs. 51.4%). Patients with the IL28B CC genotype achieved higher SVR rate, than those with CT (58.6% vs. 40.8%), or who carried T allele (41.8%). The prevalence of IL10R -1087 GG genotype was lower in patients than in controls (31.8% vs. 52.2%), that suggested a protective effect of this IL10R variant in HCV1 infection. Among patients with SVR, the IL10R -1087 GG genotype occurred with higher frequency than the AA (32.0% vs. 17.4%).

Discussion/Conclusion: Similar to IL28B CC, the IL10R -1087 GG genotype, and specific allele combinations of IL82B and IL10R -1087 can also be protective against HCV infection.
IL28B CC genotype is associated with increased Th1 but not Th2 type cytokine production by peripheral blood mononuclear cells in chronic HCV1 infection

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Introduction: IL28B CC genotype is the strongest pretreatment predictor of SVR in HCV1 patients treated with PEG-IFN and ribavirin. IL28B CC is associated with greater likelihood of rapid virological response (RVR) and immune control of HCV infection compared with CT or TT genotypes. Since cytokines are important regulators of the antiviral immune response, the aim of the present study was to compare IFN-gamma, TNF-alfa, IL-2, IL-4, IL-6, IL-10 cytokine production of patients with IL28B CC, CT and TT haplotypes.

Methods: Fourty HCV-1 patients were genotyped as CC (n = 12), CT (n = 20) or TT (n = 8) at polymorphic site of IL28B rs12979860. IFN-gamma, TNF-alfa, IL-2, IL-4, IL-6, IL-10 production of LPS stimulated peripheral blood monocytes and PMA+ionomycine stimulated lymphocytes were determined by FACS-CBA assay in each genotype group.

Results: LPS induced TLR4 activation of the monocytes resulted in significantly higher TNF-alfa production in patients with CC genotype compared to CT and TT variants. In patients with CC genotype we found increased Th1 type cytokine production of peripheral blood lymphocytes compared to non CC genotype groups. Lymphocyte TNF-alfa, IL-2, IFN-gamma production were significantly higher in CC patients compared to CT and TT groups (CC: TNF-alfa: 14.6 ng/ml, IL-2: 156.9 ng/ml, IFN-gamma: 225.7 ng/ml, CT: TNF-alfa: 7.1 ng/ml, IL-2: 40.5 ng/ml, IFN-gamma: 94.8 ng/ml, TT: TNF-alfa: 6.9 ng/ml, IL-2: 37.9 ng/ml, IFN-gamma: 109.2 ng/ml p < 0.01). IL-4, IL-6, IL-10 production did not differ between study groups.

Discussion/Conclusion: HCV1 infected patients with IL28B CC genotype had significantly increased LPS induced TNF-alfa production by monocytes, and increased TNF-alfa, IL-2 and IFN-gamma production of the lymphocytes compared to patients with CT or TT variants. Our data suggest that increased inducible Th1 type antiviral cytokine production of IL28B CC genotype patients may play a crucial role in rapid immune control of HCV infection and favour sustained virological response.
Liver stiffness measurement predicts the presence of large oesophageal varices in patients with chronic liver diseases

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Introduction: The early recognition of the oesophageal varices is of primary importance in the prevention of their bleeding in patients with liver cirrhosis. Liver stiffness (LS) measured by transient elastometry (FibroScan) may associate with portal pressure and could predict the presence of oesophageal varices. We studied the diagnostic accuracy of LS for selecting patients at risk of bearing large oesophageal varices and high risk (Paquet ≥ II grade) of bleeding.

Methods: We performed oesohago-gastro-bulboscopy and FibroScan examination in 74 patients with chronic liver disease simultaneously. We examined the relation between the presence of oesophageal varices (Paquet grade 0–IV) and LS (kPa) as well as blood hematological and biochemical laboratory parameters (INR, platelet count, ALT, AST, albumin). We analysed the predictive role of LS by FibroScan for selecting patients at high risk of variceal bleeding (Paquet ≥ II).

Results: LS values correlated to the grade of oesophageal varices (Paquet-grade) \( r = 0.67 \), \( p < 0.0001 \). The LS value was highly predictive of the presence of oesophageal varices (AUROC: 0.885, 95% CI: 0.81–0.96) and allowed to predict the high grade varices (Pq ≥ II) (AUROC: 0.85, 95% CI: 0.754–0.94). We found high measurement sensitivity: (sens) 85%, specificity (spec): 87%, positive predictive value (PPV): 85%, negative predictive value (NPV): 87% and validity: 86% at the cutoff 19.2 kPa. LS measurement value < 19.2 kPa was highly predictive of the absence of oesophageal varices grade ≥ II (sens.: 95%, spec.: 70%, PPV: 54%, NPV: 97%), thus we verified that below LS 19.2 kPa the high grade of oesophageal varices (Pq ≥ II) is not probable. The laboratory parameters did not predict oesophageal varices.

Discussion/Conclusion: The non-invasive LS measurement by FibroScan may select patients who are at high risk of bearing large oesophageal varices (Paquet ≥ II) and variceal bleeding and need endoscopic screening. LS above 19.2 kPa indicates an oesophageal-gastro-bulboscopy for the judgement of varices.
Outcomes after ileal pouch anal anastomosis in patients with primary sclerosing cholangitis

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Introduction: The function and quality of life outcomes in patients with Primary Sclerosing Cholangitis (PSC) and Ulcerative Colitis (UC) who undergo Ileal Pouch Anal Anastomosis (IPAA) are not well established. Reports conflict about the incidence of pouchitis in patients with PSC and IPAA, with few data on quality of life. This study investigated function and quality of life outcomes in patients with PSC and IPAA.

Methods: Patients with PSC-associated UC who underwent IPAA (PSC-IPAA) between 1983 and 2012 were compared to patients with UC without PSC who underwent IPAA in the same period, for pouch dysfunction, acute pouchitis, surgical complications, incidence of pouch dysplasia or cancer and biologic therapies or immunomodulators for treating pouch dysfunction. Baseline demographic and surgical characteristics were recorded. The Öresland score and Cleveland Global Quality of Life Questionnaire (CGQOL) were used to establish self-reported pouch function and the impact of IPAA on quality of life. Quality of life was also assessed by SF-36 (V1.0) questionnaire, including patients with PSC-associated UC without IPAA (PSC-UC) as a reference group.

Results: Thirteen patients with PSC-associated UC and 79 patients with UC underwent IPAA (UC-IPAA) in the period. More patients with PSC had pancolitis (62% vs. 30%) more underwent colectomy for dysplasia or cancer (23% vs. 3%). Trends for more patients with PSC-IPAA to suffer pouch dysfunction (69% vs. 47%; p = 0.231), acute pouchitis (46% vs. 20%; p = 0.073) or receive biologic or immunomodulator therapy (23% vs. 5%; p = 0.06) were not significant. Normal Q-Q plots to check the assumption of normality for the Öresland score gave a plausible linear relationship so a two sample t-test was used to compare the means of the two groups. The mean Öresland score for PSC-IPAA was 7.00 compared to 5.62 in patients with UC-IPAA (p = 0.07). The two groups had similar mean CGQOL scores (PSC-IPAA 0.73; UC-IPAA 0.75; p = 0.63). Mean Physical Health Summary (PCS) and Mental Health Summary (MCS) scores from SF-36 for PSC-IPAA vs. UC-IPAA were 41.9 vs. 48.0 (p = 0.04) and 41.4 vs. 47.6 (p = 0.03), respectively. The mean PCS and MCS scores for patients with PSC-UC were 44.4 (p = 0.65 vs. PSC-IPAA and p = 0.11 vs. UC-IPAA) and 43.5 (p = 0.33 vs. PSC-IPAA and p = 0.07 vs. UC-IPAA).

Discussion/Conclusion: Patients with PSC-IPAA have a trend to worse pouch function compared to patients without PSC, although this did not reach statistical significance, probably due to small numbers. Overall quality of life in patients with PSC-IPAA assessed by SF-36 (V1.0) and pouch function assessed by the Öresland score are significantly worse compared to patients with UC-IPAA.
Success rate of re-attempted ERCP after failed initial precut sphincterotomy for biliary cannulation

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Introduction: Selective biliary cannulation during endoscopic retrograde cholangiopancreatography (ERCP) fails in 5–10%, even in expert high volume centers. Precut sphincterotomy facilitates biliary access in most cases, but also has a failure rate. Alternative options for achieving biliary therapy, such as percutaneous-endoscopic or endoscopic ultrasound guided rendezvous procedure, percutaneous transhepatic biliary therapy, or surgical intervention are more invasive and include a considerable morbidity or are not widely available. In this retrospective cohort study, we investigated whether it is reasonable to repeat an ERCP within a few days after an initially failed precut before considering more invasive strategies such as rendezvous techniques, percutaneous transhepatic cholangiography or surgery.

Methods: Consecutive ERCPs from our endoscopy reporting database were analysed for use of precut sphincterotomy, biliary access rate, repeat ERCP rate and complications. Patients with initially failed precut sphincterotomy were identified. Precut techniques using the needle knife were exclusively performed by senior consultant endoscopists. Primary outcome was the success of repeated ERCP and precut sphincterotomy after initially failed precut sphincterotomy. Secondary outcome were procedure related complications.

Results: A total of 1839 ERCPs were performed during the study period. 187 (10.2%) patients (107F, 80M) underwent a precut sphincterotomy during the initial ERCP in attempts to cannulate a native papilla which was successful in 79 (42.2%) patients. We repeated the ERCP in 89 of the 108 patients with failed precut sphincterotomy. Repeat ERCP performed a median of 4 days after initial precut sphincterotomy allowed successful biliary cannulation in 69/89 patients (77.5%). In 5 patients with failed second attempt, we repeated ERCP a third time and gained biliary access in four. In total, repeat ERCP after failed primary precut ERCP was successful in 73/89 patients (82.0%). Complications were observed in 32/187 patients having a pre-cut sphincterotomy (17.1%). There were 24 cases of pancreatitis (12.8%), 2 retroperitoneal perforations (1.1%), one biliary sepsis (0.5%) and 5 bleeding complications (2.7%).

Discussion/Conclusion: The high success rate of biliary cannulation in a second attempt ERCP justifies repeating ERCP within 2–7 days after unsuccessful precut sphincterotomy before more invasive approaches such as percutaneous cholangiography or surgery should be considered.
The effect of hyperhomocysteinemia and associated metabolic disturbances on the liver fibrosis in patients with chronic hepatitis

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Fibrogenesis is a universal way of chronic hepatitis (CH) progression, since it leads to the organ architectural reconstruction. Hyperhomocysteinemia (HHC) induces the development of liver fibrosis in rats and accelerates the progression of its complication.

Aim of the work was to determine the prevalence of HHC in patients with CH, and study the relationship between HHC and metabolic disturbances.

Methods: The study involved 245 patients with CH. 89 patients were diagnosed with CHC, 40–CHB, 23–CHB & CHC, 38–NASH, 30–CHC & alcoholic and 25–alcoholic etiology.

Results:

Table 1: Content of phospholipids, oxidative stress markers, hydrogen sulfide, biochemical markers of hepatic fibrogenesis and adenosinedesaminase activity in patients with CH depending on the homocystein level

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Homocystein level in serum</th>
<th>Correlation with the level of homocystein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 10 mkmol/l (n = 71)</td>
<td>10–15 mkmol/l (n = 103)</td>
</tr>
<tr>
<td>Phosphatidylcholine, mg/l</td>
<td>1475 ± 20.4</td>
<td>1373 ± 15.9*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>126 ± 2.18*</td>
</tr>
<tr>
<td>Phosphatidylethanolamine, mg/l</td>
<td>963 ± 15.7</td>
<td>1008 ± 12.6**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>126 ± 2.18*</td>
</tr>
<tr>
<td>Lysophosphatidylcholine, mlg/l</td>
<td>121 ± 2.60</td>
<td>1008 ± 12.6**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>126 ± 2.18*</td>
</tr>
<tr>
<td>Malonic dialdehyde, mkmol/l</td>
<td>5.70 ± 0.24</td>
<td>6.10 ± 0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.83 ± 0.26***</td>
</tr>
<tr>
<td>Thiols groups, mkmol/l</td>
<td>7.50 ± 0.24</td>
<td>6.83 ± 0.26***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.10 ± 0.26*</td>
</tr>
<tr>
<td>Hydrogen sulfide, mkmol/l</td>
<td>71.3 ± 0.93</td>
<td>62.0 ± 0.82*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60.1 ± 4.03</td>
</tr>
<tr>
<td>Adenosinedesaminase, nmol/ml per min</td>
<td>14.3 ± 0.22</td>
<td>15.3 ± 0.24**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.7 ± 0.31</td>
</tr>
<tr>
<td>Paraoxonase, mmol/l per hour</td>
<td>159 ± 1.83</td>
<td>153 ± 1.99***</td>
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<tr>
<td></td>
<td></td>
<td>153 ± 1.99***</td>
</tr>
<tr>
<td>Transforming growth factor β1, ng/ml</td>
<td>60.1 ± 4.03</td>
<td>75.0 ± 3.77**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75.0 ± 3.77**</td>
</tr>
<tr>
<td>Tissue inhibitor of metalloproteinase 1, ng/ml</td>
<td>1076 ± 46.9</td>
<td>1500 ± 76.7**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1500 ± 76.7**</td>
</tr>
<tr>
<td>Hialuronic acid, ng/ml</td>
<td>66.9 ± 7.44</td>
<td>112 ± 8.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>230 ± 16.7</td>
</tr>
</tbody>
</table>

The difference is reliable in patients with homocystein level in serum ≤ 10 mkmol/l: *p < 0.001, **p < 0.01, ***p < 0.05.

The difference is reliable in patients with homocystein level in serum 10–15 mkmol/l: *p < 0.001, **p < 0.01, ***p < 0.05.

1. according to Pearson
Conclusion: Homocystein level in serum is increased in patients with CH and associated with increased morphological stage of liver fibrosis. HHC and associated metabolic disturbances are unfavorable factors in fibrogenesis. Correction of HHC level will slow the progression of liver fibrosis in patients with CH.
The strong expression of PRL-3 protein in liver metastases of colorectal cancer

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Introduction: The liver is the most common site of colon cancer metastasis. The mechanism of formation of these metastases is still not well known. However, an analysis of the gene PRL-3 showed that it is the only gene overexpressed in all examined liver metastasis of colorectal cancer. PRL-3 protein belongs to a family of tyrosine phosphatase. It plays a key role in physiology and pathology of various cells. Physiologically, PRL-3 is involved in the modulation of intracellular calcium transients and cell cycle regulation. In pathological conditions, PRL-3 takes part in the migration and invasion of the cancer cells, and determines the development of metastases. The aim of this study was to evaluate the immunohistochemical expression of PRL-3 protein in liver metastases of colorectal cancer.

Materials and methods: The study included a group of 30 patients diagnosed with liver metastases underwent surgery due to colorectal cancers. Tissue material was fixed in 10% buffered formalin and embedded in paraffin. The 5 μm thick sections were stained with hematoxylin-eosin (H&E) and subjected to routine histological evaluation. The expression of PRL-3 protein was assessed by using immunohistochemistry. The reaction of PRL-3 protein was observed in the cytoplasm of tumor cells and defined as a negative (reaction present in < 5% of cancer cells or its lack) and positive (expression in > 5% of cancer cells)

Results: It was noted a strong expression of PRL-3 protein in all metastases to the liver (100%). While the in primary tumor of these patients, the positive expression of PRL-3 was observed in 86% of central tumor mass, in 87% of invasive front, in 100% of tumor budding and in 100% of local lymph nodes.

Conclusion: Our study showed that PRL-3 protein is closely involved in metastases of colorectal cancer to the liver. The positive expression of PRL-3 in primary tumor can indicate a high probability of liver metastases incidence.
Polymorphism of tumor necrosis factor-α -238 G/A is associated with progression of non alcoholic fatty liver disease: A study in Dr. Kariadi Hospital Semarang Indonesia

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Introduction: Tumor necrosis factor (TNF-α) polymorphism play a role in the incidence and development of non alcoholic fatty liver disease (NAFLD). The spectrum of NAFLD is ranging from simple steatosis, non alcoholic steatohepatitis (NASH), fibrosis and cirrhosis. Non alcoholic steatohepatitis (NASH) can progress toward cirrhosis. The association between TNF-α promoter polymorphism and the incidence as well as the development of NAFLD was evaluated.

Methods: A total of 155 subjects (80 NAFLD cases and 75 healthy controls were included). Liver biopsy was performed in all NAFLD cases. Plasma TNF-α, Homa IR- and adiponectine level were evaluated. Polymorphism of TNF-α promoter gene -308 and -238 was identified using direct sequencing.

Results: Liver biopsy confirmed 29 cases with NASH. There were no differences in the incidence of NAFLD with TNF-α polymorphism at the -308 or the -238 loci .The prevalence ratio of subject having polymorphism -238 G/A was significantly higher (p < 0.02) for NASH. However there was no different prevalence ratio for subject with polymorphism -308. To our surprise a new polymorphism of -245 T/C was identified in one of subjects with possible NASH, high plasma TNF level (20.27 pg/ml), hypoadiponectinemia (1401 pg/ml) and very high value of HOMA-IR (22.73). We found double polymorphism (TNF-α -238 G/A and -308 G/A) in one of subject with highest score severity of NASH.

Discussion/Conclusion: Polymorphism TNF-α -238 is a likely risk factor for NASH in Indonesia. The identification of new possible polymorphism TNF-α -245 require further study.
Detecting of advanced fibrosis among patients with biopsy-proven NAFLD with non-invasive tests

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Evaluation of liver fibrosis is important to identify patients who may develop complications in patients with non-alcoholic fatty liver disease (NAFLD).

The aim of this study was to compare the diagnostic models of simple non-invasive tests in identifying advanced fibrosis among patients with biopsy-proven NAFLD.

Consecutive patients with biopsy proved NAFLD from 2009 to 2012. The AST/ALT ratio, AST to platelet ratio index, BARD score (BMI > 28 = 1 point, AST/ALT ratio > 0.8 = 2 points, diabetes = 1 point), FIB-4 score (age x AST (IU/l)/platelet count (x 10^9/liter) x \sqrt{ALT (IU/l)}) and NAFLD fibrosis scores were calculated from blood tests taken at time of biopsy.

In study were included 34 patients (18 male [53%), mean age 54 ± 17 years). Body mass index (mean value) was 39 ± 7 kg/m^2. Diabetes mellitus had 17 subjects (49%). Non-alcoholic steatohepatitis had 23 patients (69%). Advanced fibrosis (Kleiner stage 3–4) had 6 (21%) patients. The FIB-4 score had the best diagnostic accuracy for advanced fibrosis (area under receiver operator characteristic curve [AUROC] 0.74), followed by AST/ALT ratio (AUROC 0.73), NAFLD fibrosis score (AUROC 0.83), BARD (AUROC 0.65) and AST to platelet ratio index (AUROC 0.71). The AST/ALT ratio, BARD score, FIB-4 and NAFLD fibrosis scores had negative predictive values greater than 90% (91%, 96%, 94% and 93% respectively). Positive predictive values were modest. In order to exclude advanced fibrosis liver biopsy could potentially be avoided in 59% with AST/ALT ratio, 61% with FIB-4, 520% with NAFLD fibrosis score and 41% with BARD.

The ALT/AST ratio, FIB-4 and NAFLD fibrosis scores can reliably exclude advanced fibrosis in high proportion of patients with NAFLD, but liver biopsy still keep it place in investigation of NAFLD.
Inhibition of PECAM-1 by Plasminogen K1-5 not only inhibits orthotopic hepatoma growth but also prevents ascites

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Introduction: Plasminogen K1-5 (K1-5) is known as an angiostatic factor, inhibiting tumour angiogenesis and growth by affecting endothelial cell functions and survival. Endothelial cell survival is (at least) partly mediated by PECAM (platelet endothelial cell adhesion molecule, CD31). PECAM also mediates leukocyte-transmigration, which can cause ascites – a complication of hepatocellular carcinoma. Despite the known angiostatic effects of K1-5, effects on cell-adhesion and leukocyte-transmigration were neglected. Therefore, we tested whether K1-5 affected PECAM on endothelial cells and transendothelial leukocyte migration.

Materials and Methods: Effects of K1-5 on tumour cell (Hepa1-6, Hepa129) adhesion to endothelial cells (SVEC4-10) were analysed using a co-culture assay. Changes in PECAM expression were determined by semiquantitative real time PCR and immunofluorescence in SVEC4-10. The impact of K1-5 on leukocyte recruitment was analysed in a mouse model for acute peritonitis. Orthotopic hepatomas were established by implanting Hepa129 in the liver of C3H mice. Treatment with adenoviruses encoding K1-5 or LacZ was done seven days later. Another seven days later, animals were sacrificed and livers explanted. Tumours and ascites were measured.

Results: Adhesion of Hepa1-6 and Hepa129 to SVEC4-10 was decreased by 60% using K1-5 compared to LacZ and NaCl. This correlated to reduced PECAM expression (by 50%) and reduced PECAM-immunofluorescence on SVEC4-10. K1-5 also reduced leukocyte recruitment in an acute peritonitis model in mice by 85% compared to the controls. Orthotopic tumour growth was completely blocked (-98%) after treatment with K1-5 compared to the controls. Ascites was also strongly reduced in K1-5 treated animals (0–2 mL) compared to LacZ and NaCl (5–9 mL).

Conclusion: K1-5 not only acted on the tumour site, but also exhibited “side effects” by preventing ascites. This was due to the inhibition of transendothelial leukocyte migration. This dual therapeutic effect makes K1-5 still a promising tool in the treatment of HCC.
Real world experience of rifaximin in hepatic encephalopathy

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Introduction: Hepatic Encephalopathy (HE) is a chronic, debilitating condition secondary to portal hypertension and shunting with debated pathophysiology. Symptoms of overt encephalopathy are debilitating and decrease the ability for self-care, leading to poor nutrition and non-adherence to a therapeutic regimen, which in turn leads to further debility, frequent hospitalizations, and a poor quality of life. We looked at outcomes outwith clinical trial setting.

Methods: Rifaximin use was reviewed in a single university hospital. Patients receiving long-term treatment for HE were identified. Records were reviewed. Outcomes were total number of admissions with and without treatment and length of stay per admission. Data was divided into patient months prior and after commencement of rifaximin.

Results: 16 patients were selected. Mean age was 61 (range 42 to 73) years. Four had previously undergone TIPSS procedures. Hepatic encephalopathy was secondary to alcoholic liver disease (n = 7), non-alcoholic steatohepatitis (n = 6), hepatitis C (n = 1), autoimmune hepatitis (n = 1) and iatrogenic post splenic artery rupture repair (n = 1).
Patients had a combined 233 months on rifaximin and 203 months off. The total number of admissions decreased from 2.2 per patient (range 0–11) prior to and 1 (range 0–8) after starting rifaximin (p < 0.05), average length of stay was 7.6 nights (1–58) and 4.9 respectively (1–26) (p < 0.05). This equates to 1.35 nights per month and 0.29 nights per month respectively for each patient prior to and following commencement of treatment.

Discussion/Conclusion: In this study, rifaximin has significantly decreased the admission rates and length of stay in patients suffering from HE, supporting the use of rifaximin as an adjunct to current treatment methods. Further evaluation in larger studies and quality of life assessments are required.
Paracrine signals from liver sinusoidal endothelium regulate hepatitis C virus replication

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Introduction: Hepatitis C virus (HCV) is a major cause of global morbidity, causing chronic liver injury that can progress to cirrhosis and hepatocellular carcinoma. The liver is a large and complex organ containing multiple cell types, including hepatocytes, sinusoidal endothelial cells (LSEC), Kupffer cells and biliary epithelial cells. Hepatocytes are the major reservoir supporting HCV replication, however, the role of non-parenchymal cells in the viral lifecycle remain largely unexplored.

Methods: Primary human LSEC were isolated and studied in vitro. Co-culture models were established to define the roles of LSEC in regulating HCV replication in hepatocytes. LSEC derived factors regulating HCV replication were identified by microarray.

Results: LSEC secrete factors that promote HCV infection and transcript analysis identified bone morphogenetic protein 4 (BMP4) as a candidate endothelial expressed pro-viral molecule. Recombinant BMP4 increased HCV replication and neutralisation of BMP4 abrogated the pro-viral activity of LSEC conditioned media. Importantly, BMP4 expression was negatively regulated by vascular endothelial growth factor A (VEGF-A) via a VEGF receptor-2 (VEGFR-2) primed activation of p38 MAPK. Consistent with our in vitro observations, we demonstrate that in normal liver VEGFR-2 is activated and BMP4 expression is suppressed. In contrast, in chronic liver disease including HCV infection where there is marked endothelial cell proliferation we observed reduced per endothelial cell VEGFR-2 activation and a concomitant increase in BMP4 expression.

Discussion/Conclusion: These studies identify a role for LSEC and BMP4 in HCV infection and highlight BMP4 as a new therapeutic target for treating individuals with liver disease.
High-throughput MALDI-TOF MS as an alternative approach to HBV genotyping

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Introduction: Long-term therapy of hepatitis B (HBV) infection that is required because of the existence of extremely stable viral cccDNA in hepatocytes, can lead to the selection of drug-resistance mutations and treatment failure. Moreover it is possible to have drug-resistance mutations present already in the HBV population of treatment-naïve patients. Currently available methods detecting drug-resistance can detect mutants that constitute ≥ 5% of viral population. Furthermore drug-resistant mutants can be identified in patients with a viral load of more than 10⁴ copies/mL. The aim of this study was to assess the presence of HBV drug-resistant mutants using MALDI TOF MS and MSSCP techniques. Afterward to determine the reliability and accuracy of the routine susceptibility testing of HBV drug-resistance comparing with results obtained by MS and MSSCP.

Methods: HBV DNA was extracted from 45 serum samples of chronic HBV (CHB) patients. The 37 selected HBV variants were analyzed in 4 separate primer extension reactions on the Mass Array genotyping platform. Moreover MSSCP for identifying drug-resistant HBV mutants was developed. To compare received results INNOLipa Assay and Sanger sequencing was done.

Results: MALDI TOF MS had capability to detect mutant strains within a mixed viral population occurring with an allelic frequency even 1% (≥ 10² copies/mL). If using MALDI TOF MS it was possible to detect 100% HBV variants that were present in serum samples, with the use of MSSCP it was able for 98% species. The sensitivity of routine tests was respectively 40% and 11% for INNOLipa and Sanger sequencing.

Discussion/Conclusion: The routine HBV testings are not sensitive enough. MALDI TOF MS is a powerful technique for determination of HBV resistant variants, including quasispecies. This assay can provide a valuable insight into the understanding of how resistance develops and enables early strategic decisions of antiviral treatment in the management of CHB.
Role of FXR in controlling the expression of genes involved in the lack of response of liver cancer to chemotherapy

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Background and Aims: In addition to its role as bile acid sensor, the farnesoid X receptor (FXR) is involved in the control of several metabolic pathways, including detoxification processes. Here we have investigated FXR expression in hepatocellular carcinoma (HCC), cholangiocarcinoma (CGC) and hepatoblastoma (HPB) and its role in the expression of genes involved in mechanisms of chemoresistance (MOC).

Methods: Using RT and Taqman Low-Density Arrays and/or QPCR, biopsies from healthy livers or surgically removed tumors from naive patients and cell lines derived from HCC (SK-HEP-1, Alexander and Huh7), CGC (TFK1) and HPB (HepG2), were analyzed before and after exposure to cisplatin at IC50 for 72 h.

Results: Although FXR mRNA abundance was lower than in healthy liver, this was expressed in all liver tumors (HCC > HPB ≈ CGC). The proportion of FXR isoforms was similar to that found in the parent healthy liver cells. In contrast, expression profiles of MOC genes were characteristic of each tumor but similar to these found in parent cell lines. Incubation with cisplatin increased this resemblance. On the other hand, in human hepatocytes, GW4064 induced a significant protection against cisplatin. In human hepatoma cells, GW4064 protected against cisplatin, doxorubicin, mitomycin C and potassium dichromate, but not against non-genotoxic drugs, such colchicine, paclitaxel, acetaminophen, artesunate and sorafenib; when they had previously been transfected with FXR/RXR. Among MOC genes investigated, both in human hepatocytes and FXR/RXR-expressing hepatoma cells, only ABCB4, TCEA2, CCL14, CCL15 and KRT13 were up-regulated by FXR activation. In both models, cisplatin, even in the absence of FXR agonists, up-regulated FXR targets genes, which was due to FXR-mediated trans-activation of response elements in their promoter region.

Conclusions: Ligand-dependent and independent activation of FXR enhances MOC against genotoxic compounds, which may be involved in the lack of response of HCC, CGC and HPB to certain pharmacological treatments.
The cellular mechanism of isoniazid and rifampicin induced hepatocytes injury in cytochrome P450 2E1 over expressing cells

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Introduction: Liver injury related to anti-tubercular drugs is a multifactorial process where oxidative stress plays an important role. Induction of cytochrome P4502E1 by isoniazid (INH) is thought to play a role in this drug induced liver injury. Direct evidence of the role for CYP2E1 in this process is lacking. The aim of this study is to evaluate the molecular mechanism of cytotoxic effects of INH and rifampicin (RMP) to HepG2 cells expressing CYP2E1 and compare these effects to cells which do not express CYP2E1.

Methods: HepG2 cells that constitutively express CYP2E1 (E47 cells) or control HepG2 cells (C34 cells) were used. Cells were cultured in MEM containing 10% FBS, 0.5 mg/ml G418 with other antibiotics. Cells were co-treated with INH (5 μM) and RMP (2.5 μM) at different time points. FACS and confocal microscopy were used to measure intracellular H2O2. FACS was used to measure mitochondrial membrane potential (MMP), lysosomal membrane leakage, intracellular calcium levels and DNA analysis to quantify the apoptotic cells. The role of mitogen activated protein kinase (MAPK) of this process was evaluated by Western blotting.

Results: INH and RMP caused oxidative stress dependent toxicity in E47 cells, leakage of lysosomal membrane followed by decreased in MMP, an increase of cytosolic calcium and apoptotic death which is time dependent. The INH and RMP induced toxicity in E47 cells was blocked by antioxidants. The maximum decrease in MMP and death of the E47 cells by INH-RMP treatment were prevented only by SP600125 (JNK inhibitor).

Conclusion: We could demonstrate that INH and RMP toxicity is related to the generation of oxidative stress, and to the activation of JNK MAPK, as a result of CYP2E1-dependent production of reactive oxygen species. Activation of JNK MAPK by INH and RMP coupled to INH and RMP induced oxidative stress may synergize to cause cell toxicity by affecting mitochondrial membrane potential.
Hepatic stellate cells activation through phagocytosis of isoniazid induced necrotic hepatocytes: An in-vitro study

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Introduction: Isoniazid (INH) induced chronic hepatitis had mostly been anecdotal case reports. INH is linked with hepatocytes’ death. Engulfment of hepatocytes apoptotic bodies by hepatic stellate cells (HSCs) leads to fibrogenic response. However the relationship between necrotic hepatocytes and HSCs are not yet delineated. The aim of the present study was to evaluate whether INH induced necrotic hepatocytes are engulfed by HSCs and promote their activation.

Methods: HepG2 cells expressing CYP2E1 (E47 cells) was used to produce necrotic, propidium iodide (PI) stained, hepatocytes by treatment with INH (5 μM) for 12 hours. Necrotic hepatocytes were added to cultures of human stellate cell (LX2 cells) line. Using confocal microscopy and FACS phagocytosis of the necrotic hepatocytes and intracellular ROS in LX2 cells were assessed. Activation of LX2 cells was evaluated by mRNA expression of profibrogenic genes and their proteins by Western blotting. Pharmacological inhibition of Rac1 by NSC23766 and knockdown of Rac1 using shRNA in LX2 cells were carried out to confirm the Involvement of Rac1 signalling pathway in phagocytosis of necrotic hepatocytes.

Results: INH treated necrotic E47 cells stimulated the LX2 cells in culture. Confocal microscopy identified the engulfment of necrotic E47 cells within the LX2 cells. The phagocytic capacity of the LX2 cells towards the INH induced necrotic E47 cells was time dependent. Engulfment of necrotic E47 cells by the LX2 cells increased 10 to 12 folds of Rac1 activity, endogenous ROS formation and activation of LX2 cells. Pre-treatment of LX2 cells with NSC23766 and Rac1 silencing by shRNA also decreased engulfment of necrotic E47 cells and their activation.

Conclusion: Necrotic hepatocytes are engulfed by hepatic stellate cells that are mediated by Rac1 pathway. Engulfment of necrotic hepatocytes by HSCs is an important pathway of HSCs activation and fibrogenesis. Rac1 may be a potential therapeutic target in the treatment of fibrosis.
Hypocholesterolemia is accompanied by low level of intracellular cholesterol in PBMCs of CHC patients

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Introduction: An increasing body of data indicates that HCV exploits the lipid metabolism of hepatocytes for its own life cycle. Paradoxically, hypocholesterolemia is a well-known serological feature described in patients with chronic hepatitis C (CHC). Independently of the liver, the HCV RNA sequence was frequently evidenced in extrahepatic tissue like peripheral blood mononuclear cells (PBMCs), where, viral RNA strands can persist even after serum HCV RNA negativization. The aim of this study was to evaluate whether HCV RNA persistence in PBMCs has any effect on intracellular cholesterol level in these cells.

Methods: PBMCs and sera isolated from 30 healthy donors and 54 CHC patients were used to determine: genomic and antigenomic HCV RNA strands, expression of HMG-CoA reductase and LDL receptor, intracellular cholesterol level in PBMCs and serum lipid profile.

Results: Despite the lack of differences in HMG-CoA-reductase and LDL receptor expression in PBMCs between healthy donors and CHC patients, we found significant differences (p = 0.0000) in intracellular cholesterol level (1.8 vs. 1.15 μM) between these groups. Moreover, we observed the tendency (p = 0.06) in the group of CHC patients to have lower level of intracellular cholesterol in PBMCs when antigenomic HCV RNA (-) strand was detected in cells. The level of total serum cholesterol, LDL and HDL fractions was significantly higher (p < 0.01) in healthy donors (3.52; 2.03; 1.09 mg/L, respectively) than in CHC patients (2.73; 1.52; 0.8 mg/L, respectively).

Discussion/Conclusion: These data suggest that apart from serum hypocholesterolemia, decreased level of intracellular cholesterol in infected PBMC seems to be a typical feature of hepatitis C. Hypocholesterolemia could equally well be a direct effect of HCV infection or the result of hepatic damage caused by hepatitis. The significant decrease of intracellular cholesterol in PBMCs seems to be the result of “local” changes that facilitate viral extrahepatic persistence.
**IL-28 polymorphism and iron homeostasis disturbances in chronic hepatitis C: Preliminary Polish single center study**

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**Background:** Recently discovered single-nucleotide polymorphism (SNP) of IL-28 in infected with HCV genotype 1 proved to be one of important determinants of treatment efficacy with CC homozygosity in rs12979860 locus being the favourable prognostic factor of HCV-related liver disease. Iron overload, frequently diagnosed in chronic hepatitis C (CHC), is considered as to be a negative prognostic factor.

**Aim:** The investigation of the possible influence of IL-28 polymorphism on development of iron overload, and in future to provide the answer whether the iron burden worsens the rate of antiviral treatment efficacy among carriers of good-response IL-28 polymorphism.

**Methods:** 44 individuals, with confirmed HCV infection genotype 1, have been enrolled in this preliminary study. The frequency of CC genotype, verified by RFLP method, was at 25% (11), CT - 61% (27) and TT at 14% (6). The iron overload parameters (serum iron, transferrin saturation, serum ferritin and presence of iron deposits in hepatocytes) were assessed.

**Results:** Biochemical, serum markers of iron overload were more frequently, without evident significance, observed among CT and TT carriers, with 9/33 of CT/TT compared to 1/11 CC patients having elevated transferrin saturation, 10/33 CT/TT having elevated iron in serum compared to 1/11 CC carriers. Elevated above normal values ferritin concentrations were observed in 1/11 CC cases and in 12/33 carrying CT or TT SNP. CC carriers compared only to TT presented significant lower concentration of hemoglobin (p = 0.02), lower serum iron concentration (p = 0.03), lower transferrin saturation (p = 0.04) and lower serum ferritin concentration. Hepatocyte iron deposition was detected in 4/11CC and 10/33 CT/TT carriers.

**Conclusions:** Despite small number of cases analysed in the study, there seems to be an interesting correlation between serum iron homeostasis disturbances and CT/TT SNP at IL-28. We do hope that ongoing study of ours will serve for better understanding of some aspects of CHC pathogenesis.
Non-alcoholic fatty liver disease: Risk factors and frequency assessment

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Introduction: Risk factors and frequency of nonalcoholic fatty liver disease (NAFLD) assessment in personnel of industrial enterprise was made.

Methods: Self-questioning revealed metabolic syndrome (MS) signs in 550 people (205 men and 345 women, aged 18–59), then people with risk factors were examined to reveal NAFLD.

Results: NAFLD risk factors (no less than one) were revealed in 344 people (62.8%, 95% CI 58.7–66.9), and 16.0% reported about 2 and 13% about 3 factors. The most frequent were abdominal obesity and arterial hypertension (AH). NAFLD frequency was 146/344 (42.4%) in people with risk factors and 146/550 (26.6%) in all questioned: 117 had NAFLD signs, 29 had nonalcoholic steatohepatitis. NAFLD frequency and relative risk for different risk factors combinations were calculated.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>NAFLD</th>
<th>RR with respect to abdominal obesity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal obesity as the only factor</td>
<td>8.2 (3.5–12.9)</td>
<td>–</td>
</tr>
<tr>
<td>Abdominal obesity + AH</td>
<td>59 (47–71)</td>
<td>7.2 (4.0–12.8)</td>
</tr>
<tr>
<td>Abdominal obesity + AH + diabetes mellitus (DM)</td>
<td>93 (85–100)</td>
<td>11.3 (6.6–19.3)</td>
</tr>
<tr>
<td>Abdominal obesity + AH + increased TG and/or decreased HDL</td>
<td>92 (82–100)</td>
<td>11.2 (6.5–19.1)</td>
</tr>
<tr>
<td>Abdominal obesity + AH + DM + increased TG and/or decreased HDL</td>
<td>97 (91–100)</td>
<td>11.8 (6.8–20.3)</td>
</tr>
</tbody>
</table>

Discussion/Conclusion: NAFLD is a frequent pathology in people of working age; its severity is associated with the number of MS signs. Risk factors represent evident (abdominal obesity) or easily detectable (AH, DM) conditions. Proper attention to them not only because of adverse cardiovascular events but NAFLD possibility will enable detecting liver pathology.
Outreach service – Fostering equity and access for liver transplantation

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Introduction: The current landscape of service provision for patients with liver disease does not match that of disease burden\textsuperscript{1}. Most hepatologists are based in the transplant centres and access to tertiary liver service is not geographically equitable\textsuperscript{1}. In an attempt to improve access, we established a liver transplant outreach clinic from the regional liver unit within a large gastroenterological unit. Here, we describe the benefits of this clinic.

Methods: A dedicated monthly joint liver clinic was established in a large gastroenterological unit. Patients with complex liver disease, including pre- and post-transplant are seen by a consultant transplant hepatologist from the regional transplant unit and a local consultant gastroenterologist. Quantitative data were available from the transplant centre. A sample of patients (76) and specialists (\textit{n} = 16) were asked to complete a written questionnaire on their opinions of clinic service.

Results: Since August 2010, over 400 patients have been seen. In the 4 year prior to establishment of the clinic, there was a median of 3 (1–4) referrals annually for liver transplant assessment. This increased to 9.5 (9–10) in the subsequent 2 years. Patients were satisfied with the clinical service (Table 1) and the majority (97\%) preferred local follow up, citing it as more convenient (99\%) with easier travel arrangements (99\%). Specialist (\textit{n} = 16) agreed unanimously that the clinic was more convenient for patients, with easier to refer into and improved accessibility to liver services and communication with the regional liver unit. Most (83\%) felt that it reduced waiting times for specialist opinion.

Table 1: Patient questions and mean score 1 (low) – 5 (high)

<table>
<thead>
<tr>
<th>Patient Question</th>
<th>Mean Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall quality of care and services</td>
<td>4.5</td>
</tr>
<tr>
<td>Access to specialty care, if needed</td>
<td>4.4</td>
</tr>
<tr>
<td>Skill, experience and training of doctors</td>
<td>4.5</td>
</tr>
<tr>
<td>Respect shown to you by doctors</td>
<td>4.7</td>
</tr>
<tr>
<td>Confidence in the doctor you saw</td>
<td>4.6</td>
</tr>
</tbody>
</table>
**Discussion/Conclusion:** Establishing an outreach clinic has increased referrals for liver transplant assessment. Patients prefer to be seen locally and do not feel this affects their specialist care. They have confidence in the skills and experience of the clinicians they see and rate the quality of care, highly. Referring clinicians are also satisfied with the quality and accessibility of the outreach clinic. Overall, outreach clinic may serve to improve equity of access to transplant services.

**References:**

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Sulfatase-2 (SULF2) – A therapeutic candidate in patients with hepatocellular cancer (HCC)?

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Helen Reeves, Northern Institute for Cancer Research, Newcastle University & The Liver Unit, Newcastle upon Tyne Hospitals NHS Foundation Trust, UK

Introduction: SULF2 is overexpressed in 59% of HCC and SULF2 knockdown inhibits HCC xenograft growth. (Lai et al, 2008) It desulfates heparin sulphate proteoglycans, including adhesion molecules, cytokines, ECM molecules, and growth factors involved in hepatocyte growth and survival. Post translational modification of SULF2 is essential for its sulfatase activity, with the creation of a formylglycine in the catalytic site. SULF2 as a therapeutic target for patients with HCC has been further explored.

Methods: Immunohistochemistry for SULF2, α-sma, glypican-3 and β-catenin was performed in 61 patients with HCC graded according to Edmondson. Images were scanned digitally (Aperio) and membranous and nuclear expression quantified with Imagescope software. Survival analyses were with Cox linear regression (SPSS). Quantification of SULF2 expression in vitro was by RT-PCR and western blot.

Results: SULF2 was expressed in only 7/61 (12%) HCC but was observed in α-sma+ve non-parenchymal cells adjacent to tumour in 37/61 (61%) case. In vitro studies confirmed significant increases in SULF2 expression in activated versus quiescent hepatic stellate cells (HSC) (rat 1.82; human 1.69 fold increases). In vivo, the presence of stromal SULF2 was independently associated with a poorer survival (Median 11.2 months, versus 31 months, p = 0.043 all patients, n = 61; excluding surgically treated patients, n = 51: survival 29 months versus 9.4 months p = 0.003). Co-expression of SULF2 and tumour cell glypican-3 was present in 20/51, identifying a group with even poorer survival (median 6 months, p=0.001). This co-expression was associated with membranous rather than nuclear translocation of β-catenin.

Discussion/Conclusion: Culture activated HSC express SULF2, as do α-sma+ve HCC associated myofibroblasts in vivo. Adjacent tumour cell expression of the SULF2 modified morphogen glypican-3 is associated with a poor prognosis, supporting a role for SULF2 mediated stromal/cell HCC cross talk in HCC progression. SULF2 inhibition may be a relevant therapeutic strategy – in a biomarker driven fashion – for patients with HCC.
Low expression of hepcidin in liver tissue is not associated with an effect of treatment with pegylated interferon and ribavirin of chronic hepatitis C: A preliminary study

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Introduction: Iron overload is frequently observed in chronic hepatitis C (CHC). Based on experimental studies, iron overload in HCV infection has been associated with low hepcidin synthesis. That key regulator of iron homeostasis has an antimicrobial activity and is a part of the host innate immunity.

Methods: 31 patients with CHC that qualified for antiviral treatment were compared with 19 patients with chronic hepatitis B (CHB). Liver function tests, iron, ferritin concentration, transferrin saturation were assayed in all cases. HCV viral load and genotyping were defined in CHC group. Liver biopsy with assessment of inflammation activity, fibrosis, steatosis and presence of iron deposits in liver specimens was done in both groups. Hepcidin mRNA expression was measured in liver biopsy specimens using RT-PCR with normalization to reference genes mRNA of stable expression in liver. 19 CHC patients completed the standard therapy with pegylated interferon alfa with ribavirin, 10 of them achieved sustained viral response.

Results: Iron deposits in hepatocytes and advanced liver fibrosis were significantly more frequent with decrease of hepcidin mRNA expression in liver tissue of CHC compared to CHB patients. Hepcidin mRNA expression was positively correlated with ALT activity, serum iron concentration. Hepatocyte iron deposits in CHC were detected in 7/31 patients, they were usually mild and without association with hepcidin expression. In patients with the lowest expression of hepcidin in liver no parameters of biochemical or tissue iron overload were found. HCV viral load and efficacy of antiviral treatment were not significantly associated with hepcidin mRNA expression.

Discussion/Conclusion: Hepcidin expression was lower in CHC despite of presence of active hepatitis markers possibly as a result of mechanisms specifically activated by HCV proteins. Further studies on the influence of hepcidin on viral replication, immune response are needed to assess the potency of its use in antiviral treatment.
Serum levels of angiogenesis-related biomarkers in patients with alcoholic liver disease: Their association with liver disease complications and outcome

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Introduction: Angiogenesis seems to play an active role in the pathogenesis of alcoholic liver disease (ALD). The aim of our study was to explore the usefulness of potentially reliable, non-invasive angiogenic biomarkers for diagnosing and monitoring patients with ALD.

Methods: One hundred forty seven inpatients (pts) (40 females, 107 males) with ALD were prospectively recruited and compared with 30 healthy controls (HC). Pts were divided into subgroups based on their: 1. gender, 2. severity of liver dysfunction according to the Child-Turcotte-Pugh and MELD scores; and 3. the presence of ALD complications at the time of hospital admission (i.e. ascites, hepatic encephalopathy, esophageal varices, cholestasis, renal dysfunction and death). The plasma levels of angiogenesis-related molecules including vascular endothelial growth factor (VEGF), angiopoietins-1 and 2 (Ang1, Ang2) were assessed using immunoenzymatic ELISA test. Multivariable logistic regression was applied in order to select independent predictors of advanced liver dysfunction and the disease complications.

Results: Significantly higher plasma concentrations of two biomarkers i.e. Ang2 and VEGF in comparison with HC were found (median, 25–75 percentiles: 4.58, 3.12–9.97 versus 1.95, 1.10–2.43; p < 0.0001, and 85.27, 68.73–99.16 versus 48.47, 31.71–70.95, p = 0.001; respectively). There was no difference in serum Ang1 levels between ALD pts and HC (2.90, 0.99–5.28 versus 3.02, 1.06–6.12, p = 0.83). We found a positive correlation of Ang2 with INR, and its inverse correlation with serum albumin level. Increased Ang2 concentrations turned out to be an independent predictor of severe liver dysfunction (MELD score ≥ 20) and the development of ascites, encephalopathy, renal dysfunction, and death.

Discussion/Conclusion: Results of our study suggest that Ang2 possesses the highest diagnostic and prognostic potential among three evaluated angiogenic biomarkers. Its high blood concentrations are related to severe liver dysfunction, major liver disease complications and poor outcome in patients with ALD.
Comparison of autoimmune hepatitis patients’ characteristics in non-Caucasian and Caucasian ethnic group

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Introduction: Autoimmune hepatitis (AIH) is an immunologically mediated disease with genetic association with DR3 and DR4 allotypes, which are common in European Caucasian patients. Most studies were carried out on the European Caucasian population. There are not many studies investigating how AIH patients’ characteristics differ by ethnicity. Previous studies noted race/ethnicity-specific disparities, with late clinical presentation and poor outcomes of AIH in non-European Caucasian ethnic groups. We analysed data from AIH patients from our liver clinics, which cover a large area with a diverse ethnic population, in order to make comparisons between different ethnicities.

Methods: Retrospective review and analysis of well-defined (simplified scoring ≥ 6) Type 1 AIH patients’ records between different ethnic groups for demographics, clinical history, biochemistry, immunology, associated AIH conditions, liver histology and steroid adjustment.

Results: 199 Type 1 AIH patients with simplified scoring criteria ≥ 6 were treated in our unit between 1995–2012. These patients had median age of 53 years (quartiles: 33, 64). 157 patients (79%) were female. The patients were divided into three ethnic categories: Caucasian (83%), Asian (11%) and African (6%). Gender was not found to differ significantly by ethnicity (p = 0.773). The median age at diagnosis was found to be 56 years (quartiles: 33, 66) in Caucasian patients, compared to 50 years (33, 57) in the other two ethnicity groups (p = 0.054).

No significant differences between the groups were detected for DR3/DR4 association, liver biochemistry, immunoglobulin, autoantibodies ANA, SMA titers, immunosuppression use, number of AIH flare-ups, complications of medication use (osteoporosis), cirrhosis at presentation, serum albumin, INR or development of hepatocellular carcinoma. In addition to this, the rates of various associated autoimmune conditions did not differ significantly by ethnicity.

Prednisolone usage was found to differ significantly between the groups (p = 0.024), with usage being lowest in African patients (64%), and higher rates in those of Caucasian (92%) and Asian (95%) ethnicity. Similarly, Ig A values differed significantly by ethnicity (p = 0.026), being lower for Caucasian patients (geometric mean = 2.6) than for those of Asian or African ethnicity (geometric mean = 4.1, 3.8 respectively).

Discussion/Conclusion: Our findings suggested that the demographic, clinical presentation and response of Type 1 AIH patients are similar, despite previous reports suggesting significant diversity in disease severity. Thus impact of ethnicity may have a geographical variation. Detailed investigations on genetic, molecular and cellular biology and immunology aspects are required to understand autoimmune hepatitis patients’ natural history.
Optimising tools to stratify outcome in primary biliary cirrhosis: AST/platelet ratio index predicts clinical outcome independent of UDCA response

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Introduction: Biochemical response to ursodeoxycholic acid (UDCA) in primary biliary cirrhosis (PBC) is able to stratify patient outcome, but fails to capture all adverse events. Our aim was to identify risk-variables associated with transplant-free/overall survival in patients with PBC independent of UDCA-response.

Methods: We interrogated a clinical database of PBC patients attending the Liver Unit in Birmingham between September 1996 and January 2013. UDCA-response was defined according to Barcelona, Toronto and Paris-I/II criteria.

Results: 386 patients were followed up for a median of 79 months (IQR 48–122). On multivariate analysis, identifiable factors at diagnosis associated with liver transplant/death were age at diagnosis (HR: 1.06 per-year increase; P < 0.001), ANA-positivity (HR: 2.26; P < 0.001), male gender (HR: 2.39; P < 0.05), cirrhosis (HR: 2.81; P < 0.001) and baseline AST/platelet ratio index (APRI) (HR: 1.81 per 1-unit; P < 0.001). 12-month biochemical response to UDCA was associated with improved transplant-free/overall survival, the association being strongest when applying Paris-I criteria (HR: 0.15; P < 0.001). The only additional factors at 12 months independently associated with outcome were age (HR: 1.03; P < 0.01) and APRI (HR: 1.44; P < 0.0001). APRI > 0.54 at baseline was predictive of progression to liver transplantation/death (univariate HR: 4.08; P < 0.001), and this cut-off retained significance when applied one year following diagnosis, after adjustment for age, gender, presence of cirrhosis and UDCA-response (adjusted HR: 3.46; P < 0.001). UDCA-responders with the highest APRI (4th quartile) had poorer 10-year transplant-free/overall survival (39%) vs. UDCA-responders with a lower APRI (87%) (P = 0.002). Transplant-free/overall survival was poorer in UDCA-nonresponders with a high APRI (Q4: 12%) versus UDCA-nonresponders with a lower APRI (40%). APRI was predictive of adverse outcome with high sensitivity and specificity, at baseline (AUROC: 0.781) and at 1-year (AUROC: 0.805).

Conclusion: High APRI at baseline and/or on treatment at 12 months is associated with future risk of adverse events, independently of UDCA treatment-response. This supports efforts to refine stratification tools for use in clinical practice through large multi-centre efforts.
VAP-1 activity is elevated in PSC and modulates α4β7-dependent lymphocyte adhesion to HSEC under flow

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Introduction: Vascular adhesion protein (VAP)-1 is an adhesion molecule possessing potent amine-oxidase activity. Through this function, VAP-1 leads to the production of H2O2, NFκB activation in hepatic sinusoidal endothelium (HSEC) and expression of mucosal-vascular cell-adhesion molecule-1 (MAdCAM-1); a mechanism proposed to contribute to the homing of gut-tropic lymphocytes expressing α4β7 to the liver. Given the proposed role this pathway has in hepatic disorders complicating inflammatory bowel disease (IBD), we set out to quantify circulating/soluble (sVAP-1) and intrahepatic VAP-1 enzyme activity in primary sclerosing cholangitis (PSC), and evaluate functional consequences of its inhibition on MAdCAM-1 dependent lymphocyte recruitment to HSEC.

Methods: VAP-1 amine-oxidase activity was quantified using the amplex-red assay. Flow-based adhesion assays were performed using human HSEC isolated from liver explants, activated with TNFα and methylamine (VAP-1 substrate), and treated with VAP-1 antibody or semicarbazide (enzyme inhibitor). FAC-sorted PBMCs expressing α4β7 were perfused over HSEC under flow rates simulating physiological shear (0.05 Pa).

Results: Patients with PSC had significantly higher circulating median VAP-1 enzyme activity (114.5 pmol H2O2 produced/min/ml serum, IQR: 100.6–134.7) than patients with IBD (60.3, 38.5–73.0; P = 0.006), normal controls (84.0, 77.7–105.7; P = 0.020) and individuals with PBC (24.6, 18.7–27.8; P = 0.029) and AIH (32.3, 23.3–35.6; P = 0.028). HSEC pretreatment with semicarbazide but not antibody led to profound reduction in total α4β7+ lymphocyte adhesion (75%); however, antibody and enzyme inhibition independently reduced transmigration (~50%) compared to untreated HSEC.

Conclusion: sVAP-1 enzyme activity is greater in PSC compared to IBD, normal controls, and other immune-mediated liver diseases. Intrahepatic VAP-1 enzyme activity is significantly higher in PSC compared to AIH and PBC. Inhibition of VAP-1 leads to abrogation of α4β7-mediated adhesion to HSEC, representing a putative target for therapeutic intervention in PSC.
Impact of proton pump inhibitor (PPI) use on clinical course of cirrhosis

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Introduction: Variceal bleeding (VB) and spontaneous bacterial peritonitis (SBP) are important causes of morbidity and mortality in patients with cirrhosis. Proton pump inhibitors (PPIs) are frequently prescribed in this patient population. Gastroesophageal reflux disease might have deleterious effect on esophageal varices enhancing their rupture due to local acidic irritation. On the other hand, significant reduction of gastric acid production might increase intestinal bacterial overgrowth and hence, bacterial translocation (BT). Sustained BT has been identified as a major factor in the diseases progression in cirrhosis, either by worsening of portal hypertension, or by enhancing susceptibility to SBP.

Methods: In a follow-up cohort study, we assessed the effect of PPI administration on the clinical course of cirrhosis. 235 patients (male/female: 131/105, age: 57.1 ± 10.1 years, Child A/B/C: 86/92/58) with a confirmed diagnosis of cirrhosis were selected between May 2006 and December 2008. Medical charts of patients were reviewed for adverse outcomes including variceal bleeding, SBP and death during inpatient stays. Current medications and presence of co-morbidities were also collected. Follow-up period lasted 62 months or death/lost of follow up.

Results: 136 (57.6%) patients received regular PPI treatment. Episodes of VB were more frequent in PPI users (31.9% vs. 7.0%, \( p < 0.001 \)) and correlated with the applied dose (25.6% for standard-dose and 40.4% for high-dose PPI regimen). In patients with ascites, the occurrence of SBP was associated to PPI use (25.7% vs.12.0%, \( p < 0.01 \)), independently of the applied dose. Moreover, in a Kaplan-Meier survival analysis, PPI use was associated to higher mortality rate (HR: 2.09, 95% CI: 1.37–2.83, \( p < 0.001 \)) both in the standard-dose and the high-dose group.

Discussion/Conclusion: In our study population, PPI administration was associated to adverse outcomes in cirrhosis beyond SBP as well. Negative impact of PPI use was even proved for standard dose administration regime. Pharmacologic acid suppression enhancing BT might result in progression of cirrhosis. Further studies are needed to determine the mechanisms of these associations.
CD8αα expression, activation and terminal differentiation are shared features of the bulk CD8+ T-cell populations in chronic hepatitis B and HIV-1 infections

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Introduction: The size of the CD28-CD27-CD57+ CD8+ T-cell population in HIV-1 infection is associated with disease progression¹. Expansion of CD28-CD8 T-cells in chronic hepatitis B (HBV) infection is associated with higher HBV viral load and ALT². We have previously shown this CD28-T-cell population to be most prominent amongst T-cells which lack expression of CD161 (a molecule associated with liver homing) and to be CD8α+CD8βlow, therefore co-expressing the co-receptor, CD8αβ, and co-repressor, CD8αα. The aim of this study was to use the thymic leukaemia antigen (TL) tetramer (which binds directly to CD8αα) to define CD8αα-expression on human CD8 T-cells and to further characterize the phenotype and function of the CD161-CD8α+CD8βlow T-cell population in healthy controls and patients with chronic HBV, hepatitis C (HCV), and HIV-1 infections.

Methods: Peripheral blood mononucleocytes were obtained from 16 adult healthy controls (HC), 31 patients with chronic HBV, 23 patients with chronic hepatitis C (HCV) and 10 patients with HIV-1 infection. All study subjects were recruited following informed consent and in agreement with the Oxfordshire Research Ethics Committee. FACS analysis was performed using cell surface antibody, tetramer and intracellular cytokine staining.

Results: TL-tetramer binding (CD8αα expression) correlated with CD8β expression on CD8α+ T-cells. CD161-CD8α+CD8βlow T-cells are significantly expanded in chronic HBV and HIV-1 (mean of 47% and 40% of CD161- T-cells respectively). CD161-CD8α+CD8βlow T-cells are effector-memory cells (CD45RA-, CCR7-, CD62L-), express markers of activation/maturation (HLA-DR+, CD28-, CD27-, CD57+) and functionally distinct, expressing greater levels of TNF-α and IFN-γ on stimulation and perforin at rest than their CD161-CD8α+CD8βhigh counterparts. Antigen-specific T-cells in HLA-B*4201+HIV-1 infected patients are both CD161-CD8α+CD8βlow and CD161-CD8α+CD8βhigh.
**Discussion/Conclusion:** CD161-CD8α+CD8β<sub>low</sub> T-cells are late-differentiated, dominating the bulk CD8+ population in chronic HBV and HIV-1. Co-expression of CD8αα on CD8αβ T-cells could impact on their overall function in-vivo and contribute to the distinctive phenotype of these highly differentiated populations.

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