Symposium 204

Clinical Hepatology Practice in 2016: From Science to Therapy

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Leinenweberstr. 5
79108 Freiburg
Germany
Tel +49 (0)761/1514-0
Fax +49 (0)761/1514-321

www.falk-foundation-symposia.org
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Symposium 204

CLINICAL HEPATOLOGY PRACTICE IN 2016:
FROM SCIENCE TO THERAPY

Birmingham, Great Britain
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Scientific Organization:
D.H. Adams, Birmingham (Great Britain)
G. Hirschfield, Birmingham (Great Britain)
R. Thimme, Freiburg (Germany)
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Session I

Hepatology 2016 – The panorama
Lessons from epidemiology: The burden of liver disease

I. Rowe
University Academic Fellow & Honorary Consultant Hepatologist, Leeds Institute for Data Analytics, University of Leeds, St. James’s University Hospital, Leeds LS9 7TF, UK

Liver cirrhosis is responsible for more than one million deaths annually and the majority of these deaths are preventable. There is marked geographical variation in rates of mortality due to cirrhosis and this variation in liver disease burden exemplifies the links between population risks for liver disease and mortality. The differing geographical distribution of the major risks factors for the development of liver disease including alcohol, hepatitis C virus (HCV) infection, hepatitis B virus (HBV) infection, and obesity and the metabolic syndrome has the potential to highlight opportunities for intervention whilst the evolution of these risk factors provides insights into the future burden of liver disease.

This lecture will focus on the use of population data to identify high risk areas and populations that would benefit from preventative interventions to reduce the mortality from liver disease. Specific strategies that are effective at the policy and public health levels will be discussed to illustrate the impact these can have if widely implemented. The impact of therapies that have the potential to change the natural history of liver disease, including direct acting antivirals for HCV infection will also be described. Finally, the challenges of describing the epidemiology of non-alcoholic fatty liver disease will be highlighted to illustrate the need to understand the natural history of disease to inform the development of novel therapies.
Liver fibrosis represents highly evolved scarring response to relentless iterations of hepatic inflammation and repair. Originally considered at best irreversible and at worst relentlessly progressive; detailed studies of relevant animal models and, particularly, studies of human models following successful therapeutic intervention have demonstrated that liver fibrosis is bidirectional. Specifically, there is now a wealth of evidence that following the withdrawal of the injurious stimulus, liver fibrosis can undergo extensive remodelling characterised by degradation of the fibrillar collagen matrix and apoptosis or phenotypic reversion of the myofibroblast cellular elements.

Detailed studies of the cellular and molecular processes underlying progressive and resolving liver fibrosis have indicated that in addition to overexuberent matrix synthesis, in progressive fibrosis matrix accumulates as a result of the failure of matrix degradation. In resolving liver fibrosis, in addition to a loss of the activated myofibroblast like hepatic stellate cells macrophages derived from inflammatory monocytes may contribute to resolution through a phenotypic switch in situ and secretion of matrix degrading metalloproteinases. The pregnancy associated hormone relaxin has been identified as an agent which, through its action on the RXFP1 receptor, mediates changes in the balance of metalloproteinases and their inhibitors resulting in reduced matrix synthesis, enhanced degradation and importantly a reduction in the contractile activity of myofibroblasts. Relaxin shows promise as an antifibrotic agent, but one which may also mediate a reduction in portal hypertension and vice renal vasodilation may augment renal blood flow in the context of liver fibrosis.
Novel opportunities to image liver disease – Technologies for the future?

Guruprasad P. Aithal
NIHR Nottingham Digestive Diseases Biomedical Research Unit, Nottingham University Hospitals NHS Trust and University of Nottingham, UK

In the past decades, a number of non-invasive methods have emerged for the detection and estimation of liver fibrosis; these include both serum based panels and imaging based technology. Some of these methods are now being incorporated in clinical practice. However, the limitations of the current techniques include, lack of organ specificity, sampling errors, limited ability to reflect efficacy of interventions. Novel magnetic resonance (MR) based techniques provide an opportunity to bring about further step change in the investigations and management of patients with liver diseases. Multimodal quantitative MR techniques allow estimation of fat, iron accumulation, degree of liver injury/inflammation and fibrosis within the whole liver without the need of administration of contrast agents or breath holds. Architectural changes within the liver can be evaluated concurrently with portal haemodynamic changes allowing non-invasive assessment of portal hypertension and effects of interventions. Combination Ultra-high field (7T) provides greater sensitivity with a potential to distinguish inflammation from fibrosis on imaging and determine specific types of fats (saturated vs. unsaturated) present within the liver on MR spectroscopy. $^{13}$C magnetic resonance spectroscopy can estimate glutathione flux and rate of beta oxidation in-vivo providing novel tools for experimental studies that evaluate efficacy of interventions as well as underlying mechanisms. The $^{111}$In-hexavalent lactoside imaging has shown a potential to estimate liver functional reserve. Translational research should focus on turning potentials of these innovative methodologies into clinical applications for the benefit of patients.
Genes and environment in the development of liver diseases: Which is more important in 2016

Tom Hemming Karlsen, M.D., Ph.D.
Full Professor of Internal Medicine, Senior Consultant of Gastroenterology and Hepatology, Department of Transplantation Medicine, Oslo University Hospital Rikshospitalet and University of Oslo, Oslo, Norway, Tel. +47 91722353

The application of genetic technologies has unraveled a landscape of common and rare genetic variants that contribute to the development of various liver diseases. For liver diseases showing a Mendelian inheritance, it has often been possible to determine single genetic variants that drive disease development. Gene discoveries in these diseases have been greatly facilitated by genome-sequencing technologies, which are also increasingly being utilized in the medical genetics setting for counselling purposes. In sporadic liver diseases, the relationship between genetic variants and disease development is more complex. Often, even dozens of disease genes contribute less than 10% of the overall disease liability, leaving a potentially large space for other etiologic considerations. Disease gene discovery in these complex liver diseases is done by genome-wide association studies (GWAS), which is a study design capable of detecting associations between common (frequency > 1–5% in the general population) genetic variants and disease status. Whether the low fraction of disease liability explained by GWAS is due to genetic variation (e.g. rare variants) that cannot be determined by the study design, or by environment has been extensively debated. There is increasing appreciation for a model for disease development in complex liver diseases in which environmental factors play the predominant role. The lecture will provide a balanced perspective into this complex role of genetic contributions in a broad range of liver diseases, exemplified by recent findings.
Session II

The science of hepatitis
Co-existing with viral hepatitis – Lessons from fundamental virology studies

Jane A. McKeating
Institute of Immunology and Immunotherapy, University of Birmingham, UK

This presentation will review the highlights of research studying the molecular biology of hepatitis C virus (HCV) that developed in vitro model systems necessary to screen and identify antiviral compounds targeting multiple steps in the viral life cycle. This global effort to understand the replicative cycle of this virus has paved the way for direct-acting antiviral-based combination therapies that cure infection and highlights the success of translating basic scientific discoveries into new curative drugs. Current challenges we are facing include the development of drug-resistance, influence of host genetics and impact of other co-morbidities on the progression of advanced liver disease and hepatocellular carcinoma.
The immunology of viral hepatitis

Robert Thimme
Department of Medicine, Clinic for Internal Medicine II (Gastroenterology, Hepatology, Endocrinology and Infectious Diseases), University Hospital Freiburg, Germany

Immune responses play an important role in the natural course of viral hepatitis infection. Indeed, successful elimination of all hepatitis viruses seems to depend on strong innate and adaptive immune responses. A central role of virus-specific CD8+ T cell responses in the outcome has been suggested by close associations between the appearance of virus-specific T cells in the peripheral blood and liver, the onset of liver disease and the final elimination of the virus. The virus-specific CD8+ T cells utilize different effector functions to eliminate virus-infected cells. Specifically, they can kill infected cells after antigen recognition by perforin-dependent pathways or they can inhibit viral replication by the secretion of antiviral cytokines. In hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, depletion studies in chimpanzees further demonstrated the central role of CD8+ T cells as effector cells. CD4 T cells play a central role in the regulation of the adaptive immune response. In the absence of adequate CD4 help, CD8+ T cells are functionally exhausted and also unable to keep pace with ongoing viral evolution as has been shown in the case of HCV infection. Thus, inadequate CD4 help is linked to CD8+ T cell failure and subsequent development of viral persistence in HBV and HCV infection. The mechanisms responsible for virus-specific CD8+ T cell failure in chronic HBV and HCV infection are not completely understood. There is growing evidence that T cell exhaustion, characterized by the co-expression of inhibitory receptors, such as PD-1, the up-regulation of transcription factors, such as EOMES and a lack of antiviral effector functions, contributes to T cell failure in HBV and HCV infection. Importantly, a blockade of inhibitory receptors might partly restore virus-specific CD8+ T cell effector functions. In addition, direct antiviral therapy may also lead to a partial restoration of virus-specific CD8+ T cell immunity, pointing to an important role of ongoing replication in T cell exhaustion. Another important factor contributing to T cell failure is the emergence of viral escape mutations within targeted CD8+ T cell epitopes, as has been clearly shown in HBV and more prominently in HCV infection. The important role of T cells in the course and outcome of viral hepatitis has increased the interest to develop vaccines for the prophylaxis of HCV and the immune-therapy of HBV infection. Current concepts to overcome the exhaustion of T cell immunity in chronic infection and to stimulate effective antiviral immunity will be discussed.
The history of direct acting anti-viral therapy for hepatitis C: The molecular virology landscape

Prof Dr. Ralf Bartenschlager
Department of Infectious Diseases, Molecular Virology, Heidelberg University Hospital, Heidelberg; Division „Virus-associated carcinogenesis“, German Cancer Research Center, Heidelberg, Germany

Infections with the hepatitis C virus (HCV) are a major cause for acute and chronic liver diseases. It is estimated that ~130 million people are persistently infected with this virus, including ~500,000 people in Germany. HCV is an enveloped virus with a positive strand RNA genome that was cloned for the first time in 1989. While this allowed the rapid development of diagnostic tests to exclude HCV-contaminated blood and blood products from medical care, development of antiviral therapy was severely delayed. This was due to the lack of adequate cell culture systems allowing the reliable propagation of the virus in easy to cultivate cell lines. This hurdle was overcome in 1999 by the establishment of the so-called replicon system that became the gold standard for the development of drugs targeting HCV. Initially these targets were the viral NS3 serine-type protease and the NS5B RNA-dependent RNA polymerase. In addition to these enzymes, highly potent drugs targeting the HCV NS5A protein, which lacks known enzymatic activity, have been developed. When given in combination, > 90% of patients treated with these direct-acting antiviral drugs are able to eliminate the virus, thus presenting and as yet unprecedented success in the treatment of a chronic viral infection. The molecular mechanisms underlying this high success rate will be discussed.
How to cure hepatitis B – The hope?

Fabien Zoulim
Lyon University, Hospices Civils de Lyon, Cancer Research Center of Lyon – INSERM U1052, Lyon, France

Chronic HBV infection results in over one million deaths per year from cirrhosis and liver cancer. No known cure for chronic HBV exists, due in part to the continued presence of transcriptionally active DNA in the nucleus that is not directly targeted by current antiviral therapies. A coordinated approach is urgently needed to advance a HBV cure worldwide, such as those established in the HIV field. Current therapies of chronic hepatitis B (CHB) remain limited to pegylated-interferon-alpha (pegIFN-α) or any of the five approved nucleos(t)ide analogues (NUC) treatments. While viral suppression can be achieved in the majority of patients with the high-barrier-to-resistance new-generation of NUC, i.e. entecavir and tenofovir, HBsAg loss is achieved by pegIFN-α and/or NUC in only 10% of patients, after a 5-year follow-up. Attempts to improve the response by administering two different NUC or a combination of NUC and pegIFN-α have not provided a dramatic increase in the rate of “functional cure”. Because of this and the need of long-term NUC administration, there is a renewed interest regarding the understanding of various steps of the HBV replication cycle, as well as specific virus-host cell interactions, in order to define new targets and develop new antiviral drugs. This includes a direct inhibition of viral replication: entry inhibitors, drugs targeting cccDNA, siRNA targeting viral transcripts, capsid assembly modulators, and approaches targeting the secretion of viral envelope proteins. Restoration of immune responses is a complementary approach, which includes the restoration of innate immunity against HBV. This can be achieved, for instance, with TLR agonists or specific antiviral cytokine delivery, and/or restoration of adaptive immunity with inhibitors of negative check-point regulators, therapeutic vaccines, or autologous transfer of engineered HBV-specific T cells. Novel targets and compounds are being evaluated in experimental models of HBV infection and several are already entering the first phase of clinical evaluation. The addition of one or several new drugs to current therapies should offer the prospect of a markedly improved response to treatments and an increased rate of functional cure. This should lead to a reduced risk of antiviral drug resistance, and to a decreased incidence of cirrhosis and hepatocellular carcinoma.
Session III

Hepatitis treatment: The challenges
Treating hepatitis C in the patients with renal failure

Maria-Carliota Londoño
Hospital Clinic of Barcelona, Liver Unit, CIBERehd, IDIBAPS, Barcelona, Spain

The relationship between hepatitis C (HCV) and the kidney is bidirectional. Thus, approximately 10% to 16% of the patients with chronic hepatitis C develop renal disease (due to cryoglobulinemia, diabetes, or HCV-related gomerulonephritis), and the prevalence of HCV infection in patients with renal dysfunction is higher than that of the general population, with variations depending of the country (3–17%). In addition, HCV-positive patients on hemodialysis (HD) have higher mortality rates as compared to HCV-negative patients also on HD, not only due to liver-related complications but also owing to cardiovascular disease.

In the interferon era, treatment of HCV infection in these patients was limited because of the significant number of treatment-related adverse events including anemia, infections and graft intolerance syndrome (in patients with a previous kidney transplant). The development of direct-acting antiviral agents (DAAs) has revolutionized the field allowing viral eradication in these very sick patients. Two recently published clinical trials assessed the efficacy and safety of DAAs in patients with end-stage renal disease (ESRD). The combination of grazoprevir and elbasvir has been studied in the C-SURFER trial with 94% of the patients achieving sustained virological response (SVR). Adverse events were mild and only a very small number of patients early discontinued therapy due to adverse events. The 3D regimen was evaluated in the RUBY-I trial. Here, a 90% of SVR rate was achieved in 20 patients with ESRD, most of them on HD. The evaluation of Sofosbuvir-based regimens in patients with a glomerular filtration rate < 30 ml/min has been hampered by the pharmacokinetics of the drug (sofosbuvir' inactive metabolite (GS-331007) is eliminated by the kidney). However, several real-life data have shown good results in terms of efficacy and safety, similar to that of patients with normal or near normal renal function.

In conclusion, the use of DAAs has safely permitted the treatment of patients with renal dysfunction with excellent efficacy results.
Pre- and post-transplant treatment of viral hepatitis C

David Mutimer
University of Birmingham and NIHR Biomedical Research Unit, Birmingham, UK

Liver transplantation (LT) for hepatitis C virus (HCV) infection has undergone a transformation during the past 2 years since the introduction of effective all-oral antiviral treatment. Before 2 years ago, interferon (IFN)-based treatment was unsuitable for the majority of waiting list HCV patients, and IFN-based treatment was associated with disappointing efficacy and with transplant-specific liver dysfunction in a proportion of post-LT patients.

HCV treatment of finite duration can be given to LT waiting list patients. Relapse of HCV is not observed when SVR12 can be demonstrated prior to transplantation. Waiting list patients may also be transplanted during antiviral therapy. The antivirals might be given according to protocols that aimed to give finite duration of treatment before LT, though other protocols aim to effect and maintain indefinite suppression of HCV on the waiting list with continuation until the time of LT. In either case, and if antivirals are not continued post-operatively, the probability of SVR12 (defined as PCR-negativity 12 weeks after transplantation), is proportional to the duration of PCR-negativity on treatment before transplantation. It appears that post-LT relapse is seldom observed if at least 4 weeks of serum PCR-negativity can be demonstrated during treatment up until the date of LT.

For those patients who have received only a short duration of pre-LT treatment, and certainly if the duration of PCR-negativity before LT is less than 4 weeks, then consideration should be given to continuation of treatment without significant interruption following LT. A number of reports confirm the efficacy of this approach. However, in some cases, bridging therapy may be inappropriate. For instance, sofosbuvir continuation might be contraindicated for patients who develop acute renal injury post-LT. Also, antiviral regimens that include components with significant cytochrome 450 inhibition should be avoided in the early post-LT period.

There is a significant published experience with the use of the range of oral antiviral drugs in the post-LT patient. The majority of published series achieved SVR12 for greater than 90% of treated patients. Most patients were infected with HCV genotype 1 and received combinations of sofosbuvir with simeprevir, daclatasvir or ledipasvir. The largest series are the prospective Gilead-sponsored studies, SOLAR 1 and 2, which achieved 95% efficacy using Harvoni and ribavirin for the treatment of more than 400 post-LT patients, including 200/215 (93%) patients with graft cirrhosis, and 11/11 patients with fibrosing cholestatic hepatitis. Also, a small series showed that the AbbVie 3D regimen is highly efficacious (SVR 12 for 33/34 HCV genotype 1 patients), and reassured that cytochrome p450 interactions can be safely managed.
HBV reactivation: The controversies continue

Jordan J. Feld MD MPH
Toronto Centre for Liver Disease, Toronto General Hospital, Sandra Rotman Centre for Global Health, University of Toronto, Canada

The natural history of hepatitis B virus (HBV) infection is largely dictated by the interaction between the virus and the host immune system. Although some patients may have persistently active inflammation and viral replication requiring therapy, many, and in fact, most individuals eventually achieve immune control with suppression of viral replication and minimal or no ongoing liver injury. For such patients, HBV either never was or no longer is a major concern. However, this control is entirely dependent on immune function. With immunosuppressive therapy, whether cancer chemotherapy or immunomodulatory therapy for a host of other diseases, HBV replication can recur with severe or even fatal hepatitis. Fortunately pre-emptive therapy with oral antivirals is highly effective at preventing HBV reactivation, however to institute pre-emptive therapy, HBV must first be recognized. Awareness of HBV reactivation and screening rates for HBV among prescribers of immunosuppressive therapy, particularly oncologists, have been historically very low. There remains considerable debate in the literature on the optimal screening strategy for HBV prior to immunosuppressive therapy with differing and sometimes contradictory recommendations in guidelines from professional societies. The debate becomes even more contentious when addressing the scenario of individuals who are HBsAg-negative but anti-HBc-positive. Although the risk of reappearance of HBsAg, so-called 'reverse seroconversion' and associated HBV reactivation and hepatitis in this scenario is less frequent, the consequences can still be severe, particularly with more immunosuppressive agents like anti-B cell therapies, which are being used in an increasing number of conditions. Optimal management strategies in terms of screening and managing positive results in this setting remain unclear and have garnered much controversy in the oncology and rheumatology literature. A discussion of the issues of HBV reactivation and reverse seroconversion, including screening strategies and cost considerations will be addressed with a focus on reviewing the data and offering strategies for management of the more controversial areas.
Is hepatitis E really a problem?

Heiner Wedemeyer  
Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, 30623 Hannover, Germany, Telephone: +49 511 5326814, E-Mail: wedemeyer.heiner@mh-hannover.de

Hepatitis E virus (HEV) infection in Western and central Europe is primarily a food-borne zoonosis caused by infection with HEV genotype 3, which is highly prevalent in pigs, wild boar and a large variety of other animal species (1, 2). In Germany, an estimated 320,000 individuals become infected with HEV in Germany (3). In immunocompetent individuals, infection with HEV usually leads to a clinically silent seroconversion or to an acute self-limited inflammation of the liver. Acute hepatitis E can become life-threatening and cause fulminant liver failure in particular in distinct risk groups such as pregnant women or patients with pre-existing chronic liver diseases (4). Chronic hepatitis E can develop in immunocompromised individuals leading to rapid development of liver cirrhosis, liver failure and death (5). Importantly, HEV can also cause a variety of extrahepatic symptoms including Guillain-Barré syndromes, neuralgic amyotrophy, pancreatitis, thyroiditis, and haematological disorders (6, 7).

HEV is also endemic in tropical countries with poor sanitary conditions. Infections there are predominantly caused by genotypes 1 and 2 with humans being the most relevant hosts. Hepatitis E was thus long mistaken as an exclusively travel-associated, self-limiting liver disease that causes fulminant hepatic failure only in distinct high-risk groups. Of the estimated 70,000 yearly deaths worldwide, most are occurring in these countries (1).

Direct HEV transmissions from animal reservoirs to humans are well documented (e.g. 8, 9). However, the magnitude of this risk is largely undefined and it is unclear which pork products are the major sources of infection (10). Blood transfusions were recently identified as another important source of HEV infections in various European countries (11–12).

The pathophysiology of hepatitis E is only partially understood (13). It is possible to induce immunity against HEV by vaccination, as demonstrated in animals as well as in humans. A vaccine against hepatitis E is licensed in China which has proven long-term efficacy (14). There have been only very few studies investigating immune correlates for different courses of HEV infection (15).

Both acute as well as chronic HEV infections can be treated with ribavirin (16, 17). Ribavirin-induced viral mutagenesis has recently been identified as one major mode of action of ribavirin against HEV (18). However, treatment failure may occur associated with the selection of HEV variants characterized by a higher replication efficiency (19). Moreover, ribavirin is contraindicated in many situations as the drug can cause haemolysis, kidney failure, as well as heart and lung problems, which is a particular problem in organ transplant recipients who are at most urgent need of antiviral therapy.
References:


Session IV

Autoimmune liver disease
What comes after UDCA in PBC?

D.E.J. Jones
Centre for Liver Research, University of Newcastle, Newcastle-upon-Tyne NE2 4HH, UK

Almost since the first reports of its use there have been questions about the true value of UDCA, and what if any second line therapies are needed in PBC. This question was, to a significant degree, answered by the observation of differential response to UDCA, with some patients responding significantly more in terms of their liver biochemistry than others. The advent of very large patient cohorts such as the UK-PBC cohort of over 6000 patients have now confirmed the value of this concept and allowed us to effectively identify patients whose response to UDCA not adequate. These studies have also demonstrated that UDCA non- and under-response, and thus risk of un-controlled disease progression, are commoner in younger patients amplifying the need for further improvement in treatment options. Looking forward there are 4 areas where treatment in PBC will evolve over the next 5 years.

1) **Structured care delivery:** The broad consensus in the field is that UDCA remains first line treatment for PBC (at a dose of 13–15 mg/kg), however, non-response is a real issue and all patients commencing therapy with UDCA should go on to structured follow-up with assessment of UDCA response status after one year of therapy.

2) **Second-line anti-cholestatic therapy for UDCA non-response:** Patients failing to respond to UDCA should be considered for clinical trials of second-line anti-cholestatic therapies. The first such agent (obeticholic acid [OCA]) is now licensed in the USA. Other potential agents include other FXR agonists and fibrates and related agents, although their evidence base is currently significantly less than is the case for OCA.

3) **Symptomatic therapy:** Regardless of the response to UDCA or second-line agents, symptoms are a major issue in PBC patients and for many patients their major concern. Structured approaches to the management of pruritus are needed, together with improved therapies. Fatigue, the other major symptom type is currently untreated and a priority for research.

4) **Disease modifying therapy:** Increasingly, as our understanding of the mechanisms underpinning high risk disease in PBC increases, the question will arise as to whether we should move the whole treatment paradigm away from the current one of controlled disease deterioration towards active disease reversal. Time will tell whether this approach is possible.
Primary sclerosing cholangitis (PSC) is a devastating hepato-biliary disease most commonly associated with co-existent inflammatory bowel disease (IBD). For a significant number of patients disease progresses relentlessly and biliary strictures become symptomatic, and secondary biliary cirrhosis with liver failure develops; additionally patients harbour a lifelong ominous risk of malignancy arising from their biliary tree or colon. However equally it is now recognised that disease is more common than once imagined, and there are also patients whose clinical course is more mild. To date therapy has been very disappointing, and although widely used, evidence to demonstrate survival benefit for ursodeoxycholic acid, is very limited and contentious. In pursuit of new drug therapy for PSC a multi-faceted approach is needed. This has started with a better understanding of the basic biology underpinning development of disease, encompassing our understanding of genetic, environmental, biliary and immunologic pathways to liver injury. At the same time large-scale studies have sought to better define the clinical phenotype associated with disease presentation and course; such approaches have also facilitated stratification of individual risk of clinical events. Prospective cohort studies (replacing historic retrospective studies) are emerging that will provide both clinical and biological markers of risk, as well as much needed novel approaches to measuring early evidence of success from varied therapeutic interventions. As a result over time more stratified approaches have evolved to understanding which patients with PSC should enter clinical trials, and efforts made to evolve markers of treatment efficacy, rationalised towards the mechanism of action of any proposed therapy, and the stage of drug development. Pleasingly, and in keeping with the unmet need of patients, there are now multiple clinical trials of new agents of potential use for patients with PSC, alongside a slowly evolving but clearer pathway from rational, science driven, drug development, to hopefully ultimate drug registration and approval.
Pruritus in cholestasis – Old treatment or new treatments?

U. Beuers
Department of Gastroenterology & Hepatology and Tytgat Institute for Liver and Intestinal Research, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

Pruritus represents a major burden for patients with cholestatic liver diseases such as primary biliary cholangitis (PBC) or primary sclerosing cholangitis (PSC). Itching may be mild and tolerable, but it may also limit daily life activities and cause severe sleep deprivation resulting in lassitude, fatigue, depressed mood and even suicidal ideations. Novel insights into the pathogenesis of cholestatic pruritus have recently been achieved, including the identification of the potent neuronal activator, lysophosphatidic acid (LPA), as a potential pruritogen in cholestasis formed by the plasma enzyme autotaxin (ATX). Still, a putative factor “X” in bile and many other aspects of the complex molecular mechanisms underlying pruritus in cholestasis remain to be elucidated (reviewed in 1).

Patients with pruritus are treated following current guidelines (2) which recommend a stepwise approach and are based on only a few well-designed, randomized, placebo-controlled trials and several cohort studies. The rationale for medical and interventional therapeutic approaches is (i) to remove putative pruritogens from the enterohepatic circulation by non-absorbable anion exchange resins (e.g., cholestyramine, step 1) in mild pruritus or by invasive measures (e.g., nasobiliary and transcutaneous drainage or external biliary diversion) in most severe cases; (ii) to alter the biotransformation of the presumed pruritogen(s) in the liver and/or the intestine (e.g. rifampicin, step 2); (iii) to modulate central itch and/or pain signalling by influencing the endogenous opioidergic and serotoninergic system (e.g. naltrexone, step 3; sertraline, step 4); or (iv) to remove the potential pruritogen(s) from the systemic circulation in severe cases by invasive methods such as anion absorption, plasmapheresis or extracorporeal albumin dialysis if pruritus is otherwise intractable (2).

Novel therapeutic approaches are under investigation in randomized, placebo-controlled trials and follow the rationale described above. These include (i) inhibitors of intestinal uptake carriers such as the apical bile salt transporter (ASBT), and (ii) nuclear receptor agonists such as the peroxisomal proliferator associated receptor (PPAR) agonists. Additional approaches are under investigation.

References:


Can understanding the pathogenesis of autoimmune hepatitis (AIH) lead to rational therapy?

Ansgar W. Lohse
I. Department of Medicine, University Medical Centre Hamburg-Eppendorf, Hamburg

Present immunotherapy for autoimmune hepatitis with corticosteroids and azathioprine is relatively effective, but very non-specific thus leading to unwanted side effects. Deciphering the pathogenetic alphabet will be required in order to be able to deliver highly specific, possibly both disease and patient-specific immune intervention targeting. Present knowledge suggests that the immunopathogenesis of autoimmune hepatitis is largely governed by antigen-specific CD4^+ T-lymphocytes recognizing a peptide antigen presented on HLA-DR0301 or 0401. Ideally identification of this (or these) peptide(s) might allow antigen-specific tolerance induction. Alternatively, the usage of specific T-cell receptors on the autoimmune CD4^+ cells might allow deletion or silencing of the cellular drivers of the autoimmune reaction. Somewhat less specifically, but much more easily achievable, is targeting the specific cytokine and/or chemokine expression profile of the autoreactive and/or autoantigen-presenting cells, as monoclonal antibodies targeting many of these molecules are already available on the market. Anti-TNF therapy with Remicade®, shown to be an effective third-line drug in AIH, may be such a relatively specific immune intervention considering the over-expression of TNF in intrahepatic CD4^+ cells in active AIH. Further delineation of the characteristics and expression profiles of the intrahepatic inflammatory infiltrate will allow further specific immune interventions in the near future.
Session V

Metabolic liver disease
Is NASH a disease or a syndrome: Scientific basis for developing new therapies

Prof. Christopher Day
The Medical School, Centre for Liver Research, University of Newcastle, Newcastle-upon-Tyne NE2 4HH, UK

The original ‘two hit’ model of NASH pathogenesis, now 18 years old, suggested that steatosis - the first ‘hit’ - sensitised the liver to the injurious effects of the second ‘hits’ – fatty acid-induced oxidative stress and endotoxin induced cytokine-mediated stress. 18 years later direct toxicity of fatty acids and other lipids – so-called lipotoxicity – and endoplasmic reticulum (ER) stress have been added to the list of second hits, numerous non-inflammatory mediators of fibrosis have been identified and steatosis per se is now be considered the liver’s adaptive response to an excess supply of FFA rather than being intimately involved in the pathogenesis of progressive disease. Both obesity and the associated insulin resistance lead to an increased hepatic supply of FFA which, along with free cholesterol, have been linked directly to hepatocyte apoptosis and liver injury. Oxidative stress is thought to arise principally as a result of increased FFA oxidation in hepatocytes via non-mitochondrial pathways in peroxisomes and the ER. ER stress occurs as a physiological response to excess FFA and can lead directly to apoptotic cell death, oxidative stress and the production of pro-inflammatory cytokines. Pro-inflammatory cytokines are also produced by Kupffer cells stimulated by gut-derived endotoxin and other microbial ligands arising as a result of the altered gut microbiota and increased gut permeability associated with obesity. Failure of lipid-directed autophagy – so-called “lipophagy” may also contribute to the pathogenesis of steatohepatitis. Non-inflammatory mediators of fibrosis include insulin and glucose, oxidative stress, hepatocyte apoptosis and senescence, endotoxin, endogenous cannabinoids and adipokines including angiotensin, norepinephrine and leptin. Novel mechanisms involved in the activation of hepatic stellate cells and liver myofibroblasts in NASH include hedgehog pathway signalling, autophagy and nuclear receptors including PPARγ. Evidence that blocking triglyceride synthesis in animal models of NASH reduces steatosis but increases liver cell injury and fibrosis, suggests that the conversion of potentially toxic FFA to triacylglycerol may actually be a protective mechanism rather than part of the pathogenesis of progressive disease, with the correlation between steatosis severity and progressive disease almost certainly due to the mediators of progressive disease also causing steatosis. Based on these putative mechanisms, current therapies are based on: (a) reducing the supply of FFA to the liver (weight loss and insulin sensitizers), oxidative stress (with antioxidants), and ER stress (with molecular ‘chaperones’), (b) anti-inflammatory agents, (c) hepatoprotectants (ursodeoxycholic acid), (d) anti-fibrotic agents, (e) reducing levels of adipokines (ACE inhibitors and ARBs), (f) altering the microbiome (probiotics) and (g) activating/inhibiting autophagy, apoptosis (caspase inhibitors) and various PPAR isoforms. At present the best evidence from human studies has been provided for weight loss induced by obesity surgery, the glitazone class of insulin sensitizers, the antioxidant vitamin E, PPARγ/δ agonists and the anti-inflammatory FXR agonist obeticholic acid.
Reference:

Why you should establish a dedicated NAFLD/NASH clinic?

Philip Newsome
Centre for Liver Research, NIHR Biomedical Research Unit, University of Birmingham, Birmingham B15 2TT, UK

Patients with NAFLD/NASH will commonly have related metabolic conditions such as diabetes, hypertension, dyslipidaemia and polycystic ovarian syndrome. To avoid managing such patients in condition-specific silos it is much more efficient to manage them in a multi-disciplinary setting where their various conditions can be managed at the same-time. In addition for many such patients weight management is a key component necessitating input from relevant obesity specialists and dietitians. This approach enhances the patient experience, provides efficiencies to the healthcare provider and more importantly improves patients outcomes.
NASH is a common liver disease that increases liver-related mortality and reduces survival. The need for optimal management of NASH is therefore a priority for today’s practicing hepatologist. The rationale for specific pharmacological therapy in NASH is based on the potential for disease progression and the difficulties, in many patients, to successfully implement, in the long term, diet and lifestyle changes. Even in those that succeed to do so, limited evidence exists that severe liver injury in NASH can be reversed by diet and lifestyle measures alone, hence the need for pharmacological therapies specifically aimed at improving NASH. The PIVENS trial that compared the efficacy of pioglitazone and of vitamin E vs. placebo resulted in a shift in paradigm because it demonstrated that both an insulin sensitizer with no notable direct hepatic actions and an anti-oxidant hepatoprotectant with no direct effect on insulin resistance can improve histology in NASH. Therefore current trials are testing pharmacological agents with pleiotropic actions. Obeticholic acid (OCA), a farnesoid X receptor (FXR) agonist has metabolic as well as hepatoprotective actions. OCA reduces lipogenesis and increases fatty acid beta oxidation, it reduces neoglucogenesis and improves insulin signaling but has also anti-inflammatory and possibly anti-fibrotic effects in the liver, kidney and intestine. A large phase 2b study has demonstrated significant improvement in all histological lesions constitutive of NASH but also of fibrosis. Another prominent candidate, elafibranor, a peroxisome proliferator activated receptor (PPAR) alpha and delta agonist. This oral compound which has an extensive enterohepatic cycle and is liver-targeted does not have PPAR gamma activity and therefore is not expected to induce weight gain or be associated with unwanted cardiovascular effects of glitazones. Phase 2a trials in several hundred patients have demonstrated an improvement in hepatic and peripheral insulin sensitivity, in dyslipidemia, in systemic inflammatory markers and in liver enzymes. Importantly, animal studies in both NASH models and in liver fibrosis models have shown an improvement in experimental steatohepatitis but also in fibrosis. A large phase 2b trial of elafibranor has demonstrated that the 120 mg dose induced resolution of steatohepatitis without worsening of fibrosis more frequently than placebo. Responders according to this experienced a significant reduction in fibrosis. Another promising candidate is cenicriviroc (CVC), a dual selective inhibitor of ligand binding to C-C chemokine receptor type 2 and type 5 (CCR2 and CCR5). CVC blocks the binding of MCP1 to CCR2 and of RANTES and MIP1α and 1β to CCR5. Therefore CVC decreases recruitment, migration and infiltration of pro-inflammatory monocytes to the site of liver injury which should relieve hepatic inflammation and also decreases Kupffer cell and hepatic stellate cells activation and migration which should trigger anti-fibrotic effects. Whether CVC also has effects on adipose tissue insulin resistance through the modulation of adipose tissue inflammation, remains to be determined. A large phase 2b trial of CVC is underway in patients with histologically defined NASH. Other approaches are directed towards inhibiting hepatic lipogenesis through the inhibition of different enzymes that regulate de novo lipogenesis. One such candidate is aramchol, a fatty acid (arachidic acid)-bile acid (cholic acid) conjugate that has strong antisteatogenic effects in the rat and is able to reduce the hepatic triglyceride content in humans. A large phase 2b trial testing Aramchol vs. placebo is currently underway.
While some of these compounds might have antifibrotic effects, they are, for the most part, directed against steatohepatitis. A totally different approach would be to specifically test antifibrotic agents in trials with fibrotic end-points. Simtuzumab is a humanized monoclonal antibody that is directed against lysyl oxidase-like 2 (LOXL2) an enzyme that drives cross-linking of collagen fibers and that is key to progression of fibrosis in the human liver. Immunohistochemical studies have shown increased expression of LOXL2 in human liver fibrosis, both HCV and NASH related. Very large phase 3 trials are currently testing the parenteral administration of simtuzumab in NASH patients, both cirrhotic and non-cirrhotic. If all or some of these anti-NASH or antifibrotic drugs are effective, it might be ultimately possible to devise a personalized, tailored therapy in patients with NASH in order to avoid disease progression and the occurrence of cirrhosis.
The microbiome and the hepatologist – Will our bugs prove to be the missing link?

M. Pallen
Professor of Microbial Genomics, Head of the Microbiology and Infection Unit, Warwick Medical School, University of Warwick, Coventry CV4 7AL, UK

In recent years, the human genome has hogged the limelight, yet humans are home to a complex community of microbes that contains at least an order of magnitude more genes than our nuclear genome. This community of microbes along with their associated genes and genomes make up the human microbiome. The importance and metabolic versatility of the human gut microbiome is often summarised by saying that it represents an extracorporeal organ as complex as the liver. In fact, a range of processes link the liver and the gut microbiome, including maintenance or loss of gut barrier function, energy homeostasis, and control or induction of inflammation. There is clearly potential for cross talk, as, through the hepatic portal vein, products of the gut microbiome, whether beneficial or harmful, quickly reach the liver, while bile acids produced in the liver in turn impact on gut microbes. It is thus not surprising that the gut microbiome has been implicated in a range of hepatological conditions, including fatty liver, alcoholic liver damage and cirrhosis, hepatocellular carcinoma, primary sclerosing cholangitis and the fate of liver transplant patients. A newfound recognition of the importance of the microbiome has in turn laid the foundations for novel interventions in liver disease, including antibiotics, prebiotics and probiotics and even faecal microbiome transplants. In this talk I will aim to provide a brief guide for the perplexed focused on the human microbiome, while embracing a sceptical enthusiasm that aims to separate hype from hope in this emerging discipline.

See also: http://www.nature.com/naturejobs/science/articles/10.1038/nj7587-555a
Session VI

Liver and bile duct cancer
Treating hepatobiliary cancers: The oncology way

Peter R. Galle
University Medical Center Mainz, I. Medical Department, Mainz, Germany, E-Mail: galle@uni-mainz.de

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related deaths worldwide. HCC represents more than 90% of primary liver cancers. There is a growing incidence of HCC worldwide. In the Western world, HCC arises in a cirrhotic background in up to 90% of cases, and cirrhosis itself is a progressive disease that affects patient survival. Thus, outcome in patients with HCC and the chances for antitumoral treatment and its results are dependent not only on tumor-associated factors but also on liver function.

Assessment of tumor extension is critical for defining staging and treatment strategy and needs to be complemented by an assessment of liver function. The current EASL–EORTC GP guidelines endorse the Barcelona-Clínic Liver Cancer (BCLC) classification. It includes prognostic variables related to tumor status, liver function and health performance status along with treatment-dependent variables.

Early HCC (BCLC stage A) is defined in patients presenting single tumors > 2 cm or 3 nodules < 3 cm of diameter, ECOG-0 and Child-Pugh class A or B. Median survival of patients with early HCC reaches 50–70% at 5 years after resection, liver transplantation or local ablation in selected candidates.

Intermediate HCC (BCLC stage B): Untreated patients at an intermediate stage – BCLC B class (multinodular asymptomatic tumors without an invasive pattern) present a median survival of 16 months or 49% at 2 year. Chemoembolization extends the survival of these patients to a median of up to 19–20 months.

Advanced HCC (BCLC stage C): Patients with cancer related-symptoms (symptomatic tumors, ECOG 1–2), macrovascular invasion (either segmental or portal invasion) or extrahepatic spread (lymph node involvement or metastases) bear a dismal prognosis, with expected median survival times of 6 months, or 25% at 1 year. This outcome varies according to the liver functional status and other variables. The only available systemic treatment option to date is the TK inhibitor sorafenib which improves overall survival in the Western population by about 3 months. Recent data from the RESORCE trial suggest nother TKI, regorafenib, as second line option.

Cholangiocarcinoma (CCA) can be sub-classified as intrahepatic (iCCA), perihilar (pCCA), or distal (dCCA) where iCCA arises within the liver parenchyma. Overall the incidence of iCCA seems to be increasing globally.

Surgical resection is the treatment of choice for CCA. Patients demonstrating intrahepatic metastases, vascular invasion or obvious lymph node metastases should not undergo resection. There is no established adjuvant therapy after resection.

TACE and TARE have shown anti-tumor effects with acceptable toxicities in patients with iCCA but require further examination in appropriately designed clinical trials and therefore cannot be recommended as standard therapy.
Cisplatin and Gemcitabine is a systemic therapy practice standard for CCA in patients with ECOG performance status 0 or 1.

References:


The molecular and cellular biology of liver cancer evolution

Snorri S. Thorgeirsson M.D., Ph.D.
Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD, USA

Hepatocellular carcinoma (HCC) is the most common and deadly cause of primary liver cancer. Although the incidence of HCC is highest in Asia and sub-Saharan Africa the steadily increasing incidence of HCC in traditionally low incidence regions such as Northern Europe and the United States constitutes a significant public health care problem. Cells with “stemness,” or stem-cell properties, are referred to as cancer stem cells (CSC) or cancer-initiating cells. The concept that these cells rest at the apex of the cancer hierarchy is an evolving theme in cancer research. These cells are by definition primarily responsible for initiation and propagation of tumors as well as relapse after therapy, and they are therefore of major scientific interest. Several studies indicate that hepatocellular carcinomas that harbor phenotypic features of stem cells and progenitor cells constitute a subclass of therapeutically challenging cancers that are associated with a particularly poor prognosis. We recently showed that any hepatic lineage cell can be reprogrammed into CSC by activating diverse cell type-specific pathways. Furthermore, we identified common and cell of origin-specific phenotypic and genetic changes that accurately differentiated murine tumors according to their origin, providing an important tool to phenotypically classify morphologically diverse human primary liver cancer. These and more recent studies will be discussed in the presentation.
The recent approval of two immune checkpoint inhibitors for the treatment of malignant melanoma has sparked great interest by physicians and basic scientists searching for novel therapeutics for GI cancer. Chronic inflammation is recognized as a major risk factor for the development of hepatocellular carcinoma (HCC) and makes this type of cancer a potentially ideal target for an immune based treatment approach. Further evidence for a critical role of immune responses in patients with HCC is derived from the fact that immune signatures and profiles predict patients’ outcome as well as the fact that tumor-induced spontaneous anti-tumor immunity can be detected. In addition, ablative therapies can lead to changes in the number, phenotype and function of different immune cell subsets, which correlate with patients’ survival. Various HCC-specific mouse models have been developed, which improve our understanding of hepatocarcinogenesis and tumor-immune cell interactions. We have recently studied how non-alcoholic fatty liver disease (NAFLD) promotes hepatocarcinogenesis using different dietary NAFLD models as well as different HCC models. We made the unexpected observation that intrahepatic CD4+ T cells die within the liver through a ROS mediated mechanism leading to enhanced tumor growth. Blocking ROS production resulted in CD4+ T cell rescue and impaired tumor growth indicating a pivotal role for CD4+ T cell in immunosurveillance. Based on various studies in murine models and promising data from patients with other types of malignancies, immune based approaches are currently being evaluated in patients with HCC. Early results from clinical trials indicate that immune checkpoint blockade may be an effective way to treat patients with HCC. Different immune checkpoint inhibitors are currently being evaluated: while initial studies tested anti-CTLA4 antibodies, recent studies also included the use of anti-PD1 and anti-PDL1 antibodies. Alternate immune based approaches include adoptive immune cell therapy and vaccine approaches. Vaccines are currently being evaluated either alone or in combination with other treatments. In summary, accumulating data from studies investigating basic cancer biology of HCC as well as preclinical murine models along with early data from clinical trials evaluating novel immune based approaches in HCC clearly point towards an important role for an immunological approach as an effective treatment alternative for patients diagnosed with HCC.
Session VII

Complications of liver disease
Novel approaches to reducing the risk of variceal hemorrhage

F. Nevens, MD PhD
Hepatology – Liver transplantation, University Hospital KU Leuven, Belgium

One of the most relevant complications of chronic liver disease is portal hypertension since it is the main cause of death and liver transplantation. More than in case of ascites and hepatic encephalopathy, variceal rupture and bleeding are directly linked to the level of portal pressure (1).

I. Diagnosis of Portal Hypertension

Variceal bleeding occurs when the portal pressure gradient increases ≥ 10 mmHg: clinical significant portal hypertension (CSPH) (2). However, also a marked reduction of the risk of variceal bleeding can be achieved with less marked reduction of the portal pressure gradient (3). Hepatic venous pressure measurement is the gold-standard method to assess the presence of CSPH. It should be measured with the occlusion-balloon technique (4).

In patients with virus related compensated advanced chronic liver disease and in the absence of ALT flares, non-invasive methods are sufficient to rule-in CSPH, defining the group of patients at risk of having endoscopic sign of PH. Last Baveno consensus meeting defined this non-invasive method as a liver stiffness by transelastography of ≥ 20–25 kPa (at least 2 measurements on different days in fasting conditions). Also imaging showing collateral circulation is sufficient to rule-in CSPH in patients of all aetiologies (5)

II. Treatment of portal hypertension

a. Evolution of the treatment strategies:

Whereas direct reduction of portal pressure by portosystemic shunts, initially surgical and later on by TIPS, resulted in improved outcome of our patients, understanding of the pathophysiology of PH led to other successful treatments (6). Demonstration that increased portal flow inflow was an important contributor to PH lead to the use of non-selective beta blockers (NSBB) and vasoconstrictors (terlipressin, somatostatin and analogues) with a significant decrease of the mortality (7, 8). Further understanding that there was a partial reversible increased intrahepatic resistance in which NO dysfunction played a major role, resulted in the treatment of vasodilating nitrates in combination with NSBB (9, 10). Fibrosis is one of the main reasons of the increased intrahepatic resistance and in this regard, in several liver diseases reversal of fibrosis has been demonstrated after control or cure of the aetiology of the disease, leading to an improvement of the PH. Nevertheless, HCV patients with CSPH remain at risk for variceal bleeding regardless of response to antiviral therapy (11). Finally, recent advances in the knowledge of the pathophysiology of PH are: intrahepatic vascular thrombosis and bacterial translocation as factors worsening (12, 13, 14).

b. Old strategies wit new insights:

The NSBBs propranolol and nadolol have been used in the management of cirrhosis and PH for over 25 years. Carvedilol, a NSBB with weak anti-α adrenergic (vasodilator) activity has recently been shown to have a haemodynamic response
rate of 57% in propranolol non-responders and in lower dose, as previously used (median 12.5 mg/day) it was better tolerated (no drop in mean arterial pressure and no fluid retention) (15). In contrast to propranolol/nadolol carvedilol was effective in preventing the progression of small to large varices (16). However, the overall quality of evidence is still low to replace systematically carvedilol instead of propranolol/nadolol (17).

It has recently been suggested that patients with refractory ascites on NSBB have a higher mortality than those without (17, 18). Other retrospective studies and meta analyses could not confirm this entirely but it is nevertheless possible that in a subset of patients with refractory ascites in whom the cardiac response to hypotension may be inadequate, NSBB may lead to disturbances of the circulatory status and therefore Baveno recommended that for the given time, NSBB should be used continuously in case of a systolic blood pressure < 90 mmHg, serum sodium < 130 mEg/l or acute kidney injury (19, 33, 34, 35, 37). In this regard, a recent study demonstrated that there was no clinical or haemodynamic rebound after abrupt interruption of NSBB in patients with cirrhosis (25). Finally, prophylactic NSBB treatment is not an aggravating factor for the short-term survival in case of variceal bleeding (26).

c. New drugs to impove portal hypertension:

Preliminary clinical data suggest that simvastatin, a drug that modifies hepatic vascular tone by acting on NO signaling and improves liver fibrogenesis, results in a survival benefit when given on top of standard treatment (27).

With regard of the role of thrombosis on the development of PH, a recent clinical paper which showed a potential beneficial effect of enoxaparin in subgroups of patients with cirrhosis, supports this concept (28). In this regard, anticoagulation does not have an impact on the outcome on an upper-gastrointestinal bleeding in patients with cirrhosis (29).

d. Advances in the treatment of acute oesophageal bleeding:

TIPS became the standard therapy for variceal bleeding in case endoscopic and vasoactive therapy fails (30). Improved patency with covered versus bare stents has been established without an increase in hepatic encephalopathy. This results in a lower risk of rebleeding and a reduced cost (31). In this regard, covered TIPS was superior to endoscopic variceal ligation + NSBB for reduction of variceal rebleeding but was still associated with higher rates of early hepatic encephalopathy (32, 33).

Finally, self-expanding covered oesophageal metallic stents achieve similar or greater hemostatic rates, can be left in place for much longer periods (up to 7 days), cause fewer severe complications and are safer than balloon tamponade (34).

Conclusions

- In case of viral hepatitis transelastography allows to detect patients at risk for variceal haemorrhage (clinical significant portal hypertension).
- Several data offer indirect arguments that carvedilol (used at median dose of 12.5 mg/day) protects patients better against variceal rupture.
- Non-selective beta-blockers should be used with caution in patients with refractory ascites.
- Preliminary data suggest that simvastatin and enoxaparin improves the prognosis of patients with cirrhosis.
• Self-expanding covered oesophageal metallic stents seem to be superior to balloon tamponade in case of acute refractory variceal bleeding.

References:


Translating our current understanding of ascites management into new therapies for patients with cirrhosis and fluid retention

Andres Cardenas, MD, MMSc, PhD, AGAF, FAASLD
Institute of Digestive Diseases and Metabolism, Department of Medicine, University of Barcelona, Hospital Clinic, Barcelona, Spain

Liver disease and cirrhosis are the seventh most common cause of death in Europe. Ascites is the most common complication of cirrhosis resulting in poor quality of life, high risk of development of other complications of cirrhosis, increased morbidity and mortality associated with surgical interventions, and poor long-term outcome. Cirrhotic patients with their first onset ascites have a probability of survival of 85% at 1 year and 56% at 5 years without liver transplantation. Ascites is related to increased renal sodium retention as a result of increased activity of the renin-angiotensin-aldosterone system in response to marked vasodilation of the splanchnic circulation. The practical management of ascites involves a proper evaluation of the patient with a thorough history and physical exam. In addition, complete laboratory, ascitic fluid, radiological, and endoscopic tests should be performed. One of the most important steps in the initial assessment of patients with ascites is referral of appropriate candidates for liver transplantation as it offers a definitive cure of cirrhosis and its complications. Taking proper care of patients with ascites can be challenging because they are prone to several complications such as spontaneous bacterial peritonitis (SBP), hypervolemic hyponatremia, hepatic hydrothorax, hepatorenal syndrome (HRS) and hepatocellular carcinoma, bleeding from esophageal or gastric varices, and hepatic encephalopathy. While the initial management of uncomplicated ascites with low sodium diet and diuretic treatment is straightforward in the majority of patients, approximately 10% of patients fail to respond to diuretics and become a real therapeutic challenge. The initial treatment of choice in patients with refractory ascites is large-volume paracentesis associated with intravenous albumin; some patients also benefit from transjugular intrahepatic portosystemic shunts. The Alfa Pump system which is designed to move ascites from the peritoneal cavity to the urinary bladder where it is eliminated spontaneously through diuresis is promising, but the data is scarce and safety is still a concern. This article will focus on the practical aspects of the evaluation and treatment of patients with ascites and cirrhosis and will also discuss how to translate our current understanding of ascites pathophysiology into new treatments for patients with fluid retention.
Nutrition and liver health “Clinical hepatology practice in 2016: From science to therapy”

A.A. Jackson
Southampton General Hospital (MP 113), University of Southampton, UK

Nutrition is the set of integrated processes by which cells, tissues, organs and the whole body acquire the energy for normal structure and function. At the level of the whole body this is achieved through dietary supply and the capacity of the body to transform the substrates and cofactors necessary for metabolism. All of these domains (diet, metabolic capacity, activity of the microbiome, body composition and the level of demand for energy and nutrients) are influenced by levels of physical activity and can vary according to physiological and pathological disease states. The liver plays the central role in establishing and maintaining these regulated processes. Its capacity to achieve and maintain these functional capabilities is set during early life. When these capabilities are exceeded and the ability to maintain the milieu interieur is compromised ill-health supervenes. Stress tests which assess flow through gateway pathways can be used to determine the maximal capacity and functional reserve for critical functions. The inability of the liver to reliably integrate body lipid metabolism and the accumulation of abnormal lipid is an obvious manifestation of impaired regulation both in situations of weight loss, for example the fatty liver of severe malnutrition, and for energy excess, as in non-alcoholic fatty liver disease. The use of stable isotopic probes and more recent definition of the variability in the metabolome in different nutritional and pathological states provides an indication of the great potential for clinical tools that would enable a more precise nutritional diagnosis but these require systematic investigation and application. For the present approaches that place emphasis on being able to control the metabolic state without exposing the liver to unnecessary metabolic stress remain the basis for success nutritional support.
Session VIII

Perspectives for the future of hepatology
Liver preservation: Where we are and future perspectives

P. Dutkowski and P.-A. Clavien
Department of Surgery & Transplantation, University Hospital Zurich, Switzerland

Machine liver perfusion has significantly evolved during the last ten years to optimize extended criteria liver grafts and to address the worldwide organ shortage. Three different perfusion approaches are currently discussed for liver grafts, differing in perfusate temperature and the degree of oxygenation, e.g. normothermic, sub-normothermic and hypothermic liver perfusion. Normothermic machine liver perfusion simulates \textit{in vivo} conditions and therefore needs dual perfusion through the portal vein and the hepatic artery at physiological flow and temperature with oxygenated diluted blood and nutritional compounds as perfusate. In contrast, both sub-normothermic and hypothermic machine liver perfusion rely on the physical solved oxygen in a blood free perfusate at temperatures of 20–25°C (sub-normothermic) or 2–10°C (hypothermic). Besides the perfusate temperature and oxygenation, the optimal duration of machine liver perfusion has been repeatedly varied with either votes for continuous perfusion until implantation, or only short perfusion intervals before (pre-ischemic perfusion) or even after organ transport (end-ischemic perfusion). Current human application of normothermic perfusion, applied mostly in DBD liver grafts, showed feasibility and lower initial enzyme release after implantation. Hypothermic perfusion of human livers has been done in extended DBD and DCD livers, with no signs of cholangiopathy within 1 year after transplantation, so far. Long term outcome and RCTs in both methods are awaited. Experimental and clinical results, however, suggest a clear impact of liver perfusion methods in the clinic, with high future chances to assess and modify liver function before implantation, and also with significant extension of preservation times according to the needs of recipients or centers.
List of Chairpersons, Speakers and Scientific Organizers

Prof. David H. Adams
Centre for Liver Research
NIHR Biomedical Research Unit
University of Birmingham
Birmingham B15 2TT
Great Britain

Prof. Guruprasad P. Aithal
Nottingham University Hospitals
NHS Trust and University
E Floor, West Block
Queen’s Medical Centre
Nottingham NG7 2UH
Great Britain

Prof. Dr. Ralf Bartenschlager
Department for Infectious Diseases,
Molecular Virology
University of Heidelberg
Im Neuenheimer Feld 345
69120 Heidelberg
Germany

Prof. Dr. Ulrich Beuers
Department of Gastroenterology & Hepatology, G4-216
Tytgat Institute for Liver & Intestinal Research
University van Amsterdam
Meibergdreef 9
1105 AZ Amsterdam
The Netherlands

Dr. Andres Cardenas
Institute of Digestive Diseases and Metabolism
University of Barcelona
Hospital Clinic
Villarroel 170, Esc 3-2
08036 Barcelona
Spain

Prof. Dr. Pierre-Alain Clavien
Klinik für Viszeral- und Transplantationschirurgie
Universitätsspital Zürich
Rämistr. 100
8091 Zürich
Switzerland

Prof. Christopher Day
The Medical School
Centre for Liver Research
University of Newcastle
William Leech Building
Framlington Place
Newcastle-upon-Tyne NE2 4HH
Great Britain

Jordan J. Feld, M.D.
Liver Centre
Toronto Western Hospital
Toronto, ON M5G 2M9
Canada

Prof. Dr. Peter R. Galle
Innere Medizin I
Universitätsmedizin der Johannes Gutenberg-Universität
Langenbeckstr. 1
55131 Mainz
Germany

Tim F. Greten, M.D.
Professor of Medicine
Gastrointestinal Cancer Section
Med. Oncology, Bldg.10/Rm 12N226
National Cancer Institute, NIH
9000 Rockville Pike
Bethesda, MD 20892
USA

Dr. Gideon Hirschfield
Centre for Liver Research
NIHR Biomedical Research Unit
University of Birmingham
Birmingham B15 2TT
Great Britain
Prof. John Iredale  
University of Bristol  
5th floor, Senate House  
Tyndall Avenue  
Bristol BS8 1TH  
Great Britain

Prof. Alan A. Jackson  
Southampton General Hospital  
(MP 113)  
Tremona Road  
Southampton SO16 6YD  
Great Britain

Prof. Rajiv Jalan  
Institute for Liver and Digestive Health  
Upper Third Floor  
UCL Medical School  
Rowland Hill Street  
London NW3 2PF  
Great Britain

Prof. David E.J. Jones  
The Medical School  
Centre for Liver Research  
University of Newcastle  
3rd floor, William Leech Bldg.  
Framlington Place  
Newcastle-upon-Tyne NE2 4HH  
Great Britain

Dr. Tom H. Karlsen  
Medical Department  
Norwegian PSC Research Centre  
University of Oslo  
Rikshospitalet  
0027 Oslo  
Norway

Prof. Dr. Ansgar W. Lohse  
Medizinische Klinik I  
Universitätsklinikum Eppendorf  
Martinistr. 52  
20251 Hamburg  
Germany

Dr. Maria-Carlota Londoño  
Hospital Clinic of Barcelona  
Liver Unit  
CIBERehd, IDIBAPS  
Carrer de Villarroel 170  
08036 Barcelona  
Spain

Prof. Jane A. McKeating  
School of Immunity and Infection  
College of Medical and  
Dental Sciences  
Medical School Building  
University of Birmingham  
Birmingham B15 2TT  
Great Britain

Prof. David Mutimer  
School of Immunity and Infection  
College of Medical and  
Dental Sciences  
University of Birmingham  
Edgbaston  
Birmingham B15 2TT  
Great Britain

Prof. Dr. Frederik Nevens  
Division Liver and  
Biliopancreatic Disorders  
University Hospitals KU Leuven  
Campus Gasthuisberg  
Herestraat 49  
3000 Leuven  
Belgium

Prof. Phil Newsome  
Centre for Liver Research  
NIHR Biomedical Research Unit  
University of Birmingham  
Birmingham B15 2TT  
Great Britain

Prof. Mark Pallen  
Warwick Medical School  
University of Warwick  
Gibbet Hill Campus  
Coventry CV4 7AL  
Great Britain
Prof. Dr. Vlad Ratziu  
Hôpital Pitié Salpêtrière  
Service d’Hépatogastroentérologie  
47–83, boulevard de l’Hopital  
75013 Paris  
France

Dr. Ian Rowe  
St. James’s University Hospital  
Beckett Street  
Leeds LS9 7TF  
Great Britain

Prof. Dr. Robert Thimme  
Klinik für Innere Medizin II  
Universitätsklinikum Freiburg  
Hugstetter Str. 55  
79106 Freiburg  
Germany

Snorri S. Thorsteinsson, M.D., Ph.D.  
Laboratory of Human Carcinogenesis  
Center for Cancer Research  
National Cancer Institute, NIH  
37 Convent Drive MSC 4262  
Building 37, Room 4128B  
Bethesda, MD 20892-4262  
USA

Prof. Dr. Heiner Wedemeyer  
Klinik für Gastroenterologie,  
Hepatologie und Endokrinologie  
Medizinische Hochschule Hannover  
Carl-Neuberg-Str. 1  
30625 Hannover  
Germany

Prof. Dr. Fabien Zoulim  
Hepatology Department  
INSERM U 1052  
Lyon University  
151, cours Albert Thomas  
69003 Lyon  
France
POSTER ABSTRACTS

Poster Numbers 1 – 63

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Expression of programmed death 1 (PD-1) and its ligand PD-L1 on T and B cell subsets from peripheral blood of patients with alcoholic liver disease

Katarzyna Adamczyk¹, Beata Kasztelan-Szczerbinska¹, Agata Surdacka², Halina Cichoż-Lach¹, Jacek Rolinski², Jakub Onikijuk¹, Agata Michalak¹, Mariusz Szczerbinski¹

¹Department of Gastroenterology with Endoscopy Unit, Medical University of Lublin, 8 Jaczewski Street, 20-957 Lublin, Poland
²Department of Clinical Immunology, Medical University of Lublin, 4A Chodzki Street, 20-093 Lublin, Poland

Introduction: Activation of innate immunity system and the subsequent inflammatory cascade play a central role in the pathogenesis of alcoholic liver disease (ALD). Recent studies support the role of programmed death 1 (PD-1) and its ligand PD-L1 as a critical inhibitory signaling pathway that regulate immune responses. Therefore, we aimed to explore an expression of both molecules on T and B cell subsets from peripheral blood of patients with ALD.

Methods: 33 naïve patients (14 females, 19 males) with ALD were prospectively recruited and compared with 21 age- and sex-matched healthy controls (HC) (8 females, 13 males). Patients were divided into subgroups based on their gender and severity of liver dysfunction. The expression of PD1 and PD-L1 on T CD3, CD4, CD8, and B CD19 cell subsets was analyzed by a FACSCalibur flow cytometer (Becton Dickinson, USA) with CellQuest software.

Results: Female controls expressed significantly lower PD1 levels on T CD3 cells in comparison with males (16.9% vs. 27.6%, p = 0.04), but there were no differences in PD1 and PD-L1 expression between females with ALD and controls. In males, significantly lower levels of PD1 expression on CD3 (median 17.7% vs. 27.6%, p = 0.02), as well as PD-L1 on CD3 (0.7% vs. 1.4%, p = 0.04), CD4 (0.7% vs. 1.6%, p = 0.03) and CD8 (0.4% vs. 1.1%, p = 0.02) were found in ALD group. No differences in PD1 and PD-L1 expression between different Child-Pugh subgroups were detected.

Discussion/Conclusion: PD-1/PD-L1 signaling pathway may contribute to inflammatory activation in ALD due to its downregulation on the T cell subsets. Gender-related differences in the PD1/PD-L1 expression might have an impact on the disease course in both sexes.
The prevalence of hepatitis B sero-markers and hepatitis C antibodies in blood donors in Basra, Iraq

Ali Al-Rubaye MBChB1, Ziad Tariq MBChB, FIBFM2, Laith Alrubaiy MBChB, MSc, MRCP (UK)3
1Specialist doctor in Family Medicine, Basra General Hospital, Basra, Iraq
2Director of the Department of Public Health, Basra, Iraq
3Clinical lecturer, College of Medicine, Swansea University, Swansea, UK

Introduction: Transfusion caused hepatitis remains a major problem in Iraq. Therefore, testing for hepatitis B surface antigen (HBsAg), antibodies to hepatitis B core antigen (anti-HBc), and antibodies to hepatitis C antigen (anti-HCV) is very important to prevent transfusion caused hepatitis. The objective of this study was to establish presence of hepatitis B and C virus sero-markers among blood donors as a foundation for safe blood transfusion in Iraq.

Methods: A retrospective cohort study was conducted in the blood transfusion unit in Basra, Iraq from 1st of January to 31 of December in 2013. All blood donors during the study period were included in the study. Blood samples were collected and were tested for HBsAg, anti-HBc, anti-HCV using the standard laboratory techniques.

Results: A total of 69,915 blood donors were enrolled for the study. The prevalence of HBsAg and anti-HBc were very low at 0.22% (150 donors) and 2.29% (1600 donors) respectively. There was no significant difference between males than females (p = 0.28). The prevalence of anti-HCV was 0.12%. A total of 1475 (2.11%) donors had anti-HBc antibodies as the only serological evidence of hepatitis B virus infection.

Discussion/Conclusion: The prevalence of Hepatitis B and C among blood donors is very low in Basra. Around 2% of blood donors had anti-HBc as the only serological evidence of HBV infection. Inclusion of anti-HBc in routine screening of blood donors in Iraq should be encouraged. This is the first large population study of its kind in Basra, Iraq.
Prevalence of blood-born hepatitis (HBV & HCV) in incident hemodialysis patients in Qena University Hospital, Egypt

Mohamed Alsenbesy, Wael Haggag, Abdelrahman Elsayed
1Internal Medicine Department – Qena Faculty of Medicine, South Valley University (SVU)
2Clinical and Chemical Pathology Department – Qena Faculty of Medicine, South Valley University (SVU), Qena, Egypt

Background: Blood-born hepatitis is a major health problem in hemodialysis units. Prevalence of HCV reached up to 87.5% among Egyptian patients kept on regular hemodialysis for more than 6 months. The prevalence HBV is less than 5% in dialysis patients in Egypt which is a less prominent problem. This high prevalence of hepatitis is not clearly known whether to be due to the dialysis environment alone or also due to the common risk for hepatitis in a community with HCV prevalence of up to 14.7%.

Objectives: Was to study the prevalence of HCV and HBV among incident hemodialysis patients admitted to the recent hemodialysis unit at Qena University Hospital in Egypt.

Methods: We recruited 100 adult patients with end stage renal disease (ESRD) recently admitted to our dialysis unit in Qena. All patients were recent to initiate dialysis during the first 4 sessions. Serum samples for Hepatitis B surface antigen (HBsAg) and hepatitis C antibody (HCV Ab) were done to all patients.

Results: In our study the prevalence of HCV was 22% and the prevalence of HBV was 2% with no co-infection detected in our study group. The mean age to start dialysis was 54.33 years in our study patients with non-significant difference between males and females.

Conclusion: HCV infection is endemic among dialysis centers in Egypt with less prevalent HBV. Our dialysis unit in Qena has a prevalence of 22% and 2% of HCV and HBV respectively. A reevaluation in one year is essential to assess the impact of the dialysis process on hepatitis prevalence.
Risk factors associated with hepatitis B and C virus infection in Qena Governorate, Egypt

Mohamed A. Alsenbesy¹, Naglaa M.A. Mousa¹, Mohamed H. Maghraby², Abdelrahman A. Elsayed³, Ekram M. Abdelkhalek⁴
¹Internal Medicine Department, Qena Faculty of Medicine, South Valley University (SVU), Qena, Egypt
²Internal Medicine Department, Faculty of Medicine, Assiut University, Assiut, Egypt
³Clinical Pathology Department, Qena Faculty of Medicine, South Valley University (SVU), Qena, Egypt
⁴Public Health and Community Medicine Department, Faculty of Medicine, Assiut University, Assuit, Egypt

Introduction: Hepatitis B (HBV) and C (HCV) viruses are considered some of the major global public health problems. They are among the principal causes of chronic liver diseases, including hepatocellular carcinoma and cirrhosis-related end stage liver failure.

Aims of the study: The present study aims to investigate the most important risk factors for transmission of HBV and HCV in urban and rural areas in Qena Governorate, Egypt. Patients and Methods: A matched case control study was conducted. The study included 1000 patients aged 20–70 years. Direct interview was done for filling the questionnaire during the period from January 2014 to January 2015. The data were analyzed using SPSS version 19.

Results: This study included 100 HBV cases, 400 HCV cases and 500 controls. Odds of HBV and HCV infection are significantly higher among cases with some risk factors: injection by reused needle, sharing razors with others, blood products transfusion, dental procedures or oral surgery. The majority of these risk factors were more frequent among cases from rural areas than urban cases.

Conclusion: The common risk factors of hepatitis B and C infection included blood transfusion, dealing with blood, hospital admission, surgery, accidental stick with a blood contaminated needle, intravenous catheterization and dental treatment. Living in rural areas was associated with significantly increased risk of behavioral attitude associated with HCV and HBV infection.
Hepatic steatosis associated with lipidic and nutritional disorders – Oxidative stress biomarkers assessment and therapy

M.C. Bezna, C. Deliu, M. Bezna, C. Pitis
University of Medicine and Pharmacy, Emergency Hospital, Craiova, Romania

Hepatic steatosis, through lipidic metabolic perturbations, may remain stationary or may evolve to steatohepatitis, hepatic cirrhosis and even hepatic carcinoma.

Aim: To present the importance of oxidative stress biomarkers in the presence of hepatic steatosis and especially, in its evolution towards steatohepatitis, with multiple future complications.

Patients and methods: The study was conducted on 10 patients, with suggestive images for hepatic steatosis, associating dyslipidemia and obesity, without other clinical or biological hepatic manifestations.

Determination of oxidative stress biomarkers (malondialdehyde as marker for lipids peroxidation, nitric oxide) showed an increased level in 4 patients; in the last 6 months, at these patients, a turn into steatohepatitis was observed, with cytolyses lesions due to hepatic inflammation and necrosis.

Results: In patients with lipids, hepatic and weight disorders, initial and control determinations of oxidative stress biomarkers are important. This fact monitors evolution towards complications: steatohepatitis, and rarely, towards non-alcoholic hepatic cirrhosis, viral or autoimmune. Also, there is an increased risk of neoplasia development, including hepatic carcinoma. Pathophysiological mechanisms of oxidative stress actions, expressed by determinable biomarkers are complex and target: mitochondrial dysfunction, the occurrence of mutations in mitochondrial DNA, increased production of reactive oxygen species, disrupting cellular DNA and apoptosis, disrupting cell signaling pathways, increased proinflammatory cytokines, decreased antioxidant defense capacity, chronic inflammation. In association with these markers, leptin level determination, a circulating protein encoded by obesity gene, located on chromosome 7q31, with role in "peroxisome proliferator activated gamma" receptor regulation is useful.

Conclusions:
1. In patients with modified metabolic profile, hepatic steatosis, dyslipidemia, obesity changes of oxidative stress biomarkers and oxidants-antioxidants imbalance may occur.
2. Modification of oxidative stress biomarkers profile is important in assessing the evolution towards complications (sometimes severe), including carcinogenesis.
3. Food correction through a diet rich in antioxidants and liver protection drugs is a highly recommended therapy.
Clinical aspects in interdisciplinary current hepatology practice: 
Cardiac disorders in the evolution of young patients with autoimmune hepatitis

M.C. Bezena, C. Deliu, M. Bezena, N. Gard, C. Pitis
University of Medicine and Pharmacy, Emergency Hospital, Craiova, Romania

Introduction: Multiple manifestations of patients with autoimmune hepatitis may associate cardiac perturbations, requiring proper assessment and therapy.

Aim: Observation of cardiac disorders occurring in the evolution of autoimmune liver disease in young people, without pre-existing cardiac disease.

Methods: The study was conducted on 22 patients, aged 18–50 years, diagnosed by clinical and laboratory findings with autoimmune hepatitis and who presented cardiac disorders in onset or evolution. Were recorded: risk factors of cardiac perturbations occurrence, their persistence, heart condition and staging of liver disease. The assessment was achieved during a 3-years period, both cardiac and immune-hepatic, as well as extrahepatic perturbations and therapy.

Results: Abnormalities of cardiac rhythm were observed in autoimmune hepatitis evolution in young patients. Cardiac disorders profile was associated with various rhythm troubles: sinus tachycardia, observed in 6 patients, sinus bradycardia in 8, atrial extrasystoles in 6, ventricular extrasystoles in 4, supraventricular tachycardia in 2, atrioventricular 1st degree block in 1, paroxystic atrial fibrillation in 2 patients. Some patients manifested simultaneously two types of arrhythmias. Risk factors associated with cardiac perturbations and hepatic-immune disease were: inflammatory immune activity, hepatic cytolysis, anemia, long-term immunosuppressor therapy, cortizonic cardiomyopathy, hormonal modifications (mostly thyroid), jaundice, hypocalcemia, dyselectrolytemia, arterial pressure oscillations, dyslipidemia, anxiety. Most arrhythmias were paroxysmal or persistent.

Conclusion:
1. In current hepatology practice, in the evolution of patients with autoimmune hepatitis, cardiac disorders may be recorded.
2. Profile of observed cardiac perturbations was: sinus tachycardia and bradycardia, atrial and ventricular extrasystoles, supraventricular tachycardia, paroxystic atrial fibrillations, related with risk factors of immune disorders and prolonged immunosuppressor therapy (cortizonic cardiomyopathy).
3. Risk factors can trigger cardiac dysrhythmia in liver diseases, requiring adapted monitoring and assessment.
4. Cardiac manifestations may worsen the quality of life, needing control and also treatment, associated with the main autoimmune hepatitis therapy, in order to evolve from science to proper and complex therapeutic management.
Current assessment of chronic viral hepatitis C: Immune manifestations – Cryoglobulinemia

Marinela Bezna, Mariana Balasoiu, Maria Cristina Bezna, Cristina Deliu
University of Medicine and Pharmacy, Emergency Hospital, Craiova, Romania

Introduction: Chronic infection with hepatitis C virus induces many immune abnormalities, experiencing a variety of manifestations.

Aim: To observe the translational relationship between chronic hepatitis C and the presence of cryoglobulinemia and its manifestations.

Patients and methods: The study was conducted on a group of 32 patients, aged 34–66, who were diagnosed with hepatitis C and cryoglobulinemia by clinical and laboratory markers. Other causes for the presence of cryoglobulinemia were excluded.

Results: A significant markers level, with aspect of mixed cryoglobulinemia was present in all patients of the group diagnosed with hepatitis C in an active form. Clinical manifestations were: cutaneous vasculitis with recurrent purpura in 22 patients, polyneuropathy in 6 patients, nephritis in 3 patients and asymptomatic – 1 patient. Many immune tests, associated to cryoglobulinemia presented abnormalities (the presence of CIC, hypocomplementemia, hypergammaglobulinemia). The pathologic appearance at skin biopsy was of leukocytoclastic vasculitis and necrotizing cutaneous vasculitis with fibrinoid necrosis of the vessel wall and transmural inflammatory reaction. This is correlated with deposition of CIC in vessel wells, immune dysfunctions, elevated values of liver function tests and increase of interleukine-1 (IL-1) and tumor necrosing factor (TNF-α) which are potent inductors for ELAM-1 and VCAM-1 which intensify leukocytes adhesion at endothelial cells.

Conclusions:
1. Cryoglobulinemia is an important extrahepatic complication of hepatitis C, observed with recurrence in liver disease.
2. It is associated with the increase of IL-1, TNF-α, perturbation of immune and liver tests and frequently leukocytoclastic vasculitis.
3. The increased risk of developing cryoglobulinemia as interdisciplinary manifestation of hepatitis C make necessary the monitoring and proper therapy management.
Hepatic and extrahepatic aspects in practice and therapy of liver disease: Megaloblastic anemia – Diagnosis difficulties and a rare association in autoimmune hepatitis in a young patient

M. Bezna, C. Deliu, C. Pitis, M.C. Bezna
University of Medicine and Pharmacy, Emergency Hospital, Craiova, Romania

**Introduction:** Extrahepatic manifestations associated in evolution of patients with autoimmune hepatitis may describe various aspects induced by autoimmunity abnormalities, some frequent, other rare, needing proper assessment and combined therapy.

**Aim:** The description of a rare aspect observed in clinical practice and management of hepatic disease (autoimmune hepatitis), such as associated megaloblastic anemia, in a young patient.

**Methods:** It is presented a clinical case of a woman aged 34 years, which had a slowly progressive start of disease, with fatigue, asthenia, weight loss, intermittent diarrhea, low degree fever, palpitations, diffuse abdominal pain, paresthesia of extremities and difficulties in walking. At physical examination the patient was underweight, pale, jaundice, with moderate hepatomegaly, first degree splenomegaly, diminished tendon reflexes and cutaneous hypoesthesia.

**Results:** Laboratory and imaging investigations (biochemical, biological, immunological, endoscopic, ultrasound, computer tomography, neurological, bone biopsy) revealed the existence of autoimmune hepatitis type I and the association with severe megaloblastic anemia, with neurological manifestations and decreased B12 vitamin. Other diseases were excluded: cancers, inflammatory bowel disease, infectious diseases, viral or toxic hepatitis. Corticosteroid therapy associated with vitamin B12 supplementation improved manifestations of both diseases, with slowly, but favorable response.

**Conclusion:**
1. Megaloblastic anemia, most common in elders, may be linked, in young people, to a liver disease.
2. Manifestations of severity of megaloblastic anemia, especially neurological and hematological, require complex investigations for exclusion of other severe illnesses. After that, can be considered as autoimmune extrahepatic disturbance associated with autoimmune liver disease, in the presence of vitamin B12 decrease.
3. Treatment combination, immunosuppression and vitamin B12 supplementation leads to clinical improvement of both diseases, with hematologic data correction, improved hepatic and immunologic abnormalities and decreased neurological manifestations.
4. Precocious diagnosis is necessary because complicated megaloblastic anemia worse the hepatic disease and disturbs the quality of life, requiring intensive and urgent treatment.
Interdisciplinary link between hepatic and extrahepatic observations in autoimmune liver diseases associated with Sjögren syndrome

M. Bezna, C. Deliu, S. Bezna, O. Diaconu, M.C. Bezna
University of Medicine and Pharmacy, Emergency Hospital, Craiova, Romania

Introduction: In interdisciplinary evolution of autoimmune liver diseases, immune mediated disorders can be observed, with destruction of liver as target organ, but also, sometimes, at distance, extrahepatic disturbances, in salivary-tear glands may be associated.

Aim: To present Sjögren syndrome as immune extrahepatic manifestation and to describe risk factors associated with autoimmune liver diseases.

Methods: The study was conducted on 15 patients divided in: a group of 5 patients with autoimmune liver disease without Sjögren syndrome, a group of 5 with autoimmune liver disease and Sjögren syndrome and other group of 5 with Sjögren syndrome without autoimmune liver disease. The methods for diagnosis were: clinical, biological, immunological, bioptic, ultrasound, and endoscopic. Sicca tests were performed, as well as tests for determining Helicobacter pylori antibodies presence.

Results: Sjögren syndrome was associated in the translational evolution of autoimmune liver diseases characterized by autoimmune disorders, as extrahepatic manifestation. It was present before hepatic specific features in 2 patients and after the actual liver disease diagnosis, in 3. The antibodies G and M against H. pylori were frequently present in patients with Sjögren syndrome and autoimmune liver disease (3 patients). The IL-6, IL-1, TNF-α were increased in Sjögren syndrome and autoimmune liver diseases. Sjögren syndrome was described in 3 patients with autoimmune hepatitis and in 2 with primary biliary cirrhosis.

Conclusions:
1. Sjögren syndrome associated with active autoimmune liver disease is regarded as connected autoimmune manifestation, weather it was diagnosed prior or after the hepatic disease.
2. Risk factors were: immune perturbations of active liver diseases, increase of immunoglobulins G, M for H. pylori infection and of cytokines (TNF-α, IL-1, IL-6).
3. In the presence of autoimmune liver disease, extrahepatic manifestations must be taken into account; complex diagnosis and therapy management must be established, in order to think outside the box and to include interdisciplinary pathology.
Assessment of dietary risk factors for hepatic encephalopathy in patients with liver cirrhosis

M. Bezna, C. Deliu, M. Balasoiu
University of Medicine and Pharmacy, Emergency Hospital, Craiova, Romania

Introduction: Diet is very important in patients with hepatic cirrhosis, especially the protein intake, having the risk of hyper ammonia and development of hepatic encephalopathy (HE).

Aim: The investigation of protein nutrition role in hepatic encephalopathy onset, in patients with liver cirrhosis.

Patients and methods: The study was conducted on a group of 128 patients diagnosed with liver cirrhosis, who presented latent or clinical obvious hepatic encephalopathy. The assessed risk factors were: diet (high intake of protein), ammonia level, gastrointestinal hemorrhage, azotemia, diuretic intake, sedative drugs, infections, constipations, renal failure, hypokaliemia, stage of cirrhosis. Investigations were clinical, imagistic (electroencephalography, evoked potentials, ultrasound, endoscopy, x-ray) and biological.

Results: The high protein intake was involved in HE in 56% of patients. It was correlated with other risk parameters in 69% of patients. The decrease of hyper protein nutrition led to normalization of psychometric abnormalities in 42% patients and it improved the clinical manifestations of HE in 48%. The association of rich protein diet with hyper ammonia was correlated in 70% of patients. The efficient therapeutic recommendations were: to reduce protein intake to 30 g/day for about 3 days when HE appeared, then, as the symptoms subside, to increase the amount by 10 g/day, every third day until a daily protein diet of 1 g/kg-body weight.

Conclusions:
1. The rich protein nutrition is correlated with increase of ammonia level by reducing detoxification in liver cirrhosis, leading to latent or obvious encephalopathy.
2. The improvement of nutritional status is one of the goals of HE treatment in liver cirrhosis.
3. The avoidance of protein, however, led to a protein-catabolic state in which breakdown of protein occurred in the organism, especially in skeletal muscles; is important to maintain regular proper nutrition for catabolic processes.
4. Decrease of HE risk involves poor protein diet.
Translational particularities and therapy of chronic hepatitis C in elderly

Marinela Bezna, Cristina Deliu, Maria Cristina Bezna, Camelia Pitis, Mariana Balasoiu
University of Medicine and Pharmacy, Emergency Hospital, Craiova, Romania

Introduction: Hepatic affection, caused by viral hepatitis C infection may be diagnosed at any age, including in elderly patients.

Aim: Presentation of clinical and therapeutic particularities in the evolution of hepatitis C in elderly – over 80 years.

Patients and methods: The study was conducted on eight male patients, aged 82–86 years, diagnosed for the first time with hepatic disease, post-viral C infection. Three patients presented viral liver cirrhosis-portal decompensated and with liver failure signs, with onset between 2 weeks–2 months; they had a history of moderate alcohol consumption. Four patients presented chronic hepatitis manifestations, with asthenia and moderate hepatic cytolysis, associating anti-virus C antibodies presence. One patient was asymptomatic, with steatosis-type hepatomegaly, having also present anti-virus C antibodies.

Results: In these patients, liver disease caused by C virus evolved insidious, subclinical, until later in life; progression towards decompensate cirrhosis was favored, possibly by nutritional and debilitating age-related factors; the required treatment management provided both proper medication (taking into account age-related issues, tolerance, renal function-failure) for hepatic disease complications and also correction of nutritional and metabolic disorders status. For the patients with chronic hepatitis, the therapy was based on hepatic-protection, being still well tolerated. For the asymptomatic patient, periodic control and liver-protection medication were recommended, in particular, nutritional. Psychological support was required. Evolution of patients with cirrhosis was unfavorable in 2 cases, within approximately one year.

Conclusions:
1. Viral C etiology of a hepatic disease can be detected even at elder age, over 80 years.
2. Hepatomegaly in elders requires complex investigations, including viral etiology.
3. Being a translational and age-related issue, therapy must be established by a team of specialists in hepatology, geriatrics, pharmacology, psychology, nutrition, internal medicine.
4. Treatment management is complex, associating liver protection medication, proper nutritional dietary programs, psychological support, and if complications occur, their specific-targeted therapy.
Major histocompatibility complex (MHC) class I associated phosphopeptides and tumor immunity in hepatocellular carcinoma (HCC)

Nico Buettner¹,², Stuart Curbishley², Paisley D. Trantham³, Sarah A. Penny², Lora G. Steadman², David G. Millar²,⁴, Oliver C. Goodyear², Michael Russel², Mirka Blahova², Ellen Speers³, Nicola Ruth², Gabriel Wong², David Adams², Robert Thimme¹, Donald F. Hunt³, and Mark Cobbold²,⁴
¹Universitätsklinikum Freiburg, Abteilung für Innere Medizin II – Gastroenterologie/Hepatologie, Hugstetter Str. 55, 79106 Freiburg, Germany
²University of Birmingham, Immunity and Infection/Centre for Liver Research, Edgbaston, Birmingham, B15 2TT, United Kingdom
³University of Virginia, Department of Chemistry, McCormick Road, PO Box 400319, Charlottesville, VA 22904-4319, USA
⁴Massachusetts General Hospital, Center for Cancer Immunology, 55 Fruit Street, Boston, MA 02114, USA

Introduction: The identification of specific tumour antigens provides the basis for the development of an efficient immunotherapy. Only few specific tumour antigens have been characterized for hepatocellular carcinoma (HCC). Dysregulation of signalling pathways in cancers leads to aberrant and augmented protein phosphorylation and in such way modified proteins can be degraded to generate cancer-specific phosphopeptides. These are presented by MHC class I molecules and recognized by T cells. The aim of this study was to identify HCC-associated, MHC class I-bound phosphopeptides (MHC-I-pP) and to assess immunity against this novel class of antigens in patients with chronic liver disease and HCC.

Methods: For identification, MHC class-I complexes were affinity purified and bound phosphopeptides were characterized using mass spectrometry. Immunity against MHC-I-pP was assessed in PBMCs and liver-derived lymphocytes from healthy donors, patients with chronic liver disease and HCC and analysed using intracellular cytokine staining. MHC-I-pP-specific CD8+ T cells were expanded in a large scale using a rapid expansion protocol (REP) with anti-CD3, IL-2 and irradiated feeder cells.

Results: We have identified over 300 HCC-associated MHC-I-pP. Many of the novel identified MHC-I-pP were derived from proteins, which can be directly linked to important cancer-associated signalling-pathways. More MHC-I-pP were displayed on HCC in comparison to non-cirrhotic and cirrhotic liver tissue. CD8+ T cell responses against this novel class of tumour-antigens were comparable in quantity and quality to those seen against viral control epitopes. CD8+ T cell responses were found in a large fraction of patients with chronic liver disease, were rarer in patients with end-stage liver cirrhosis and could not be detected after HCC formation. Expansion of MHC-I-pP-specific CD8+ T cells in a large scale was achieved from a combination of REP and repeated rounds of MHC-I-pP-specific stimulation.

Discussion/Conclusion: MHC-I-pP represent an attractive target for future cancer immunotherapies with a possible application in adoptive T cell therapy.
Stabilin-1 expression in hepatocellular carcinoma is associated with adverse histological features

O. Cain, S. Shetty, S. Hübscher
Centre for Liver Research, University of Birmingham, Birmingham, B15 2TT and Queen Elizabeth Hospital Birmingham, B15 2TH, United Kingdom

Introduction: There is growing interest in the immune response to cancer, including hepatocellular carcinoma (HCC). However, the mechanisms of recruitment of tumour infiltrating lymphocytes to the inflammatory micro-environment are not well understood. The aim of this study was to investigate the pattern of expression of the lymphocyte recruitment molecule stabilin-1 in HCC and its association with histological markers of prognosis.

Methods: Explant liver tissue from patients with a single nodule of HCC was studied (n = 48). Immunohistochemistry for stabilin-1 was semi-quantitatively scored and correlated with established histological markers of prognosis. Dual immunofluorescence staining was used to confirm the identity of stabilin-1 positive cells.

Results: Stabilin-1 was expressed by sinusoidal vessels within the tumour (tumoural blood vessels) and by blood vessels in the fibrous stroma directly investing the tumour nodule (peritumoural vessels). Stabilin-1 expression in peritumoural vessels was significantly correlated with tumour grade (p = 0.003), such that poorly differentiated tumour had the highest levels of expression. Stabilin-1 expression was also significantly associated with vascular invasion (p = 0.022) and tumour size (p = 0.049).

Discussion/Conclusion: Stabilin-1 has been associated with adverse features and outcomes in a limited number of cancers. However, this is the first study to demonstrate an association between stabilin-1 expression and adverse histological features in HCC. These findings may provide insight into the way in which lymphocyte recruitment molecules shape the inflammatory microenvironment of tumours. We next plan to test the hypothesis that stabilin-1 selectively recruits regulatory T cells to the tumour microenvironment, and to supplement this data with measurements of stabilin-1 mRNA and protein levels.
Is the decompensated cirrhosis care bundle an essential checklist for acute admissions

Z. Cargill¹, T. El Menabawey¹, S. Phillpotts¹, K. Tang¹, A. Alisa¹
¹Gastroenterology Department, Barnet Hospital, Royal Free NHS Foundation Trust, London, UK

Introduction: Acute Decompensated Cirrhosis (ADC) is a medical emergency that carries high mortality (10–20%). The British Society of Gastroenterology/British Association for the Study of the Liver has published an evidenced based Decompensated Cirrhosis Care Bundle (DCCB) checklist to be completed during the initial six hours of admission for all patients with ADC and for expert care to be implemented within 24 hours.

Methods: Patients were identified by the gastroenterology team inpatient referral system, admission notes and electronic records. It included 40 consecutive patients with confirmed cirrhosis between 14/12/2015 and 12/2/2016. Our current performance was compared to the standards set out in the DCCB.

Results: Our institution is a District General Hospital which serves a population of 500,000 in North London. Forty admissions with ADC were identified. All had radiologically confirmed cirrhosis.

Compliance with the standard can be seen in table 1 and demonstrates areas in which the target standard of 100% is not reached. All weekday admissions had gastroenterology review within 24 hours. Ten admissions (25%) were over the weekend and were seen by the on-call medical team initially and by the Gastroenterology team the following Monday. Inpatient mortality was seen in 18% of our inpatients.

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<th>Demographics, n = 40</th>
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<td>Average length of stay</td>
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<th>Aetiology of cirrhosis, n = 40</th>
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<td>Alcoholic liver disease</td>
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<td>NASH</td>
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<th>Early investigations, n = 40</th>
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<td>New early warning scores</td>
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<td>FBC, U&amp;E, CRP</td>
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<td>Bone profile and magnesium</td>
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<td>Coagulation profile</td>
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<td>Ascitic tap if clinical ascites present</td>
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<td>Ultrasound abdomen</td>
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Discussion/Conclusion:
As patients admitted with ADC in our hospital have morbidity and mortality levels similar to national level, the adoption of the DCCB is definitely warranted which may save lives and reduce hospital stay. It is to be completed within 6 hours of admission with the investigations and treatment offered within 24 hours. A repeat study of our performance following the implementation of the DCCB is now underway.

<table>
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<th>Recent excessive alcohol consumption, n = 25</th>
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<td>IV Pabrinex</td>
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<td>CIWA score + alcohol reducing regimen</td>
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<th>Suspected infection, n = 19</th>
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<td>Started on antibiotics</td>
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<td>Blood cultures</td>
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<th>Acute kidney injury and/or hyponatraemia, n = 19</th>
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<td>Fluid resuscitation with sodium chloride</td>
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<td>Fluid balance chart</td>
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<td>Weight chart</td>
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<th>Suspected GI bleeding, n = 14</th>
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<td>Endoscopy within 24 hours</td>
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<td>Terlipressin administration</td>
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<td>Antibiotics</td>
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<td>Red blood cells transfusion</td>
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<td>FFP transfusion</td>
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<th>Encephalopathy, n = 40</th>
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<td>Signs of encephalopathy</td>
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Tab. 1: Decompensated cirrhosis care bundle compliance
CLEC2 dependent platelet activation drives acute inflammatory hepatitis

Abhishek Chauhan David H. Adams, Steve P. Watson, Patricia F. Lalor
Center for Liver Research, Institute of Biomedical Research, College of Medical and Dental Sciences, University of Birmingham, Birmingham B15 2TH, UK

Introduction: Platelets are fundamental players in liver pathobiology; driving inflammation, fibrosis, cancer and even aiding regeneration. Platelet interaction with the sinusoidal endothelium drives leukocyte recruitment, thus initiating and perpetuating cycles of iterative inflammation or acute hepatitis. The specific molecular basis of platelet activation in the context of liver inflammation and thus damage remains elusive. We have been investigating the platelet ITAM receptor CLEC-2 in this regard. Platelet based CLEC-2 mediates powerful platelet activation on meeting its only known natural ligand podoplanin. Interestingly homozygous loss of CLEC-2 does not give rise to the bleeding diathesis seen with traditional platelet inhibitors.

Methods: For our studies we used carbon tetrachloride to induce a toxic liver injury in transgenic mice. These mice have been engineered in such a manner that their platelets are selectively deficient in CLEC-2 (CLEC1bfl/fl PF4cre) or its ligand podoplanin (VAV1 icre), thus abrogating platelet activation due to this receptor.

Results: Our data shows that hepatic necroinflammation post CCL4 injection is markedly less in CLEC2 deficient mice (mean serum ALT 105+/- 14 IU/l vs. wild type mice 230 ± 10 IU/l). We next demonstrate that in the inflamed liver, resident and non-resident macrophages (F480+CD11b+) up-regulate the only known naturally occurring CLEC-2 ligand-podoplanin and that by interacting with podoplanin expressing macrophages in the inflamed liver, platelet activation drives liver damage. We then demonstrate that abrogating any part of the CLEC2-podoplanin axis i.e.

1) The platelet based CLEC-2 signal (in CLEC1bfl/fl PF4cre mice) or
2) Removing podoplanin (in Vav1 icre mice) or
3) Blocking podoplanin using a selective function blocking antibody, blocks platelets activation resulting thus reducing liver injury.

We subsequently demonstrate that mechanism that underlies the observed hepatoprotective effect in CLEC2 deficient mice is TNF-alpha dependent enhanced hepatic accumulation of the liver healing myeloid derived suppressor cells (WT 40677 cells/gm ± 14593, n = 8 vs. CLEC2 KO 137211 cells/gm ± 30039, n = 6, p < 0.01).

Discussion/Conclusion: These findings together indicate that platelets and specifically the CLEC-2 podoplanin axis plays an important role in acute inflammatory liver disease and thus presents an exciting avenue for further research into potential therapies for acute hepatitis.
Entecavir in the treatment of fulminant hepatic failure patients who did not receive a liver transplant

Konstantinos J. Dabos*, Georgios Dimos, Eleni Arvaniti, Gerasimos Aravanis, Nikolaos Akritidis
*Royal Infirmary of Edinburgh, Edinburgh, United Kingdom
Department of Medicine and Hepatology, Ioannina General Hospital Hatzikosta, 45001 Ioannina, Greece

Introduction: The definite treatment for fulminant hepatic failure (FHF) is Liver Transplantation. However the availability of cadaveric donors is limited and in Greece in particular very few FHF patients receive a liver transplant. Alternative therapies need to be sought therefore.

Methods: A series of case reports have shown that entecavir might be beneficial in patients with FHF due to Hepatitis B (Hep B). We present 3 cases of FHF due to Hep B who were not transplanted, were treated with entecavir and recovered.

Results: Case 1. A 33 year old male an inmate at the local prison presented with jaundice at A&E. His bilirubin rose to 20.6, his INR to 8.21 and he became encephalopathic. He had a History of drug abuse and he was not listed for psychiatric reasons. HBV DNA was present at 175,000 copies/ml. He was started on entecavir 1 mg daily along with best supportive care (BSC). From day 7 of the treatment his mental state improved and LFT’s started to recover. He made a full recovery and remains well 18 months post FHF.

Case 2. A 23 year old male presented with jaundice at A&E. His bilirubin rose to 42.1 and his INR was raised at 14.9. He became encephalopathic was listed but never received a liver transplant because he had no insurance in Greece. HBV DNA was present at 226,000 copies/ml. He was started on entecavir 1 mg daily along with BSC from day 3 of the treatment his mental state improved and LFT’s started to recover. He was able to leave hospital on day 46 but his liver still suffers from chronic liver failure 11 months after the FHF incident.

Case 3. A 39 year old male presented with jaundice at A&E. He became encephalopathic 12 days after the onset of jaundice and his bilirubin rose to 25.8. HBV DNA was present at 44,500 copies/ml. He was listed for transplantation but was never transplanted. He was started on entecavir 1 mg daily along with BSC. From day 3 of the treatment his mental state improved and LFT’s started to recover. He has made a full recovery and remains well, 10 months post FHF.

Conclusion: This small case series suggest that the use of entecavir early in FHF from HepB might be beneficial. A multicenter trial although difficult will be very beneficial.
Cell-in-cell structures in the liver: Implications for hepatocellular carcinoma

S. Davies¹, G. Reynolds¹, S. Nakum, N. Weight, B. Wiggins¹, Y. Liu¹, R. Bhogal¹, C. Weston¹, R.C. May¹, S. Hubscher², D. Adams¹, Z. Stamataki¹
¹University of Birmingham, Birmingham, UK
²QE Hospital NHS Trust, Birmingham, UK

Introduction: Hepatocytes are competent phagocytes, clearing away dead cells during parenchymal liver damage in a process called efferocytosis, which is critical for liver homeostasis. We discovered that hepatocytes and hepatoma cell lines also capture live CD4+ T cells in a process similar to entosis, described by Overholtzer et al, Cell 2007. We hypothesised that capture of live and dead cells by hepatocytes were distinct processes and investigated their implications for inflammation and hepatocellular carcinoma (HCC).

Methods: CD4+ T cells were isolated from healthy blood or liver, and cultured with primary hepatocytes or hepatoma cell lines (HepG2, Hep3B, Huh-7). Co-cultures using fluorescently-labelled live, apoptotic or necrotic T cells were imaged using time-lapse confocal and scanning electron microscopy in the presence of antibodies or chemical inhibitors, to determine the mechanism/kinetic of cell capture.

Formalin-fixed, paraffin embedded tissue sections from patients with interface hepatitis and HCC were stained for Hepatocyte Nuclear Factor 4-alpha by immunohistochemistry, and we measured nuclear surface areas and multinucleation using ImageJ.

Results: Internalisation of live CD4+ T cells by hepatocytes was distinct to phagocytosis of dead cells with regards to capture mechanism, efficiency, kinetic and the outcome of cell internalisation.

Cell-in-cell structures induced multinucleation in hepatoma cell lines in vitro. Multinucleation is a feature of more aggressive tumours.

Neoplastic tissue was more multinucleate than adjoining healthy hepatocytes. Hepatocyte multinucleation correlated with their proximity to the inflammatory infiltrate in interface hepatitis.

Discussion/Conclusion: Cell-in-cell structures in the liver alter hepatocyte ploidy and may contribute to the pathogenesis of hepatocellular carcinoma.
The Scottish look back on acute liver failure: Are we as good as the best?

Mhairi C. Donnelly¹, Janice Davidson¹, Kirsty Martin¹, Andrea Baird¹, Peter C. Hayes¹, Kenneth J. Simpson¹
1. Department of Hepatology and Scottish Liver Transplant Unit, Royal Infirmary of Edinburgh, Edinburgh, UK

Introduction: Acute liver failure (ALF) results from sudden loss of hepatic parenchyma and metabolic function. The Scottish Liver Transplant Unit (SLTU) is the single national referral centre for a population of 5 million, and offers specialist management to appropriate patients with acute liver injury (ALI) and ALF. We aim to describe our patient cohort, focusing on evolution of disease over time.

Methods: Retrospective analysis of SLTU database of patients admitted with ALI/ALF from 1992–2014. To assess trends, data were analysed in 5 year subgroups. Primary outcome measure was spontaneous (transplant free) survival.

Results: 1164 patients were admitted (56% [n = 652] female, median age 37 years [IQR 27–48]). The number of admissions peaked in 1997–2001 and subsequently declined. Paracetamol overdose (POD) was the commonest aetiology (73.7%, n = 858). There was no significant change in the number of POD admissions over time (1992–1996 n = 188; 2007–2011 n = 173; p > 0.05). Significantly less patients with POD developed HE compared with other aetiologies (POD 54.6%, non-POD 65.7%; p < 0.001).

Spontaneous survival rates did not significantly increase over time (1992–1006 61.7%, 2012–2014 65.3%; p 0.25). Those with POD were significantly more likely to spontaneously survive than those with a non-POD aetiology (POD 61.7%, non-POD 50.3%; p < 0.0001). Overall survival rates significantly increased from 71% to 84% (p 0.024), predominantly due to an increase in percentage of patients surviving with transplantation.

In patients meeting the Kings College Hospital Poor Prognostic Criteria, spontaneous survival increased from 0% in 1992–1996 to 31.2% in 2007–2011 (p < 0.001). Overall survival also increased in this group of patients, from 28.6% to 66.6%.

Discussion/Conclusion: There has been a decrease in total number of admissions over the past 20 years, with no change in the number of admissions with POD. Spontaneous survival rates improved significantly in the group meeting KCC. Overall survival rates increased due to more patients surviving with transplantation.
The role of the inflammatory response in non-POD ALF: Are serial measurements of SIRS, SOFA scores and neutrophil-lymphocyte ratio clinically relevant markers of prognosis?

Mhairi C. Donnelly¹, Kenneth J. Simpson¹

¹. Department of Hepatology and Scottish Liver Transplant Unit, Royal Infirmary of Edinburgh, Edinburgh, UK

Introduction: The systemic inflammatory response syndrome (SIRS), sequential organ failure assessment (SOFA) score and neutrophil-lymphocyte ratio (NLR) have been studied as prognostic markers in paracetamol (POD) induced acute liver failure (ALF). We aimed to determine the utility of these scores as prognostic markers in non-POD ALF.

Methods: Retrospective analysis of 50 cases of non-POD ALF admitted to the Scottish Liver Transplant Unit. SIRS, SOFA and NLR were obtained for all patients during the first 7 days of admission, and for the 24 (24FO) and 48 (48FO) hours prior to the patient reaching their final outcome (spontaneous survival [SS] or death/transplantation [DT]).

Results: 46% (n = 23) met the combined outcome of death/transplantation, at a median of 6.5 days. 84% (n = 42) of patients developed a SIRS response by day 7; 60% (n = 30) within 24 hours. There was no significant difference in mortality rate depending upon the presence or absence of SIRS at 24 hours (43% vs. 50%; p 0.77), 48 hours (47% vs. 42%; p 1.0), 72 hours (48.6% vs. 38%; p 0.75) or 96 (51% vs. 27%; p 0.19). The number of SIRS components present was a predictor of outcome at 48FO (AUC 0.827) and 24FO (AUC 0.925) time points.

Mean SOFA score was significantly different between the 2 groups on day 1 (DT 7.5 vs. SS 5.17; p 0.041), 48FO (CT 7 vs. SS 4; p 0.037) and 24FO (DT 7.6 vs. SS 3.7; p 0.002). SOFA scores at 48FO and 24FO were predictors of outcome (AUC 0.827 and 0.925 respectively).

There was no significant difference in mean NLR between the 2 groups from day 0 to day 7. There was a significant difference in mean NLR at 48FO (DT 26.1 vs. SS 3.7; p 0.043) and 24FO (DT 16.5 vs. SS 5.2; p 0.047). Mean NLR in the 48FO and 24FO were predictors of outcomes (AUC 0.864 and 0.833 respectively).

Discussion/Conclusion: SIRS, SOFA and NLR are useful in predicting prognosis in non-POD ALF at 48 hours and 24 hours prior to the patient reaching their final outcome. These scores require further validation to confirm their utility.
The results of treatment of gastric cancer with liver metastases

D. Egamberdiev, A. Yusupbekov, M. Djuraev, S. Khudoyarov
National Cancer Center, Tashkent, Uzbekistan

The aim: To improve long-term results of surgical treatment of the stomach cancer with metastases in liver.

In 48 patients the stomach cancer with metastasizes in liver was performed palliative gastrectomy (GE) and distal subtotal resection (DSR). The patients were in the age of from 32 till 74 years. The diagnosis was established by conventional testing methods with usage of computed tomography and laparoscopy. Histological in 34 patients was established intestinal form and in 34 – diffuse form of cancer. In 32 (66.6%) patients tumor located in the antrum 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. The tags of a tumoral bleeding were marked at 26 (54.1%) patients, the metastases in both hepatic lobes are established in 29 (60.4%) patients, in the dextral lobe in 12 (25%) and in the left lobe in 10 (20.8%) patients. Quantity of metastasizes 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 metastasizes 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 lethality has compounded – 8.3%. The cause of lethality - inconsistency of esophago-intestinal anastomosis – 1, thromboembolism of pulmonary artery – 1, renal-hepatic failure – 1, and eventeration with high intestinal fistula – 1.

The full regressions of metastatic locuses, 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).
Endothelial injury and oxidative stress in patients with hepatitis C virus-related cirrhosis: Relation to renal function and hemodynamics

H. El Aggan¹, S. Abodeya², S. Mahmoud²
¹Department of Medicine and ²Department of Medical Biochemistry, Faculty of Medicine, University of Alexandria, Alexandria, Egypt

Introduction: Endothelial injury plays an important role in the pathogenesis of chronic renal diseases and may be related to oxidative stress. The present work was designed to study markers of endothelial injury and oxidative stress in patients with hepatitis C virus (HCV)-related cirrhosis in relation to renal function and hemodynamics.

Methods: Thirty patients with HCV-related cirrhosis and 15 healthy subjects were included in the study. The severity of liver disease was assessed using Child-Pugh classification and the Model for End Stage Liver Disease (MELD) score. Glomerular injury was assessed by urinary albumin excretion rate and tubular damage was determined by 24-hour urinary leucine aminopeptidase (LAP) levels. Endothelial injury was evaluated by plasma von Willibrand factor (vWF) activity and serum angiotensin converting enzyme (ACE) levels. Serum malondialdehyde (MDA) levels were measured as a marker for oxidative stress. Renal hemodynamics were assessed using Duplex-Doppler ultrasonography by calculating the diastolic/systolic renal flow velocity ratio (d/s), intrarenal resistive index (RI) and hilar renal blood flow (RBF).

Results: Patients with HCV-related cirrhosis showed significant increases in plasma vWF activity, serum levels of ACE and MDA, urinary LAP levels and RI and significant decreases in d/s ratio and RBF compared with healthy subjects (p < 0.05). Plasma vWF activity and serum ACE levels were positively correlated with serum MDA levels, urinary albumin excretion rate, urinary LAP levels and RI and were inversely correlated with RBF (p < 0.05). No statistically significant correlations were found between severity of liver disease and plasma vWF activity, serum ACE levels and urinary LAP levels in patients with cirrhosis (p > 0.05).

Discussion/Conclusion: Endothelial injury, possibly due to oxidative stress, plays an important role in the pathogenesis of renal dysfunction and increased renovascular impedance in patients with HCV-related cirrhosis. Pharmacological approaches to enhance endothelial function could improve renal function in these patients.
Toll-like receptor 7 and interferon-lambda 1 in chronic hepatitis C: Relation to virus replication and liver injury

Hoda El Aggan¹, Nahla Farahat², Nevine El-Deeb³, Ahmed Zeid¹, Assem El-Shandidi¹
¹Department of Internal Medicine (Hepatobiliary Unit), ²Clinical Pathology and ³Pathology, Faculty of Medicine, University of Alexandria, Alexandria, Egypt

Introduction: Toll-like receptor 7 (TLR7) recognizes single-stranded RNA viruses like hepatitis C virus (HCV) with subsequent induction of interferons (IFN) including IFN-lambdas (IFN-λs), which play an important role in antiviral innate immunity. The present work was designed to study the role of TLR7 and IFN-λ1 in chronic hepatitis C (CHC) in relation to virus replication and liver injury.

Methods: Forty-two treatment-naïve patients with CHC and 20 healthy subjects were included in the study. Liver biopsies were examined to assess METAVIR histological activity grade and fibrosis stage and steatosis grade. Immunohistochemical staining was performed using antibodies against TLR7 and IFN-λ1 and was scored semi-quantitatively (0–3). Hepatic expression of TLR7 and IFN-λ1 was further classified into: low expression (score 0 or 1) and high expression (score 2 or 3). The TLR7-expressing peripheral blood mononuclear cells (PBMCs) were identified by color flow cytometry. Quantification of IFN-λ1 levels in serum was performed using enzyme-linked immunosorbant assay.

Results: In patients with CHC, the expression of TLR7 and IFN-λ1 in the liver was low in 35.7% and 23.8% of patients respectively and high in 64.3% and 76.2% of patients respectively. Patients with low hepatic TLR7 and IFN-λ1 expression showed significant decreases in the percentages of TLR7-expressing PBMCs and serum IFN-λ1 levels and significant increases in serum levels of aminotransferases, viral load and METAVIR histologic activity grade and fibrosis stage compared with patients with high expression ($p < 0.01$). TLR7 expression in the liver showed positive correlations with the percentages of TLR7-expressing PBMCs and serum levels and hepatic expression of IFN-λ1 ($p < 0.001$). The percentages of TLR7-expressing PBMCs and serum IFN-λ1 levels were positively correlated ($p < 0.001$).

Discussion/Conclusion: Dysregulation of TLR7/IFN-λ1 pathway in CHC seems to play an important role in viral replication and HCV-related liver injury and could be a potential therapeutic target in chronic HCV infection.
Chronic hepatitis C – Predictive factors in the response to antiviral treatment

A. Gaman¹,², A. Ungureanu¹, A. Drocaș¹, M. Dobritoiu³, A. Turculeanu¹
¹University of Medicine and Pharmacy, Craiova, Romania
²Filantropia University Hospital, Craiova, Romania
³Emergency County Hospital, Craiova, Romania

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

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

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

Discussion/Conclusion: Our research demonstrates that all the parameters defining insulin resistance are negative predictors for achieving both EVR and SVR. The high viral load is a strongly negative predictor for therapeutic success. The low pre-treatment level of HCV RNA was statistically significantly correlated with virologic response in patients with chronic hepatitis C treated with α-IFN and ribavirin.
New arising of focal fatty sparing in non-alcoholic fatty liver disease

Iva Hoffmanová, MD, PhD; Michal Anděl, MD, PhD, Prof.
2nd Department of Internal Medicine, Third Faculty of Medicine, Charles University, Prague, Czech Republic

Introduction: Focal fatty sparing (FFS) - areas with decreased fat content in otherwise steatotic liver – could mimic hepatocellular carcinoma or other malignant lesion in ultrasonography evaluation. The mechanisms of development of FFS is related to aberrant venous liver supply (especially an aberrant course non-portal veins e.g., left gastric vein, gallbladder veins), which leads to localized dilution of insulin and nutrient levels in the blood flowing into the FFS; therefore hepatocytes are spared from steatosis.

Case report: A 49-year-old woman with obesity, non-alcoholic fatty liver disease (NAFLD), and a two years history of metformin-treated type 2 diabetes mellitus. Her regular annual abdominal ultrasonographic examination revealed diffuse homogeneous fatty liver. In May 2012, 100 mg daily of sitagliptin was added to her medications. 11 months after initiation of sitagliptin, an ultrasonographic examination showed 7.55 cm large FFS in liver segment 2 (Fig. 1). The FFS was confirmed using MRI, as patient refused a liver biopsy. Four months later, the patient refused treatment with sitagliptin. Moreover, 16 months after the discontinuation of sitagliptin, a reduction in the maximum diameter of FFS (from 7.55 to 3.51 cm) in liver segment 2 was detected (Fig. 2).

Discussion: Treatment with sitagliptin is known to decrease fat content in hepatocytes. We hypothesize, that this effect could occur rapidly in areas with aberrant venous liver circulation than in the rest of liver parenchyma. Other influences than sitagliptin treatment (such as weight reduction, better diabetes compensation or improvement in insulin resistance) is excluded in our patient.

Conclusion: To our knowledge, this is the first report that a benign complication, such as FFS, may appear in the clinical course of NAFLD due to sitagliptin medication. Diagnosis of FFS could be challenging in differential diagnosis to HCC; however, with widespread use of sitagliptin and other DPP-4 inhibitors, this challenge will need to be met.
MR1-dependent activation of intrahepatic MAIT cells by bacterially exposed biliary epithelial cells and liver B cells

1Centre for Liver Research and NIHR BRU in Liver Disease, Institute of Immunology and Immunotherapy, University of Birmingham, UK
2Peter Medawar Building of Pathogen Research, Oxford
3Institute of Immunology and Immunotherapy, University of Birmingham
4University Hospital of Birmingham NHS Foundation Trust, Birmingham

Introduction: Mucosal-associated Invariant T-cells (MAITs) are innate-like T-cells characterised by the invariant TCR-chain, Va7.2-Ja33 and high CD161 expression. They are activated by bacterial vitamin B metabolites presented on MR1, and function in antibacterial immunity at mucosal sites. MAITs are found in the human liver but the detailed characteristics of liver-infiltrating MAITs (LI-MAITs) in health and chronic liver diseases and their role in liver immune surveillance remained unexplored and were investigated in this study.

Methods: Flow cytometry, ELISA, cell co-culture, immunohistochemistry, immunofluorescence

Results: LI-MAITs were present at reduced frequency (% total CD3+ T-cells) in diseased compared to normal livers and ratios of CD4+/CD8+CD4-CD8- subsets altered. In normal and diseased livers LI-MAITs localized predominantly around bile ducts in portal tracts as shown by immunohistochemistry for TCR-Vα7.2 and 4-colour confocal microscopy for CD3/CD161/TCR-Vα7.2/nucleus. Consistent with this distribution, in flow cytometry analysis they expressed biliary-tropic chemokine receptors CCR6, CXCR6, and integrin αEβ7. LI-MAITs were also present at lower density in the hepatic sinusoids and possessed tissue-homing chemokine receptor CXCR3 and integrins LFA-1 and VLA-4, suggesting their recruitment via hepatic sinusoids. LI-MAITs had an activated, effector memory phenotype, expressed integrin α4β7 and receptors for IL-12, IL-18 and IL-23 and produced cytokines IFN-γ, TNF-α, and IL-17. Importantly, in co-culture assays, in response to E. coli-exposed macrophages, liver B-cells and biliary epithelial cells, MAITs up-regulated IFN-γ, TNF-α and CD40-Ligand and degranulated in an MR1-dependent, cytokine-independent manner.

Discussion/Conclusion: Our findings provide the first evidence of an immune surveillance effector response for MAITs towards biliary epithelial cells and B-cells in human liver that might be targeted therapeutically in the future.
Potential benefits of low-dose proleukin in autoimmune liver diseases via selective induction of phospho-STAT5, regulatory phenotype and Bcl-2 in Treg

Hannah C. Jeffery1, Louisa E. Jeffery2, David H. Adams1,3, Ye Htun Oo1,3
1Centre for Liver Research and NIHR BRU in Liver Disease, Institute of Immunology and Immunotherapy, University of Birmingham
2Institute of Metabolism and Systems Research, University of Birmingham
3University Hospital of Birmingham NHS Foundation Trust, Birmingham, UK

Introduction: CD4+CD25highCD127lowFOXP3+ regulatory T cells (Treg) are essential for the maintenance of peripheral tolerance. Impaired Treg function and an imbalance of effector and Treg cells contribute to the pathogenesis of autoimmune diseases. We recently reported that the hepatic microenvironment is deficient in IL-2, a cytokine essential for Treg survival and function. Consequently, few liver-infiltrating Treg demonstrate STAT5 phosphorylation.

Methods: Flow cytometry, ELISA, cell co-culture.

Results: To establish the potential of IL-2 to enhance Treg therapy, we investigated the effects of very low dose proleukin (VLDP) on phosphorylation of STAT5 in Treg and T effector cells from the peripheral blood and liver of patients with autoimmune hepatitis and on the survival and function of these T cells. VLDP, less than 5 IU/ml, induced selective phosphorylation of STAT5 in Treg but not effector T cells or natural killer cells and promoted expression of regulatory CTLA-4, FOXP3 and CD25 as well as the anti-apoptotic protein Bcl-2 in blood and liver Treg. VLDP also maintained liver-homing CXCR3 expression on Treg. These findings suggest that VLDP therapy could restore the functional properties and enhance the survival of Treg to re-establish a state of self-tolerance within the liver and may prove beneficial as an adjuvant in GMP Treg therapy in autoimmune liver diseases.

Discussion/Conclusion: These findings suggest that VLDP therapy could restore the functional properties and enhance the survival of Treg to re-establish a state of self-tolerance within the liver and may prove beneficial as an adjuvant in GMP Treg therapy in autoimmune liver diseases.
Human intrahepatic Tregs are functional, require IL-2 from effector cells for survival and are susceptible to FAS ligand mediated apoptosis

Yung-Yi Chen1&3*, Hannah C. Jeffery1&3*, Stuart Hunter1, Ricky Bhogal1, Jane Birtwistle2, Manjit Kaur Braitch1, Sheree Roberts1, Mikaela Ming1, Jack Hannah1, Clare Thomas1, Gupse Adali1, Stefan Hubscher5, Wing-Kin Syn4, Simon Afford1&3, Patricia F. Lalor1&3, David H. Adams1&3, Ye Htun Oo1&3

1Centre for Liver Research & NIHR Birmingham Liver Biomedical Research Unit, University of Birmingham, 2Clinical Immunology Department, UHB NHS Foundation Trust, Birmingham, United Kingdom, 3Institute of Immunology and Immunotherapy, 4The Institute of Hepatology, London, United Kingdom, Department of Cellular Pathology, Queen Elizabeth Hospital Birmingham5, United Kingdom

Introduction: Regulatory T cells (Treg) suppress T effector cell proliferation and maintain immune homeostasis. Autoimmune liver diseases (AILD) persist despite high frequencies of Treg in the liver suggesting that the local hepatic-microenvironment might affect Treg stability, survival or function. We hypothesized that interactions between Treg and endothelial cells during recruitment and then with epithelial cells within the liver affect Treg stability, survival and function.

Methods: Flow cytometry, ELISA, cell co-culture, immunohistochemistry, immunofluorescence.

Results: We explored the function of Treg after migration through human hepatic sinusoidal-endothelium (post-endothelial migrated or PEMTreg) and the effect of subsequent interactions with cholangiocytes and local proinflammatory cytokines on survival and stability of Treg. Our report that, intrahepatic-microenvironment is enriched with proinflammatory cytokines but deficient in IL-2. Migration through endothelium into the inflamed liver microenvironment did not affect Treg stability, however functional capacity was reduced. Furthermore, the addition of exogenous IL-2 enhanced PEMTreg phosphoSTAT5 signaling compared with PEMCD8. CD4 and CD8 T cells are the main source of IL-2 in the inflamed liver. Liver infiltrating Tregs reside close to FASL expressing bile ducts. Treg from diseased livers expressed high levels of CD95 and co-culture with cholangiocytes or their supernatants induced preferential apoptosis of Treg compared to CD8 effector cells and this apoptosis was inhibited by IL-2 or blockade of CD95.

Discussion/Conclusion: These results provide a mechanism to explain Treg dysfunction in inflamed tissues and suggest that IL-2 supplementation, particularly if used in conjunction with Treg therapy, could restore immune homeostasis in inflammatory and AILD.
The effect of B-cell depletion on B-cell activating factor (BAFF) in primary biliary cholangitis/cirrhosis (PBC)

Laura Jopson, Jeremy Palmer, Achilleas Floudas, David Jones
Institute of Cellular Medicine, Newcastle University, Newcastle, UK

Introduction: B-cells are implicated in the pathogenesis of PBC and targeting B-cells with Rituximab has been trialled although efficacy has been limited to date. B-cell activating factor (BAFF), a cytokine and member of the TNF superfamily is involved in B-cell maturation and survival and levels are raised in many autoimmune diseases including PBC. The natural history of BAFF following B-cell depletion has not been explored in PBC.

Methods: PBC patients enrolled in a placebo controlled trial of rituximab (RitPBC) had BAFF measured by ELISA and 10 pro-inflammatory cytokines assessed using the MSD V-PLEX panel at baseline and 3, 6, 9 and 12 months post treatment. B-cell depletion was quantified using flow cytometry (CD19) whilst patients remained blinded to the therapeutic arm.

Results: Serum BAFF was significantly higher in PBC than controls (1392 ± 393.2 vs. 722.5 ± 152.9 pg/ml p < 0.0001). There was a significant increase in serum BAFF in the B-cell depleted group between baseline and 3 months (1381 ± 368.4 vs. 3186 ± 1172 pg/ml, p < 0.0001). There was no significant change in pro-inflammatory cytokines in either cohort. There was a significant correlation between BAFF and IL-6 at 3 months in the B-cell depleted group ($r^2 = 0.48$ p = 0.019). BAFF elevation following B-cell depletion was sustained, levels at 12 months remained significantly elevated.

Discussion/Conclusion: The BAFF elevation in PBC further supports the concept that B-cells play a key role in disease pathogenesis. Following B-cell depletion there was significant further elevation in BAFF, with a strong correlation between BAFF and IL-6. Significant and sustained elevation of BAFF following B-cell depleting therapy may limit the efficacy of this treatment because of effects on immuno-regulation; indirectly on B-cell reconstitution and, potentially, directly on Treg function. This may explain the limited therapeutic response to rituximab seen in PBC to date. The combination of B-cell depletion and BAFF-targeting therapy holds promise in PBC.
High blood concentrations of IL-6 and alterations in systemic inflammatory and immune balance are poor prognostic indicators in alcoholic liver disease

Beata Kasztelan-Szczerbinska1, Agata Surdacka2, Halina Cichoz-Lach1, Jacek Rolinski2, Katarzyna Adamczyk1, Jakub Onikijuk1, Agata Michalak1, Mariusz Szczerbinski1

1Department of Gastroenterology with Endoscopy Unit, Medical University of Lublin, 8 Jaczewski Street, 20-954 Lublin, Poland
2Department of Clinical Immunology, Medical University of Lublin, 4A Chodzki Street, 20-093 Lublin, Poland

Introduction: Mechanisms of inflammation in alcoholic liver disease (ALD) are still unclear. Th17 and regulatory T (Treg) cells are critically linked to immune response. While Th17 lymphocytes exert pro-inflammatory effects, Treg cells are potent immune suppressors. Human Th17 differentiation is IL-1beta, IL-6, IL-23 and IL-17A-dependent and suppressed by TGF-beta1. TGF-beta1 enhances the differentiation of human Treg cells. Women develop more severe alcohol-associated liver injury at lesser ethanol intake and fewer years of exposure. The aim of our study was to determine an impact of the Th17/Treg cell balance and its corresponding cytokine profile on the ALD outcome. Possible gender-related differences in the alcohol-induced inflammatory response were also assessed.

Methods: 147 patients with ALD were prospectively recruited, assigned to subgroups based on their gender, severity of liver dysfunction and presence of ALD complications, and followed for 90 days. Peripheral blood frequencies of Th17 and Treg cells, and IL-1beta, IL-6, IL-17A, IL-23, and TGF-beta1 levels were investigated. Flow cytometry was used to identify T cell phenotype and immunoenzymatic ELISAs for the corresponding cytokine concentration assessment. Multivariable logistic regression was applied in order to select independent predictors of advanced liver dysfunction and the disease complications.

Results: IL-17A, IL-1beta, IL-6 levels were significantly increased, while TGF-beta1 decreased in ALD patients. The imbalance with significantly higher Th17 and lower Treg frequencies was observed in non-survivors. IL-6 and TGF-beta1 levels differed in relation to the patient gender in ALD group. Concentrations of IL-6 were associated with the severity of liver dysfunction, development of ALD complications, and turned out to be the only independent immune predictor of 90-day survival in the study cohort.

Discussion/Conclusion: IL-6 revealed the highest diagnostic and prognostic potential and was related to the fatal ALD course. Gender-related differences in immune regulation might influence the susceptibility to ethanol-associated liver injury.
Reduced expression of TJP-2 is associated with chronic liver disease and hepatic malignancy

Sivesh K. Kamarajah1, Daniel A. Patten1, James C.R. Wadkin1, Chris Coldham1, Ricky H. Bhogal1, Shishir Shetty1, Chris J. Weston1
1NIHR Centre for Liver Research, Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, UK

Introduction: Gene alterations in Tight Junction Protein-2 (TJP-2) have been discovered in patients with progressive familial intrahepatic cholestasis leading us to hypothesise that TJP-2 is differentially regulated in chronic liver disease and liver cancer. Our aim was to characterise the expression of TJP-2 in liver disease with a particular focus on malignancy.

Methods: The cellular localization and expression of TJP-2 in human liver tissue sections was studied using immunohistochemistry, multi-colour confocal immunofluorescence and immunocytochemistry. TJP-2 mRNA expression was quantified by qRT-PCR and protein expression was determined by western blotting of tissue lysates from pathological control and diseased liver samples.

Results: TJP-2 expression in human tissue sections was significantly downregulated in chronic liver disease and primary liver cancer when compared to pathological control tissue. The majority of TJP-2 protein was restricted to cellular junctions of biliary epithelial cells and hepatocytes in both pathological control and chronically diseased livers. In primary liver cancer tissue sections, the protein was found to adopt a characteristic perinuclear distribution, consistent with previous studies on pancreatic cancer cell lines. Western blotting and qRT-PCR studies revealed a significant decrease in TJP-2 expression in both chronic liver disease and primary liver cancer when compared to pathological control livers. Preliminary analysis of tissue samples taken from patients who had undergone hemihepatectomy for intrahepatic cholangiocarcinoma, and who were matched for anatomical location and tumour histology, suggested a correlation between TJP-2 expression and overall survival.

Conclusion: This is the first report on TJP-2 expression in liver disease. TJP-2 was expressed on epithelial cells, with reduced mRNA and protein levels detected in chronic liver disease and liver cancer, suggesting that progressive loss of this molecule may contribute to altered epithelial function in hepatic injury and malignancy. Our data suggest that measurement of TJP-2 expression might have utility in the staging of liver cancer.
CTLA-4 is a negative regulator of T-cell activation in acute liver failure


1Division of Digestive Diseases, Imperial College London, UK; 2Institute of Liver Studies, King’s College London, UK; 3Institute of Immunity and Transplantation, University College London, UK; 4Institute of Cellular Medicine, Newcastle University, Newcastle, UK; 5Institute of Reproductive and Developmental Biology, Imperial College London, UK; 6Centre for Liver Research and National Institute for Health Research, Biomedical Research Unit, University of Birmingham, UK; 7MRC Centre for Transplantation, King’s College London, UK

Background and aims: Central to the pathogenesis and outcome of acute liver failure (ALF) is activation of systemic inflammatory responses, immune paresis and susceptibility to recurrent infections. Pilot data showed dysfunction in adaptive immune responses in liver failure. This study aims to evaluate whether negative regulation of CD4+ T-cell responses could account for immune paresis in ALF.

Methods: Circulating CD4+ T-cell subsets from 35 ALF patients, 22 pathological and 20 healthy controls (HC) were determined using flow cytometry. Naïve, memory and regulatory subsets were identified. Expression of CTLA-4, a negative regulator of T-cell activation was analysed. The effect of ALF sera on CTLA-4 expression was assessed. CD4+ T-cell antigen-recall responses were examined in the presence of anti-CTLA-4 blocking antibody. Furthermore, the efficacy of suppression by CTLA-4 was tested in the presence of CTLA-4 Ig and anti-CTLA-4 antibody. CD4+ T-cell proliferation was assessed by flow cytometry and IL-2 levels were measured using ELISA. Soluble costimulatory molecules were measured in ALF sera samples and in Pre and Post plasma exchanged samples using ELISA.

Results: Percentages of circulating CD4+ T-cells were higher in ALF (p = 0.0028) with a significant increase in CTLA-4 expression in the memory subset (p = 0.01). Compared to control groups, % CTLA-4+CD4+ T-cells were significantly higher in ALF patients on day 1 of admission (p < 0.0001) and remained elevated until day 14. CTLA-4 expression was significantly higher in patients who developed culture-positive infections compared to those who did not (p = 0.04). CTLA-4 expression correlated positively with disease severity MELD score (r = 0.792, p = 0.001). In comparison to HC, antigen-recall proliferative responses are impaired in ALFs (p = 0.008). We show that blocking CTLA-4 restores the antigen-recall responses particularly in the memory CD45RO+RA- subset (p = 0.01) as defined by T cell proliferation (p = 0.01) and IL-2 secretion (p = 0.01). Levels of sCD80/sCD86 in ALF sera were significantly elevated (p < 0.0007). Purified HC CD4+ T-cells cultured in the presence of ALF sera significantly increase CTLA-4 expression (p = 0.01). Neutralisation of sCD80/sCD86 diminished the levels of CTLA-4 (p = 0.007). A significant reduction in the levels of circulating sCD80/sCD86 (p = 0.03) was detected in post-plasma exchange samples.
Conclusions: Our novel data identify defects in function of circulating CD4$^+$ T-cell subsets in ALF with significant increase in CTLA-4 positive population, a CD4$^+$ T-cell phenotype with an inhibitory functional aspect. We suggest a potential role for sCD80/86 in the induction of CTLA-4 expression in ALF. Plasma exchange represents a potential immunomodulatory therapy to promote adaptive immune responses in ALF.
Long-term therapy with fenofibrate and ursodeoxycholic acid does not improve projected survival in primary biliary cholangitis

Amardeep Khanna¹, Vinod S. Hegade¹, Lin-Lee Wong¹, Jessica K. Dyson¹, David E.J. Jones¹
¹Institute of Cellular Medicine, Newcastle University, United Kingdom

Introduction: Fenofibrate (FF) has been suggested as an adjuvant treatment for PBC patients with incomplete response to ursodeoxycholic acid (UDCA). We aimed to evaluate the safety and efficacy of long-term treatment with FF+UDCA in PBC patients in relation to the UK-PBC Risk Score (a novel validated scoring system for long-term prediction of end-stage liver disease in PBC), liver biochemistry and renal function.

Methods: We performed a retrospective study of all PBC patients treated with FF+UDCA between 2007 and 2015 in Newcastle. We recorded the 5-year, 10-year, and 15-year UK-PBC Risk Score, serum ALP, creatinine and eGFR at baseline and at 12, 24, 36, 48 and 60 months after FF+UDCA therapy. Non-parametric tests were applied for statistical analysis.

Results: 24 patients (all females, median age 56 years) formed the study cohort. Median follow-up period was 21 months (range 1–123) and median dose of FF was 200 mg/day (range 110–200). There was no significant improvement in the UK-PBC risk scores at any time during the 12–60 months of FF+UDCA treatment (p = n.s for all comparisons). Significant decrease in serum ALP levels were seen at 12 (p = 0.07), 24 (p = 0.0029) and 36 months (p = 0.03). No significant changes in renal function were seen with FF. Two (8%) patients experienced nausea and dizziness and two (8%) developed transient transaminitis.

Conclusion: Long-term treatment with fenofibrate did not improve predicted prognosis using the UK-PBC risk scores possibly suggesting no overall survival benefit with fibrate therapy. The focus on ALP alone may have led in the past to overestimate the true value of fibrates in PBC.
Bile salt signaling and the FGF19 response in early liver regeneration in humans

Kiran V.K. Koelfat1, Kim C. van Mierlo, Johanne G. Bloemen1, Albert K. Groen3, Peter L.M. Jansen1, Cornelis H.C. Dejong1,13, Frank G. Schaap1, Steven W.M. Olde Damink1,4

1Department of Surgery, Maastricht University Medical Center and NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, The Netherlands;
2Department of Pediatrics, Laboratory of Medicine, University of Groningen, University Medical Center Groningen, The Netherlands;
3GROW School for oncology and developmental biology, Maastricht University, Maastricht, The Netherlands;
4Institute for Liver and Digestive Health, University College London, London, United Kingdom

Introduction: Bile salts (BS) and the bile salt-induced enterokine FGF19 play an important role in liver regeneration following surgical resection. Little is known about involvement of these signaling molecules in liver regeneration in humans. The aim of this study was to i) assess short-term changes in flux of BS and FGF19 across the remnant liver, portal drained viscera (PDV) and splanchnic area and ii) assess the impact of liver resection on genes engaged in bile salt homeostasis.

Methods: Arterial, portal and hepatic venous blood were sampled in patients undergoing liver resection for colorectal liver metastasis (n = 29) shortly before (within 1 hours) and directly after (within 2 hours) liver resection. BS and FGF19 fluxes were calculated and hepatic BS measurements and quantitative real-time qPCR have been performed on liver specimens obtained at both time-points. To assess the post-operative course, BS and FGF19 levels in plasma were assessed from the same cohort of patients at postoperative day (POD) 1, 2 and 3.

Results: Partial hepatectomy induced an immediate increase in BS levels in both arterial (2.7 vs. 5.9 μmol/l, p < 0.001) and portal venous plasma (7.3 vs. 12.3 μmol/l; p < 0.001). The release of BS by the PDV (+1.2 vs. +1.6 mmol·kg-1·h-1; p = 0.02) and the hepatic uptake (-1.0 vs. -1.5 mmol·kg-1·h-1; p = 0.005) were also increased. In contrast, FGF19 levels were decreased in arterial (0.13 vs. 0.07 ng/ml, p = 0.03), portal (0.16 vs. 0.08 ng/ml, p = 0.03) and hepatic venous blood (0.14 vs. 0.08 ng/ml, p = 0.04) following liver resection. Prior to liver resection, FGF19 flux across the PDV was positive (+3.9 ng·kg-1·h-1) this remained stable after liver resection, indicating release of FGF19 from the PDV. Following liver resection gene expression of CYP7A1 was decreased (-2.1 fold; p < 0.001) and FXR gene expression was upregulated (+6.6 fold; p < 0.001). Systemic BS concentrations were elevated from POD1 onwards. Subgroup analysis revealed that BS levels were higher in patients undergoing major hepatectomy (-> 3 segments) compared with patients undergoing minor hepatectomy, on POD1 (10.2 vs. 5.0 μmol/l; p = 0.002) and POD2 (22.2 vs. 8.7 μmol/l; p = 0.03). Plasma FGF19 levels transiently peaked at POD1, with no differences in levels between the hepatectomy subgroups at any of the time points.
Discussion/Conclusion:
Liver resection results in prompt effects on portal levels of signaling molecules participating in liver regeneration. Elevated BS flux across the remnant liver, decreased BS synthesis, upregulated FXR expression and post-operative rise of BS levels implicate early involvement of BS signaling in the regenerative process.
A family outbreak of hepatitis A – Friend or foe

R. Komitova1,4, M. Atanasova2, A. Kevorkyan3, M. Nedeva2
1“St. George” University Hospital, Plovdiv, Bulgaria
2Microbiology Department, Medical University, Plovdiv, Bulgaria
3Faculty of Public Health, Medical University, Plovdiv, Bulgaria
4Infectious Diseases Department, Medical University, Plovdiv, Bulgaria

Although not generally accepted, hepatitis A superimposed on chronic hepatitis B runs more severe course and has worse outcome.

Objectives: To evaluate the overall impact of hepatitis A on health status of family members during an outbreak.

Methods: Two brothers (aged 27 years and 31 years respectively) and their 54 year-old mother were simultaneously diagnosed with hepatitis A in February 2016. Their close family contacts were traced and tested accordingly. Serology markers for hepatitis A (anti-HAV IgM), hepatitis B virus (HBsAg, HBeAg, anti-HBcIgM, anti-HBc total, anti-HBs) and hepatitis C (anti-HCV) were tested by third generation enzyme-linked immunosorbent assay (ELISA) using commercial kits. HBV DNA was detected by transcription-polymerase chain reaction (PCR).

Results: The clinical course of hepatitis A in both brothers was quite severe with unusually prolong temperature necessitating chest X-ray and initial antibiotic treatment. Their mother was first treated for acute cholecystitis and later was transferred to our infectious diseases department. In addition, the younger brother was diagnosed with inactive chronic hepatic B one year ago on an attempt to donate blood. At that time the family contacts were not tested for HBsAg.

On further evaluation serology of the elder brother went back with HBsAg(+), HBeAg(-), anti-HBeAg(+) and anti-HBcIgM(-) results. The mother was only positive for anti-HBc total (isolated anti-HBc). Their HBV DNA PCR assay results were pending. The only non-immune in the extended family was the wife of the elder brother, thus she received the first shot of hepatitis B vaccine.

Conclusion. Although with not too friendly behavior it was hepatitis A that enabled diagnosis of salient hepatitis B infection in the elder brother and initiation of hepatitis B vaccination of his wife.
Advanced liver disease and insulin resistance – Is there a connection?

Iva Košuta¹, Sara Šundalić¹, Maro Dragičević¹, Ana Višnjić¹, Marijana Vučić-Lovrenčić¹, Anna Mrzljak¹,²
¹University Hospital Merkur, Zagreb, Croatia; ²School of Medicine, University of Zagreb, Zagreb, Croatia

Introduction: Glycemic alterations are a hallmark of liver cirrhosis due to both beta-cell dysfunction and impaired insulin sensitivity. Loss of liver function seems to play an important role in development of glucose intolerance and diabetes, as liver transplantation usually ameliorates aforementioned disturbances. The prevalence of insulin resistance (IR) and its relation to the stage of liver disease in euglycemic patients, was investigated in this study.

Methods: One hundred and eighty-three euglycemic patients with cirrhosis of varying etiology were included. Cirrhosis was proven either histologically or clinically. Patients were classified into Child-Pugh class A, B or C, and MELD/MELD-Na score was calculated. IR was assessed by the Homeostasis Model Assessment (HOMA) model. The cut off value of 1.64 was used in this study.

Results: Overall, IR was observed in 77.8% of euglycemic cirrhosis. IR and non-IR patients were not equally distributed among MELD/MELD-Na classes (p = 0.0054; p = 0.0057, χ²-test), with IR more frequently observed in MELD/MELD-Na ≥ 10. However, no significant difference was found between IR occurrence and CP classes. The findings were not supported by further analysis, as Pearson correlation coefficients between HOMA-IR and CP/MELD/MELD-Na scores, as well as mean HOMA-IR values among different CP/MELD/MELD-Na classes (ANOVA test) were found to be non-significant.

Discussion/Conclusion: IR is common in euglycemic cirrhosis and with advancement of liver disease, however no correlation between liver disease stage and IR was found in our study sample. A possible explanation is that loss of liver function affects primarily beta-cell function, which exerts a more important role than IR on the development of ‘hepatogenous diabetes’.
Liver transplantation in patients with neuroendocrine tumors: Single center experience

Nino Kunac*, M.D.; Petra Dinjar Kujundžić* M.D.; Nikola Sobočan*, M.D. Ph.D.
Clinical Hospital Merkur, Zagreb, Croatia

Introduction: Neuroendocrine tumors are a rare and heterogenous group of neoplasms with variable biological behavior. With large proportion of them being diagnosed with synchronous liver metastases, the necessity for active, multimodality treatment has emerged. Surgical resection is possible in minority of cases, and other treatment modalities have not yet proven to accomplish long-term effects. In this abstract we present the results of liver transplantation due to neuroendocrine tumors with liver metastases performed at our University hospital.

Methods: Pre-transplant selection criteria included: metastatic disease exclusively presented in the liver, Ki67 proliferation index < 10% and time interval between primary tumor resection to liver transplantation ≥ 1 year.

Results: Between year 2009 and 2015, four patients (0.4% of all liver transplantations) underwent liver transplantation due to neuroendocrine tumors with solitary hepatic metastases. Two women and two men were included, with a median age of 42.5 years (range 34–54). The primary tumor was resected in three patients, along with liver metastases resection and radiofrequent ablation in one patient. Concerning medical treatment in the pretransplantation period, one patient was treated with somatostatin analogue and chemotherapy consisting of cisplatin and etoposide, while two patients were given either somatostatin analogue or chemotherapy. Time between the diagnosis of liver metastases and liver transplantation ranged between 13 and 145 months. At a median follow-up 13.5 months (range 2–53 months) the entire group of are patients is alive, and without signs of recurrence.

Discussion/Conclusion: Being the only metastatic disease indication for liver transplantation, neuroendocrine tumors were widely evaluated throughout the literature. Despite the recognition of several prognostic factors and selection protocols clear recommendations have not yet been fully implemented in routine clinical practice. With the advent of new and sophisticated diagnostic modalities and prospectively evaluated selection criteria, liver transplantation should be increasingly referred to as a long-term cure for this group of patients.
Metabolic profiles of alcoholic and non-alcoholic steatohepatitis

Zoe Schofield¹, Michelle A.C. Reed², Karen Atkins², Philip N. Newsome³, David H. Adams³, Ulrich L. Günther², Patricia F. Lalor³
¹Sci-Phy-4-Health, EPSRC Research and Training Centre in Physical Sciences for Health, College of Engineering & Physical Sciences; ²School of Cancer Sciences and ³Centre for Liver Research and National Institute for Health Research (NIHR) Birmingham Liver Biomedical Research Unit, University of Birmingham, Birmingham, UK

Introduction: Rising rates of obesity have led to a dramatic increase in non-alcoholic fatty liver disease (NAFLD), a manifestation of the metabolic syndrome that occurs as a spectrum from fatty liver (steatosis) through inflammation (non-alcoholic steatohepatitis, NASH) to cirrhosis. Urgent strategies are required to reduce the burden of fatty liver disease but there are currently no licensed therapies. Furthermore robust markers that can accurately identify patients at risk of progression from steatosis to NASH are lacking, as are tests that discriminate between (alcohol-related liver damage) ARLD and NASH.

Methods: We have used high throughput, non-destructive ¹H-NMR to derive metabolomic signatures from human liver tissue from patients with either steatosis, NASH or ARLD to identify species that can predict outcome and discriminate between alcohol and metabolic-induced liver injuries.

Results: Changes in branched chain amino acid homeostasis, TCA cycle and purine biosynthesis intermediates along with betaine were associated with the development of cirrhosis in both ARLD and NAFLD. Species such as propylene glycol and as yet unidentified moieties that allowed discrimination between NASH and ARLD samples were also detected using our approach.

Discussion/Conclusion: Our technique for multiple analyte quantification in human liver specimens has identified potential biomarkers with prognostic and diagnostic significance in metabolic liver disease.
T cells are vehicles for HCV and HCVcc infection

Y. Liu1, D. Niesen1,2, N. Fletcher1, P. Balf1e, L. Meredith1, R. Thimme2, D. Mutimer1, D. Adams1, J. McKeating1, Z. Stamatakis1

1Institute of Immunity and Immunotherapy, University of Birmingham, UK
2University Hospital Freiburg, Germany

Introduction: Microbial delivery from one cell type to another is known as trans-infection, and can lead to efficient spread to poorly permissive target cells. Hepatitis C virus (HCV) is notoriously difficult to propagate in vitro, with few variants replicating efficiently in cell culture (HCVcc). We show that activated T cells captured and transmitted authentic HCV and HCVcc in vitro.

Methods: T cells from healthy donors and HCV infected patients were used to transmit HCVcc JFH-1 and SA13/JFH-1 or authentic HCV as described for B cells (Stamatakis et al. Blood. 2009). Captured virus was estimated by real time qPCR and transmission to liver and brain cells by NS5A staining. Transmission was inhibited by IgG HCV, a-CD81, direct acting antiviral VX-950 and IFN-alpha.

Results: T cells captured HCV via the low-density lipoprotein receptor (LDL-R). Ligation of CD81 on T cells boosted trans-infection by increasing the capture and release of virus, and by enhancing T cell association with hepatocytes.

HCV exists as swarms of multiple variants within each patient (quasispecies). CD81 ligation on T cells by HCV envelope glycoproteins and pseudoparticles from diverse quasispecies promoted HCVcc transmission, revealing that viral quasispecies could facilitate trans-infection.

T cell transfer of infection was amenable to therapeutic interventions including direct acting antivirals, interferon and neutralizing antibodies.

HCV delivery via T cells was the optimal route for infection of less permissive polarized hepatic and brain cell targets.

Discussion/Conclusion: This work puts forward a key role of T cells as vehicles for HCV transmission, using authentic HCV from patients of various genotypes.
The relative PMN count in the ascites is related to different forms of ascites infections

Philipp Lutz1*,3,4, Felix Goeser1,3, Dominik J. Kaczmarek1,3, Stefan Schlabe1,3, Hans Dieter Nischalke1,3, Jacob Nattermann1,3, Achim Hoerauf2,3, Christian P. Strassburg1,3, Ulrich Spengler1,3
1Department of Internal Medicine I, University of Bonn, Sigmund-Freud-Str. 25, 53127 Bonn, Germany
2Institute for Medical Microbiology, Immunology and Parasitology, University of Bonn, Sigmund-Freud-Str. 25, 53105 Bonn, Germany
3German Center for Infection Research
4Centre for Liver Research and NIHR Biomedical Research Unit in Liver Disease, Institute of Immunology & Immunotherapy, University of Birmingham, United Kingdom
*Corresponding Author: Phone: +49 228 287 15507, Fax: +49 228 287 51419, E-Mail: philipp.lutz@ukb.uni-bonn.de

Introduction: Bacterial ascites infections in patients with liver cirrhosis comprise spontaneous bacterial peritonitis (SBP) and bacterascites. SBP is diagnosed if the ascites polymorphonuclear (PMN) cell count exceeds 250 cells/µl. Bacterascites describes detection of bacteria in the ascites in the absence of SBP. We investigated if the relative ascites PMN may distinguish uninfected from infected ascites.

Methods: Patients with liver cirrhosis who received paracentesis at our department were stratified for the different forms of ascites infections and prospectively followed-up for one year in this observational cohort study. The relative ascites PMN count was calculated by dividing the ascites PMN by the ascites leukocyte count.

Results: We included 269 patients, of whom 198 (74%) had uninfected ascites, 28 (10%) bacterascites and 43 (16%) SBP at inclusion. Median age was 60 (interquartiles: 53; 68). Most patients had liver cirrhosis due to alcohol abuse (n = 169; 63%) or viral hepatitis (n = 30; 11%). Child-Pugh stage was B in 143 (53%) and C in 125 (47%) patients. 16 patients developed bacterascites and 25 SBP during follow-up. At inclusion, the median relative ascites PMN count was lower in patients with uninfected ascites (0.12; interquartiles: 0.08; 0.20) compared to patients with bacterascites (0.22; interquartiles: 0.13; 0.30) (p = 0.001) or SBP (0.76; interquartiles: 0.55; 0.84) (p < 0.001). The same pattern was noted when the median relative ascites PMN count in bacterascites (0.27; interquartiles 0.14; 0.39) or in SBP (0.75; 0.56; 0.81) developed during follow-up was compared to the relative PMN count in uninfected ascites of the same patient at inclusion (p = 0.001 and p < 0.001, respectively).

Discussion/Conclusion: The relative ascites PMN differs between different forms of ascites infections. It may help to identify a subgroup of patients without SBP but at risk for bacterascites who should be closely observed till results from microbiological culture are available.
Orthotopic liver transplantation and reversal of hypersplenism – What can we expect in the long-term?

Matea Majerović¹, Marina Premužić¹, Agata Ladić¹, Ivana Knežević Štromar¹, Davor Radić¹, Nadan Rustemović¹, Rajko Ostojić¹
¹Department of Gastroenterology and Hepatology, University Hospital Centre Zagreb, Zagreb, Croatia

Introduction: Thrombocytopenia (platelets < 150 x 10⁹/l) due to hypersplenism carries a significant burden of disease in cirrhotic patients namely regarding bleeding risk and platelet transfusions prior to invasive procedures. Even though orthotopic liver transplantation (OLT) significantly restores portal hemodynamics a certain degree of hypersplenism persists post-transplant but data on this are scarce and heterogeneous. The aim of our study was to assess the rate of platelet count recovery after OLT.

Methods: Our study included 24 patients (7 female, 17 male, average age at OLT 52 years, average MELD 14.3) that did not undergo splenectomy nor were treated with IFN prior or post-OLT. Data were obtained from their medical records. Patients were divided into 3 groups according to etiology of liver disease (hepatitis C (HCV) 5, alcoholic liver disease (ALD) 8, other 12). Platelet count was noted before OLT and at 6, 12 and 18 months post-OLT. Descriptive statistics were used to interpret results.

Results: Overall average platelet count before OLT was 89.3 x 10⁹/l and rose by 34.7% in first 6 months post-OLT (120.3 x 10⁹/l) remaining relatively stable in consecutive 6-month periods (at 12 months 129.2 x 10⁹/l, +7.4%, at 18 months 131.2 x 10⁹/l, +1.5%). Hepatitis C patients had lower pre-OLT platelet values (44.2 x 10⁹/l) when compared to patients with ALD (105.5 x 10⁹/l) and other etiology (93.5 x 10⁹/l). At 6 months post-OLT HCV patients displayed most notable rise (93.3 x 10⁹/l, +111.3% vs. other 131.3 x 10⁹/l, +40.3% and ALD 117.2 x 10⁹/l, +11.4% ALD). In all groups platelet count at 12 (HVC 97 x 10⁹/l, +3.7%; other 133.7 x 10⁹/l, +1.8%; ALD 132.5 x 10⁹/l, +13%), and 18 months (HCV 79.3 x 10⁹/l, -18.2%; other 136.5 x 10⁹/l, +2.1%; ALD 139.3 x 10⁹/l, +5.9%) remained relatively stable.

Discussion/Conclusion: OLT improves hypersplenism however absolute reversal of thrombocytopenia cannot be expected in all patients. The rate of platelet count recovery at 6 months post-OLT indicates expected long-term platelet count and depends on pretransplant values regardless of the etiology of liver disease.
Exhaled breath profiling by electronic nose as a novel non-invasive method for assessment of chronic liver disease: Proof of principle study

Natasha McDonald¹, Rohit Sinha², Rianne de Vries³, Peter Hayes², Robert Chamuleau⁴, Jonathan Fallowfield¹, John Plevris²
¹MRC Centre for Inflammation Research; ²Hepatology Laboratory, University of Edinburgh, Edinburgh, United Kingdom; ³Department of Respiratory Medicine; ⁴Surgical Laboratory and Tytgat Institute for Liver and Intestinal Research, Academic Medical Centre, Amsterdam, The Netherlands

Introduction: Exhaled breath analysis by electronic nose (eNose) is a novel non-invasive point-of-care diagnostic tool based on pattern recognition of volatile organic compounds (VOC). Metabolic derangements and imbalance of gut microbiota observed in liver disease result in production of VOC signatures that are different from healthy controls; a distinct ‘breath print’ of acute liver failure in a rat model has recently been described². The aim of this pilot study was to evaluate the potential for eNose in stratifying liver disease stage in patients from a well-characterised clinical cohort.

Methods: Consecutive unselected adult patients who underwent a clinically-indicated liver biopsy within the previous 8 months (median 5 months), were recruited for eNose assessment. Reference eNose data was also collected from 5 subjects with normal liver biopsy findings and 4 healthy volunteers (HV) with no known liver/metabolic disease and BMI < 25 kg/m². Exhaled breath was collected in triplicate by an eNose based on 5 identical and exchangeable arrays of 4 metal-oxide-semiconductor sensors. Data analysis involved signal processing and environment correction based on alveolar gradients. Resulting dataset was reduced to 4 principal components (PCs) that explained 96.8% of the total variance, followed by discriminant analysis (Matlab2014/SPSS20).

Results: Study subjects were divided into 3 groups:
   a) Normal (n = 9; n = 5, normal biopsy; n = 4, HV)
   b) Liver disease (n = 16; multiple aetiologies and fibrosis stages)
   c) Advanced fibrosis/cirrhosis (n = 7; 4 non-alcoholic fatty liver disease, 1 cryptogenic, 1 chronic hepatitis C, 1 alcohol-related).

Against a gold-standard of liver biopsy, eNose was able to differentiate between normal subjects and patients with advanced fibrosis/cirrhosis (Ishak stage > 4) (p = 0.031) and between normal subjects and patients with any liver disease (p = 0.022, cross validation value (CVV) 74%), potentially leading to avoidance of further testing if normal while streamlining the referral to secondary care. In addition, eNose was able to separate patients with advanced fibrosis/cirrhosis from lesser stages of liver disease and irrespective of aetiology (p = 0.048, CVV64%), thus facilitating decision-making regarding hepatoma and variceal surveillance.

Discussion/Conclusion: eNose shows promise in detecting and staging chronic liver disease irrespective of aetiology. Larger prospective studies will be required to validate the role of point-of-care exhaled breath diagnostics in clinical practice.
QT interval prolongation and QRS voltage reduction in the course of liver cirrhosis

Agata Michalak¹, Halina Cichoż-Lach¹, Michał Tomaszewski², Emilia Lis-Janczarek¹, Beata Kasztelan-Szczerbińska¹, Katarzyna Adamczyk¹
¹Department of Gastroenterology, Medical University of Lublin, Poland
²Department of Cardiology, Medical University of Lublin, Poland

Introduction: Liver cirrhosis (LC) involves various alterations of cardiovascular system termed cirrhotic cardiomyopathy (CC). Heart failure development might be reflected by coexisting electrocardiographic (ECG) abnormalities. The aim of presented study was to analyze ECG changes in patients with cirrhosis to assess whether alcoholic etiology of liver damage and ascites have an impact on ECG curve.

Methods: The study was conducted on 81 patients with previously untreated LC (64 patients with ascites and 17 without ascites), 41 patients with previously untreated LC due to the hepatitis C (HCV) (3 patients with ascites and 11 without ascites), 42 patients with alcoholic steatohepatitis and 46 patients with alcoholic steatosis. The control group involved 32 healthy volunteers. Twelve-lead ECG recordings were performed and selected parameters were measured.

Results: Remarkably longer QT and QTc intervals were detected in patients with alcoholic and HCV LC compared to the controls. Significantly lower QRS voltage was found in subjects with ascites than in those without ascites. Removal of ascites noticeably increased QRS voltage.

Discussion/Conclusion: Presented study revealed the correlation between liver cirrhosis and ECG changes. In cirrhosis, irrespective of etiology, ECG abnormalities involved prolonged QT and QTc intervals. The study also showed the correlation between ascites in the course of cirrhosis and QRS voltage reduction. Reports in the literature regarding the effects of LC on the ECG is scarce (especially data concerning QRS voltage) and involves mainly QT changes. ECG alterations in patients with decompensated LC have essential practical importance. Electrophysiological abnormalities in this group occur in the absence of any known cardiac disease. By mean of this fact a multi-disciplinary approach is vital in the course of LC to exclude potential coexisting conditions which may alter cardiac function and even accompany CC.
Relationship between controlled attenuation parameter and liver stiffness measurements obtained by FibroScan® with red blood cell distribution width in patients with one or more components of metabolic syndrome

I. Mikolasevic1*, L. Orlic2, D. Stimac1, N. Kristo3, I. Jakopcic3, V. Mavrinac2, S. Milic1
1Department of Gastroenterology, UHC Rijeka, Croatia
2Department of Nephrology, Dialysis and Kidney Transplantation, UHC Rijeka, Croatia
3School of Medicine, Rijeka, Croatia
*Corresponding author: E-Mail: ivana.mikolasevic@gmail.com

Aim: Our aim was to investigate the correlation between Controlled Attenuation Parameter (CAP) and liver stiffness measurements (LSM) values obtained by FibroScan® as measurements of liver steatosis/fibrosis and red blood cell distribution width (RWD) values in patients with one or more components of metabolic syndrome (MS).

Materials and methods: In this prospective study we have analyzed 360 patients between April 2014 and October 2015. The HIS, FIB-4 and BARD scores as well as CAP and LSM were obtained to assess liver steatosis and fibrosis.

Results: RDW showed significant positive correlation with age, presence of diabetes, hypertension and MS, waist circumference, CRP and uric acid as well as with LSM, HIS and BARD scores as indicators of fibrosis (all p < 0.05). Patients with higher RDW values (> 13.4%), had statistically significantly higher incidence of diabetes, hypertension, MS, higher values of waist circumference. CRP, uric acid, as well as CAP, LSM and FIB-4 and BARD scores in comparison to the patients with lower RDW (≤ 13.4%) values. In multivariate analysis, RDW continued to be statistically significant and an independent predictor of elevated LSM (OR = 1.88, 95% CI: 1.0226–3.4774, p = 0.04)

Conclusion: RDW, is an inexpensive, non-invasive parameter that can be used for assessment of liver steatosis/fibrosis in NAFLD patients, especially in those with MS. Our results give a possibility that RDW in combination with FibroScan®-CAP or in combination with some other noninvasive markers could identify patients with NAFLD that have a risk for progression of liver disease.
Non-alcoholic fatty liver disease – A multisystem disease

I. Mikolasevic1*, L. Orlic2, V. Lukenda Zanko3, D. Stimac1, M. Colic2, S. Milic1
1Department of Gastroenterology, University Hospital Center (UHC) Rijeka, Rijeka, Croatia
2Department of Nephrology, Dialysis and Transplantation, UHC Rijeka, Rijeka, Croatia
3Department of Internal Medicine, General Hospital “Dr. Josip Bencevic”, Slavonski Brod, Croatia
*Corresponding author: E-Mail: ivana.mikolasevic@gmail.com

Aim: Our aim was to investigate the incidence of NAFLD in patients with chronic kidney disease (CKD), patients with adenomatous polyps and colorectal cancer (CRC). We were interested to explore the association between NAFLD and decreased kidney function in patients CKD grade III and IV, as well as in renal transplant recipients (RTRs). Furthermore, we were interested to analyze whether the presence of NAFLD is associated with a higher cardiovascular (CVD) risk in hemodialysis (HD) patients and RTRs.

Methods: We examined 206 CKD patients; 62 CKD patients grade III and IV, 71 HD patients and 73 RTRs. Fifty patients with adenomatous polyps and 13 with CRC take a part in this study as well. Anthropometric measurement, biochemical test for liver, kidney and metabolic function, and transient elastography (Fibroscan®-CAP) were assessed.

Results: Out of 62 CKD patients grade III and IV, 85.5% had NAFLD. Furthermore, NAFLD was present in 57.5% of RTRs. The severity of liver steatosis was negatively correlated with kidney function in CKD and RTRs. NAFLD was found in 52.1% of HD patients. RTRs and HD patients with NAFLD shows more carotid atherosclerosis than RTRs and HD patients without NAFLD. Fourthly-five (90%) patients with adenomatous polyps and 13 (84.6%) patients with CRC had NAFLD. Thirty-eight out of 50 patients with polyps had advanced histological findings of their polyps; 36 of them had NAFLD. Twenty-one patients had polypus of 10 mm or more in diameter and all of them had NAFLD.

Conclusion: Our results support the idea that NAFLD is a multisystem disease.
Many faces of childhood Wilson disease – 12 year experience of a hepatology center in Romania

Alexandra Moraru*, Daniela Pacurar*, Dumitru Oraseanu*  
*“Grigore Alexandrescu” Emergency Hospital for Children, Bucharest, Romania

Introduction: Wilson disease (WD) is a rare genetic inherited pathology, due to mutations in ATP7B gene, which can lead to copper accumulation in tissues. WD is difficult to diagnose, especially in children. The spectrum of clinical pictures is highly variable from asymptomatic patients up to patients with hepatic cirrhosis and acute liver failure. Even if the diagnosis is challenging, it must be established soon, to prevent disease complications.

Methods: We have conducted an observational retrospective study, between 2004 and 2015, in which we included patients diagnosed in our clinic with WD. For diagnosis we used serum ceruloplasmin, 24-hour urinary copper with D penicillamine administration, WD scoring system, for some cases gene testing and for several patients liver biopsy.

Results: Between 2004 and 2015 we had 30 patients with WD with mean age 11.4 years and sex ratio M/F = 2.7/1. From the study group 13 patients were from the rural areas and 17 from urban areas. Thirteen patients had relatives with WD, nine of them had more than one relative with WD. Clinical picture at diagnosis was as follows: twenty patients (66.6%) were asymptomatic or only with biochemical abnormalities (hepatic cytolysis), 5 patients (16.6%) from the study group presented with jaundice. We had one patient (3.33%) with hemolytic anemia, one patient (3.33%) with extrapyramidal symptoms, 1 patient (3.33%) with hepatic cirrhosis. Two patients from the study group presented with hepatic failure.

Discussion/Conclusion: Age and symptoms in the onset of WD can vary widely, and the diagnosis can be difficult. Event tough WD is a rare condition, it must be considered in the differential diagnosis of hepatic pathology in children. Early diagnosis can improve the outcomes.
**HLA complex genes as a risk factor in non-alcoholic fatty liver disease**

Daniela Neagoe, Gabriel Ianosi, Mihaela Popescu, Anca Amzolini, Anca Farmazon, Mihaela Dutescu  
University of Medicine and Pharmacy, Craiova, Romania; C.T. Nicolau Institute, Bucharest, Romania

**Introduction:** HLA complex is a genetic factor that may be involved in the pathogenesis of non-alcoholic liver fatty disease (NAFLD) or may contribute to disease susceptibility. The aim of our study was to evaluate the relationship between HLA complex and NAFLD and, for the first time in Romania, to compare HLA frequencies in NAFLD group with healthy population.

**Methods:** In NAFLD group we included 46 patients with high level of amino-transferases and a bright liver at abdominal ultrasound and we excluded the patients with other conditions known to be associated with hepatic steatosis with or without microinflammation. In control group we included 300 healthy candidates. In both groups we performed HLA class I – A, B and HLA class II – DR-, DQ- using ARROW BLOOD DNA method and molecular technique SSO-HISTO-SPOT.

**Results:** We compared the antigen frequencies of NAFLD group with control group and we found that HLA A24 (31.12% in NAFLD group vs. 19.5% in controls), A31 (11.12% vs. 3.6%), A32 (13.3% vs. 8.4%), B18 (26.6% vs. 9.2%), B49 (8.89% vs. 3.6%) and B53 (6.67% vs. 0.08%, p < 0.0001) were significantly high expressed in NAFLD group than in controls. In study group we found 2 combinations of genes that appear to influence the disease: HLA A24, HLA B15, HLA DR15, DR16, HLA DQ5, HLA DQ3 in patients without other risk factors and HLA A2, HLA A32, HLA B18, HLA B49 and HLA B53 in patients with obesity or metabolic syndrome. The prevalence of HLA A1, A3, DR1, DR3, DR4 and DR7 were less frequent in NAFLD group than in controls. Moreover, HLA DQB1*03:01 and DQB1*05:02 were high statistical in NAFLD group. All the patients with HLA B53 associated hepatic steatosis and metabolic syndrome.

**Discussion/Conclusion:** Our study demonstrate that some HLA complex genes (A24, A31, A32, B18, B49, B53) are related with the disease and others (DR1, DR3, DR4 and DR7) seems to be protective for development of NAFLD.
Genes and environmental factors involvement in development of NAFLD

Daniela Neagoe, Anca Amzolini, G. Ianosi, Mihaela Popescu, Anca Farmazon, Cristina Deliu, Mihai Ioana
University of Medicine and Pharmacy, Craiova, Romania

Aim: Non-alcoholic fatty liver disease is considered a major public health and the natural course of the disease is possible influenced by the interaction of genetic and environmental factors. Aim of our study was to identify the 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, amino-transferases level, presence of diabetes mellitus or metabolic syndrome, insulin resistance and the PNPLA3 gene polymorphism. Metabolic syndrome was defined according to IDF criteria and for insulin resistance we used HOMA-IR index. The genotyping assays were performed using predesigned TaqMan SNP Genotyping Assays.

Results: All 138 patients with hepatic steatosis (39men and 99 women, mean age 49 ± 13 years) 107 (77.53%) were obese, 120 patients (86.95%) had metabolic syndrome, 53 (38.4%) were diabetics and 81% (58.69%) had elevated liver enzymes. 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 [C] 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 vs [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.
Diagnostic difficulties in an infant with jejunal atresia, failure to thrive, acute cholecystitis and hepatitis – A case report

I.V. Nenciu1,2, R.E. Smadeanu1,2, C. Jurjiu1, S. Mosescu1
1“Grigore Alexandrescu” Emergency Hospital for Children, Bucharest, Romania
2“Carol Davila University” of Medicine and Pharmacy, Bucharest, Romania

Introduction: Intestinal atresia is a common cause of bowel obstruction in the newborn. The incidence of jejunal and ileal atresia ranges from 1:1500 to 1:12,000 births. About 10% of patients with intestinal atresia have cystic fibrosis.

Methods and results: We report a case of a male infant admitted to our hospital after the first 30 hours of life with intestinal obstruction, abdominal distention and agitation. The infant was a full-term, born with 4030 g, coming from uninvestigated pregnancy. Exploratory laparotomy has been performed, and, based upon histopathological features, it was established the diagnosis “jejunal atresia”. During hospitalization the infant had recurrent bilious vomiting and he developed sepsis, acute cholecystitis and hepatitis. Enteral feeding via nasogastric tube was initiated after 24 days of life with extensively-hydrolyzed formula, and oral feeding after 36 days. He was discharged at the age of two months, with the weight of 3450 g. Afterwards, the infant has been admitted to the hospital for acute gastroenteritis and acute pneumonia with acute respiratory failure; every time he presented with poor weight gain. At the age of five months, when the infant’s weight was 4660 g, he was tested positive for cystic fibrosis (sweat conductivity test: 98 mmol/l, homozygous positive for delta F508). The infant received chest physiotherapy and treatment with inhaled hypertonic saline, inhaled dornase alfa, inhaled beta-2-adrenergic receptor agonist, pancreatic enzyme replacement therapy and vitamins A, D, E and K. The weight gain has improved, and at the age of one year the infant had 8500 g.

Discussion/Conclusion: Giving this important association, all the infants with intestinal atresia should be tested for cystic fibrosis, in order to improve the patient’s outcome.
Albendazole induced acute hepatitis – A consideration on 18 patients

Mihaela-Claudia Nistor¹, Andreia-Florina Niță¹,², Irina Dijmărescu¹, Luciana Zah¹, Daniela Păcurar¹,², Dumitru Orășeanu¹,²
¹“Grigore Alexandrescu” Emergency Children’s Hospital, Bucharest, Romania
²Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Introduction: Albendazole is a broad-spectrum anthelmintic drug known for hepatotoxicity. In Romania is widely used for intestinal infections, often based on clinical criteria only, without laboratory confirmation.

Methods: A retrospective study was conducted in “Grigore Alexandrescu” Emergency Children’s Hospital, between January 2009 and February 2016. We identified 18 patients with hepatic involvement after they received albendazole for a supposed intestinal parasitosis. The diagnostic was supported by Roussel Uclaf Causality Assessment Method (RUCAM). Other causes of acute hepatitis were excluded, without liver biopsy.

Results: The median age of the study group was 6 years, 77.77% were female, 52.62% cases came from rural areas. In 38.88% of cases was no medical recommendation regarding indication for parasitic treatment. A period of three weeks to four months was noticed between the intake of albendazole and the moment of the diagnosis. Eight patients had symptoms suggestive for liver involvement: 6 jaundice, 5 dyspepsia (nausea, vomiting, abdominal pain). Elevated liver enzymes were an incidental discovery. Hepatic tests showed liver cytolysis in all patients, cholestasis in 71.22% of cases, no coagulopathy. Liver ultrasonography was normal, except for one case with increased liver echogenicity. The RUCAM scale showed a causal relationship between albendazole intake and the liver injury event as highly probable in 2 cases, probable in 8 cases and possible in other 8 cases. The liver enzymes returned to normal in a period of time ranging from 15 to 90 days. All patients had good clinical evolution after non-specific symptomatic treatment.

Conclusion: Our study showed a correlation between albendazole use and hepatic involvement. Over 50% cases for acute drug induced hepatitis caused by albendazole use presented with asymptomatic liver cytolysis. Albendazole should be used only in cases with laboratory confirmed diagnosis of parasitosis.
Challenges of autoimmune hepatitis in children: What we need to learn from a retrospective view

Andreia Florina Nita¹², Mihaela Nistor¹, Andra Radulescu², Irina Dijmarescu¹², Daniela Pacurar¹², Dumitru Oraseanu¹²
¹“Grigore Alexandrescu” Clinical Emergency Hospital for Children, Bucharest, Romania
²Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Introduction: Though is the most prevalent cause of chronic hepatitis among children in USA and Western countries, autoimmune hepatitis is still incompletely understood, being both a challenge and a mystery which requires immediate treatment yet there is no unique treatment approach. Therefore, early recognition and diagnosis leading to inspired choice of management strategy is the key of a favorable outcome.

Methods: We conducted a retrospective longitudinal study during 1st of January 2010–29th February 2016 which included 23 children diagnosed with AIH in “Grigore Alexandrescu” Clinical Emergency Hospital for Children in Bucharest. The viral, metabolic and toxic etiology was excluded in all cases. A complete medical history, physical examination and laboratory evaluation were performed and recorded in all patients on repeated regular intervals.

Results: With a mean of 23,305 children admitted to hospital/year, the prevalence of AIH was 0.035%, and a total of 23 patients were included in the study. Among them, 69.5% (15) were female and median age was 8.5 years (STDEV = 4.71). Regarding the classification, 52.17% (14) were diagnosed with AIH type I, 17.3% with AIH type II. Seven cases had unclassified autoimmune hepatitis which was associated with autoimmune disorders: cholangitis, thyroiditis, pancreatitis, medullar aplasia or hemolytic anemia. The patterns of onset were as follows: acute hepatitis in most of the cases, insidious onset in 3 cases and decompensated cirrhosis in 3 cases. Remission after immunosuppressive therapy was obtained in 3 cases at 5 years follow-up, 1 patient was lost, 2 were noncompliant and the rest are still in treatment. We report 4 deceases and a patient who underwent liver transplant during the study.

Discussion/Conclusion: Rapid initiation of immunosuppressive therapy is the cornerstone of treatment and it depends on the ability of the physician to diagnose the disease without delay. Patient compliance is crucial for survival and good outcome.
The acceptability of cohort screening for blood borne viruses amongst sex offenders in a male prison in Scotland

Karen Prentice, Lorraine Moffat, Karen Gibb, Wendy Mitchell, Pete Bramley
Forth Valley Hepatology Service, Forth Valley NHS, Stirling, Scotland, UK

Introduction: Within the Scottish Prison Service, testing and treatment of hepatitis has high priority, and the introduction of “Opt-Out” testing for new prisoners has concentrated on diagnosing Hepatitis C early in order to facilitate treatment. We report on a pilot cohort screening program to offer counselling and BBV testing to established sex offender (SO) prisoners who are in the target population over 45 years old, in an adult male Prison containing SO and mainstream short term, long term and life sentence offenders.

Methods: Glenochil Prison has an average of 660 prisoners ranging from 20 to 90 years old, approximately 50% being sex offenders. An invitation letter was sent with information about BBV and a pre-booked appointment to see a hepatology nurse specialist during in-reach clinics. The oldest prisoners in the establishment were contacted first, then younger cohorts were invited. Data including numbers refusing to attend, or declining testing following discussion of risk factors were collected.

Results: Overall the oldest 204 prisoners were contacted, of whom 160 were sex offenders. Amongst SO, 135 (85%) attended for discussion of risks and 116 (73%) agreed to testing. 20 (13%) prisoners refused and 5 liberated prior to clinic. 85% of the over 60 year total cohort agreed to testing compared to 60% of the 45–50 year total cohort.

In total three SO prisoners were newly diagnosed as HCV antibody positive with two PCR negative, and 1 PCR positive who underwent treatment and achieved SVR. The prevalence of HCV Ab positivity in this tested cohort of sex offenders was 2.6%.

Discussion/Conclusion: Whilst the older sex offender population appears keen to engage with screening, they demonstrate a lower HCV positivity than predicted from established prison prevalence rates. These data indicate that older prisoners readily engage with BBV screening programs.
Mapping the B cell compartment in human liver health and disease

S. Purswani1, G. Reynolds1, J. Harrison1, K. Aliazis1, B. Guevel1, Y. Liu1, S. Davies1, E. Triantafyllou2, B. Wiggins1, G. Webb1, H. Antoniades2, G. Hirschfield, E. Liaskou1, D. Adams1, Z. Stamataki1

1Institute of Immunity and Immunotherapy, University of Birmingham, 2Institute of Liver Studies, Imperial College London, UK

Introduction: B cells exist in the liver in proportions similar to blood, yet little is known about their subset composition and role in health and inflammation (autoimmunity, viral infection and dietary injury). We set out to define the phenotype, tissue distribution and functional significance of B cell subsets in human liver inflammation.

Methods: We defined eleven subsets of B cells isolated from human liver tissue explants by flow cytometry (n = 60 livers): Naïve Mature (CD19+IgM+IgD+CD27-CD38-), Memory non-switched (CD19+IgD+CD27+CD38-), Memory-switched (CD19+IgD-CD27+CD38-), Double negative (CD19+IgD-CD27-), Plasmablasts (CD19+IgD-CD27+CD38hi), Plasma cells (CD19+IgD-CD27+CD38hiCD138+), B10-like (CD19+CD5+CD1dhi), Human B1-like (CD19+CD70-CD43+CD27+), Centroblasts (CD19+CD77+), Centrocytes (CD19+CD77-IgD-CD38hi) and Transitional/Regulatory B cells (CD19+CD38hiCD24hi).

We compared healthy and diseased liver to blood (flow cytometry) and perihepatic lymph nodes (immunohistochemistry) and characterised B cell aggregates in tissue sections from diseases of various aetiologies n = 70 livers.

Results: B cells in the liver contained subsets that were distinct in numbers and in phenotype compared to matched blood in end stage liver disease. We identified two novel B cell populations that were enriched in the liver. CD24- B cells were subdivided into two subsets that were a) increased in cholestatic liver diseases (PBC, PSC) compared to dietary injury (NASH, ALD) and b) capable of producing IFN-gamma and IL-6 or IL-10 following CpG stimulation.

Discussion/Conclusion: Our experiments demonstrate that the human liver is home to a diverse B cell compartment, including B cells with innate immune cell characteristics that have not been previously defined in humans.
Differences in sequences between HBV relaxed circular (RCDNA) and covalently closed circular DNA (cccDNA) forms

Magda Rybicka¹, Anna Woziwodzka¹, Tomasz Romanowski¹, Piotr Stalke², Marcin Dręczewski², Krzysztof Piotr Bielawski¹

¹Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Poland, Abrahama 58, 80-307 Gdansk, Poland
²Department of Infectious Diseases, Medical University of Gdansk, Smoluchowskiego 18, 80-214 Gdansk, Poland

Introduction: The HBV genome exists in two different forms: cccDNA and RCDNA. There are some reports that free cccDNA can occur in the serum as an early signal of liver damage. The aim of this study was to investigate the presence of cccDNA in serum and liver biopsy samples of chronically infected patients (CHB). Another goal of this study was to compare polymorphisms in cccDNA and RCDNA forms.

Methods: Serum and liver biopsy samples were collected from 67 CHB patients at the same time point. Genotyping of RCDNA form was done directly after DNA extraction. For the cccDNA analysis samples were treated with the T5 Exonuclease which degrades ssDNA and linear or circular dsDNA with gaps and nicks. cccDNA was present in all liver samples and in none serum sample. Next, the mass spectrometry analysis was performed to compare RC and cccDNA sequence. HBV mutations associated with drug resistance located in the HBV pol (P) region and mutations located in the HBV basal core promoter/pre-core region (BPC/PC) were included.

Results: The BPC/PC and P sequence of RCDNA extracted from liver and blood samples were different in 38% and 11% of patients, respectively. Differences were also found between RC and cccDNA extracted from the same liver specimen. 60% of these samples have differed in the BPC/PC region and 40% in the pol region. The most frequently found differences were at codon 1764, 1899, 1762. The BCP/PC mutations were associated with HBeAg negativity and lower viral load.

Discussion/Conclusion: We have demonstrated that there are differences in the sequence of RCDNA and cccDNA extracted from the same liver specimen. However, further investigations are needed to analyze if mutations in cccDNA are conserved and whether cccDNA serves as a ‘mutation storage’ pool for HBV. This could have profound implications for subsequent therapy choice for the treatment-experienced patients.
Association of serum adiponectin levels and tumor stage in biliary tract cancer

Saray Aida¹, Papovic Vedad¹, Gogov Bisera¹, Nahodovic Kenan¹, Glavas Sanjin¹, Mehemdovic Amila¹, Vukobrat-Bijedic Zora¹
¹Department of Gastroenterology and Hepatology, University Clinical Center Sarajevo, Sarajevo, Bosnia and Herzegovina

Introduction: Multiple recent studies have indicated that some of adipose tissue-derived hormones may significantly influence the growth and proliferation of GI tumors including liver cancer (1, 2). However, the role of adipokines such as adiponectin and leptin in biliary tract cancer have not been well studied before. The aim of the study was to analyze plasma concentrations of adiponectin and leptin in cholangiocarcinoma (CC) patients and to compare these concentrations to clinicopathological parameters.

Methods: Baseline levels of adiponectin and leptin were determined in 27 consecutive patients with newly diagnosed cholangiocarcinoma and 27 healthy control subjects. The association between adiponectin and leptin and tumor stage was evaluated using nonparametric Spearman’s correlation test. Control subjects were matched to case patients by smoking status, age and BMI.

Results: Overall median adiponectin concentrations were lower in CC patients versus control subjects (5.3 vs. 9.1 µg/ml, \( p = 0.001 \)). In CC patients with T stage 2–4 (n = 16) median adiponectin concentrations were significantly lower than in CC patients with T stage 1 (n = 11) (4.3 vs. 6.5 µg/ml, \( p = 0.001 \)). The mean leptin levels were not significantly decreased in CC patients (\( p = 0.45 \)). Adiponectin concentrations were inversely correlated with tumor T stage (\( r = -0.821, p = 0.01 \)) of CC patients.

Discussion/Conclusion: This study identified an association between adiponectin levels and tumor stage suggesting a potential role for adiponectin in progression of cholangiocarcinoma. Our results support the hypothesis linking adipokines levels to malignant tumor growth (3) and suggest that adipokines might exert an adjunctive tool in risk prediction and management of cholangiocarcinoma patients.

References:


Molecular mechanism of action of ursodeoxycholic acid in hepatitis C virus (genotype 1b) resistance to interferon-based antiviral therapy

I. Sarvilina, PhD, ScD
Medical Centre “Novomeditsina”, Rostov-on-Don, Russia

Introduction: 5 million people in Russia are infected with hepatitis C virus (HCV), subtypes 1a, 1b (52.8%), 2, 3a of HCV. Interferon (IFN)-based therapy is burdened by a sustained virological response (50% of patients with genotype 1). The aim of the study was the research of molecular mechanism of action of ursodeoxycholic acid (UDCA) in HCV (genotype 1b) resistance to IFN-based antiviral therapy.

Methods: The study included 65 patients with HCV genotype 1b (22–45 years old, female – 31, male – 34, the duration – 2.5 years) according to inclusion/exclusion criteria. We used HCV RNA detection/quantification by RT-PCR, anti-HCV Ab by EIA, the IL28B genotype test, FibroTest, histological activity index (Knodell,1981), methods of proteomics and bioinformatics. All patients received PegIFN-α2a (180 μg/week, s.c.) and RBV (1000 mg/day, p.o.) during 12 weeks, next 36 weeks-similar scheme+UDCA (1000 mg/day, p.o.). "Statistica 12.0" was applied.

Results: All patients had HVL > 800,000 IU/ml, F0 (n = 45) and F1 (n = 20), an IL28B CC (n = 32), CT (n = 13), TT (n = 20, non-responders) genotype. The reduction of clinical symptoms was in 45 patients. We registered undetectable HCV RNA (≤ 15 IU/ml, n = 35), LVL < 800,000 IU/ml (n = 10) and HVL > 800,000 IU/ml (n = 20) after 12 weeks of treatment. The decrease of number of patients with HVL (n = 9), the increase - with LVL (n = 15) and undetectable HCV RNA (n = 41) were revealed after the treatment. We noticed the increase of fetuin-A, vitamin D binding protein, complement C5, STAT 3, eukaryotic initiation factor 2 in blood serum after PegIFN-α2a+RBV+UDCA-therapy.

Discussion/Conclusion: The dynamics in the proteome-map of blood serum revealed the molecular mechanism of action of UDCA in hepatitis C virus (genotype 1b) resistance to IFN-based antiviral therapy.
Mer tyrosine kinase regulates the activation of myeloid cells and innate immune responses in acute decompensated cirrhosis and acute-on-chronic liver failure

Arjuna Singanayagam¹²³, Evangelos Triantafyllou¹²³, Vishal C. Patel², Christine Bernsmeier², Christ Weston³, Stuart Curbishley³, Chris Willars², William Bernal², Georg Auzinger², Michael Heneghan², David Adams³, Mark Thursz¹, Julia Wendon², Charalambos G. Antoniades¹²³
¹Imperial College London, London, United Kingdom
²Kings College London, London, United Kingdom
³Centre for Liver Research, Birmingham, United Kingdom

Introduction: Immuneparesis and monocyte dysfunction are central to the pathogenesis of acute decompensated cirrhosis (AD) and acute-on-chronic liver failure (ACLF), accounting for increased susceptibility to infection and mortality. Mer tyrosine kinase (MerTK), expressed on monocytes/macrophages, dampens innate immune responses to microbial stimuli. We determined functional and migratory characteristics of circulating and tissue-derived myeloid cells expressing MerTK.

Methods: Using flow cytometry, immunophenotype and functional responses (TNFα/IL-6 production) of peritoneal macrophages (pMΦ) in ascites and circulating monocytes were measured in patients with AD (n = 10). pMΦ pathogen uptake was assessed using a phagocytosis assay (n = 4). Migratory characteristics of circulating monocytes were assessed using a transendothelial migration (TEM) assay in healthy control (HC) and ACLF (n = 6). Effects of ACLF-derived monocytes on neutrophil function were assessed after culture of healthy neutrophils in supernatants derived from LPS-stimulated monocytes using Annexin V and LPS-induced TNFα/IL-6 production (n = 6).

Results: pMΦ exhibit an anti-inflammatory (MerTK⁹²CD163⁹²) phenotype. Compared with circulating monocytes, MerTK expression is markedly elevated (82.1 vs. 16.6%, p = 0.03). In ACLF, circulating MerTK-expressing monocytes had reduced TNFα secretion compared with MerTK negative (6.22 vs. 24.9%, p = 0.094). Whilst pMΦ demonstrated preserved phagocytosis, LPS-stimulated TNFα (p = 0.4) and IL-6 (p = 0.05) secretion was attenuated. Compared to HC, monocytes in ACLF had higher MerTK expression (65.7 vs. 42.4%, p = 0.002) and lower TNFα secretion (48.6 vs. 25.3%, p = 0.03) after TEM; MerTK expression inversely correlated with TNFα secretion (r = -0.49, p = 0.053). Compared to HC, neutrophils cultured with the microenvironment from LPS-stimulated healthy monocytes conditioned in AD/ACLF plasma demonstrated increased apoptosis (AD: 51.2 vs. 20.4%, p = 0.055; ACLF: 79.8 vs. 20.4%, p = 0.003) and reduced TNFα (23.5 vs. 29.7%, p = 0.35) and IL-6 (31.4 vs. 42.6%, p = 0.01) secretion.

Discussion/Conclusion: Our data shows progressively enhanced MerTK expression as monocytes enter and then exit tissues, associated with impaired activation of circulating and tissue-specific myeloid cells in AD/ACLF. This highlights the regulatory role of MerTK signalling in innate immune responses, and need for future work to elucidate its mechanism to develop immunotherapeutic targets.
Ursodeoxycholic acid in advanced polycystic liver disease: A multicenter randomized controlled phase 2 trial (CURSOR)

CURSOR: Controlled trial of ursodeoxycholic acid to reduce liver volume in polycystic liver disease

Hedwig M.A. D’Agnolo¹, Wietske Kievit², R.B. Takkenberg³, Ioana Riaño⁴, Luis Bujanda⁴, Myrte K. Neijenhuis¹, Ellen J.L. Brunenberg⁵, Ulrich Beuers³, Jesus M. Banales⁴, Joost P.H. Drenth¹

¹Department of Gastroenterology and Hepatology, Radboud University Medical Center, Nijmegen, The Netherlands
²Radboud University Medical Center, Radboud Institute for Health Sciences, Nijmegen, The Netherlands
³Department of Gastroenterology and Hepatology, Amsterdam Medical Centre, Amsterdam, The Netherlands
⁴Department of Liver and Gastrointestinal Diseases, Biodonostia Research Institute – Donostia University Hospital, University of the Basque Country (UPV/EHU), IKERBASQUE, CIBERehd, San Sebastián, Spain
⁵Department of Radiation Oncology, Radboud University Medical Center, Nijmegen, The Netherlands

Background and aims: Ursodeoxycholic acid (UDCA) has been shown to inhibit proliferation of polycystic human cholangiocytes in vitro and hepatic cystogenesis in a rat model of polycystic liver disease (PLD). Our aim was to test whether UDCA may beneficially affect liver and cyst volume in patients with advanced PLD.

Methods: We conducted an international, randomized controlled trial in symptomatic PLD patients between 18 and 80 years old, in 3 tertiary centers. PLD was defined as the presence of ≥ 20 liver cysts with total liver volume (TLV) ≥ 2500 ml and underlying diagnosis of autosomal dominant polycystic liver disease (ADPLD) or autosomal dominant polycystic kidney disease (ADPKD). Patients were randomly assigned to UDCA treatment (15–20 mg/kg/day) for 24 weeks, or to no treatment. Primary endpoint was proportional change in TLV, as measured by computer tomography volumetry at baseline and 24 weeks. As secondary outcome measures we assessed change in symptoms using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core-30 (EORTC). We also performed a post-hoc analysis of liver cyst volume (LCV). Analyses were done on a modified intention-to-treat basis.

Results: We included 34 patients with advanced PLD and were able to assess the primary endpoint in 32 patients (50% ADPKD, mean age 51 ± 10 years). Mean liver volume increased by 4.6% ± 7.7% after 24 weeks of UDCA treatment (6697 ml to 6954 ml) compared to 3.1% ± 3.8% (5512 ml to 5724 ml) in the control group (p = 0.493). TLV did not increase significantly during UDCA treatment in ADPKD patients (p = 0.066) compared to controls (p = 0.021), though the absolute increase was not significantly different between both groups (p = 0.789). The increase in LCV did not change between UDCA and control groups (p = 0.848). In addition, UDCA treatment
inhibited LCV growth in ADPKD patients compared to controls ($p = 0.049$). Moreover, UDCA treatment improved EORTC scores ($p = 0.039$).

**Discussion:** UDCA administration for 24 weeks did not reduce TLV in patients with advanced PLD. Notably, UDCA reduced LCV growth in a subgroup of ADPKD patients. This randomized clinical trial suggests that the effect size we can expect from UDCA on TLV is below the range of detection.
Secretory leukocyte protease inhibitor (SLPI) drives hepatic resolution responses in acute liver failure

E. Triantafyllou\textsuperscript{1,2}, A. Wilhelm\textsuperscript{2}, L. Possamai\textsuperscript{2}, O. Pop\textsuperscript{1}, E. Liaskou\textsuperscript{3}, C. Bernsmeier\textsuperscript{1}, W. Khamri\textsuperscript{2}, S. Davies\textsuperscript{3}, Z. Stamataki\textsuperscript{3}, Y. Ma\textsuperscript{1}, A. Quaglia\textsuperscript{1}, S. Curbishley\textsuperscript{3}, J. Wendon\textsuperscript{1}, M. Thursz\textsuperscript{2}, D. Adams\textsuperscript{3}, C. Weston\textsuperscript{3}, C. Antoniades\textsuperscript{1,2}

\textsuperscript{1}Institute of Liver Studies, King’s College London
\textsuperscript{2}Division of Digestive Diseases, Imperial College London
\textsuperscript{3}Institute of Immunity and Immunotherapy, University of Birmingham, UK

Introduction: Acute liver failure (ALF) is an immune-driven disorder characterized by hepatocyte death and monocyte/macrophage (Mo/M\(\text{\textregistered}\)) liver infiltration. Following tissue injury, MERTK activation promotes resolution while SLPI suppresses innate immune responses in ALF. Here, we examined the mechanisms underlying hepatic resolution and the role of SLPI in regulating this response.

Methods and results: Using flow cytometry and confocal imaging we reveal an expanded pro-resolution MERTK\textsuperscript{high}CD163\textsuperscript{high}HLA-DR\textsuperscript{low} Mo/M\(\text{\textregistered}\) population in ALF (\(n = 15\)), compared to CLD (\(n = 10\)) and HC (\(n = 10\)). Transmigration assays showed that MERTK+ Mo readily migrate across hepatic endothelium, secrete regenerative mediators, suppress neutrophils and promote efferocytosis. Cholangiocyte-derived SLPI and Mo exposure to apoptotic cells induce pro-resolution Mo/M\(\text{\textregistered}\). \textit{In vitro}, SLPI (0.5 \(\mu\)g/ml) promoted resolution through Mo/M\(\text{\textregistered}\) and neutrophil suppression, increased HGF/IL-10 secretion and enhanced efferocytosis. \textit{In vivo}, SLPI effects on hepatic myeloid cells were determined using WT C57BL/6J mice dosed with APAP (300 mg/kg), rh-SLPI (16.5 \(\mu\)g/kg) or both. SLPI administration accelerated hepatic resolution (necrosis/ALT) and increased the proportion of F4/80\(\text{\textregistered}\)+MERTK+Ly6C\textsubscript{low} M\(\text{\textregistered}\) while decreased CD11b+Ly6G+ neutrophils in APAP-mice.

Discussion/Conclusion: SLPI is a key micro-environmental mediator in ALF promoting hepatic resolution through induction of pro-resolution MERTK+ Mo/M\(\text{\textregistered}\), innate immune suppression and enhancement of resolution responses, thus it may be a novel immunotherapeutic target in ALF.
Transplantation for autoimmune liver disease in the UK and USA 1995–2014: A 20-year review

Gwilym Webb1, Abbas Rana2, Mohammed Zeeshan Akhtar3, David Jones4, John Vierling2, Gideon Hirschfield1
1National Institute for Health Research (NIHR) Birmingham Liver Biomedical Research Unit (BRU) Centre for Liver Studies, University of Birmingham, UK
2Baylor College of Medicine, Houston, Texas, USA
3Churchill Hospital, Oxford, UK
4Newcastle University, Newcastle, UK

Introduction: Autoimmune liver disease (AILD) primarily comprises primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH). In each, listing for liver transplantation (OLT) may be considered representative of failed medical therapy. The only major therapeutic innovation in the last twenty years has been the widespread introduction of ursodeoxycholic acid (UDCA) in 1994. Recent work has highlighted variability in disease course by gender, ethnicity and age.

Methods: We examined UK and USA transplant registries to assess trends in listings for AILD 1995–2014 (n = 3065 and 17,367).

Results: Overall, total, PSC, and AIH listings increased; PBC listings decreased in both the UK and USA. This decrease in PBC listings was only present 1995–2004 but not 2005–2014. Changes in UKELD/MELD score and age at listing did not explain this decrease. Over time, the female over-representation in PBC listings decreased in both countries. The number of white ethnicity listings for PBC decreased but non-white listings did not. Proportionally fewer PBC listings were of black ethnicity and fewer PSC listings were Hispanic. Patients of non-white ethnicity were listed at younger ages for all conditions.

Discussion/Conclusion: There is an unmet therapeutic need in AIH and PSC evidenced by a persisting requirement for OLT. For PBC, the temporary decline in listings may be associated with UDCA but there is also continued unmet need. PBC patients requiring OLT are becoming more likely to be male and non-white, consistent with reported variable UDCA response and disease course between genders and ethnicities. The stability of the median age of those requiring OLT, and the OLT cohort having a lower median age than that reported for diagnosis elsewhere, supports the assertion that younger patients have more aggressive disease. Observations of variability in ethnicity and in age of listing between ethnicities support further research into genetic and environmental factors.
Model systems to study the consequences of CD4+ T cell interactions with liver cells

B. Wiggins1, D. Niesen1,2, Y. Liu1, K. Aliazis1, F. Ahmad1, S. Davies1, G. Reynolds1, R. Bhogal1, R. Thimme2, P. Lalor1, D. Adams1, Z. Stamataki1

1Institute of Immunity and Immunotherapy, University of Birmingham, UK
2University Hospital Freiburg, Germany

Introduction: The generation and phenotype of liver resident CD4+ T cells remains unclear. Capitalising on our access to liver and blood from well-characterised patient cohorts, we devised novel in vitro and ex vivo systems to interrogate CD4+ T cell phenotype and function in the context of the liver microenvironment.

Methods: CD4+ T cells were isolated from blood and liver of patients with end stage liver disease, and cultured with liver cells in models ranging from static co-cultures, transwell and trans-gel (collagen matrices) migration assays, precision-cut liver slices and pump-perfused liver wedges.

The phenotype and function of CD4+ T cells were established by flow cytometry for CD69, HLA-DR, CD25, CD38, CD62L, PD-1L, CCR-7 and CD45RA and intracellular cytokine expression for IL-2, IL-4, IL-10, IL-17, TNF-alpha, IFN-gamma.

Results: T cells upregulated CD69 but not other activation markers in co-culture with hepatocytes. Unlike activation through the T cell receptor or by phorbol ester, hepatocyte-induced CD69 expression was not linked to migration and resulted in sustained, intermediate levels of CD69. Spontaneous migration through hepatocytes was a useful tool to reveal the potential for activation in T cells from PBC and HCV patient blood.

Discussion/Conclusion: Our ex vivo models demonstrated that CD4+ T cells adopted a liver resident-like phenotype following co-culture with liver epithelia. Assessment of T cell function in the context of migration revealed previously unappreciated differences in CD4+ T cells from the blood of PBC compared to HCV infected patients and healthy donors.
The results of chemoembolization and endoarterial chemotherapy in primary hepatic carcinoma

A. Yusupbekov, M. Djuraev, D. Egamberdiev
National Cancer Center, Tashkent, Uzbekistan

**Purpose:** Determination of results of chemoembolization (ChE) and endoarterial chemotherapy (EChT) in primary hepatic cancer (PHC).

Was analyzed the results in 108 patients with PHC. The T3N0–1M0 stage of PHC was established in 37 patients, T4N0–1M0 in 41 and T4N0–1M1 in 30. Tumor was localized in one part of liver in 42, in both parts in 66. Hepatocellular carcinoma (HCC) was determined in 53, cholangiocellular carcinoma (CCC) – in 39, hepatic hemangi-endothelioma (HHE) in 16 cases. ChE of hepatic artery spent in 47 patients with doxorubicinum 60–80 mg and jodolypol with metallic spiral. In 61 patients we made EChT by scheme FAC during 120 hours.

Chemotoxicity after ChE was 0 degree in 23.4% patients, in 51.0% 1st degree, in 25.5% 2nd. After EChT in 45.9% determined 0 degree of toxicity, in 37.7% 1st and in 16.4% 2nd degree. The partial regression of tumor size observed in 55.3% and stabilization in 38.3%, but in 6.4% patients was progression. The regression was observed in all patients with HHE and in 10 from 23 patients with HCC. The toxicity results after EChT was better than after ChE, but partial regression was increase at EChT in comparison with ChE.

We made punction liver biopsy in 3–4 weeks after treatment in 31 with ChE and in 37 patients with EChT. The 1st stage of pathomorphose was turn out in 13 and 14 patients corresponded. The 2nd stage observed in 15 after ChE and in 19 after EChT.

Therefore analysis was shown that ChE is method of choice in the treatment of HHE. The methods ChE and EChT is more effective in PHC. So after treatment we can observed deep pathomorphose degrees in HCC and HHE.
Liver function correction in pancreatic cancer patients with obstructive jaundice

A. Yusupbekov
National Cancer Center, Tashkent, Uzbekistan

Purpose: To study the influence of conservative therapy in patients with pancreatic cancer complicated with obstructive jaundice.

Materials: A prospective analysis of liver function correction therapy using in 45 patients with pancreatic cancer complicated with obstructive jaundice T3–4N0–1 M0–1. Percutaneous decompression of the biliary tract was performed in 32 cases, retrograde stenting with plastic prosthesis in 13. Postoperative therapy included colloid plasma substitutes based on hydroxyethyl starch and protein emulsions with antibacterial and local hemostatic therapy background. In addition to improve the rheological properties of bile and liver function liver protectors and ursodeoxycholic acid medications were used.

Results: Relative hypovolemia and hypoproteinemia with hypercoagulation was observed on second postoperative day. Basically it is most clearly expressed in patients with baseline bilemia > 150 mkmol/l. In addition, hepatocytes autolysis with more than 2.5 times intracellular enzyme rates increasing was observed. The average volume of allocated bile via external drainage was 317 ml/day.

After therapy application there was a positive trend towards the normalization of enzymatic indicators. The average volume of allocated bile was increased up to 533 ml/day.

In the study of bile biochemical composition and viscosity was found that the viscosity of the bile completely normalized on the tenth postoperative day, but with the preservation of the relative unbalance between free and bound bilirubin components, which indicates the presenting of latent hepatic insufficiency.

Thus, our analysis shows the effectiveness of multicomponent conservative therapy in patients with pancreatic cancer after biliary tract decompression. Using of hepatotropic therapy improves the rheological properties of the bile and restores of hepatocyte functional activity.
The Doppler flow study for the assessment of the metabolic liver lesion

Grzegorz Zabielski, Marlena Broncel, Iwona Marczyk, Justyna Zabielska
Department of Internal Diseases and Clinical Pharmacology, Medical University, Łódź, Poland

Introduction: Physical examination, laboratory tests, 2D sonographic analysis are not fully sufficient for the diagnosis of the liver lesion. The Doppler flow study of the hepatic vessels gives new non-invasive possibilities for assessment of the liver, especially in NAFLD/NASH disease.

The aim of the study was the Doppler flow examination of the liver vessels in patients with documented metabolic liver lesion (hepatomegaly, intensive hyperechogenic liver, abnormal hepatic tests) and to compare results to healthy subjects.

Patients and methods: 40 patients suffering different metabolic disorders (diabetes mellitus, dyslipidemia, metabolic syndrome, toxins, obesity) were examined by Doppler method. Following parameters of liver vessels were analyzed: portal and arterial flow, relationships portal/arterial flow and portal flow/2D USG presentation, the spectrum of hepatic veins flow, presence of pathological flow. 40 healthy subjects were control group.

Results: We have observed numerous Doppler flow pathologies. The most useful for the grade of the liver abnormalities were: decreased portal flow and increased arterial flow, pathological relationship portal flow/2D USG presentation, lack of undulation of hepatic veins flow.

Comments: The prognostic importance for the assessment of the metabolic liver lesion has detection of periportal fibrosis. The liver biopsy remains essential method for diagnose it. The advanced fibrosis disturbs flow in hepatic vessels: decrease or reverse portal flow, pathological portal/arterial relationships, the formation of collaterals. The spectral Doppler of hepatic vessels flow allows to measure: portal flow velocity, systolic and diastolic hepatic artery flow, hepatic circulation index (systolic artery flow/portal flow), pulsatility or resistive indexes of hepatic artery, congestive index (portal vein surface/mean portal flow) and vascular index (portal flow/hepatic arterial pulsatility index). The considerable decrease of portal flow usually below 10 cm/s and/or hepatic circulation index above 3.5 may be used in patients with hyperechogenic liver as positive indicators for periportal fibrosis.

Conclusions:
1. Numerous Doppler flow disturbances give possibilities to assess the metabolic liver damage
2. The analysis by Doppler method relationship of portal and arterial flow of the liver, has the essential significance for the assessment of metabolic liver lesion
3. The Doppler flow should be analyzed as part of the duplex and triplex USG examination of the liver
4. The range of Doppler flow abnormalities is often independent from the grade of clinical and biochemical liver tests
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