Falk Workshop

Liver and Pancreatic Diseases: Consequences of Chronic Alcoholic Consumption

October 5 – 6, 2010
Freiburg, Germany

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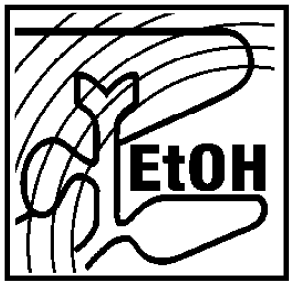
Poster Abstracts

Falk Workshop

IV Falk Gastro-Conference

LIVER AND PANCREATIC DISEASES: CONSEQUENCES OF CHRONIC ALCOHOLIC CONSUMPTION



Freiburg (Germany)
October 5 – 6, 2010

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Opening Session

Evolutionary origin and development of alcohol consumption

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It is suggested, that the evolution of animals including humans proceeded for millions of years in a world rich in drugs and that drug intake by mammals has always been an everyday occurrence. In fact, anecdotal reports of wild animals describe occasional drug intake, however, evidence for their everyday consumption as it occurs in humans with often devastating consequence is lacking. We discovered, that pentailed tree shrews *Ptilocercus lowii* show chronic high alcohol intake in their natural environment. This discovery was made in a primary tropical rainforest in West-Malaysia. The ecosystem is old and involves alcohol in the floral display of the bertam palm *Eugeissona tristis* and pentailed tree shrews feeding on it. We suggest that alcohol intake through sugar-rich plant products susceptible to fermentation like nectar, sap, and fruit was a recurrent, and maybe a constant, feature linking ancestral forms that lived thousands of generations ago with today's mammals. Under a long selective regime of natural alcohol challenges specific evolutionary responses can be expected. Some of the hidden mechanisms in nature's bigger-than-thought arsenal to counter adverse effects of drugs may eventually help combat human pathologies related to alcohol abuse.

System biology to understand alcohol dependent liver disease

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There is increasing recognition that alcoholic liver disease is best interpreted as a systemic disorder. Chronic alcohol use induces adaptations in the liver that help maintain tissue function, but affect the capacity to respond to other challenges, e. g. toxin exposure or viral infection. However, it is difficult to characterize in a comprehensive manner the systemic changes in the liver that affect the capacity to respond appropriately to such perturbations. The tools of systems biology developed over the past decade cast a wide net, but the massive amounts of experimental data obtained in high-throughput genomics or proteomics screens do not readily enhance understanding of the mechanistic underpinnings of disease conditions associated with chronic alcohol use. The challenge of systems biological analysis is to develop tools that can help characterize the nature of these adaptive changes and their impact on the tissue responses that leads to alcoholic liver disease.
We are developing approaches to characterize how transcriptomic regulatory dynamics is affected by alcohol intake. We use an animal model of chronic alcohol treatment and evaluate the response to an acute challenge of partial hepatectomy (PHx). Liver regeneration after PHx is a characteristic repair response that is deregulated by alcohol, thereby contributing to liver damage. Liver regeneration requires a large scale reorganization of tissue function, which helps hepatocytes emerge from quiescence and enter the cell cycle while maintaining liver-specific differentiated functions. A broad range of gene expression changes can be detected with characteristic temporal response patterns through the course of regeneration. Alcohol adaptation by itself involves relatively modest changes in gene expression profiles, but there are characteristic changes in the dynamic response patterns to PHx, which can be linked to key pathways in the liver regeneration response that are adversely affected.
The upstream transcriptional regulation that contributes to these changes in gene expression profiles can be analyzed through the identification of common response elements in the promoter regions of co-expressing clusters, indicating potential combinatorial interactions in the early dynamic response and helping to identify candidate mediators affected by adaptation to alcohol exposure. Additionally, microRNA expression profiles affected by alcohol contribute to the regulation of gene expression. Predicted target genes of specific microRNAs are of little evidence in the alcohol-adapted resting state, but appear in gene expression dynamics in response to the acute challenge of PHx. Thus, these and related approaches facilitate the task of identifying the mechanisms by which alcohol adaptation affects liver repair and regeneration.

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Session I

Clinical research

Genetics of pancreatitis: A guide for clinicians

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In 1896 Hans Chiari postulated that pancreatitis results from pancreatic autodigestion. An inappropriate conversion of pancreatic zymogens to active enzymes within the pancreatic parenchyma was proposed to initiate the inflammatory process. A key role has been attributed to the activation of trypsinogen to trypsin, converting all proteolytic proenzymes to their active form.
In 1996 a gain-of-function mutation in the cationic trypsinogen gene *(PRSS1)* has been identified as underlying defect in hereditary pancreatitis. In following studies, numerous other *PRSS1* alterations have been reported. These mutations are thought to lead to an enhanced intrapancreatic trypsinogen activation. Beside point mutations, a triplication of a ~605-kb segment containing *PRSS1* and *PRSS2* has been reported in families with hereditary pancreatitis. Thus, a gain of trypsin through a gene dosage effect may also contribute to the disease pathogenesis. In addition, a p.G191R variant in the anionic trypsinogen *(PRSS2)* gene has been described that mitigates intrapancreatic trypsin activity and thereby plays a protective role against chronic pancreatitis.
Since gain of function mutations in *PRSS1* leading to a "super trypsin" cause pancreatitis it was hypothesised that pancreatitis may also raised by "loss of function" mutations in pancreatic trypsin inhibitors such as *SPINK1*. In 2000, a p.N34S variant in *SPINK1* was associated with chronic pancreatitis (CP), mostly found in patients without a family history – 15–40% of patients with so-called idiopathic CP carry N34S on one allele or on both alleles. Thus, *SPINK1* mutations represent so far the strongest genetic risk factor in idiopathic CP.
In 1998 two studies described an association between CP and variants in *CFTR*, the gene mutated in cystic fibrosis. Albeit the association between *CFTR* and idiopathic CP is now well established, the pathogenic mechanisms are poorly understood. Subsequent studies analysing the complete *CFTR* coding sequence as well as *PRSS1* and *SPINK1* found that 25% to 30% carried at least one *CFTR* mutation, but few patients only were compound-heterozygous. Several CP patients, however, were trans-heterozygous for a *CFTR* alteration and a *SPINK1* or *PRSS1* variant, respectively, illuminating the significance of the combination of mutations in different genes in the disease pathogenesis.
Because trypsin degradation serves as a protective mechanism against pancreatitis, we hypothesised that loss of function in trypsin degrading enzymes increases the risk for pancreatitis. Recently it was demonstrated that chymotrypsin C (CTRC) degrades all human trypsin and trypsinogen isoforms with high specificity. We analysed *CTRC* in 1249 Germans affected with idiopathic, hereditary, or alcohol-related chronic pancreatitis and in more than 3000 German control subjects by DNA sequencing. Two alterations, p.R254W and p.K247_R254del, were significantly over-represented in the pancreatitis group (30/901 [3.3%] vs. 21/2,804 [0.7%]). A replication study on subjects with alcohol-related diseases identified these two variants in 10/348 (2.9%) individuals with CP but only in 3/432 (0.7%) subjects with liver disease. Functional analysis of the CTRC variants revealed impaired activity and/or reduced secretion. The data indicates that loss-of-function alterations in *CTRC* predispose to pancreatitis by diminishing its protective trypsin degrading activity.

Alcohol and pancreatic cancer: A pooled analysis of fourteen cohort studies

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More than 250,000 patients die each year of pancreatic cancer. Epidemiologic studies found a positive association with family history and cigarette smoking. There is an association with diabetes mellitus and chronic pancreatitis. The data are inconsistent for red meat, sugar, fat, body mass index, gallstones, and H. pylori. The protective effect of parity, dietary folate, aspirin, and statins has not been demonstrated. There is no evidence for linking alcohol or coffee consumption with an increased risk of pancreatic cancer.
The International Agency for Research on Cancer concluded that there was little evidence to support casual associations (1). Since these statements, similar data have been reported in the US Health Professionals Follow Up Study (2), The Nurses Health Study (2), The Japan Collaborative Cohort Study for Evaluation of Cancer Risk (3), and The Swedish Twin Registry Cohort Study. In addition other studies did not find an association of moderate alcohol consumption and pancreatic cancer (4,5).
However, The Iowa Women's Health Study, reported a nonsignificant positive association with increased alcohol intake (p trend = 0.11) and a doubling of the risk with an increased coffee consumption (RR, 2.2; 95% CI: 1.1–4.3) (6,5–9). Other have shown that very high alcohol consumption might contribute to pancreatic cancer development (10).
The mechanistic understanding of the effect of alcohol on the pancreas is limited. A direct toxic effect of alcohol on pancreatic acinar cells has been demonstrated in model systems. In addition alcohol induced changes have been shown on duct cells and pancreatic secretion. These toxic effects seem to be dose-related. Since pancreatitis develops in only a minority of alcoholics, additional cofactors have to be discussed to triggert this disease. Chronic pancreatitis is mostly induced by alcohol abuse. Chronic alcohol intake triggers a necrosis fibrosis sequence which in combination with increased gut permeability leads to a progressive acinar atrophy and fibrosis.
Chronic inflammation promotes cancer by a variety of mechanisms and inflammation orchestrates the tumor microenvironment. This has been shown fort he development of esophageal cancer in patients with gastrointestinal reflux and Barrett's esophagus, gastric cancer in patients with chronic gastritis, hepatocellular carcinoma in patients with chronic hepatitis, cholagniocarcinoma in patients with sclerosing cholangitis, colon cancer in patients with ulcerative colitis and pancreatitis cancer in patients with chronic pancreatitis. This process usually takes decades since multiple hits seem to be necessary to inactivate tumor suppressor genes and activate oncogenes to finally drive cancer development. The association between chronic pancreatitis and pancreatic cancer is not very strong since patients with long standing chronic pancreatitis have a high morbidity and mortality. In fact in almost all studies more than 90% of patients with chronic pancreatitis die not from pancreatic cancer. Moreover most cases of pancreatic cancer develop in patients without clinical evidence of chronic pancreatitis.

In summary, moderate alcohol consumption is not linked to pancreatic cancer but a risk factor of chronic pancreatitis. Chronic pancreatitis being a risk factor for pancreatic cancer has been overextimated.

References:

1. International Agency for Research on Cancer. Alcohol drinking. IARC Monogr Eval Carcinog Risks Hum. 1988; 44: 1–378.

2. Michaud D, Giovannucci E, Willett W, et al. Coffee and alcohol consumption and the risk of pancreatic cancer in two prospective United States Cohorts. Cancer Epidemiol Biomarkers Prev. 2001; 10: 429–437.

3. Lin Y, Tamakoshi A, Kawamura T, et al. Risk of pancreatic cancer in relation to alcohol drinking, coffee consumption and medical history: findings from the Japan Collaborative cohort study for evaluation of cancer risk. Int J Cancer. 2002; 99: 742–746.

4. Silverman DT. Risk factors for pancreatic cancer: a case-control study based on direct interviews. Teratog Carcinog Mutagen. 2001; 21: 7–25.

5. Brown LM. Epidemiology of alcohol-associated cancers. Alcohol. 2005; 35: 161–168.

6. Harnack L, Anderson K, Zheng W, et al. Smoking, alcohol, coffee, and tea intake and incidence of cancer of the exocrine pancreas: The Iowa Woman's Health Study. Cancer Epidemiol Biomarkers Prev. 1997; 6: 1081–1086.

7. Ye W, Lagergren J, Weiderpass E, et al. Alcohol abuse and the risk of pancreatic cancer. Gut. 2002; 51: 236–239.

8. Talamini G, Bassi C, Falconi M, et al. Alcohol and smoking as risk factors in chronic pancreatitis and pancreatic cancer. Dig Dis Sci. 1999; 44: 1303–1311.

9. Harnack L, Anderson K, Zheng W, et al. Smoking, alcohol, coffee, and tea intake and incidence of cancer of the exocrine pancreas: The Iowa Woman's Health Study. Cancer Epidemiol Biomarkers Prev. 1997; 6: 1081–1086.

10. Hart AR, Kennedy H, Harvey I. Pancreatic cancer: a review of the evidence on causation Clin Gastroenterol Hepatol. 2008; 6: 275–282.

Long-term, clinical follow-up in fatty liver patients

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The first and most predictable hepatic change attributable to alcohol is development of large droplet (macrovesicular) steatosis (fatty change, fatty liver). This characteristically is most prominent in centrilobular regions, but in more severe cases may involve the entire lobule. Lipid vacuoles occupy much of the hepatocyte cytoplasm, pushing the nucleus and other organelles to the periphery of the cell. The lipid vacuoles comprise triglycerides, fatty acids, monoglycerides, and diglycerides. A minor component of the alcoholic fatty liver may be small droplet (microvesicular) steatosis. The condition is histologically indistinguishable from Non-alcoholic-fatty-liver-disease (NAFLD). It is therefore important to have reliable informations on alcohol consumption in studies dealing with fatty liver disease. A Danish cohort of 240 patients with histological verified alcoholic fatty liver disease (AFLD) without signs of alcoholic hepatitis were followed for a mean period of 12.8 years. Patients with an alcohol intake above the limits set by The Danish National Board of Health (21 drinks per week for men (1 drink = 12 g alcohol) and 14 drinks per week for women) or an alcohol-related diagnosis according to the International Classification of Diseases (ICD-8 and ICD-10) at any time from biopsy until 31 December 1999 were considered to have alcoholic fatty liver. All surviving patients were contacted in February 2003 and invited to attend a clinical follow-up visit. In the AFLD group, 54 patients (22%) developed cirrhosis, all prior to clinical follow-up. A significantly higher prevalence of cirrhosis was observed in female alcoholic patient and time to cirrhosis was significantly associated with female gender, but not with histological parameters, although an insignificant increase in the risk of cirrhosis development with the degree of fatty liver was observed. The AFLD cohorte was compared to a cohorte of NAFLD patients, and the comparison showed a highly significant excess in death rate ($p < 0.001$) in the AFLD group, mainly attributable to liver disease or other alcohol-related diseases.

Session II

Pathomechanisms of alcohol induced damage (Part I)

Innate immunity and pathogenesis of alcohol dependent liver injury

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The development of alcoholic liver disease (ALD) is a complex process involving both parenchymal and non-parenchymal cells resident in the liver, as well as innate and adaptive immune pathways. The contribution of innate immunity to ALD is mediated, at least in part, by an increased sensitivity of hepatic macrophages (Kupffer cells) to activation by TLR-4 ligands. Recent evidence in mouse models has also implicated the complement system in the pathogenesis of ALD. However, it is not yet known whether ethanol activates complement via the classical, lectin and/or alternative pathways. C1q, the recognition subunit of the first complement component, associates with apoptotic cells and thereby initiates complement activation. Since ethanol exposure increases hepatocellular apoptosis, we hypothesized that ethanol-induced apoptosis acts as a signal to activate complement via C1q. If C1q were required for ethanol-induced activation of complement, then *C1q-/-* mice should be resistant to ethanol-induced complement activation, and, as a consequence, protected from ethanol-induced liver injury. In recent studies, we have found that ethanol exposure activates C1q and is required for the activation of complement by ethanol. These studies therefore demonstrate that C1q and the classical pathway of complement activation contribute to the development of ethanol-induced liver injury.

TGF-β as amplifier of adverse alcohol effects

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The damaging effect of alcohol is mediated via complex interactions between liver cells involving oxidative/non-oxidative metabolism, fat deposition and increased production of fibrogenic cytokines, among which TGF-β plays pivotal roles. Accordingly TGF-β is elevated in serum of patients with alcoholic liver disease and TGF-β signaling is increased in fibrotic areas of the liver in these patients. Hepatocytes are the predominant liver cell type and are strongly responsive to TGF-β. We therefore analyzed possible interactions between ethanol intoxication and TGF-β signalling in hepatocytes.
Ethanol and TGF-β, both display a dose-dependent cytotoxic effect on hepatocytes as shown by release of LDH and ROS measurements and this was further increased by combined treatment. Microarray data implicate that TGF-β regulates the expression of genes involved in fibrogenesis as well as lipid-, and oxidative stress metabolism. We found that TGF-β decreases expression and activity of alcohol dehydrogenase 1 (ADH1) and thereby augmented a more toxic route of alcohol metabolism. Downregulation of ADH1 was confirmed *in vivo* in a transgenic mouse over-expressing active TGF-β. The mild cytotoxic effect of ethanol or TGF-β alone was strongly enhanced when both substances were added simultaneously to mouse hepatocytes, resulting in strong apoptosis with cytochrome-C release from mitochondria and activation of caspases 9 and 3. Ethanol (or the combination of ethanol and TGF-β) reduced basal activity of p-Akt accompanied by increased TGF-β receptor type II (TβRII) expression. Blunting PI3K with small molecule inhibitor LY294002 (40 μM) similarly led to increased TβRII expression, indicating a functional link between loss of activated Akt and TβRII expression. Further, acute treatment of mice with alcohol also resulted in upregulation of TβRII expression in liver lysates, suggesting *in vivo* relevance of the finding. TGF-β alone had contrary effects towards hepatocytes, that is increasing pAkt levels and reducing TβRII expression, which might explain why TGF-β alone does not induce apoptosis. It remains to be investigated how pAkt suppresses TβRII expression.
In summary, ethanol decreased phospho-Akt levels in hepatocytes leading to increased TβRII expression and strongly enhanced TGF-β dependent apoptosis. Our results further show that TGF-β acts as pro-steatotic cytokine in ALD by down-regulating the ethanol metabolizing enzyme ADH1, which augments a more toxic route of alcohol metabolism leading to increased fat deposition and ROS formation. Our findings suggest that in hepatocytes exists an intracellular cross-talk between ethanol and TGF-β which most likely contributes to enhanced organ damage.

Induction of crosslinking and silencing of Sp1 by transglutaminase during liver injury in ASH and NASH via different ER stress pathways

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Alcoholic steatohepatitis (ASH) and non-alcoholic steatohepatitis (NASH) share many histological similarities, including accumulated fat, hepatic apoptosis and fibrous tissues in the liver, but the molecular mechanisms responsible for hepatic apoptosis remain unclear. We previously reported that transglutaminase 2 (TG2), a protein crosslinking enzyme, is induced in the nucleus of ethanol-treated hepatocytes, and crosslinks and inactivates a general transcription factor Sp1, which eventually leads to reduced expression of c-Met and caspase-independent hepatic apoptosis (Tatsukawa et al. *Gastroenterology* 2009; 136: 1783–1795). In this study we investigated, if a similar change might be observed also in NASH and if yes, how TG2 and cross-linked Sp1 (CLSp1) would be induced in NASH and ASH. We obtained elevated nuclear TG2 and CLSp1 formation in NASH patients, as well as in HepG2 cells treated with free fatty acids (FFAs). Biochemical analyses on this culture model revealed that both ethanol and FFAs provoked fat accumulation, endoplasmic reticulum (ER) stress, increased nuclear factor kappa B (NFκB) and nuclear TG2, but the synergistic effect was not obvious between FFA and ethanol. However, salubrinal, a selective inhibitor against dephosphorylation of eukaryotic initiation factor-2α in ER stress-induced pancreatic ER kinase (PERK) signal pathway, inhibited NFκB activation, nuclear TG2 expression and apoptosis only induced by FFAs, but not those induced by ethanol. Moreover, FFAs-induced apoptosis accompanied increased or released apoptosis inducing factor (AIF) and cytochrome *c*, which was reduced with decreased TG2 by salubrinal, insuring the PERK-TG2 dependency. These results suggest that FFA and ethanol may increase ER stress and lead to nuclear NFκB activation and TG2 induction through respectively distinctive pathways, leading to TG2-mediated apoptosis via crosslinking and inactivation of Sp1 and reduction in c-Met.

Special Lecture I

New advances in cell physiology and pathophysiology of the exocrine pancreas

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Numerous scientific groups studied the mechanisms of pancreatic digestive enzyme synthesis and secretion by the acinar cell and the regulation of pancreatic enzyme and bicarbonate secretion. I would like to especially mention the groups of John A. Williams and Ole H. Petersen. The pancreas synthesizes and secretes digestive enzymes and secretes HCO_3. Both are necessary for normal digestion. The pancreas is stimulated acutely by feeding via neuronal mechanisms. The vagus mediates a small cephalic phase. Vagal afferents are important. There are vagal-vagal reflexes. Acinar cells have M1 and M3 receptors. There may be some direct innervation of the pancreas from the gut. Acetylcholine is the main transmitter and stimulates acini, ducts, and islets. Norepinephrine is the main transmitter for the stimulation of islets and blood vessels. CCK is a peptide hormone of the gastrointestinal system and brain. It is synthesized by I-cells in the mucosa of small intestine and secreted from the first segment of the small intestine. Its release from I-cells is stimulated by oligopeptides, certain amino acids (phenylalanine), and fatty acids. Probably CCK releasing peptides present in pancreatic secretions and duodenal mucosa are involved. According to new data CCK-receptors exist on human acinar cells not only in rodents. Thus, CCK may stimulate enzyme secretion not only indirectly by acting on CCK-B-receptors on intrapancreatic nerves which release acetylcholine but also directly. The group of OH Petersen could demonstrate that CCK-8 and human CCK-58 at physiologic concentrations (1–20 pmol/L) caused rapid, oscillatory increases of the cytosolic Ca^{2+} ion concentration, showing apical to basal progression, in acinar cells from 14 patients with unobstructed pancreata. The cytosolic Ca^{2+} ion concentration increases were followed by increases in mitochondrial ATP production and secretion. Secretin is the major stimulant of HCO_3 secretion.
Secretion is probably terminated by exit of food from upper small intestine, the so called ileal brake. PYY is one of probably several hormones and neurotransmitters involved in the ileal brake. Whether negative feedback inhibition via destruction of CCK releasing peptides by trypsin exists in humans, is still discussed controversially.

In stimulus secretion coupling acetylcholine stimulates muscarinic receptors followed by the cascades in which phospholipase C, diacylglycerol, inosito trisphosphate (IP3), calcium release from the endoplasmatic reticulum, stimulation of protein kinase C etc. are involved. At least in rodents there are further membrane receptors on the acinar cell involved in enzyme secretion such as VIP, bombesin, CCK, secretin. VIP and secretin stimulate cyclic-AMP formation which leads to stimulation of protein kinase A. According to JA Williams et al. small GTP-binding proteins are molecular switches in the regulation of numerous cellular processes, including vesicular transport. The biochemical steps which lead to fusion of enzyme granules with the apical cell membrane are still not fully clarified. Proteins such as Rab3D, Rab27B, and Rap1 are present on the granule

membranes and are involved in the steps leading up to exocytosis. Rho family small G proteins such as RhoA and Rac1 regulate secretion through remodelling of the actin cytoskeleton.
Secretin stimulates via its own receptor cAMP formation which in turn is responsible that finally bicarbonate is secreted by the cystic fibrosis transmembrane conductance regulator (CFTR).
Besides stimulation of secretion, CCK is like insulin involved in stimulation of protein synthesis. Numerous proteins such as kinases, proteases etc. are involved in these cascades from the receptor to the DNA and finally mRNA such as PI3-1, Akt/PKB, mTOR-Complex 1.
The discovery of a mutation of the cationic trypsinogen gene (PRSS1) in patients with hereditary chronic pancreatitis supported Hans Chiari's theory that chronic pancreatitis is the result of autodigestion of the pancreas. A genetic basis for chronic pancreatitis had to be assumed in a pedigree described by Comfort and Steinberg already in 1952 it needed additional 44 years to identify this first genetic association. Thereafter, research had its focus on proteases and anti-proteases that are assembled in the digestive enzyme cascade, an approach that identified serine protease inhibitor, Kazal type 1 (SPINK1) as another pancreatitis gene. Aside the digestive enzyme cascade, CFTR that is responsible for cystic fibrosis, was investigated. An enrichment of CFTR variants in patients with chronic pancreatitis has been found. Further aspects in the field of genetics in chronic pancreatitis emerged in the last years. A variant of anionic trypsinogen (PRSS2) was found to be overrepresented in controls and protects against chronic pancreatitis. Additionally, variants of the calcium sensing receptor (CASR) seem to influence the pathogenesis in SPINK1 p.N34S carriers. Triplication and duplication of the trypsinogen locus represents a completely new disease causing mechanism that predisposes to chronic pancreatitis by a so-called gene dosage effect. Identification of chymotrypsin C (CTRC) by our group in collaboration with H. Witt and M. Sahin-Toth displayed another reasonable candidate gene. Investigations of CTRC found low penetrance loss of function variants that diminish secretion and/or activity of CTRC and thereby contribute to the development of the disease. Taken together recent data further support the importance of a balanced digestive enzyme cascade in that trypsin captures a key role. According to the findings of O.H. Petersen et al. toxic Ca^{2+} signals generated by excessive liberation of Ca^{2+} from both the endoplasmic reticulum and the secretory granules are involved in the pathogenesis of pancreatitis.

Special Lecture II

Animal models of alcohol-induced liver disease

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The risk of alcohol-induced liver disease (ALD) increases dose- and time-dependently with consumption of alcohol. ALD ranks among the major causes of morbidity and mortality in the world, and affects millions of patients worldwide each year. Progression of the disease is well characterized and is actually a spectrum of liver diseases, which ranges initially from simple steatosis, to inflammation and necrosis (steatohepatitis), to fibrosis and cirrhosis. Although the progression of alcohol-induced liver injury is well characterized, there is no universally-accepted therapy available to halt or reverse this process in humans. With better understanding of the mechanism(s) and risk factors that mediate the initiation and progression of this disease, rational targeted therapy can be developed to treat or prevent it in the clinics. Several models for experimental ALD exist, including nonhuman primates, micropigs and rodents. However, owing to the prodigious costs of the more complex (albeit, arguably more relevant) models of ALD, most researchers employ rodent models of ALD. Enteral models of alcohol delivery that bypass rodents' natural aversion to alcohol-containing liquids have also increased the severity of damage that alcohol causes in these species. Furthermore, the advent of genetically modified strains of rodents (e.g., 'knockout' mice) have increased the specificity of the hypotheses that can be directly tested. Based on these models systems, several plausible hypotheses to explain the mechanism(s) by which alcohol leads to liver damage have been proposed, including consequences of alcohol metabolism (e.g., acetaldehyde production and CYP2E1 induction), oxidative/nitrosative stress, altered inflammatory responses (i.e., 'priming'), and increased sensitivity to cytotoxic stimuli (i.e., 'sensitization'). Indeed, several genetic/pharmacologic modulations that focus on these general mechanisms have been shown to blunt the progression of experimental ALD. These studies have also identified candidate genes for polymorphism studies to explain potential increased genetic risk in some individuals. However, despite significant advances in our understanding of the mechanisms by which ALD develops based on studies with these models, this work has yet to translate to a viable therapy for ALD in the clinics. This talk will also discuss potential reasons for these limitations to date and suggest future prospects to improve the translational utility of modeling ALD.

Session III

Pathomechanisms of alcohol induced damage (Part II)

Gut-liver axis and sensing microbes

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Increasing evidence suggests that in healthy individuals, maintenance of homeostasis between gut microbiota and the host plays an important role in health and disease. It is estimated that there are multiple times more microbial cells in the gut than the total number of cells in the human body. Microbes in the gut play important roles in digestion of food, vitamins and shaping immunity. Changes in the composition of the microbiome or alterations in gut permeability, however, can promote translocation of microbes into the portal circulation that delivers blood directly to the liver. Thus, the liver is constantly exposed to varying extent of gut-derived microbes and microbial products. Previous studies showed that one of the roles of the liver is “detoxification” of gut-derived toxins and microbial products. While the full repertoire of gut-derived microbial products that reach the liver in health and disease is yet to be explored, the levels of bacterial lipopolysaccharide (LPS), a component of Gram-negative bacteria, is increased in the portal and/or systemic circulation in several conditions that lead to liver disease. Increased gut permeability and LPS plays a role in alcoholic liver disease where alcohol impairs the gut epithelial integrity through alterations in tight junction proteins. In addition, non-alcoholic fatty liver disease is also associated with increased serum endotoxin levels where activation of the pro-inflammatory cascade plays a central role in disease progression.

Pathogen-associated molecular patterns (PAMPs) represent danger signals sensed by the host through pattern recognition receptors. Toll-like receptors, expressed on the cell surface or in the endosomes, are specific for recognition of bacterial, viral or fungal pathogens and induce a coordinated cascade of signal transduction events to stimulate pro-inflammatory cytokine and/or type I interferon production. TLR4, a receptor recognizing LPS, is expressed in the liver not only in Kupffer cells and cells of the immune system but also on hepatocytes, stellate cells, endothelial and biliary epithelial cells. Thus, the overall effects of gut-derived LPS on the liver is determined not only by the activation of the individual cell types by LPS but also by the coordinated interaction between the various cell types residing in the liver. Increasing evidence suggests that TLR4-mediated signaling via the MyD88-dependent or MyD88-independent pathways may play different roles in liver diseases associated with increased LPS exposure of the liver as a result of gut permeability. For example, in alcoholic liver disease, the MyD88-independent, IRF3-dependent TLR4 cascade plays a role in steatosis and inflammation. Recent evidence suggests that the contribution of hepatocyte-specific IRF3 expression might be different from the role of IRF3 in immune cells in alcoholic liver disease. In contrast, our data suggest that non-alcoholic liver disease is attenuated in TLR4- or MyD88-deficient mice suggesting a role for the TLR4/MyD88 signaling events in this liver disease. Thus, understanding the cell-specific recognition events in sensing gut-derived microbes will help to better understand the role of gut-liver axis in liver disease and health.

Zinc and alcoholic liver disease

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Zinc is the second most abundant trace element in the body next to iron. Zinc participates in cell functions mainly through binding to a large number of zinc proteins, including metalloenzymes. Zinc coordination has catalytic, structural or regulatory roles in zinc proteins, and the removal of zinc can exert deleterious effects on cell function. Zinc deficiency is one of the most consistent nutritional/biochemical observations in alcoholic liver disease (ALD), but the mechanistic link between zinc deficiency and ALD has not been well defined. The objectives of our research are to determine if zinc deficiency is a causal factor in the development of ALD, and how alcohol interferes with cellular zinc homeostasis. Long term alcohol feeding mouse model and hepatoma cell culture model were used in our studies. Chronic alcohol feeding caused a significant decrease in hepatic zinc levels in association with pathological changes, including lipid accumulation, neutrophil infiltration and hepatocyte injury. Dietary zinc supplementation normalized alcohol-induced zinc depletion and attenuated the pathological changes in the liver. Several mechanisms were involved in zinc action against alcoholic cytotoxicity. Alcohol exposure reduced cellular antioxidant capacity and induced lipid peroxidation, which was prevented by zinc supplementation. Alcohol exposure caused a shift of alcohol metabolic pathway from alcohol dehydrogenase (ADH) to cytochrome P4502E1 (CYP2E1), and zinc supplementation corrected the imbalance between ADH and CYP2E1. Alcohol caused apoptotic cell death via up-regulation of TNF-α receptor- and Fas-mediated pathways, which were attenuated by zinc supplementation. Lipid accumulation in the liver (alcoholic steatosis) is one of the earliest pathological changes in the progression of ALD. Hepatocyte nuclear factor 4-α (HNF-4α) and peroxisome proliferators activated receptor-α (PPAR-α) are zinc finger transcription factors, which play a major role in the regulation of lipid metabolism in the liver. Alcohol exposure inactivated both HNF-4α and PPAR-α in association with the inhibition of hepatic secretion of low density lipoproteins (VLDL). Zinc supplementation not only normalized hepatic VLDL secretion, but also accelerated fatty acid β-oxidation. Hepatic genes related to fatty acid β-oxidation and VLDL secretion were up-regulated by zinc supplementation, which was accompanied by restoration of alcohol-inhibited DNA binding activity of HNF-4α and PPAR-α. Hepatoma cell cultures showed that zinc deprivation significantly suppressed the DNA binding activities of HNF-4α and PPAR-α, and reduced HNF-4α and PPAR-α target proteins. Consequently, zinc deprivation caused lipid accumulation. Knockdown of HNF-4α in hepatoma cells resulted in lipid accumulation and inhibition of cell growth in association with the down-regulation of genes related to lipid metabolism and cell growth. To determine how alcohol exposure interferes with zinc homeostasis, gene expression of zinc transporters was measured. Alcohol exposure down-regulated a large number of zinc transporter genes which act for positive cellular and organelle zinc balance, including ZnT3, ZnT4, ZnT5, ZnT6, ZnT10, Zip1, Zip8, and Zip9. However, alcohol exposure did not affect ZnT1, the major zinc transporter gene for negative cellular zinc balance. These results suggest that alcohol exposure interferes with hepatic zinc homeostasis, leading to cellular zinc deprivation. Inactivation of zinc proteins due to zinc deprivation is likely an important molecular mechanism underlying the pathogenesis of ALD.

The role of the endocannabinoid system in alcoholic liver disease

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Hepatic fibrosis is the response of the liver to chronic liver injury of various etiologies including alcoholic liver disease viral hepatitis, non-alcoholic steatohepatitis and autoimmune diseases. The development of hepatic fibrosis is associated with portal hypertension, progression to hepatic cirrhosis, liver failure and high incidence of hepatocellular carcinoma (1). Although the main mechanisms of fibrogenesis are independent of the etiology of liver injury, alcoholic liver fibrosis is distinctively characterized by a pronounced inflammatory response due to elevated gut-derived endotoxin plasma levels, an augmented generation of oxidative stress with pericentral hepatic hypoxia and the formation of cell-toxic and profibrogenic ethanol metabolites (e.g. acetaldehyde or lipid oxidation products) (2). These factors, based on a complex network of cytokine actions, together result in increased hepatocellular damage and activation of hepatic stellate cells, the key cell type of liver fibrogenesis (2). Recent evidence suggests that the endocannabinoid system is a signaling system that also plays an important role in the pathogenesis of alcoholic liver disease. This signaling system consists of mainly two G-coupled receptor types (cannabinoid receptor 1 and 2; CB1, CB2), several arachidonic acid-derived lipid-like ligands (endocannabinoids, e.g. arachidonoyl-ethanolamine or 2-arachidonoyl-glycerol) and different production and degradation enzymes (3) Generally, the hepatic endocannabinoid system becomes activated under pathophysiological conditions on ligand-, receptor- and metabolic levels. Hepatic cell populations become highly responsive to the effects of endocannabinoids and modulation of the endocannabinoid system affects proliferation and cell death of fibrogenic cell types such as hepatic stellate cells, and the development of hepatic fibrosis (4–9). The crucial role of the endocannabinoid system in hepatic fibrosis has been highlighted by recent genetic and pharmacologic approaches that targeted CB1 and CB2 receptors. Interestingly, CB1 and CB2 exert opposing effects on fibrogenesis suggesting that the endocannabinoid system regulates both pro- and antifibrogenic responses in the liver (4, 9). CB1-deficient mice showed a strong decrease in fibrogenesis induced by CCL_4, thioacetamide or bile duct ligation (9), whereas CB2-deficient mice displayed an increase in fibrogenesis following CCL_4 treatment (4). The pro-fibrogenic function of the CB1 receptor in the liver is further emphasized by pharmacologic studies in which the CB1 inhibitor rimonabant reduced profibrogenic markers such as TGF-β1 and α-SMA as well as histological fibrosis (9). These findings could be confirmed by our group in an animal model of chronic alcohol administration comparing CB1 and CB2 knockout versus wild type mice (11). After 7 months of 16% ethanol solution as the only fluid source, we found that CB1 signaling aggravated hepatic steatosis and fibrogenesis whereas CB2 protected the liver from alcoholic liver injury (11). Louvet et al. also suggested a protective role of CB2 in alcoholic liver disease (12). Similar results could be demonstrated by Jeong et al., since global or hepatocyte-specific CB1 knockout mice were resistant to ethanol-induced steatosis (10). Ethanol feeding increased the hepatic expression of CB1 receptors in wild-type mice and upregulated the endocannabinoid 2-AG and its biosynthetic enzyme diacylglycerol lipase beta selectively in hepatic stellate cells (10). Moreover, the pro-fibrogenic CB1-receptor becomes up-regulated in alcoholic liver disease by acetaldehyde (13). Thus, the hepatic endocannabinoid system might offer options for therapeutic exploitation not only for liver disease in general, but also for alcoholic liver disease.

References:

1. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest. 2005; 115: 209–218.

2. Siegmund SV, Brenner DA. Molecular pathogenesis of alcohol-induced hepatic fibrosis. Alcohol Clin Exp Res. 2005; 29: 102S–109S.

3. Di Marzo V, Bifulco M, De Petrocellis L. The endocannabinoid system and its therapeutic exploitation. Nat Rev Drug Discov. 2004; 3: 771–784.

4. Julien B, Grenard P, Teixeira-Clerc F, Van Nhieu JT, Li L, Karsak M, Zimmer A, et al. Antifibrogenic role of the cannabinoid receptor CB2 in the liver. Gastroenterolgy. 2005; 128: 742–755.

5. Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Batkai S, Harvey-White J, et al. Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. J Clin Invest. 2005; 115: 1298–1305.

6. Siegmund SV, Uchinami H, Osawa Y, Brenner DA, Schwabe RF. Anandamide induces necrosis in primary hepatic stellate cells. Hepatology. 2005; 41: 1085–1095.

7. Siegmund SV, Seki E, Osawa Y, Uchinami H, Cravatt BF, Schwabe RF. Fatty acid amide hydrolase determines anandamide-induced cell death in the liver. J Biol Chem. 2006; 281: 10431–10438.

8. Siegmund SV, Qian T, de Minicis S, Harvey-White J, Kunos G, Vinod KY, Hungund B, et al. The endocannabinoid 2-arachidonoyl glycerol induces death of hepatic stellate cells via mitochondrial reactive oxygen species. Faseb J. 2007; 21: 2798–2806.

9. Teixeira-Clerc F, Julien B, Grenard P, Tran Van Nhieu J, Deveaux V, Li L, Serriere-Lanneau V, et al. CB1 cannabinoid receptor antagonism: a new strategy for the treatment of liver fibrosis. Nat Med. 2006; 12: 671–676.

10. Jeong WI, Osei-Hyiaman D, Park O, Liu J, Batkai S, Mukhopadhyay P, Horiguchi N, et al. Paracrine activation of hepatic CB1 receptors by stellate cell-derived endocannabinoids mediates alcoholic fatty liver. Cell Metab. 2008; 7: 227–235.

11. Trebicka J, Racz I, Siegmund SV, Cara E, Granzow M, Klein S, Wojtalla A, Hennenberg M, Huss S, Fischer P, Zimmer A, Sauerbruch T. Role of cannabinoid receptors in alcoholic hepatic injury: Steatosis and fibrogenesis are increased in CB2 deficient mice and decreased in CB1 knockouts. Submitted 2010.

12. Louvet A, Teixeira-Clerc F, Deveaux V, Chobert MN, Pavoine C,. Mallat A, Lotersztajn S. Beneficial effects of cannabinoid receptor 2 on alcoholic liver disease. J Hepatol. 2010; 52 (Suppl 1): S307–S308.

13. Patsenker E, Wissniowski T, Millonig G, Mueller S, Stoll M, Ocker M, Seitz HK, Stickel F. Cannabinoid receptor type 1 expression modulates alcohol-induced liver fibrosis: Role of acetaldehyde. J Hepatol. 2010; 52 (Suppl 1): S376.

MnSOD overexpression prevents liver mitochondrial DNA depletion after an alcohol binge, but worsens this effect after prolonged alcohol consumption in mice

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Background andaims: There are two types of alcoholism, either excessive amounts of alcohol ingested daily, or frequent and chronic episodes of binge drinking separated by short periods of relative temperance. Both types of alcohol consumption increase reactive oxygen species (ROS) formation and lipid peroxidation, whose products can damage mitochondrial DNA (mtDNA), alter mitochondrial function and damage the liver. A possible role of manganese superoxide dismutase (MnSOD) on these effects has not been investigated.

Methods: To test whether MnSOD overexpression modulates **acute** alcohol-induced mtDNA lesions and mitochondrial alterations, transgenic MnSOD-overexpressing ($MnSOD^{+++}$) mice, heterozygous knockout ($MnSOD^{+/-}$) mice and their wild type (WT) littermates were given a single intragastric ethanol dose (5 g/kg) and sacrificed 2 or 24 hours later. Other groups of these mice were given ethanol in their drinking water for 7 weeks to test whether MnSOD modulates also **chronic** alcohol-induced mtDNA lesions.

Results: A single **high dose** of alcohol administration further increased MnSOD activity in $MnSOD^{+++}$ mice, but further decreased it in $MnSOD^{+/-}$ mice. In WT mice, this acute alcohol administration transiently increased mitochondrial ROS formation, decreased mitochondrial glutathione, depleted and damaged mtDNA, and decreased complex I and V activities; alcohol durably increased inducible nitric oxide synthase (NOS) expression, plasma nitrites/nitrates and the nitration of tyrosine residues in complex V proteins. These effects were prevented in $MnSOD^{+++}$ mice and prolonged in $MnSOD^{+/-}$ mice. In alcoholized WT or $MnSOD^{+/-}$ mice, mtDNA depletion and the nitration of tyrosine residues in complex I and V proteins were prevented or attenuated by co-treatment with tempol, a superoxide scavenger, L-NAME and 1400W, two NOS inhibitors, or uric acid, a peroxynitrite scavenger.
Chronic alcohol consumption further increased the activity of MnSOD in $MnSOD^{+++}$ mice, but decreased cytosolic glutathione as well as cytosolic glutathione peroxidase activity and peroxisomal catalase activity. Whereas chronic ethanol increased cytochrome P-450 2E1 and mitochondrial ROS generation in both WT and $MnSOD^{+++}$ mice, hepatic iron, lipid peroxidation products and respiratory complex I protein carbonyls were only increased in ethanol-treated $MnSOD^{+++}$ mice but not in WT mice. In chronic ethanol-fed $MnSOD^{+++}$ mice, but not in chronic ethanol-fed WT mice, mtDNA was depleted, and mtDNA lesions blocked the progress of polymerases. The iron chelator, DFO prevented hepatic iron accumulation, lipid peroxidation, protein carbonyl formation and mtDNA depletion in chronic alcohol-treated $MnSOD^{+++}$ mice. Alcohol markedly decreased the activities of complexes I, IV and V of the respiratory chain in $MnSOD^{+++}$, with absent or lesser effects in WT mice.

Conclusions: MnSOD over-expression prevents, and MnSOD deficiency prolongs mtDNA depletion after an acute alcohol binge in mice. However, MnSOD over-expression aggravates the effects of prolonged alcohol consumption, which selectively triggers iron accumulation in $MnSOD^{+++}$ mice but not in WT mice.
In the model of acute alcohol binge, the protective effects of MnSOD, tempol, NOS inhibitors and uric acid point out a role of the superoxide anion reacting with NO to form mtDNA-damaging peroxynitrite. In the model of prolonged ethanol consumption, the protective effects of DFO suggested the role of iron reacting with hydrogen peroxide to form mtDNA-damaging hydroxyl radical.

Keywords: mtochondria; liver; alcohol; oxidative stress; manganese superoxide dismutase, mitochondrial DNA

Investigating the pathobiology of alcoholic pancreatitis

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Alcohol abuse is one of the most common causes of pancreatitis. The risk of developing alcohol-induced pancreatitis is related to the amount and duration of drinking. However, only a small portion of heavy drinkers develop disease indicating that other factors contribute to disease initiation. Epidemiologic studies suggest roles for cigarette smoking and dietary factors in the development of alcoholic pancreatitis. The mechanisms underlying alcoholic pancreatitis are starting to be understood while there is little known about the how smoking and dietary factors influence disease pathogenesis. Studies from animal models are revealing that alcohol sensitizes the pancreas to key pathobiologic processes that are involved in pancreatitis. Current studies are focused on investigating the mechanisms responsible for the sensitization effect of alcohol and are revealing disorders in organelles including endoplasmic reticulum and mitochondria. As our understanding of alcohol's effects continue to advance to the level of molecular details, insights into potential therapeutic strategies will emerge providing opportunities for clinical benefit.

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Alcoholic and non-alcoholic steatohepatitis: Pathophysiological similarities, differences and synergisms

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The pathophysiological significance of hepatic lipid accumulation in the absence of significant alcohol consumption is increasingly recognized. Thus, non-alcoholic fatty liver disease (NAFLD) is now considered the most common cause of liver enzyme elevations in Western countries. It is regarded the hepatic manifestation of the metabolic syndrome, characterized by central obesity and insulin resistance, and resulting diabetes type 2, dyslipidemia and hypertension. Similarly to alcoholic liver disease, NAFLD encompasses mild hepatic steatosis to steatohepatitis (NASH) with significant necroinflammation and progressive fibrosis, and, in its advanced form, believed to account for a large fraction, if not entirely for what was previously termed "cryptogenic cirrhosis".
Still, there are only few NAFLD patients who entirely abstain from drinking while the “obese drinker” is clearly more frequent. Furthermore several clinical studies suggest a strong causative link between the consumption of alcohol and progressive liver disease in individuals with high fat intake and/or diabetes. However, it is incompletely understood how alcohol and obesity interact and whether the combined effects on the progression of liver injury are additive or synergistic.
Since alcohol is predominantly metabolized in hepatocytes, which also accumulate dietary lipids, interactions between alcohol- and lipid-metabolism are very likely. Actually, in vitro and in vivo models indicate synergistic effects of alcohol and (dietary) lipids on the generation of oxidative stress and secretion of proinflammatory and profibrogenic factors by hepatocytes.
In addition to direct effects on hepatocytes/the liver, the view has to be expanded to other organs to understand the pathophysiological mechanisms involved in hepatocellular injury caused by alcohol and/or (components) of the metabolic syndrome. Thus, it is known that alcohol consumption increases gut permeability and leads to higher levels of plasma endotoxin levels, and patients with NAFLD show a loss of the integrity of epithelial tight junctions in the gut, which leads to increased intestinal permeability. It is well known that the progression of hepatic inflammation and fibrosis is crucially affected by gut derived endotoxin, and most recently, the central role the endotoxin receptor TLR4 in hepatic fibrosis has been highlighted. Moreover, it has been shown that free fatty acids (FFA) can activate the pattern recognition receptor TLR4 by mimicking pathogens, and FFA are elevated in obese patients and patients with NAFLD, respectively.
Undoubtedly, alcohol and obesity appear as a dangerous mix, and there are important synergistic effects of either condition with regard to crucial triggers of liver injury. When translating these findings to humans, obese individuals should therefore adhere to lower amounts of regular alcohol consumption than lean subjects, and those who drink within safe margins should make sure they do not become overweight.

Session IV

Pathomechanisms of alcohol induced damage (Part III)

Protective role of HO-1 for alcohol dependent liver damage

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Alcohol-dependent liver damage is continuously increasing in developed countries and is a leading cause of death worldwide. Chronic alcohol uptake is reported to induce synthesis and activation of TGF-β. The resulting signal transduction in liver cells is critically required for progression of liver disease.
Ethanol, as well as TGF-β, rapidly induces oxidative stress in primary hepatocytes, as measured by increase in reactive oxygen intermediates (ROI) and reduction of cellular glutathione (GSH). This leads to a significant release of LDH (~ 45%) into the culture supernatant 72 h after treatment with ethanol or TGF-β. AnnexinV staining identified the majority of the cells affected to be apoptotic. Treatment of hepatocytes with anti-oxidative flavonoids (e. g. quercetin) results in nuclear translocation of NF-E2-related factor-2 (Nrf2), which is mediated by MAPK signaling pathways. Addition of SB203580 (p38 inhibitor) and PD98059 (ERK inhibitor) resulted in the inhibition of the quercetin-derived HO-1 induction and Nrf2 translocation. Similar results were obtained by treatment of hepatocytes with various antihypertensives, e.g. nifedipine and verapamil. Nuclear Nrf2 induces expression of hemeoxygenase-1 (HO-1) for buffering oxidative stress. Consequently, hepatocytes treated with quercetin, nifedipine or verapamil produced significantly less ROI when treated with either of the substance. At the same time, reduction of cellular GSH was less pronounced. This effect was even more pronounced, when cells were pre-treated with these substances. Thus, flavonoids as well as certain antihypertensives protect hepatocytes from ethanol or TGF-β induced damage. Blocking of HO-1 activity, by the chemical inhibitor ZnPP9, was able to reduce the observed protective effect significantly, proving its HO-1 dependence.
Our results suggest that increasing HO-1 activity in liver cells protects them from ROI dependent damage by increasing cellular GSH. Under these conditions GSH favors formation of non-toxic products from ROI. Therefore, food supplementation with anti-oxidants, such as the polyphenol quercetin, support liver function by interfering with increased ROI production observed during chronic alcohol consumption.

HCV, HBV and alcohol – The Dionysos study

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The available knowledge on the natural course of chronic viral liver disease is based on studies performed on blood donors, military recruits, and secondary or tertiary care series. Population-based studies on the natural history of chronic viral liver disease that consider co-morbidity factors, such as alcohol or metabolic diseases are lacking. We report here on the natural history of chronic HCV and HBV infection and the contribution of ethanol intake and non-organ specific autoantibodies (NOSA) to the course of chronic viral disease in the Dionysos cohort. As reported in detail elsewhere (2), the Dionysos study was performed in two towns of Northern Italy, started in 1992 with a 10 years follow up in 2002, and allowed us to quantify the burden of chronic viral liver disease and the contribution of alcohol intake and NOSA to morbidity and mortality in a representative sample of subjects from these towns. We followed up 139 subjects with chronic hepatitis C virus (HCV) infection and 61 with chronic hepatitis B virus (HBV) infection for a median (IQR) time of 8.4 (1.0) and 8.3 (0.9) years, respectively. Ethanol intake was evaluated using a validated semi-quantitative food-frequency questionnaire, fatty liver (FL) was diagnosed by ultrasonography, and liver cirrhosis and hepato-carcinoma (HCC) were diagnosed by liver biopsy.
The incidence and remission rates of steatosis were 9.0 and 29.7 per 1000 person-years (PY) in the HCV cohort and 4.0 and 30.4 per 1000 PY in the HBV cohort. Progression to cirrhosis and HCC was more common in the HCV than in the HBV cohort (4.5 vs 2.0 and 2.7 vs 2.0 per 1000 PY, respectively). Ethanol intake was an independent predictor of liver cirrhosis in the HCV cohort (rate ratio [RR]= 4.15 [95% CI: 1.02–41.2] for every increase of 30 g/day of ethanol intake at baseline) and of death rate in both cohorts (RR = 8.53 [95% CI: 1.40–24.61] and 3.56 [1.34 to 26.50] for every increase of 30 g/day of ethanol intake at baseline). We found no association between baseline NOSA and 8.4-yr mortality. We conclude that the morbidity and mortality rate of HBV and HCV infection in the general population is lower than that reported in secondary-care populations, blood donors, or clinical series. Ethanol intake is an independent predictor of cirrhosis in subjects with chronic HCV infection and an independent predictor of death in subjects with either HCV or HBV infection.

References:

1. Bedogni G, Miglioli L, Masutti F, Ferri S, Castiglione A, Lenzi M, Crocè LS, Granito A, Tiribelli C, Bellentani S. Natural Course of Chronic HCV and HBV Infection and Role of Alcohol in the General Population: The Dionysos Study. Am J Gastroenterol. 2008; 103: 1–6.

2. Bellentani S and C.Tiribelli. The spectrum of liver disease in the general population: lesson from the Dionysos study. J Hepatol. 2001; 35: 531–537.

Alcohol and regulation of iron metabolism

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More than 50% of patients with alcoholic liver disease (ALD) show pathological deposits of iron in their livers. The known toxicity of iron with cellular oxidative stress (e. g. H_2O_2) via the Fenton chemistry suggests that these iron deposits are a major factor in the progression of fibrosis and the development of hepatocellular carcinoma.

Besides other potential mechanisms, a dysregulation of hepcidin seems to be causative for hepatic iron accumulation in ALD patients. The 25 amino acid and liver secreted peptide hepcidin is the major systemic iron sensor in humans and typically induced under conditions of iron overload. Hepcidin blocks duodenal iron absorption and the release of iron by inflammatory cells via the iron export pump ferroportin. In addition, cytokine-mediated upregulation of hepcidin could be identified as key mechanism for the commonly observed ‘anemia of chronic disease’ during chronic inflammation.

Suppressed hepcidin levels have been recently found in mice chronically exposed to alcohol. Our studies on well characterized ALD patients confirmed that hepcidin is inadequately expressed in ALD patients despite iron overload. Alcohol-mediated oxidative stress has been suggested to mediate the suppression of hepcidin, however, the exact molecular mechanisms are still incompletely understood. Interestingly, we could recently demonstrate that hepcidin expression is very sensitive to the central reactive oxygen species H_2O_2 in a complex manner. Thus, high and toxic dosages suppress hepcidin, while submicomolar and sustained H_2O_2 levels drastically upregulate hepcidin. The upregulation of hepcidin by H_2O_2 is independent of the IL6 and BMP6 response. Promotor studies confirmed that H_2O_2 induces hepcidin solely via the unique STAT3 element. The effects of H_2O_2 are abrogated by N-acetylcysteine (NAC) and catalase in vitro and partially in vivo. Thus, apart from cytokines such as IL6, H_2O_2 is a potent inducer of hepcidin.

It is expected that novel analytic tools such as molecular fusion redox probes (e.g. HYPER) and novel H_2O_2-models such as the GOX/CAT system will allow us to directly analyze the interaction of ROS and alcohol in exposed tissues. Finally, a better mechanistic understanding of hepatic iron accumulation could help to develop novel targeted approaches to prevent cancer and progression in alcoholic liver disease.

Session V

Alcohol drinking associated factors

Role of CYP2E1 in ethanol-induced oxidant stress, fatty liver and hepatotoxicity

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A major pathway for chronic ethanol-induced liver injury is ethanol-induced oxidant stress. Several pathways contribute to mechanisms by which ethanol induces oxidant stress. While some studies support a role for cytochrome P450 2E1 (CYP2E1), others do not. Most previous studies were conducted in the intragastric infusion model of ethanol administration. There is a need to develop oral models of significant liver injury and to evaluate the possible role of CYP2E1 in ethanol actions in such models. We evaluated chronic ethanol-induced liver injury, steatosis and oxidant stress in wild type (WT) mice, CYP2E1 knockout (KO) mice and in humanized CYP2E1 knockin (KI) mice, where the human 2E1 was added back to mice deficient in the mouse 2E1. WT mice and the CYP2E1 KO and KI mice (both provided by Dr F. Gonzalez, NCI) were fed a high fat Lieber-DeCarli liquid diet for 3 weeks; pair-fed controls received dextrose. Ethanol produced fatty liver and oxidant stress in WT mice but liver injury (transaminases, histopathology) was minimal. Ethanol-induced steatosis and oxidant stress was blunted in the KO mice (no liver injury) but restored in the KI mice. Signicant liver injury was produced in the ethanol-fed KI mice with elevated transaminases, necrosis, and increased levels of collagen type 1 and smooth muscle actin. This liver injury in the KI was associated with elevated oxidant stress and elevated levels of the human CYP2E1 compared to levels of the mouse 2E1 in WT mice. Activation of JNK and decreased levels of Bcl-2 and Bcl-XL were observed in the ethanol-fed KI mice compared to the other groups. Fatty liver in the WT and the KI was associated with elevated levels of lipogenic stearoyl CoA desaturase-1 and lower levels of lipolytic PPAR alpha. No such changes were found in the ethanol-fed KO mice. These results show that CYP2E1 plays a major role in ethanol-induced fatty liver and oxidant stress. It is the absence of CYP2E1 in the KO mice responsible for the blunting of steatosis and oxidant stress since restoring the CYP2E1 restores the fatty liver and oxidant stress. Moreover, it is the human CYP2E1 which restores these effects of ethanol which suggests that results on fatty liver and oxidant stress from rodent models of ethanol intake and mouse CYP2E1 can be extrapolated to human models of ethanol intake and to human CYP2E1 and that the ability of human CYP2E1 to promote ethanol injury can be studied in the total absence of mouse CYP2E1.

Pancreas – Non-alcoholic constituents and their effects

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Alcoholic beverages contain numerous non-alcoholic compounds that could have beneficial or pathological effects. For example, more than 2000 organic and inorganic constituents in beer and more than 1000 in wine were identified to date. Whereas the role of alcohol (ethanol) in the development of pancreatic diseases – in particular acute and chronic pancreatitis – has been intensively investigated, only little is known about the effects of non-alcoholic compounds in this context. Present data indicate that natural phenolic compounds (e. g. quercetin, resveratrol), which are contained in alcoholic beverages exert different effects on the pancreas *in vitro*, such as inhibition of pancreatic enzyme output, of pancreatic stellate cell activation and of pancreatic cancer growth as well as protective effects against oxidative stress and on experimentally induced acute pancreatitis in rats (1, 2). However, one may assume that the effect/s of the extremely complex mixtures of bioactive substances in alcoholic beverages will differ from the effect of these single components.
Studies on human pancreatic secretion suggest different effects of alcoholic beverages when compared to appropriate ethanol solutions. Intragastric administration of beer in a volume (250 ml) that does not alter plasma ethanol concentrations causes a significant stimulation of basal pancreatic enzyme output, whereas ethanol in concentrations similar to beer (4% v/v) has no effect (3). Therefore, non-alcoholic constituents might be responsible for the stimulatory effect of beer on pancreatic enzyme secretion in humans. The intragastric administration of an higher amount of beer (850 ml) or white wine (400 ml) elevates plasma ethanol concentrations, but does not affect the basal pancreatic enzyme output (4). It was suggested that the direct inhibitory effect of circulating ethanol neutralized the stimulatory effect of the non-alcoholic components of beer (3).
In a study with healthy humans, Chari et al. (1996) found that isotonic glucose solution (5.76% w/v) as well as glucose solution with a concentration found in finished wort (11.5% w/v) were strong releasers of cholecystokinin (CCK), but stimulated the exocrine pancreatic secretion only moderately as compared to beer. These results suggested that CCK is one, but not the exclusive, mediator of pancreatic secretion induced by beer (3). However, direct effects of beer on enzyme secretion have not been examined, and cannot necessarily be inferred from infusion or injection studies because of potential interaction with enteropancreatic reflexes, other hormones or regulators *in vivo*.
Therefore, in recent studies we investigated the direct effect of alcoholic beverages, especially beer, on pancreatic secretion of freshly isolated rat pancreatic acinar cells and the rat pancreatic acinar cell line AR4-2J (5, 6). The cell line retains many of the characteristics of pancreatic acinar cells in its differentiated state and is a general model to study stimulus-secretion coupling (7). Stimulation of AR4-2J cells with different alcoholic beverages and appropriate ethanol solutions showed that increasing beer doses enhance amylase release, whereas pure ethanol, wine or alcoholic beverages produced by distillation (e. g., whisky, tequila) have no effect. This effect was reproducible in freshly isolated pancreatic acinar cells. Lactate dehydrogenase measurement after long-term treatment (24 h) of AR4-2J cells indicated that beer-induced amylase release is not due to protein release due to cell membrane damage (6). Pre-treatment of AR4-2J cells with selective inhibitors of known mediators of stimulus-

secretion coupling in pancreatic acinar cells showed that beer-induced amylase secretion is mediated by activation of phospholipase C (PLC), binding of IP_3 to its receptor (IP_3-R) and subsequent calcium release from intracellular stores. The participation of calcium in beer-induced amylase release was confirmed by pre-treatment of the cells with the calcium chelator BAPTA/AM and measurement of fluorescence after loading the cells with the calcium indicator Fura-2 AM. However, further results indicated that additional, yet unknown, signaling pathways are involved in beer-induced amylase release (5). Concerning the characterization of the compounds that are responsible for the stimulatory effect of beer *in vitro*, we have shown that barley is the source of the stimulants and malting as well as fermentation have just a marginal effect on the stimulatory action. In addition, fermented and unfermented glucose in concentrations found in finished wort, as well as the known stimulants of gastric acid secretion, maleic acid and succinic acid, had no effect on pancreatic secretion of AR4-2J cells. Stimulation of cells with treated beer (distillation, lyophilization, dialysis and protease digestion) suggested that the stimulatory compound/s is/are heat-stable, non-volatile substance/s with a molecular weight higher than 15 kDa (5).
In summary, there is accumulating evidence that non-alcoholic compounds of alcoholic beverages exert different effects on the pancreas. The effects and mechanisms of most single compounds and their combinations are still unknown and thus, caution is required in attempting to draw firm conclusions on the effect of non-alcoholic compounds of alcoholic beverages on defining alcoholic etiology of pancreatitis.

References:

1. P. Feick, A. Gerloff, M.V. Singer. Pancreatology. 2007; 7: 124.

2. A. Gerloff, M.V. Singer, P. Feick. Int J Environ Res Public Health. 2010; 7: 1093.

3. S.T. Chari et al. Dig Dis Sci. 1996; 41: 1216

4. F. Hajnal, M.C. Flores, J.E. Valenzuela. Pancreas. 1989; 4: 486.

5. A. Gerloff, M.V. Singer, P. Feick. Alcohol Clin Exp Res. 2009; 33: 1638.

6. A. Gerloff, M.V. Singer, P. Feick. Alcohol Clin Exp Res. 2009; 33: 1545.

7. J.B. Dietrich. Biosci Rep.1996; 16: 273.

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POSTER ABSTRACTS

The epidemiology of survival in alcoholic hepatitis among Chilean population

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Introduction: Alcoholic hepatitis (AH) represents a severe form of alcoholic liver disease with high in-hospital mortality. Since there are no studies of survival after discharge, our aim was to describe demography, survival and cause of death in these patients.

Methods: Follow-up research is possible in Chile because there is a national registry of mortality (Civil Registry). Discharged patients with AH, excluding dead ones, were identified from the hospital database between 1996 and 2009 and their survival status was obtained in the civil registry at May 2010. We analyzed age, gender, mortality and cause of death of these patients. We divided them in two groups, mild and severe, according to prognostic scores (Maddrey, MELD) and presence of encephalopathy during hospitalization. The Kaplan Meier survival analysis was used to compare both groups.

Results: The age of 53 patients ranged from 30 to 75 years (mean ± SD, 48.98 ± 10,87) with a gender proportion of 44 males (83%) and 9 females (17%). The mild group had a 1- and 5-year survival of 0.95 and 0.75, whereas the severe group had a rate of 0.5 and 0.15 respectively (RR 4.37; SE 2.18; p-value 0.0031). The causes of death were liver failure (27%), pneumonia (23%), gastrointestinal bleeding (23%), a new AH episode (17%) and other infections (10%).

Conclusion: Survivors of severe AH are at higher risk of mortality than those who suffered a mild episode. Diseases related to cirrhosis determine death during follow-up.

2

Incidence of acute pancreatitis in North Adriatic Region in Croatia during last ten years (2000–2009)

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Introduction: Incidence rate and the etiology of acute pancreatitis vary in different parts of Europe. There are few studies showing discrepant incidence rate for different countries in Europe but in the most of them it is between 20 and 30 new cases per 100.000 habitants annually. The aim of this study was to present incidence rate of acute pancreatitis for North Adriatic Region in Croatia as well as to make epidemiology analysis concerning etiology, mean age, gender and number of patients with a severe disease.

Methods: During a ten year period (2000–2009) we analysed 922 patients admitted in our hospital with confirmed diagnosis of acute pancreatitis by history, clinical findings, laboratory and imaging methods. An epidemiologyc analysis is carried out on incidence, demographic data and etiology as well as severity of a disease based on a Ranson score.

Results: Incidence rate varied from 24 to 35 new cases per 100.000 annually. Mean age was 60 ± 15.77 years. There were 53.2% of men and 46.8% of a woman. Most common etiology was biliary in 60.6%, and alcohol in 19% of patients. According to the Ranson score, pancreatitis was considered to be severe in 43.5% of the cases.

Discussion/Conclusion: In our region incidence rate of acute pancreatitis was around 30 per 100.000 habitants annually during last ten year period. The mean age at admission was 60 years, there were no gender differences and etiology was dominantly biliary. Our study showed epidemiological characteristics typical for Mediterranian countries.

Chronic pancreatitis in children – Three clinical cases

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Introduction: Chronic pancreatitis in childhood is a rare disease. The resulting symptoms of abdominal pain, steatorrhea and weight loss may be debilitating and complicated by psychosocial problems. The causes of pancreatitis in children tend to be more related to heredity, trauma or idiopathic, as opposed to lifestyle choices (alcohol abuse) in the adult.

Methods: We present three cases of chronic pancreatitis that were rapidly progressing and poorly responsive to medication.

Results: The first case is a boy with a history of recurrent acute pancreatitis since the age of 6. At the age of 10 he underwent laparotomy with removing of a pancreatic pseudocyst, Roux-en-Y pancreatojeunostomy was done. At the age of 16 he expressed episodes of recurrent acute pancreatitis. Pancreatic lithiasis was found in ductus pancreaticus and in ductus choledochus. We performed ERCP, papillotomy with removal of the stone in the papilla. We performed a lot of investigations to seek the etiology but we were not able to find the cause of lithiasis and recurrent pancreatitis.
The second case is a girl with a history of choledochal cyst at the age of one year which was operated without a complete surgical excision. Since the age of 13 she had recurrent episodes of acute pancreatitis. At the age of 15 we performed MRT, MRCP and ERCP and diagnosed a pancreatic cyst related to ductus choledochus as well as ductus Santorius. Both of the children suffered severe pain, they needed frequent admissions to the hospital. Their quality of life was much disturbed.
The third case is a girl at the age of 17 who suffered 2 episodes of jaundice and acute pancreatitis. Cholelithiasis was found as a causative factor.
The three patients were referred to surgery.

Discussion/Conclusion: Chronic pancreatitis is rarely seen in Bulgarian children. It takes a lot of time to find the etiology. Ultrasound scan is useful in making the diagnosis. Surgery was the most useful treatment.

4

Multiple cancers of liver and pancreas associated alcohol drinking in Czech population

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The relations of patients with hepatocellular cancer (HCC) and pancreatic cancer (PC) to alcoholic consumption is unclear. A retrospective study was performed with comparison to 1,573 multiple HCC and 3,632 PC by their primary and subsequent diagnoses from Czech Cancer Registry in 1976–2005. Long-time trend of high alcoholic consumption in the Czech population is compared to a population of EU.

Introduction: In 2007 in Czech population there were 558 (11.1 per 100,000) in men, 326 (6.2) in women cases of HCC and 964 (19.1 per 100,000) in men, 962 (7.8) in women cases of PC, reported to the Czech Cancer Registry. Their prevalence increased between 1989 and 2005 from 181 to 527 cases i. e. by 191% of HCC and from 786 to 1,266 i. e. by 61% of PC. The risk of suffering a second primary neoplasm after HCC and PC has not been evaluated till now in the Czech population.

Methods: Czech Cancer Registry recorded 355,624 new GI cancers (54.4% in men, 45.6% in women) during 1976–2005, of which there were 19,998 (1.6%) cases of HCC and 40,922 (11.5%) of PC. From national database we analysed 330 primary and 1,243 subsequent cases of HCC and 562 primary and 3,070 cases of PC.

Results: Generally 8.2% HCC in men and 7.3% in women, 8.9% PC in men and 8.9% in women of newly diagnosed cases of HCC and PC were associated with multiple neoplasms. The results present the proportion of duplicities and multiplicities, the average length 6 years of HCC and 6.2 years of PC between the primary and subsequent neoplasms, the ratio of synchronous and metachronous cancers, early and advanced clinical stages and the over-all risk of new malignancies among survivors following both diagnoses. The available data compared the increasing trend of alcohol consumption in Czech population to a population of EU.

Discussion/Conclusion: Gastrointestinal cancers present long-time serious health problem among the Czech population alike the alcohol drinking as the considerable risk factor of primary and subsequent HCC and PC. The dispensary care for patients with HCC and PC is decisive in view of multiple malignant neoplasms especially in expected prevalence 684 of HCC and 1.478 of PC cases in 2015 as well as reduce alcohol consumption especially among teenagers. The expenditure nearly 10.7 million Euro i. e. 8% in GDP on health in 2009, giving evidence of not only more developed but also more expensive health care, including patients with previous high alcohol consumption, induced damage of multiple HCC and PC.

Study of the relationship between chronic hepatitis C virus, non-alcoholic fatty liver disease and insulin resistance

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Background: Hepatitis C infection is considered the leading cause of chronic liver disease in Egypt. Hepatic steatosis (NAFLD) has been reported in 30–70% of patients with chronic hepatitis C (CHC).

Aim of the work: Assess the relationship between chronic hepatitis C infection, NAFLD and insulin resistance in Egyptian patients.

Material: 20 Patients with chronic HCV infection and hepatic steatosis were proven by liver biopsy were included in the study. Other 20 healthy adults were enrolled as a control group.

Methods: Liver function tests, HCV antibodies by third generation ELISA, HCV-RNA by PCR, fasting serum insulin and serum ferritin. Insulin resistance was evaluated using the homeostasis model assessment (HOMA-IR) method. Also, ultrasound examination of the abdomen.

Results: Insulin resistance (IR) by (HOMA) index was significantly higher in patients than in control group (mean ± SD) 8.36 ± 6.61 and 2.17 ± 0.08), respectively). Fasting insulin level was significantly higher in chronic HCV patients than in control group ($P = 0.000$). Serum ferritin level was significantly higher in patients (124.04 ± 123.18 ng/ml) than in control group (65.22 ± 46.27 ng/ml). HOMA-IR positively correlated with staging ($P = 0.034$, $r = 0.477$) and with grading ($P = 0.022$, $r = 0.509$) in HCV infected patients.

Conclusion: HCV infected patients have high degree of IR. IR facilitates fibrogenesis in chronic HCV patients. High serum ferritin may be implicated in (IR).

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The QT interval in alcoholic patients

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Introduction: The aim of the study was to assess the QT interval in alcoholic patients with and without liver cirrhosis, and to identify QT interval prolonging factors.

Methods: We included 39 alcoholic patients, aged 57 ± 13 years, and 20 age-matched healthy controls in our study. The patients underwent 12-lead ECG and: QT interval (QTmax), heart rate corrected QT interval (QTc) and mean QT interval duration in all 12 ECG leads (QTm) were assessed. The patients were divided in two groups: 19 with and 20 without liver cirrhosis. Complete blood count, inflammatory markers, liver function tests and glycaemia were also assessed. Laboratory tests and QT interval parameters were compared between the two groups.

Results: QTmax (435 ± 35 ms vs. 358 ± 23 ms) was significant prolonged in alcoholic patients compared to healthy controls ($p < 0.001$). There were no significant differences for QTmax and QTc between the two groups of alcoholic patients. Only gammaglutamyltranspeptidase (GGT) and platelet count (PLT) were significant different between the two groups of alcoholic patients. A good correlation was found between GGT (56 ± 17 U/l) and QTmax ($r = 0.566$) and between PLT ($267 \pm 10^1 \times 10^3/\mu l$ and QTc in alcoholic patients without liver cirrhosis ($r = 0.719$).

Discussion/Conclusion: Chronic alcohol consumption significantly prolongs the QT interval, independent of the existence of liver cirrhosis. Increased GGT and PLT are QT interval prolonging factors in alcoholic patients without liver cirrhosis.

The prevalence of Th17 cells in patients with alcoholic liver disease

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Introduction: Th17 cells have been identified to play a crucial role in inflammatory immune dysregulation. Their significance in pathogenesis of alcoholic liver disease (ALD) remains unclear. The aim of our study was to assess the possible involvement of Th17 cells in ALD.

Methods: We studied the frequency of Th17 cells in peripheral blood of 28 patients with ALD in comparison with 11 healthy controls (HC). The study population consisted of 8 women and 20 men, mean age 50.7 ± 11.0 (range 28–69). Pts were divided according to their Child-Pugh score into 3 groups: A- 6 pts; B- 14 pts; C- 8 pts. Flow cytometric analysis FACS Calibur with CellQuest software was used to identify T cell phenotype. CD3+CD4+IL17+ cells were considered Th17 cells and expressed as the percentage of all CD3+CD4+ cells. All data were analysed using Statistica 8.0 software.

Results: The mean MELD score of the study population was 16.1 ± 6.8 (range 6–32). The frequency of Th17 cells in peripheral blood was mildly increased without statistically significant difference in pts with ALD in comparison with HC (1.38 ± 1.40 versus 1.01 ± 0.44; p = 0.41). The highest level of Th17 cells was observed in Child B group, the lowest level in Child C (1.79 ± 1.85 versus 0.91 ± 0.51; p = 0.32), the correlation with the severity of disease did not reach statistical significance. The mean number of that subset was higher in men than in women (1.47 ± 1.59 versus 1.13 ± 0.79; p = 0.56). We found no correlation of CD3+CD4+IL17+ cells with serum aminotransferases levels.

Discussion/Conclusion: Patients with ALD tend to have mildly higher proportion of IL-17-producing cells among circulating CD3+CD4+ lymphocytes. The highest frequency was observed in Child B group, the lowest in Child C group.

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Intestinal permeability is increased in patients with alcoholic liver cirrhosis

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Introduction: To study the intestinal permeability (IP) in patients with liver cirrhosis (LC) and to analyze its association with the aetiology of the disease.

Material and Methods: 31 patients with LC (19 alcohol-induced and12 viral-induced) and 25 healthy controls consented to participate in the study. IP was assessed by using of a contrast medium Omnipaque (iohexol), which was administrated orally (25 ml, 350 mg/ml) 2 hours after having breakfast. Six hours later serum iohexol concentrations (Se IOH mg/l) and its urine recovery (U IOH g/mol) were determined by a validated high-performance liquid chromatography technique.

Results: Abnormal IP was found in 22.6% of LC patients compared with 8% of controls. The median Se IOH 6 h after its ingestion in patients with LC (1.92 ± 1.58 mg/l) was significantly higher in comparison to the controls (1.11 ± 1.10 mg/l), ($p < 0.05$). No significant difference was found in terms of excretion of the administered dose of iohexol between patients (20.65 ± 10.78 g/mol) and controls (18.10 ± 19.57 g/mol), ($p > 0.05$). The IP was significantly increased in the subgroup of patients with alcoholic LC (2.33 ± 1.91 mg/l), compared with the one with viral LC (1.27 ± 0.68 mg/l) and control subjects (1.11 ± 1.10 mg/l), ($p < 0.05$). An altered IP were established more frequently in the patients with alcoholic LC (31.6%) than in those with viral LC (8.3%).

Conclusion: The IP has been increased in patients with alcoholic LC compared to those with viral LC. The results have suggested the role of permeability alterations as a possible contributing factor for the development of chronic liver damage in heavy drinkers.

Gender differences in the development of hepatic steatosis after acute ingestion of ethanol and beer in mice

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In a setting of chronic alcohol intake, women and female rodents are more susceptible to alcohol-induced liver disease (ALD) than men and male mice.
The aim of the present study was to determine if female mice are also more susceptible to acute alcohol-induced steatosis than male mice and to investigate whether this is due to alterations in hepatic lipid export.
Male and female C57BL/6J mice received one single dose of ethanol (6 g/kg b.w.), isocaloric maltose-dextrin solution (control) or two different kinds of beer intragastrically. Hepatic triglycerides, lipid accumulation, mRNA expression of microsomal triglyceride transfer protein (MTP) as well as MTP activity were measured 12, 24, and 48 h after alcohol intake. In both genders acute alcohol ingestion caused a significant hepatic steatosis; however, fat accumulation was ~2-fold higher and more persistent in livers of female than in male mice. MTP activity was increased only in male mice 12 h after ethanol ingestions; whereas expression of MTP mRNA was reduced only in female alcohol-treated animals compared to controls at this timepoint. Contrary to female mice hepatic triglyceride concentrations were higher in beer treated male animals compared to animals treated with plain ethanol.

Our data suggest that beer gender-specifically induces hepatic steatosis and that the markedly more pronounced susceptibility to acute alcohol-induced liver steatosis of female mice results at least partly from a gender-specific regulation of hepatic lipid export.

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High echogenicity of pancreas is an obligatory sign in patients with alcoholic steatohepatitis and metabolic risk factors

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Introduction: It is a common situation when alcoholic steatohepatitis occurs in patients with metabolic risk factors and risk of non-alcoholic fatty liver disease. At the same time the interaction of alcoholic steatohepatitis and non-alcoholic fatty liver disease as well as their influence on functions of different digestive organs are almost uninvestigated. **The aim** of this study was to estimate such ultrasound characteristics as echogenicity of the pancreas in patients with alcoholic steato-hepatitis and metabolic risk factors.

Methods: A total of 38 patients aged 32–65 with alcoholic steatohepatitis and metabolic risk factors (overweight/obesity, glucose intolerance, dyslipidemia and hypertension) were investigated. Alcohol consumption in all patients exceeded 80 ml/day for men and 40 ml/day for women. All patients demonstrated consistently elevated serum levels of alanine aminotransferase and aspartate aminotransferase. Liver biopsy was taken in four patients. In addition, 25 normal subjects were observed as a control group. Pancreatic echogenicity was the main sign to be investigated, which was subdivided into normal and mild or high (moderate or severe) degrees of increasing.

Results: On the whole, moderate or severe increasing of pancreatic echogenicity was revealed in 37 patients with steatohepatitis and in 1 patient the pancreatic echogenicity was mildly increased. In the control group, 4 patients demonstrated a mild increase of pancreatic echogenicity and in one patient the pancreatic echogenicity was moderately increased (for moderate/severe echogenicity: chi-square = 51.09; $p < 0.001$).

Discussion/Conclusion: In patients with alcoholic steatohepatitis and metabolic risk factors the echogenicity of pancreas is always elevated. As a rule, a moderate or severe degree of elevation is observed.

Discovery of the missed link between schistosomiasis and HCV infection

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Introduction: Schistosomiasis is a term covering infestation of man by blood fluke trematode parasite. Egypt S. mansoni occurs intensively in the Nile Delta, while S. hematobium is endemic in Nile Valley. Studies in Egypt found the highest risk of HCV infection in whom infested with schistosomiasis. HCV-Ab prevalence reported 70% in adults suffering from Schistosomiasis and without history of blood transfusion.

Aim: Determine the link between schistosomal infestation and HCV, whether the parasite could be vector of transmission of the virus to human.

Methods: Different stages of life cycle of S. mansoni provided by Theodore-Bilharzias-Institute. Specimens of living S. mansoni worms and Biomphalaria alexandria snails were grounded separately in sterile mortar after adding 5 ml sterile saline. After centrifugation sterile supernatant tested for:
– Detection of HCV-RNA by RT-PCR
– HCV-RNA quantitation.

Results:
HCV-Antigen: Worms, miracidia, snails and cercariae of S. mansoni were positive for HCV-Ag. The snails gave strong positive result. Eggs gave negative result.
HCV-RNA by RT-PCR: Worms, miracidia, snails and cercariae of S. mansoni tested for HCV-RNA by qualitative RT-PCR were positive. The eggs gave negative result.
HCV-RNA Quantitation: Miracidia were positive (800 copies/ml) and snails were positive (1100 copies/ml) other specimens gave negative results.

Conclusion: Existence of virus and its replication in parasite S. mansoni parasite carries HCV and considered as a non-human vector for transmission of HCV infection parasitic and viral co-infection change pathology of hepatic schistosomiasis from periportal fibrosis to cirrhosis and the development of HCC.

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Laboratory abnormality in HCV associated B NLH

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Introduction: Hepatitis C virus infection, known to cause hepatitis, cirrhosis and liver cancer, but is also associated with lymphomas (NHL). Molecular mechanisms by which HCV infection promotes B-cell NHL development remain unclear, but indicate that HCV-associated lymphomas may be a distinct entity.

Methods: A total of 258 patients with B malignant lymphoproliferations were tested for HVC infection. It was also rated the transaminases, bilirubin, INR, serum protein electrophoresis and other haematological parameters.

Results: The frequency of HCV virus infection was detected in 13.56% of B NLH patients. The histologic distribution in our lot was: 8 (22.85%) lymphoplasmacytoid, 5 (14.28%) follicular, 2 (5.71%) mantle, 10 (28.56%) large B cell, 3 (8.57%) diffuse small cell, 1 (2.86%) Hodgkin lymphoma, 3 (8.57%) multiple myloma and 3 (8.57%) acute lymphoblastic leukemia. The age of patients with HCV infection and B NLH was between 18 and 84 years, with an average of 55 years, which is not significantly different from the general B NLH group. Sex distribution favors females 62.45% and 54% of patients was from urban areas (51.7%) presented at least 1 extrahepatic laboratory abnormality, including mixed cryoglobulinemia (37.1%), anemia (31.43%), thrombocytopenia (27.6%), thyroid autoimmunity (16.2%), dermatological disorders (4.1%) and type 2 diabetes (4.1%). The pathogeny of this abnormality will be discussed. The sicca syndrome, nephropathy and polyneuropathy were observed in single cases for each manifestation.

Discussion/Conclusion: The B NLH associated with HVC infections may be a distinct entity with frequent extrahepatic abnormality which can interfere with disease evolution and therapy.

Malignant B lymphoproliferative diseases associated with HCV infections

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Introduction: In the last decades, it has been demonstrated that patients infected by hepatitis C virus (HCV) are more likely to develop B-cell non-Hodgkin's lymphoma (NHL) than those uninfected.

Methods: A total of 258 patients with B malignant lymphoproliferative disorder were tested for HVC infection. It was also rated the transaminases, bilirubin, INR, serum protein electrophoresis and all these parameters were monitored during the disease evolution.

Results: The frequency of HCV virus infection was detected in 13.56% of B NLH patients. The histology distribution in our lot was: 8 (22.85%) lymphoplasmacytoid, 5 (14.28%) follicular, 2 (5.71%) mantle, 10 (28.56%) large B cell, 3 (8.57%) diffuse small cells, 1 Hodgkin lymphoma, 3 (8.57%) multiple myeloma and 3 (8.57%) acute lymphoblastic leukemia. The age of patients with HCV infection and B NLH was between 18 and 84 years, with an average of 55 years, which is not significantly different from the general B NLH group. Sex distribution favours females 62.45% and 54% of patients was from urban areas. 56.66% of these patients have extranodal lesions, compared with 19% for group of B NLH without HCV infection (Chi2 $p < 0.05$). Extranodal involvement refers mainly to the liver, spleen, salivary gland and digestive tract.

Discussion/Conclusion: The absolute risk of developing lymphoma when infected with hepatitis C appears to be low. This group of NLH has more frequent extranodal involvement and viral reactivation with chemotherapy and immune reconstitution hepatitis can complicate antineoplastic treatment.

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Hepatic encephalopathy after TIPS

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Introduction: The *transjugular intrahepatic portosystemic shunt* (TIPS) is recommended for the treatment of portal hypertension (PHT) complications. The risks and secondary complications are still only presumed. Hepatic encephalopathy (HE) *de novo* or aggravated by the procedure is described in 20–30% with some studies identifying factors associated with an increased risk.

Objectives: Determination of HE incidence, predisposing factors and relevance of infection as one.

Methods: Retrospective study of the patients submitted to TIPS between December 1999 to January 2008. Statistic analysis with Excel-2007.

Results: 24 patients (2 excluded). Final sample: 10 male and 12 female, mean age 51 years (range: 18–71). Cause of cirrhosis: alcohol 59.1% (n = 3), viral 4.6% (n = 1); other causes 36.4% (n = 8). TIPS´s indication: PHT without further specification n = 6, refractory ascites n = 14, variceal bleeding n = 4, other n = 2. HE-pre-TIPS 6/16 (37.5%), HE-post-TIPS 10/16 (62.5%), both more frequent in the alcoholic hepatic cirrhosis. With MELD ≥ 17, HE-pre-TIPS 27.3% vs HE-post-TIPS 42.9%; and with Child-Pugh C, HE-pre-TIPS 85.7% vs HE-post-TIPS 42.9%. In the HE-pre-TIPS group, the mode was in 40–49 years group. An increase of HE-post-TIPS occurred in > 40 years.

	Pre-TIPS (%)	Post-TIPS (%)
Esophageal varices or hypertension gastric disease (EV/HGD)	31.6	47.4
Ascite with evacuative paracentese necessity	26	47.4
Variceal bleeding	44.4	55.6
Refractory ascite	20	46.7
Concomitant infection	16.7	30

The urinary tract infection was the most frequent infection, followed by spontaneous bacterial peritonitis, respiratory infection and infection associated with catheter. Mortality rate: 27.3% (n = 6). Most frequent death cause: HE (n = 2).

Discussion/Conclusion: Hepatic encephalopathy is a serious complication associated to TIPS, increasing after this procedure. Ascites and EV/HGD are risk factors for the development of HE post-TIPS, being predictive in this sample. Age (> 40 years) seems to be a risk for development of HE. The infection, the MELD and the Child-Pugh class are not predictive factors of HE-post-TIPS.

The relationship between bile duct lesions and the severity of biomorphologic parameters in patients with viral chronic hepatitis

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Introduction: Hepatic bile duct lesions (BDL), lymphoid aggregates or lymphoid follicles (LA/LF) and hepatic steatosis (HS) are common histological features, often observed in patients with chronic hepatitis C (VHC). The pathogenesis and clinical significance of hepatic BDL remain unclear.

Methods: We evaluated the prevalence and clinical significance of BDL on a group of 189 liver biopsies from patients with chronic viral hepatitis (125 patients with VHC and 64 with VHB), trying therewith to compare clinical, biochemical and morphologic data in patients with and without BDL.

Results: The mean age of patients with BDL was 50.5 years, slightly higher than the one of patients without BDL (48.61 years). Analyzing the frequency and pathological significance of BDL and portal lymphocyte infiltrate in patients with VHC we noted: a higher frequency of BDL in patients with VHC than in those with VHB (55/125; 44% vs. 14/64; 21.8%); a high level of serum ALT (93.83 U/l), alkaline phosphatase (AF) (175.83 U/l) and γ-GT (99.76 U/l) in patients with BDL vs. 71.73 U/l, 146.76 U/l and 71.24 U/l, respectively, in those without these kind of lesions; serum triglyceride (TG), cholinesterase (CHE) and total bilirubin (TB) levels were found to be significantly higher in patients without BDL. We did not observe a relationship between the sex of the patients and the presence of blood transfusions in their history, between the two groups of patients.
For the group of patients with VHC and BDL we observed a higher score of necroinflammation (9.87 vs. 7.38), portal inflammation (3.01 vs. 2.44) and fibrosis (1.65 vs. 0.97) and a higher frequency of LA/LF (49 vs. 36), as compared to patients without BDL. HS was found in 96 from the 125 (76.8%) patients with VHC, in 44 from 55 (80%) patients with BDL and in 52 from 70 (74.3%) of those without BDL.

Discussion/Conclusion: The incidence of hepatic BDL is highly related to the severity of the histopathological lesions, being higher in patients with advanced liver disease and cholestasis. The implication and molecular role of hepatitis C virus in the pathogenesis of BDL requires further studies.

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Pseudocysts in chronic pancreatitis

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Introduction: The pathology of chronic pancreatitis is reviewed in order to study the histology and incidence of pseudocysts in relation to the degree of pancreatic fibrosis and calcification.

Material and Methods: The series consisted of 50 resection specimens and 5 autopsy pancreata. The histology of cystic lesions observed in the specimens was found to be identical to that of pseudocysts in acute pancreatitis. In 19 of 50 there was concomitant occurrence of focal autodigestive (fat) necrosis and pseudocysts.

Results: Pseudocysts were more common in specimens with focal fibrosis and few calcifications (12/24) than in those with diffuse advanced fibrosis and numerous calcifications (14/40). The size of the pseudocyst and the length of time the cyst has been present are poor predictors for the potential of pseudocyst resolution or complications, but in general, larger cysts are more likely to be symptomatic or cause complications. The findings indicate that sequelae of acute pancreatitis are frequently present in chronic pancreatitis, particularly in an early stage when fibrosis is still focal and calcification rare. This suggests that chronic pancreatitis may result from relapses. Pseudocysts were located primarily in the cephalic pancreas in groups (a) and (b) (58–71%) and in the pancreatic tail in group (c) (61%). This finding and the primary location of pseudocysts in the cephalic pancreas (groups (a) plus (b)) are compatible with the 'necrosis-fibrosis' pathogenetic hypothesis. Pseudocysts were less frequent (36%) than in the early stage of the disease. Endoscopic ultrasound with fine needle aspiration has become the preferred test to help distinguish pseudocyst from other cystic lesions of the pancreas. Most pseudocysts resolve spontaneously with supportive care. On the basis of these findings it is suggested that pancreatitis, if it is severe and also affects the intrapancreatic fat deposits, may evolve into chronic pancreatitis. As a result, the management varies based on local expertise, but in general, endoscopic drainage is becoming the preferred approach because it is less invasive than surgery, avoids the need for external drain, and has a high long-term success rate.

Conclusion: These data suggest that the progression of acute to chronic pancreatitis is closely related to the incidence and severity of acute attacks. A tailored therapeutic approach taking into consideration patient preferences and involving multidisciplinary team of therapeutic endoscopist, interventional radiologist and pancreatic surgeon should be considered in all cases.

Immune investigations in primary sclerosing cholangitis (PSC)

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Introduction: Although the etiology of PSC is not completely understood, many studies suggest that is related to immunologic damage. Our study examines involvement of autoantibodies in the pathogenesis of PSC.

Methods: Sera from 28 adults patients (17 males and 10 females) with various stages of PSC and of 18 normal controls were investigated for the presence of autoantibodies: antinuclear (ANA), antismooth muscle (ASMA), perinuclear antineutrophilc cytoplasmic (pANCA) and antimithocondrial (AMA). We have used indirect immunofluoresscent technique (tissue sections-rat Trinity biotech) to measure ANA, ASMA and AMA. Autoantibodies pANCA were evaluated by ELISA technique. Serum level of IgM was investigated by single radial immunodiffusion.

Results: A high frequency of autoantibodies (42% ANA, 28% ASMA, 32% pANCA) has been found in sera from patients with PSC. The presence of pANCA was significantly more frecquent in patients who had PSC with biliary tract complications or with an inflammatory bowel disease. AMA were found in 5%. ANA and ASMA were more rarely detectable to PSC-patients with signs of autoimmune hepatitis (possible overlap syndrome); they had present AMA and higher concentrations of IgM.

Discussion/Conclusion: The detection of serum autoantibodies offers possibilities for rapid population detection of preclinical PSC. In patients with PSC, pANCA should be sought as a marker of prognosis. Our results no exclude the involvement of AMA in pathogenesis of autoimmune hepatitis-PSC overlap syndrome, when autoimmune signs predomine.

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Chronic ethanol-induced alterations to the liver mitochondrial respiratory chain proteome: Protection by betaine

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Introduction: Mitochondrial damage and disruption in oxidative phosphorylation contributes to the pathogenesis of alcoholic liver injury. We have shown that S-adenosylmethionine (SAM) confers hepatoprotection via maintaining mitochondrial function and the proteome. Herein, we tested the hypothesis that the hepato-protective actions of betaine against alcoholic liver injury occur at the level of the mitochondrial proteome.

Methods: Male Wister rats were pair-fed control or ethanol-containing liquid diets supplemented with or without betaine (10 mg/mL) for 4–5 wks. Liver was examined for histopathology, levels of methionine cycle metabolites, and alterations in mitochondrial proteins.

Results: Chronic ethanol ingestion resulted in the accumulation of micro- and macro-vesicular fat in liver from ethanol-fed animals, which was attenuated in the ethanol + betaine group. Blue native gel electrophoresis (BN-PAGE) proteomics revealed a significant decrease in the content of the intact oxidative phosphorylation complexes I, III, IV, and V in mitochondria from ethanol-fed animals, with no change observed for complex II. Two-dimensional BN-PAGE revealed that the chronic alcohol-dependent loss in many of the low molecular weight oxidative phosphorylation proteins was prevented by betaine supplementation. This protection by betaine was associated with normalization of SAM:SAH ratios and attenuation of the ethanol-induced increase in inducible nitric oxide synthase, nitric oxide generation, and protein nitration in liver.

Discussion/Conclusion: In summary, betaine prevents alcoholic steatosis and alterations to the oxidative phosphorylation system. Therefore, preservation of mitochondrial function may be another key molecular mechanism responsible for betaine hepatoprotection.

Hepatic dysfunction is evident in zebrafish larvae exposed to ethanol: A model for acute alcoholic liver disease

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Introduction: Over 90% of people who binge drink will develop steatosis. While this condition is reversible, repeated bouts of steatosis may predispose to more severe forms of alcoholic liver disease (ALD) including steatohepatitis, fibrosis, and cirrhosis. Our goal is to elucidate the pathways that are required for steatosis in response to acute alcohol exposure. We have thus developed zebrafish as a model for the study of acute ALD.

Methods: Zebrafish are a useful vertebrate model for the study of ALD as they possess fully-functional livers by 4 days post-fertilization (dpf) and molecular pathways necessary for alcohol metabolism. Larvae were exposed to 2% ethanol for 32 hours at 4 dpf. Incidence of steatosis was determined by oil red O staining. Several aspects of liver function were assessed by histological (H&E staining, PAS staining, immunofluorescence) and ultrastructural (transmission electron microscopy, TEM) analyses. Gene expression changes in the liver were examined by qPCR and *in situ* hybridization.

Results: Exposure to 2% ethanol for 32 hours caused pericardial edema, lordosis, hepatomegaly, and steatosis in zebrafish larvae. Furthermore, hepatocytes in fish exposed to ethanol have glycogen loss, mitochondrial swelling, and alterations in canaliculi. Vascular congestion was also observed in ethanol-treated larvae. These findings suggest a decrease in hepatocellular function. Despite activation of the unfolded protein response (UPR), endoplasmic reticulum structure in hepatocytes of treated larvae appeared unaltered; UPR effectors that were upregulated in the liver by ethanol include *bip, dnajc3, canx, derl1* and *atf4*.

Discussion/Conclusion: These findings demonstrate the complexity of responses to acute alcohol exposure and underscore the utility of zebrafish as a tractable vertebrate model for the study of acute ALD. As in humans, we show that larvae exposed to ethanol develop steatosis and experience moderate hepatocellular dysfunction. Future studies will focus on understanding the role of UPR activation in the development of acute alcoholic steatosis.

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Association of chronic alcohol consumption with hepatitis C virus infection: Interactions and consequences

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Introduction: The aim of this comparative study was to evaluate the effect of association between chronic alcohol consumption and hepatitis C virus (HCV) infection and risk of development hepatocellular carcinoma (HCC).

Methods: We studied 88 patients structured in three groups. A group consist of 29 heavy alcohol drinkers (intake-over 80 g ethanol/day for more than 10 years) with chronic HCV, B group consist of 34 heavy alcohol drinkers with alcoholic liver disease (ALD) without HCV and C group composed of 25 non-alcoholic patients with chronic HCV. We monitor and assess the clinical manifestation, alcohol consumption, biochemical parameters, liver function tests and histological aspects of liver biopsy (HAI score) at baseline, after 12, 24 and 36 months.

Results: Mean levels of alcohol intake were similar in A and B group (116.25 g/day vs. 109.03 g/day) and duration of consumption was longer than 12 years. In C group all patients was non-alcoholic in the last three years, but 17 patients are history of medium or low alcohol consumption.
At baseline and after 12 months, the mean value of AST/ALT ratio was < 1 in A group, > 2 in B group and between 1 and 1.2, in C group. This level of AST/ALT ratio was maintained whole period. Sub unitary AST/ALT ratio was correlated with the presence of histological active hepatitis and exclusively with the presence of the C viral infection. More elevated transaminases are present in A group than in the group of alcoholic patients without chronic HCV ($p = 0.002$).
After 12 months, the steatosis was present in all groups, but most frequent in A group (89.65% of cases), comparative with B group (73.5%) and C group (72.0%). At 24 and 36 months, the steatosis grade was significantly higher in A group and indicate a quickly progression of steatosis. The score of fibrosis was more severe in patients with HCV chronic infection and alcohol intake. The degree of fibrosis was significantly higher in A group, comparative with patients with HCV infection or ALD alone.
The incidence of cirrhosis after three years was significantly increased in alcoholic patients: 37.93% in A group, 47.05% in B group and 16.00% in C group. HCC was developed in 9 cases (10.22% of whole group), but significantly higher incidences were observed in patients with chronic hepatitis C infection: 6 cases in A group and 3 in C group.

Discussion/Conclusion: Association of HCV infection with alcohol abuse was correlated with high steatosis grade and severe fibrosis. In patients with chronic hepatitis C infection, the risk of quickly development of HCC was higher in heavy-drinkers.

Impact of moderate alcohol consumption on histological liver lesions in patients with chronic hepatitis C

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Introduction: Alcohol use and hepatitis C are major risk factors of liver injury. The aim of the present study was to evaluate the effects of moderate alcohol consumption on the severity of histological liver lesions in patients with chronic hepatitis C (CHC).

Methods: The authors have retrospectively studied two groups of patients with CHC hospitalized in Clinic of Infectious Diseases in Timisoara, Romania: group A (40 patients) comprised abstinent patients and group B (41 patients) included individuals with moderate alcohol consumption (30–50 g/day). The positive diagnosis was established on the epidemiological elements, the physical examination, biochemical tests (ESR, ALT, AST, DB, TB, ALP, Gamma-GT, summary urine examen, electrophoresis, Ab HCV etc.), along liver echography examination, using a 3.5 MHz transductor. Liver biopsy samples were graded for histological activity and fibrosis according to the METAVIR scoring system.

Results: The number of patients with mild (A1), moderate (A2) or marked (A3) activity decreased gradually in abstinent patients and increased in moderate alcohol consumption: 18 patients from group A and 7 from group B were included in A1 subgroup ($p = 0.04$); the A3 subgroup comprised 8 patients from group A and 21 from group B ($p = 0.04$); The number of patients with moderate or marked fibrosis increased gradually with alcohol intake: 16 patients from group A and 1 from group B had F0–F1 ($p = 0.0007$); 4 patients from group A and 14 from group B had F4 ($p = 0.03$); There was a positive relationship between the level of alcohol intake and the degree of steatosis: 11 patients with moderate steatosis in group A and 26 patients in group B ($p = 0.04$); 3 patients with marked steatosis in group A and 12 cases in group B ($p = 0.03$).

Discussion/Conclusion: The results demonstrate that even moderate alcohol consumption may aggravate the histological liver lesions in patients with CHC.

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Low-level ethanol intake impairs liver angiogenesis and exacerbates hepatic fibrosis in mice in response to CCl_4

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Introduction: The mechanisms by which chronic alcohol consumption directly causes liver fibrosis or exacerbates the progression of fibrotic injury in the setting of underlying non-alcoholic liver diseases are not well understood. Ethanol impairs hepatic wound healing responses in the liver. Since the precise and timely induction of angiogenesis is critical to a normal wound healing response, here we have tested the hypothesis that ethanol impairs angiogenesis in response to hepatotoxic injury, thus leading to increased fibrosis.

Methods: Female C57BL/6J mice were fed with 2% (vol/vol) ethanol (11% calories) or pair-fed control diets for 4 days or 2 weeks. Liver fibrosis was induced by a single intraperitoneal administration of CCl_4 (acute) or 4 doses over two weeks (chronic).

Results: Expression of CYP2E1, required for bio-activation of CCl_4 was not increased by feeding 2% ethanol for 4 days or 2 weeks. CCl_4-induced increases in AST/ALT activity, markers of CCl_4 toxicity, were not increased by ethanol, indicating that the low concentration of ethanol used here did not increase the direct hepatotoxicity of CCl_4. In control mice, acute CCl_4 increased expression of intermediate biomarkers of angiogenesis, with a peak at 4–8 h after challenge with CCl_4. Ethanol exposure for 4 days impaired this angiogenic response in liver, decreasing expression of ANGP-1 and TIE-2 mRNA compared to pair-fed mice, but not VEGF mRNA. After chronic CCl_4, ethanol feeding increased hepatic stellate cell activation, sinusoidal angiogenesis and hepatic fibrosis in response to CCl_4.

Discussion/Conclusion: These data indicate that ethanol consumption, even at low concentrations, exacerbates hepatic fibrosis induced by CCl_4. Increased fibrosis in the presence of ethanol was associated with an impaired angiogenic response to the initial hepatic injury induced by CCl_4. Taken together, these data suggest that therapies to normalize angiogenesis may be useful in preventing ethanol-induced hepatic fibrosis.

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Association of overweight and obesity worsens the evolution of alcoholic liver cirrhosis

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Introduction: The particular features of the body contribute to the constitution and evolution of the alcohol aetiology liver pathology. This paper aims to evaluate the influence of overweight and obesity on the morbidity and mortality in alcoholic liver cirrhosis.

Methods: At a group of 9109 consecutive patients who were hospitalised in the Emergency Clinical County Hospital from Sibiu, there was studied the association of overweight and obesity at the alcoholic liver cirrhosis. The patients with other pathologies associated with alcoholic cirrhosis were excluded from the study.

Results: The study group was formed by 5360 women (58.85%) and 3749 men (41.15%) with a medium age of 53.13 years. At a number of 588 patients the diagnosis of alcoholic liver cirrhosis was established at their first hospitalisation: 518 were men (88%) and 70 were women (12%). From these, 398 patients (67%) were overweight or obese, with no significant difference regarding gender. In a two years observation, a number of 113 patients with liver cirrhoses died; 85 of these (75.22%) were diagnosed with alcohol related liver cirrhosis: 72 men (84.7%) and 13 women (15.3%). Overweight and obesity were present at 58 patients (68.23%) from the patients who have died due to alcohol liver cirrhosis.

Discussion/Conclusion: At an adult population of hospitalised patients from Southern Transylvania, the study of morbidity and mortality in liver alcoholic cirrhosis reveals a statistical significant association of overweight and obesity. These data suggest that the weight excess represents an aggravator factor for the evolution of this liver disease.

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Chronic pancreatitis after lead contaminated alcohol consumption

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Introduction: In the geographical area from Southern Transylvania there is often met the habit of manufacturing concentrated alcohol with improvised equipment. In this way of manufacture, the alcohol can be contaminated with lead. The lead intoxication, unrelated with profession, raises difficult diagnostic problems, especially in the context of alcohol consumption. This paper aims to evaluate the influence of the chronic alcohol consumption in association with the lead intoxication on the pancreatic level at the patients who have suffered an acute episode of pancreatitis.

Methods: We have take into consideration a group of 9 consecutive male patients, who have presented an episode of acute pancreatitis which was due to lead contaminated alcohol consumption and who have continued this habit. The mean duration of patients follow-up was of 13.3 years. The parameters which were analysed were the following: mean daily quantity of alcohol consumption, clinical manifestations, provoked hyperglycaemia, steatorrhea, imagistic changes (ultra-sound, computer tomography), provoked urinary lead, delta-aminolevulinic acid and porhobilinogen. As a controlled group, we have used a group of 10 consecutive patients who have presented an episode of acute pancreatitis which was due to alcohol consumption and who have continued this habit. The mean duration of these patients follow-up was of 14.6 years.

Results: 7 out of 9 patients who have consumed lead contaminated alcohol (with a mean daily quantity of 110 g) have developed chronic pancreatitis during the follow-up. From the 10 patients who have consumed pure alcohol, only one patient developed chronic pancreatitis.

Discussion/Conclusion: The association between lead intoxication at the chronic alcohol consumption can aggravate the process of developing chronic pancreatitis. The lead is acting directly on the pancreas, but also through the porphyria which it induces.

Complication of chronic pancreatitis associated with obesity in heavy-drinkers patients

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Introduction: The aim of this study was to describe the relationship between alcohol consumption, obesity and the incidences of complications in chronic pancreatitis (CP).

Methods: We included in this study 79 patients with chronic pancreatitis. The diagnosis was established by clinical picture, biochemical and imagistic investigations (US, CT, ERCP). The analysis was performed on two groups: A group consist of 42 patients with alcoholic chronic pancreatitis and B group composed of 37 patients with non-alcoholic pancreatitis (biliary 18 cases, hypercalcemia 2 cases, hypertriglyceridemia 7 cases, idiopathic 8 cases and trauma 2 cases).

Results: The mean value of BMI was elevated (27.88 ± 4.79 kg/m^2) and the incidences of obesity was higher (70.88%, 56 cases with BMI > 30 kg/m^2), comparative with only 23 normoponderal patients. The patients with alcohol consumption was grouped, function by frequency and quantity, in 3 categories: 22 cases with more than 80 mg alcohol-intake in every day, 12 cases with 40–80 mg alcohol-intake, 3–4 times/week and 8 cases with 20–40 mg 1–3 times/ week.
In 57.14% of the obese patients (32 cases) and in 26.08% of the normoponderal patients (6 cases) the chronic pancreatitis presented complications. In A group, the complications were: pancreatic cancer (16 cases), pseudocysts (4 cases), abscess (2 cases), hemorrhage of the digestive tract (4 cases) and diabetes (15 cases). Multiple complications with severe evolution were observed in patients with alcoholic CP. Calcifications was developed in 42.85% of cases in alcoholic pancreatitis. Liver cirrhosis was associated with CP in 14 heavy-drinkers patients. In B group, the incidences of complications were significant reduced: 32.43% (12 cases).
Frequency of complications was associated with BMI values and alcohol consumption categories. We identified a relationship between risk of complication, frequency of alcohol consumption and the age of starting drinking ($r = 0.39$, $p < 0.01$).

Discussion/Conclusion: Alcohol consumption and obesity remains the main risk factors for develop complications in chronic pancreatitis. The complications of CP were more severe in heavy drinkers.

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Resveratrol inhibits amylase release and protein tyrosine phosphorylation in rat pancreatic AR4-2J cells by an increase of protein tyrosine phosphatase activity

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Introduction: Chronic excessive consumption of alcoholic beverages is clearly associated with acute and chronic pancreatitis. While the role of alcohol in pancreatitis has been studied since more than thirty years the role of non-alcoholic compounds of alcoholic beverages was hardly investigated. One of these compounds is resveratrol a polyphenol which is also found in fruits and vegetables. Resveratrol has been recently reported to exhibit anti-inflammatory, cell growth modulatory and anticarcinogenic effects. The aim of this study was to determine the effects of resveratrol on protein secretion and signal transduction in the rat pancreatic acinar cell line AR4-2J.

Methods: Amylase released by the cells into the supernatant within 60 min of incubation was determined using the Phadebas Amylase Test (Pharmacia). Western blot analysis was applied to assess protein tyrosine phosphorylation after 15 min stimulation of cells.

Results: Incubation of AR4-2J cells with resveratrol (10–300 µM) had no effect on basal protein secretion. However, amylase secretion stimulated by 100 nM bombesin was dose-dependently inhibited at resveratrol concentrations of more than 50 µM. Resveratrol inhibited also both time (15–60 min) and dose-dependently (50–300 µM) bombesin-induced tyrosine phosphorylation of proteins with apparent molecular masses of 120–130 and 70 kDa. Furthermore, resveratrol led to rounding off of AR4-2J cells indicating changes in cell cytoskeleton. Since disruption of the actin cytoskeleton leads to an increased protein tyrosine phosphatase activity and subsequently to reduced tyrosine phosphorylation, the effect of resveratrol was compared to those of tyrosine kinase inhibitor genistein and actin cytoskeleton disrupting toxins Cytochalasin B or Latrunculin B. Incubation of the cells with the protein tyrosine phosphatase inhibitor pervanadate induced strong tyrosine phosphorylation which could be inhibited by genistein but not by resveratrol, Cytochalasin B or Latrunculin B.

Discussion/Conclusion: Our data suggest that resveratrol induces activation of an actin cytoskeleton-controlled protein tyrosine phosphatase leading to a decrease of protein tyrosine phosphorylation in AR4-2J cells which is involved in bombesin-stimulated amylase secretion.

Echovirus pancreatitis and parotitis following infliximab and prednisolone therapy in a patient with ulcerative colitis

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Introduction: Anti-tumor necrosis factor alpha inhibitors and corticosteroids are effective in treatment of inflammatory bowel disease but association with serious infections has generally been attributed to increased risk of bacterial and opportunistic fungal microorganisms. In recent years studies have reported increased risk of serious viral infections. Hereby, we present a patient who developed in same time echovirus parotitis band pancreatitis following therapy with infliximab and prednisolone.

Case report: In May 2009 a 19-year old girl, with ulcerative colitis, was admitted with 10-day history of diarrhea occurring seven-to-ten times per day with stools with mucus and blood. In April she received last infusion of infliximab. After that she had been taking prednisolone but had ongoing active disease. Second day of admission she got severe acute abdominal pain, nausea and vomiting. She suffered also of pain band swelling in infraauricular areas. Examination revealed pyrexia, swelling parotid-glands band diffuse tenderness of abdomen. Laboratory test showed lymphocytosis band increased inflammatory biomarkers. Amylases band lipase was significantly elevated. Abdominal computed tomography revealed a mild swelling of the pancreas. Ultrasonography of parotid glands showed diffuse enlargement. Serological test of commonly infection agents were all negative, but antibody titer against echoviruses was markedly elevated. After diagnosis, patient's immunosuppression was reduced and clinical course was satisfactory.

Discussion/Conclusion: Infliximab and corticosteroids have been associated with an increased risk of viral infection, but to our knowledge our case for the first time describes synchronous echovirus pancreatitis band parotitis.

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Analysis of the etiology in 458 pancreatitis patients according to the M-ANNHEIM multiple risk factor classification

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Introduction: Chronic pancreatitis (cP) represents a complex disorder with interaction of various risk factors. The M-ANNHEIM classification addresses this issue and stratifies multiple (M) etiological risk factors such as Alcohol (A), Nicotine (N), Nutrition (N), Heredity (H) (including idiopathic disease), Efferent duct factors (E), Immunity (I), Miscellaneous factors (M), differentiates various disease stages and defines different degrees of disease severity. The aims were to identify the interaction and frequency of known etiological risk factors in pancreatitis patients.

Methods: Patients (n = 458 with sufficient data, exclusion of biliary pancreatitis) from our clinic in Germany (1997–2008) were classified according to the M-ANNHEIM classification.

Results: In n = 172/458 patients (38%, n = 49 borderline cP, n = 123 definite or probable cP), only up to one etiological risk factor was found. In n = 286/458 patients (62%, n = 62 borderline cP, n = 224 definite or probable cP), two or more risk factors were identified. Alcohol was observed as a risk factor in n = 266/458 (58%) patients. Nicotine was found in n = 311/458 (68%) individuals. Alcohol was found in n = 25/172 (15%) and Nicotine in n = 44/172 (26%) patients with up to one risk factor. Alcohol was detected in n = 241/286 (84%) and Nicotine in n = 67/286 (93%) patients with multiple risk factors. Interaction of Alcohol with Nicotine was found in n = 236/286 (83%) patients with multiple risk factors. Idiopathic disease without any known etiological risk factor was identified in only n = 56/458 (12%) patients.

Discussion/Conclusion: Nicotine is more frequently found as a risk factor of pancreatic inflammation than Alcohol. Idiopathic pancreatitis with absence of any known risk factor is rare.

Clinical and morphological elements influencing health related quality of life in patients with chronic pancreatitis

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Introduction: The aim of the study was to examine the quality of life (QOL) in patients with chronic pancreatitis (CP) of varying severity and to determine the factors associated with poor physical and mental health, using SF-36 (a generic questionnaire for the assessment of health status with 36 questions grouped in eight scales: physical functioning [PF], role physical functioning [RP], bodily pain [BP], general health [GH], vitality [VT] social functioning [SF], role emotional functioning [RE] and mental health [MH]).

Methods: We studied 104 patients, (93.2% male and 6.8% female) from a tertiary care gastroenterology clinic. The diagnosis of CP was made by a combination of morphologic, functional and clinical findings. Data were obtained either by face-to-face interview (31%) or by telephone (69%).

Results: Scores for PF, RP, BP, GH, VT, SF, RE and MH were as follows: 58.7, 65.1, 77.6, 65.7, 54.4, 53.6, 69.4, and 66.9. When compared to the general population (values of mean SF-36 scores published by The National Institute of Statistics), all domains of health-related quality of life were reduced in CP, mainly for PF ($p = 0.08$), symptoms' severity ($p = 0.005$) and cognitive function ($p = 0.004$). Regarding the severity of CP assessed by imagistic (CT, EUS and ERCP) and functional means (fecal elastase test), the 64 (61,7%) patients meeting the stage III Cambridge criteria scored significantly worse then those in stage II (n = 28, 27.1%); or I (n = 12,11.1%) ($p < 0.005$). Out of the various variables that we considered as possible factors related to CP (pancreatic calcifications, Wirsung duct dilatation, pseudocysts, pancreatic insufficiency, diabetes), only pain significantly impaired all eight domains (ANOVA, $p < 0.0005$ for all scales).Regarding the changes in pancreatic morphology, main pancreatic duct dilatation was the element that influenced the most QOL. Between clinical variables patients presenting diabetes mellitus, pancreatic insufficiency and BMI < 19 scored worse mainly for physical domains.

Discussion/Conclusion: Patients with CP presented an impaired QOL witch was more important in the physical component than in the mental component. In our study, pain was the clinical element that impaired all 8 domain of SF-36, whereas pancreatic morphological changes and other clinical elements influenced only some of these domains.

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Alcohol abuse as a risk factor for hepatocarcinoma in non-cirrhotic liver

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Background: Hepatocellular carcinoma (HCC) usually develops in patients with liver cirrhosis or chronic liver disease. The etiology of HCC is variable among geographical regions, depending on the prevalence of chronic hepatitis B, C and alcohol intake.

Aim: The aim of this study was, to analyze the role of alcohol intake as risk factor for HCC in patients without cirrhosis.

Methods: A total of 121 patients, histological diagnosed with HCC, admitted in a tertiary center between 2005–2009, was retrospectively evaluated.
Serorogical profiles for HCV and HBV infections, alcohol consumption (> 80 g/day) and also histological findings of nontumoral liver tissue (fibrosis, necroinflammatory activity, steatosis, alcoholic hepatitis, iron overload and hepatocellular displasia) were registed. Chronic hepatitis was classified by grading and staging according to Knodell's histological activity index.

Results: Were evaluated 121 patients with HCC and noncirrhotic liver (26% of all HCC). The median age was 61 years and 78.1% were male. The etiology of hepatic injury was alcoholic 10.7%, HCV 49.5%, HBV 27.2% and in 35.5% mixed etiology (alcoholic and viral).
The histological changes in nontumoral tissue were: Chronic hepatitis (predominant severe form 60.8%), fibrosis (predominant F2–F3 in 79.3% cases), typical histological findings of alcohol intake (6.6% of patients), steatohepatitis (30.5%), steatosis (52%), iron overload (43.8%) and small liver cell dysplasia (only 15.7%).

Conclusions: Our study showed that majority of patients with HCC are cirrhotics, males and ethanolic etiology has the second place, after the viral etiology, probably because of a high prevalence of viral infections in our area. HCC occurs in histologically abnormal liver and the premalignant lesions could start before the development of cirrhosis. Advanced liver fibrosis without cirrhosis was found in patients with HCC and history of heavy alcohol intake.

Mortality risk factors in alcoholic hepatitis

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Introduction: Alcoholic hepatitis (AH) represents a severe form of alcoholic liver disease with potential mortality. The aims of our study were to describe demographic, clinical and laboratory findings and to identify mortality risk factors in a country with a high prevalence of alcoholism.

Methods: Patients with AH were identified from the database of hospital discharges between 2004 and 2009. We analyzed age, gender, clinical findings, liver and renal functions tests, treatment and causes of death. Fisher's exact test was used to compare gender, presence of cirrhosis, prognostic scores (Maddrey, MELD) and encephalopathy with mortality.

Results: The age of 95 patients ranged from 26 to 87 years (mean ± SD, 50 ± 10.9) with a gender proportion of 81 males (85%) and 14 females (15%). 80 patients (84%) had cirrhosis and 32 patients died (33%). The dead group had a mean age of 50 (± SD 11), while survivors were 49.4 years old (± SD 10.3), all were cirrhotic ($p = 0.0011$; LR = 13.7), 10 were female ($p = 0.0018$; RR = 2.6), all had Maddrey score > 32 ($p = 0.000$) and a MELD score > 18 ($p = 0.000$) and 16 presented encephalopathy ($p = 0.015$, RR = 1.96). The most common cause of death was hepatorenal syndrome (43%), followed by liver failure (22%), variceal bleeding (12.5%) and pneumonia (12.5%). Spontaneous bacterial peritonitis was detected in three patients. No benefit was found in severe AH (n = 71) treated either with pentoxyfilline (n = 41) or steroids (n = 12).

Conclusion: The majority of patients with AH are middle-aged cirrhotic men. Most of cases are severe. The presence of cirrhosis, female gender and development of renal and/or liver failure determine high mortality in AH patients.

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Hepatic stellate cell apoptosis is mediated through differential aquaporin expression

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Introduction: Alcohol consumption is a major risk factor for the development of fibrosis, which is characterized by accumulation of secreted type I collagen by hepatic stellate cells (HSCs). Resolution of fibrosis, in part, is attributed to induction of HSC apoptosis and inhibition of HSC activation. Aquaporins (AQP) play a critical role in the initiation of apoptosis by controlling the apoptotic volume decrease.

Methods: Primary HSCs were isolated from Sprague-Dawley rats. Parallel studies were conducted using human HSCs (LX-2). AQP expression in quiescent (dQ) and activated (d14) HSCs was determined by RT- and RealTime PCR, immunoblot analyses and immunohistochemistry. An ethanol-LPS fibrotic liver rat model was used to investigate AQP expression in vivo. AQP function was determined by cell swelling. Apoptosis was assessed by morphology, caspase-3 activity and PARP cleavage with or without $HgCl_2$, an AQP inhibitor.

Results: AQP 0, 1, 5, 8, 9, 11 and 12 mRNAs were detected in dQ, while d14 and LX-2 cells exhibited a marked decrease in expression. AQP 0, 1, 8 and 9 protein expression was decreased in d14 versus dQ, while AQP 11 expression increased in d14. Conversely, AQP 11 protein was not detected in LX-2 cells. Results were confirmed in vivo by dual immunofluorescent staining. Osmotic challenge significantly increased cell swelling in dQ, which was abolished by $HgCl_2$ pre-treatment, while d14 and LX-2 cells did not swell. D14 and LX-2 cells exhibited decreased cellular viability after one hour exposure to the apoptotic agent gliotoxin, whereas dQ responded within 20 minutes. Finally, apoptosis was abrogated by inhibition of AQP-dependent water movement.

Discussion/Conclusion: HSCs express AQPs and down-regulation of AQP expression/function contributes to resistance to apoptosis and thus persistent activation and cell survival. Modulation of HSC AQP expression may lead to the development of novel therapeutics for treating hepatic fibrosis.

Ethanol suppresses proteasome function in HCV+ hepatocytes via induction of oxidative stress and impaired protein methylation

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Introduction: Proteasome is a multi-catalytic enzyme involved in intracellular degradation of aged or oxidatively modified proteins, peptides and signal transduction factors. The goal of this study was to characterize the composition and activity of 26S proteasome isolated from hepatocytes of HCV core+ and HCV- mice fed with control diet or 20% of ethanol in water for 5 weeks.

Methods: Proteasome was precipitated from cytosols obtained from liver homogenate and then isolated on 10–40% of glycerol gradient. The same amount of protein from each analyzed 33 fractions with the highest chymotrypsin-like proteasome activities was used to perform proteasome enzyme assays and Western blots.

Results: Ethanol feeding decreased the amount of 20S proteasome. These changes in 20S proteasome content (within 26S proteasome particle) were consistent with ethanol-elicited suppression of Ecm29, a protein that stabilizes 26S proteasome. Ethanol feeding also decreased the level of de-ubiquitinating enzyme, UCHL5, which interacts with 19S proteasome and dissembles Lys48-linked poly-ubiquitin from proteins. In addition, *in vivo* ethanol exposure slightly suppressed incorporation of immunoproteasome subunits, LMP7 and LMP2, to 20S proteasome. Also, the level of 20S proteasome activator, PA28α, was lower in hybrid proteasome from ethanol-fed mice, and these changes were more prominent in HCV+ mice. The latter was accompanied by the reduction in methyl lysine in proteasome isolated from HCV+ mice, suggesting that HCV core protein expression may be responsible for proteasomal hypomethylation. Interestingly, ethanol-mediated alterations in proteasome composition combined with normal methylation of proteasome on lysine residues provided very low inhibition of chymotrypsin-like proteasome activity in ethanol-fed HCV- mice. However, changes in proteasome composition combined with hypomethylation status of proteasome caused a reduction in proteasome activity of HCV+ mice fed with ethanol. These mice showed the highest oxidative stress (based on the elevation of ROS, lipid peroxidation and low GSH levels) in hepatocytes from ethanol-fed vs control group or HCV- littermates.

Discussion/Conclusion: We conclude that the combination of impaired proteasome methylation on lysine residues with high oxidative stress mediated by both HCV and ethanol, suppresses proteasome activity, thereby negatively affecting downstream proteasome functions.

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Impaired protein and lipid trafficking: Its role in the development of alcoholic liver injury

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Introduction: Research in our laboratory has focused on alcohol-impaired protein trafficking in the liver, with special attention to the role of impaired endocytosis by the hepatic asialoglycoprotein receptor (ASGP-R). Recently we have broadened our study to include impaired trafficking of lipid droplets (LDs), neutral stores of fat which accumulate during both non-alcoholic steatohepatitis (NASH) as well as alcoholic steatohepatitis (ASH). Fatty liver or steatosis is a hallmark of both NASH and ASH, and altered trafficking and utilization of LDs, in addition to altered protein trafficking, may contribute to the alcoholic injury process.

Methods: We are studying impairments in lipid and protein trafficking in several models. These include alcohol feeding to rats and mice with subsequent use of cultured primary cells and/or isolated LDs from these animals, as well as cultured, polarized, ethanol-metabolizing hepatoma cells (WIF-B cells), which are a well-characterized model for protein trafficking.

Results: In rats, we have shown that ethanol-impaired ASGP-R function causes decreased uptake of apoptotic cells, as well as of ligands such as cellular fibronectin (cFn, a known fibrogenic protein). Accumulation of both apoptotic cells and cFn promote production of pro-inflammatory cytokines, thereby contributing to the liver injury. Studies with ASGP-R deficient mice showed enhanced injury after toxic insults by alcohol, as well as anti-Fas antibody (CD95), endotoxin (LPS)/galactosamine, and carbon tetrachloride, which link impaired receptor function to liver injury. In cultured cells, we have identified ethanol-induced cell injury in WIF-B cells. Our recent studies show that ethanol exacerbates lipid droplet accumulation and impairs efflux of loaded fat from cultured WIF-B cells. Similar to studies with animal models, ethanol metabolism is a necessary component of this accumulation and impaired efflux in WIF-B cells.

Discussion/Conclusion: These results indicate a significant role of altered protein trafficking and lipid droplet accumulation and efflux in alcohol-induced liver injury.

Alcohol-loading and obesity regulate the expression of transferrin receptor 1 and hepcidin in mice liver

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Introduction: Both chronic alcohol intake and obesity frequently cause to hepatic iron overload and may contribute to hepatic carcinogenesis through production of free radicals. Previously, we reported that the expression of transferrin receptor 1 (TfR1), which mediates cellular transferrin-bound iron uptake, was increased in hepatocytes in patients with alcoholic liver disease (ALD). Recently it has been reported that alcohol metabolism down-regulates the expression of hepcidin (Hepc) both in vivo mouse model and in vitro hepatoma cell lines. However the mechanism of iron overload in metabolic steatosis due to obesity, frequently merges with ALD, is still unclear. In this study, we investigated the gene expressions of TfR1 and Hepc in the liver and the serum concentration of mature Hepc peptide in obesity and/or alcohol-loaded mouse model.

Methods: C57BL/6 and ob/ob mice were fed on a regular rodent chow diet. 10% ethanol was added in the drinking water to alcohol-loaded group for 7 days. Hematoxylin-eosin and oil red O stains were performed for histopathologic evaluation. Hepatic iron concentration was estimated by atomic absorption spectrophotometry method. The gene expressions of TfR1 and Hepc in the mice liver were evaluated by quantitative real-time RT-PCR method. The serum concentration of mature Hepc peptide was evaluated by Liquid chromatography/electrospray ionization tandem mass spectrometric technology (LC/ESI-MS/MS).

Results: Total iron content in the whole liver in ob/ob mice was significantly higher than in wild type ($p < 0.005$). The expression of TfR1 mRNA in the liver was significantly higher in ob/ob mice and/or alcohol-loaded mice than in control ($p < 0.05$). The expression of Hepc mRNA in the liver was significantly lower in ob/ob mice and/or alcohol-loaded mice than in control ($p < 0.05$). Furthermore, the serum concentrations of mature Hepc in ob/ob mice with water and with alcohol-loaded were significantly lower than in control ($p < 0.01$).

Discussion/Conclusion: Our data suggest that both metabolic steatosis and alcohol load might up-regulate TfR1 expression and down-regulate Hepc expression respectively, following iron absorption from the small intestine and increased iron uptake into hepatocytes.

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Expression of angiopoetin-2 (Ang-2) and vascular endothelial growth factor (VEGF) in duodenal tissue of patients with alcoholic liver cirrhosis

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Introduction: Laboratory animals with experimentally-induced portal hypertension (PH) show significant alteration in expression of pro-angiogenic factors. Molecular mechanisms involved in formation of varices secondary to PH in humans remain to be fully understood. Such knowledge could be of importance in developing new therapies aimed at amelioration of dangerous complications of PH. The aim of this study was to analyze the expression of pro-angiogenic factors including Ang-2, VEGF and VEGFR2 in duodenal tissues from a homogenous group of patients with alcoholic liver cirrhosis.

Methods: Eleven consecutive patients with alcoholic liver cirrhosis (6M, 5F; age 50 ± 3) were included. Duodenal biopsies were obtained during routine endoscopy. Ten subjects (5M, 5F; age 59 ± 5), without cirrhosis, who underwent endoscopy for other reasons were used as controls. Blood samples were obtained before each endoscopic procedure. 6 patients with cirrhosis received treatment with beta-blockers. Analysis of mRNA and protein expression were performed by real-time PCR and ELISA. The study was accepted by ethics committee and consents were obtained from all included subjects.

Results: Seven patients with cirrhosis had esophageal varices on endoscopy. Real-time PCR analysis of duodenal tissues showed the increased expression of Ang-2 mRNA (3.8-fold increase vs. controls, $p = 0.007$) but no changes in VEGF and VEGFR2 mRNA levels. Treatments with beta-blockers had no significant effects on the transcript levels of the examined genes. Protein levels of Ang-2 and VEGF in patients' duodenum showed 3.5-fold and 2-fold increase, respectively (both $p < 0.05$ vs. control). In serum samples VEGF protein levels were enhanced in cirrhotic patients (2-fold increase, $p < 0.05$ vs controls).

Discussion/Conclusion: Ang-2 and VEGF contribute to the formation/modulation of a network of portosystemic collateral vessels induced by alcoholic liver cirrhosis and PH. Increased blood flow and pressure within the vessel are important hemodynamic triggers initiating angiogenesis within splanchnic circulation.

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Iron overload in ALD: Deregulated expression of hepcidin in liver cells?

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Introduction: Iron overload is an often observed phenomenon in livers of ALD patients. Since hepcidin represents the major regulator of iron homeostasis we investigated how hepcidin expression is controlled in liver cells in response to either transforming growth factor (TGF)-β or bone morphogenetic proteins (BMPs).

Methods: Hepatocytes and hepatic stellate cells (HSC) were isolated from mouse livers and treated with different TGF-β family ligands in vitro (monolayer cultures). HSC were allowed to transdifferentiate to myofibroblast-like cells in culture and expression of hepcidin was monitored by real-time PCR.

Results: TGF-β as well as BMPs 6 and 9 rapidly and strongly induce hepcidin expression in hepatocytes. While induction by TGF-β is transient and disappears within 24h that by BMPs is constant and long lasting. Interestingly, BMP-9 was a more potent inducer of hepcidin in hepatocytes than BMP-6. In addition, BMP-9 induced hepcidin in short-time cultured HSC, although this was observed at later time-points (24h) than in hepatocytes. Furthermore, HSC strongly induce hepcidin expression during early stages of fibrotic transdifferentiation, while fully established myofibroblasts do not express it any more.

Discussion/Conclusion: We show that TGF-β which is known to be high in fibrotic livers of ALD patients can induce hepcidin expression and it remains to be investigated how this is inhibited in vivo and thereby allows hepcidin reduction and consequent deposition of iron. HSC have so far not been considered as participants in the regulation of iron. One future aim will be to investigate whether HSC-derived hepcidin is indeed active. Taken together the results demonstrate that TGF-β and BMPs regulate hepcidin expression in both, hepatocytes and HSC, implying that there exist complex interactions between pathways and cell types.

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Metabolites of arachidonic acid – Lipidic kiss of death in steatohepatitis?

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Background: Eicosanoids are key players in inflammation. Their precursors – fatty acids originating from membrane phospholipids – are metabolized by cyclooxygenases to prostaglandins, prostacyclins and thromboxanes, and by lipooxygenases (LOX) to leukotriens, hydroxy-metabolites of arachidonic acid – HETE and linolenic acid – HODE. Phospholipases such as cytoplasmatic $cPLA_2$ and secretory $sPLA_2$ release fatty acids from membranes and $sPLA_2$ is responsible for ROS synthesis. HETE and HODE can act as PPAR agonists and stimulate inflammation. Markers of oxidative stress intensity are prostaglandins isomers i.e. isoprostanes. They also activate platelets and myocytes in a similar fashion to thromboxan A2.

Aim: To evaluate the intensity of inflammatory processes in healthy individuals, and patients with alcoholic liver disease (ALD).

Patients and methods: The analyzed cohort consisted of 20 healthy volunteers and 63 patients with ALD. All analyzed subjects were Caucasians. Plasma 12-, 15-, and 5-HETE as well as 9-, 13-HODE were assessed with HLPC using LiChrospher 100-RP18 column. $sPLA_2$ and isoprostane 8-i-PF_2 were evaluated with immunochemistry using commercial tests (Cayman, USA). Statistica 7.1 software was used for the statistical analysis.

Results: We found the most significant difference between the levels of isoprostane, both products of $sPLA_2$ and 5LOX in analyzed groups of patients, but no difference in $sPLA_2$ activity. The results of U Mann-Whitney correlations ($p < 0.05$) are showed in table.

	Healthy vs. ALD
8-i-$PF_2\alpha$III	P < 0.0001
$sPLA_2$	NS
DiHETE	P < 0.0001
9+13 HODE	P < 0.0001
15-HETE	P < 0.0001
12-HETE	P < 0.007
5-HETE	P < 0.0001

Conclusion: Our data support the previous results obtained in animal models according to which 5LOX pathway may trigger the significant number of Kupffer cells and increase inflammation, steatosis and fibrosis in liver disease. Apoptotic hepatocytes may thus send the kiss of death, activating cyclooxygenases to increase ROS synthesis.

S-Adenosylmethionine regulates ubiquitin-conjugating enzyme 9 protein level and sumoylation

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Objectives: Sumoylation is a post-translational modification that can alter function and stability of the target proteins. Ubiquitin-conjugating enzyme 9 (Ubc9), the sole SUMO E2 conjugating enzyme, is overexpressed in several cancers but has not been reported in hepatocellular carcinoma (HCC). Genotoxic stress occurs in cancer and induces sumoylation of many proteins. Mice lacking methionine adenosyl-transferase 1a (*Mat1a* KO) have chronic hepatic S-adenosylmethionine (SAMe) deficiency, increased genotoxic stress and develop HCC. Ethanol feeding also results in hepatic SAMe deficiency. The aim of this study was to examine whether SAMe regulates Ubc9 expression.

Methods: Studies were conducted using livers from *Mat1a* KO and ethanol-fed mice, HCC specimens, HepG2 and Huh-7 cells.

Results: Ubc9 protein but not mRNA levels increased in livers of *Mat1a* KO and ethanol-fed mice compared to controls by 2 and 1.7-fold, respectively. Ubc9 protein levels also more than doubled in human HCC samples. SAMe treatment in *Mat1a* KO mice for 7 days normalized Ubc9 protein levels and reduced overall protein sumoylation. SAMe and its metabolite methylthioadenosine (MTA) treatment lowered Ubc9 protein levels by 30–60% in HepG2 and Huh-7 cells without affecting mRNA level. SAMe and MTA are selectively pro-apoptotic in liver cancer cells and Ubc9 knockdown by RNAi also promoted apoptosis and reduced Bcl-2 expression.

Conclusions: Ubc9 protein levels increase when hepatic SAMe levels fall and in human HCC. SAMe treatment decreases Ubc9 protein level and sumoylation. SAMe and MTA's pro-apoptotic effects in liver cancer cells may also be partly mediated by inhibiting Ubc9 expression.

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Role of the hepatic asialoglycoprotein receptor in lymphocyte-associated liver injury in concanavalin A-mediated acute murine hepatitis

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Introduction: We have previously shown that the asialoglycoprotein receptor (ASGPR), an endocytic hepatic lectin, is markedly impaired in models of alcoholic liver disease (ALD). It has also been shown that ethanol administration results in enhanced susceptibility to concanavalin A (Con A)-induced T cell-mediated hepatitis, demonstrating the importance of infiltrating lymphocytes in ALD. Recently, it has been suggested that ASGPRs may be involved in the regulation of intrahepatic T cells as the binding of desialylated lymphocytic proteins to the receptor may trigger the elimination of lymphocytes. Here we investigated whether altered ASGPR activity could be related to the dysregulation of intrahepatic T lymphocytes and enhancement of T-cell mediated hepatitis.

Methods: ASGPR deficient (RD) and counterpart wild-type (WT) mice were intravenously administered Con A (5–15 mg/kg). The extent of hepatitis and liver-associated lymphocytes were analyzed up to 16 hours later.

Results: Con A-mediated hepatotoxicity was induced within 4 hours of treatment as indicated by elevated serum transaminase and TNF-alpha levels in proportion with the concentration of Con A administered. In the RD mice however, these effects were significantly enhanced as indicated by increased liver transaminases, liver to body weight ratios, and levels of inflammatory mediators (TNF-alpha and IL-4). Additionally, histological evidence of necrosis and expression of Fas ligand were elevated in ASGPR-deficient livers compared to WT mice. Strikingly, a significant increase (2-fold, $p < 0.01$) in CD8+ T cells was also observed in the RD livers that was sustained up to 16 hours post Con A treatment.

Discussion/Conclusion: The lack of hepatocyte ASGPRs results in an exaggerated response to the T-cell mitogen, Con A, evident by cytokine upregulation and increased leukocyte accumulation in the liver. Our findings support the suggestion that ASGPRs have a role in the regulation of liver-associated lymphocytes involved in the pathogenesis of alcohol and Con-A mediated liver disease.

Features of copper homeostasis in patients with chronic hepatitis C

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Introduction: Increased concentrations of copper can serve as prooxidant and profibrogenetic agents in patients with CHC.

Methods: Were examined basic liver function tests, the HCV markers, serum copper and ceruloplasmin levels. Control group constituted 29 healthy individuals.

Results: In patients with CHC was found higher level of copper (39.29 ± 2.08 μmol/l), respectively versus the same data in healthy individuals (15.96 ± 3.12 μmol/l) ($p = 0.001$). The increased level of ceruloplasmin was determined in patients with CHC (180.42 ± 4.50 mg/l) in comparison with healthy individuals (116.42 ± 2.66 mg/l) ($p < 0.001$). Copper level in patients with moderate/high level grade of liver inflammation (47.52 ± 4.84 mmol/l) was found higher than that in patients with minimum grade (36.78 ± 2.23 mmol/l) ($p < 0.05$). In patients with reactivation phase of viral infection C was detected hipercupremia more expressed compared to patients with latent phase ($p < 0.05$).

Discussion/Conclusion: A direct interconnection was established with the maximum copper and ALT level ($r_{xy} = 0.41$) and disease duration more than 5 years ($r_{xy} = 0.55$). Certifying indirect correlation between the maximum level of ceruloplasmin and viral load ($r_{xy} = -0.69$). Lasting hipercupremia in patients with CHC can serve as a negative factor for progress in fibrosis or cirrhosis of liver, due to prooxidant and profibrogenetic action of hipercupremia.

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Glypican-3 amino terminal marker for early detection of HCC

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Introduction: HCC is the 6th common cancer. Global increase of hepatitis B and C infection, the incidence of HCC has been steadily increasing. Egypt seroprevalence of HCV in Nile delta was 20–35%. AFP had limited sensitivity 60% and specificity 90% for small HCC. GPC-3 oncofetal protein over expressed in HCC.

Aim: Evaluating the validity of Glypican-3 as an early detector of HCC.

Material: 10 healthy controls and 40 HCV positive patients: 10 patients with chronic hepatitis C virus infection.10 patients with compensated cirrhosis [Child-Pugh class A and B]. 10 patients with decompensated cirrhosis [Child-Pugh class C]. 10 patients with HCC.

Methods: Liver functions: ALT, AST, Bilirubin (T), Albumin, γGT. Tumor markers: AFP and GPC-3. Viral markers: HCV antibodies, HBs Ag and HBc Ab.

Results: The median value of GPC-3 in HCC, DC, CC was significantly higher than chronic hepatitis and control groups. No significant correlation found between AFP and GPC-3. AUROC of AFP was 0.85 & AUROC of GPC-3 was 0.84. The diagnostic Sensitivity of AFP (20 ng/ml) was 70% with PPV 53.8%. The specificity was 85% with NPV 91.9%. While the diagnostic Sensitivity of GPC-3 (2 ng/ml) was 100% with PPV 27%. The specificity was 42.2% with NPV 100%. Combined serial approach of AFP and GPC-3 improved the specificity to 87.5%.

Conclusion: GPC-3 although it is a serological test for early detection of HCC, it showed limited specificity, where It is detected in different stages of chronic liver disease, as it is an oncofetal protein produced by regenerating liver cells. The diagnostic signature approach for simultaneous determination of AFP and GPC-3 may improve the prediction accuracy of HCC patients in those showing seronegativity to AFP.

The ultrasound study of the portal circulation and spleen in alcoholic cirrhosis: Relationship between noninvasive parameters and the severity of portal hypertension

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Introduction: The aim of our study was to analyze the value of the gray-scale and Doppler ultrasound (US) as a noninvasive parameter for the diagnosis of portal hypertension in alcoholic liver cirrhosis.

Methods: The study comprised 35 patients (21 males, mean age 52.57 years) diagnosed with alcoholic liver cirrhosis who had different degrees of esophageal varices (EV), assessed with endoscopy and no ascites. Our examinations included an abdominal US with spectral and Color Doppler, for portal flow evaluation, mean portal vein velocity (PVV) and portal vein dimension, spleen size measurement and the splenic index (SI) calculation.

Results: All patients presented low PVV, elevated dimension of the portal vein and elevated SI. The mean speed was 9.53 cm/sec, and the mean SI was 88.4 square centimeter. There was a negative correlation between PVV and the SI ($r = -0.46$), as well as between PVV and portal vein dimension ($r = -0.22$). From the 35 patients, 13 (37.14%) presented under-mean velocity and 3 or higher degree of EV, with the exception of two patients who had 2 degree EV. All of the 22 patients (62.86%) with upper-mean velocity had EV of 2 degree or lower. From the 21 patients with under-mean SI 20 presented small grade EV. From the 14 patients with upper-mean SI 10 had large grade EV. Our data suggest that the reduction of the PVV is correlated with the degree of EV ($p < 0.0001$) and the increase of the SI is significantly associated with the degree of EV ($p < 0.0001$).

Discussion/Conclusion: In patients with alcoholic liver cirrhosis the reduction of the portal velocity is correlated with the degree of esophageal varices and the splenic index is correlated with the severity of the portal hypertension respectively with the degree of EV. The ultrasound examination can be a complementary method to endoscopy in the follow-up of portal hypertension and correlation with esophageal varices.

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Assessment for liver fibrosis in alcoholic patients using Fibroscan

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Introduction: Because chronic, excessive alcohol consumption is a major public health problem, screening for liver fibrosis in chronic alcohol-drinking patients is mandatory. The transient elastography (Fibroscan) allows evaluation of liver fibrosis by measuring liver stiffness. The aim of this study was to assess Fibroscan for non-invasive diagnosis of liver fibrosis in alcohol abuse patients and to compare performance of Fibroscan with other laboratory tests.

Methods: Ninety five alcoholic patients were studied (patients aged more than 18 years old, who admitted to chronic consumption of more than 50 g/day of alcohol for more than 5 years). Fibroscan and aspartate aminotransferase (ASAT) to platelet ratio index (APRI) tests were performed. Spearman's correlation coefficient, univariate analysis and multiple regression analysis were used to study the relationship between liver stiffness and laboratory parameters.

Results: The recorded liver stiffness values were between 3.9 and 68 kPa, with a mean of 15.4 ± 19.1 kPa. The mean measurement success rate was 85.1 ± 28.4%, with an average of 11.4 ± 2 valid measurements. In univariate analysis, 9 parameters were significantly correlated with liver stiffness. In a multiple regression analysis only prothrombin time ($p < 0.01$) and alkaline phosphatase ($p < 0.01$) were associated with liver stiffness. The correlations between Fibroscan and APRI tests was 0.74 (0.51–0.94) for F1, 0.51 (0.38–0.57) for F2, 0.41 (0.31–0.49) for F3 and 0.52 (0.35–0.69) for F4 ($p < 0.01$ for F2, F3 and F4).

Discussion/Conclusion: The diagnostic performance of Fibroscan was better than the performance of APRI. The various combinations of the Fibroscan with non-invasive laboratory tests did not improve diagnostic performance relative to Fibroscan alone. Ultrasound-based transient elastography can be used on its own for the non-invasive diagnosis of alcoholic fibrosis.

Diagnosis of focal lesions of the liver – Visualizating examinations or biopsy

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Introduction: Owing to wide availability of modern visualizing diagnostic methods focal liver lesions are often diagnosed.

Methods: The retrospective study was conducted involving the patients with focal liver lesions hospitalized in the Department of Gastroenterology, Medical University of Lublin between 1 Jan 2003 and 1 Jul 2009.

Results: In the analyzed period 8307 patients were treated. Focal liver lesions were diagnosed in 1000 of them. In 901 patients focal lesions were diagnosed on the basis of visualizing methods. Cysts of hepatis occurred in 337 patients and hemangiomas in 477 cases. In 99 patients guided liver biopsy was necessary to establish the univocal diagnosis. Non-neoplasm and benign neoplasm constituted 58.5% of all biopted focal lesions. Metastases were the most frequent among malignant neoplastic lesions. In 7.01% of the patients who underwent the guided liver biopsy immunohistochemistry was absolute necessary to univocally diagnose the kind of focal lesion.

Discussion/Conclusion: The observation data demonstrated that focal liver lesions were diagnosed in every 8th hospitalizated patient. Cysts and hemangiomas occurred the most frequently and constituted 4.1% and 5.7% of all hospitalized patients, respectively, and 33.7% and 47.7% of patients with focal liver lesions. In the majority of cases the diagnosis was based on the visualizing methods. Every 10th focal lesion required the guided liver biopsy. Every 3rd biopted focal lesion was malignant neoplasm. Among them metastases occurred the most frequently.

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The adequacy of liver biopsies can be predicted from the length of fresh tissue obtained at the time of biopsy

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Introduction: An adequate liver biopsy should contain ≥ 10 portal tracts. We asked: what length of fresh tissue would guarantee enough microscopic tissue for an adequate liver biopsy after allowing for tissue shrinkage during fixation and processing?

Methods: Data for liver biopsies taken between 2004–2008 was analysed for the following: macroscopic length – length of tissue before processing/embedding, microscopic length on the pathology slides, number of portal tracts in each biopsy and diagnosis. We also conducted a prospective pilot study to correlate length of fresh tissue at the time of biopsy with the length of macroscopic formalin-preserved tissue at the time of arrival in the lab.

Results: There were 412 ultrasound-guided liver biopsies taken with 18 Fr needles in 195 females (F) and 217 males (M). Portal tracts could not be counted in the following: 98 cases of malignancy, 42 cases of cirrhosis and 14 insufficient biopsies. Of the remaining 258 biopsies, statistical analysis was performed on 192 (75%) samples where all of macroscopic length, microscopic length and number of portal tracts were recorded. The diagnoses were: 119 viral hepatitis (F = 45, M = 74 mean 37 yrs) 35 fatty liver disease (F = 14, M = 21 mean 49 yrs) 10 haemochromatosis (F = 2, M = 8 mean 59 yrs) 7 autoimmune hepatitis (F = 6, M = 1 mean 50 yrs) 6 normal liver (F = 3, M = 3, mean 43 y) 4 cholestatic liver disease (F = 2, M = 2 mean 54 yrs) 4 drug induced liver injury (F = 3, M = 1 mean 39 yrs) 7 others (F = 5, M = 2 mean 51 yrs). Tissue shrank consistently ($r = 0.837$, $p < 0.01$) from a mean length of 23.8 mm to 21.0 mm after processing. Using linear regression analysis, macroscopic length was found to be highly predictive of microscopic length (variance 95.1%) and the number of portal tracts (variance 85.2%). Chi-square analysis showed that age and sex were non-significant parameters for an adequate liver biopsy. **We calculated that 17.55 mm of macroscopic tissue equating to 15.89 mm of microscopic tissue ($p < 0.01$) would guarantee an adequate liver biopsy with at least 10 portal tracts in any diagnosis excluding cirrhosis and malignancy. Similarly, 30 mm of fresh tissue would guarantee ≥ 15 portal tracts in > 99% of cases.** Our pilot study of 10 consecutive biopsies showed no tissue shrinkage during transport in formalin to the lab.

Discussion/Conclusion: The length of fresh tissue from a liver biopsy obtained using an 18 Fr needle directly correlates to the number of portal tracts in all diagnoses excluding cirrhosis and malignancy. To ensure that patients have an adequate diagnosis and avoid a repeat biopsy we suggest that operators ensure **at least 18 mm** of fresh liver tissue at a cut-off of 10 portal tracts and 30 mm of tissue at a cut-off of ≥ 15 portal tracts. We advise operators to actively measure the length of tissue at the time of the procedure.

The abdominal duplex-Doppler sonography on alcoholic liver disease patients

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Consequences of alcohol abuse are difficult for prognosis. The early diagnosis of visceral organs destruction is important for choice mode of treatment and sometimes for patients live.

The aim of the study: The duplex-Doppler examination of patients with documented alcoholic liver disease (ALD). The control group were 40 healthy patients.

Patients and methods: 22 alcoholic patients (pts) with different abdominal complains were examined by duplex-Doppler method. Following parameters were estimated by two-dimensional presentation: The liver shape, abdominal organs and vessels size, liver echogenicity. The visceral spectrum size and shape flow has been assessed by Doppler presentation. During the clinical observation other diseases (HCV infection) which could influence the usg picture of the liver and/or bile ducts, were excluded.

Results: 6 alcoholic pts had not pathological USG symptoms. The rest had numerous sonographic symptoms important for disease grade and prognosis: Visceral vessels abnormality (portal/arterial flow pathological distribution, mesenteric arteries increased flow, portal vein dilatation, monophasic-flat hepatic flow), liver lesions signs (from hyperechogenic to cirrhotic liver), presence of ascites, bile ducts and/or pancreatic pathology, others.

Comments: There is no good correlation between severity of ALD on the one hand and the alcohol ingestion, clinical symptoms and laboratory data on the other hand. The clinical manifestation of following liver lesions periods – steatosis, steato-hepatitis, compensated and decompensated cirrhosis not always are visible. Liver biopsy often is essential to determine severity of disease. But it not always is possible – tendency for bleeding, thrombocytopenia. The use of duplex-Doppler examination in ALD pts may facilitate early diagnosis and sometimes eliminate invasive diagnostic procedures.

Conclusions:
1. The duplex-Doppler examination is the valuable diagnostic tool in alcoholic liver diseases pts.
2. Numerous pathological symptoms observed in duplex-Doppler examination allow to grade advancement of ALD independently from others diagnostic methods.
3. Results of duplex-Doppler examination may constitute important suggestion for therapy and prognosis in ALD patients.

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Assessment of severity of alcoholic steatohepatitis

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Introduction: Excepting histologically proven alcoholic steatohepatitis (ASH), the indication for corticotherapy is given by a Maddrey index ≥ 32 or on the presence of hepatic encephalopathy. However, this score neglects the 15% mortality rate when it is under 32.

Methods: We performed a retrospective study over a period of 3.5 years, performed on 224 patients with histologically proven ASH (mean age 53 yrs {29–70}; M/F = 152/72; acute alcoholic hepatitis 93 pts of which 43 with severe form, the remaining patients with cirrhosis). All patients who had a Maddrey index ≥ 32 or who presented hepatic encephalopathy received steroids in the form of methylprednisolon 32 mg over a period of 28 days.

Results: 72% of patients received steroids. Global survival at 3 months was 74%. 60 pts died after a mean period of 26 days (1–88) due to liver failure (N = 29), infection (N = 14), bleeding (N = 6) and other causes (N = 11). In univariate analysis, Maddrey ≥ 32 ($p < 0.001$), Child-Pugh > 10 ($p < 0.001$), MELD > 19 ($p < 0.001$), hepatic encephalopathy ($p < 0.001$), digestive bleeding ($p < 0.001$), age > 50 ($p < 0.05$), C reactive protein > 30 ($p < 0.02$), creatinine > 1.5 mg/dl ($p < 0 .001$), factor V ($p < 0.04$) were associated with mortality at 3 months. In multivariate analysis, age > 50 (0.003; 4 {1.45–6.25}), presence of hepatic encephalopathy (0.007; 2.3 {1.25–4.25}), creatinine > 1.5 mg/dl (0.001; 4.6 {2.5–8.7}), MELD score > 19 (0.001; 4 {1.8–9.25}) are independent predictive factors of mortality. In ROC curve analysis, the AUC of MELD was 0.846 (SE 0.027). A cut-off of 19 for the MELD score predicts mortality at 3 months, with a sensitivity of 86% and a specificity of 59%.

Discussion/Conclusion:

1. The performance of the MELD score is superior to the Maddrey index for predicting death at 3 months, with a good sensitivity and specificity.
2. The decrease of serum bilirubin levels after 7 days of corticotherapy is a predictive factor of survival at day 30, with a negative predictive value of 88.2%.

Potential role of serum adiponectin for diagnose of NAFLD or ALD

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Aim of our study was to evaluate the relationship beetwen serum adiponectin level and the progression of non-alcoholic liver disease (NAFLD) or alcoholic liver disease (ALD).

Methods: We included patients with bright liver on US and high levels of aminotransferases. We excluded the patients with other known cause of liver disease: Viral, autoimmune, genetic or drug-induced. The patients were divided into 2 groups: group A – 47 patients with ALD (daily alcohol intake more 20 g) and group B – 54 patients with NAFLD (daily alcohol intake less than 20 g). In all patients we measured waist circumference, BMI, aminotransferases, fasting blood glucose, cholesterol, HDL-cholesterol, triglycerides and adiponectin level. The liver US was performed by the same doctor. Steatosis was graded using a semi-quantitative scale of 1 (mild) to 3 (severe).We used Matteoni classification for histological samples in patients with NAFLD. Only 23 patients with alcoholic liver disease benefit for liver biopsy because the others patients were already diagnosed with liver cirrhosis.

Results: 31 males and 16 females in group A with mean age 45.6 years and 32 females and 22 males in grouop B with mean age 48.5 years. In group A, BMI was 27.5 kg/msq ± 5 and waist circumference was 109 ± 5 cm in males and 99 ± 5 cm in females. In group B, BMI was 30.4 kg/msq ± 6.9 and waist circumference was 106 ± 7 cm in males and 109 ± 9 cm in females. Waist circumference in females was statistically significant lower in group A compared with group B. No statistical significant differences between the two groups in fasting blood glucose levels, cholesterol levels but triglycerides levels were higher in group B compared with group A. In group B, adiponectin levels were decreased in hepatic steatosis, but elevated in cirrhosis (5 patients), and correlated negatively with US steatosis and positively with HDL-cholesterol. In group A serum adiponectin levels were increased in all patients according with the progression of ALD.

Conclusion: In our study, serum adiponectin levels were increased in accordance with the progression of liver disease, like non-invasive serum marker for hepatic fibrosis, unconcerned the etiology of liver disease.

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Significance of mTOR signaling proteins for prognosis of chronic alcohol hepatitis

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Introduction: Signals through the protein complexes generated by energy-dependent nutrient-sensitive kinase mTOR may have a prognostic value under chronic alcoholic hepatitis. Alcohol is known to modulate a phosphorylation of some components mTOR complexes to regulate the protein synthesis and cell growth in non-malignant cells. We have found that expression of proteins from mTOR complexes and its signaling targets are diverse from grade of fibrosis under alcoholic hepatitis.

Methods: We investigated 34 liver biopsy materials from patients with chronic alcoholic hepatitis and from 10 healthy donors. Grades of fibrosis and inflammation were estimated by Metavir. Separated cells from liver tissues were obtained by homogenizing, fixed in paraformaldehyde, stained with antibodies to intracellular proteins (TSC1, TSC2, protein 14-3-3, mTOR, Raptor, Akt, p70S6K1, 4EBP1, etc.) which expressions were measured by flow cytometry. Cell proliferation and apoptosis were measured by staining with propidium iodide and bromo-deoxyuridine.

Results: Median of mTOR protein expression was higher in cells from alcoholic hepatitis tissues compared with healthy donor. Under pronounced fibrosis, very high expression of mTOR protein was found in some cell populations which expressed also high level of kinase p70S6K1, factor 4EBP1, activation markers and possessed an increased proliferation activity. Diverse expression in upstream mTOR inhibitors TSC1 and TSC2 have been found in the half of clinical cases. Hepatic inflammation was not found to be correlated with mTOR expression rather with the apoptosis elevation.

Discussion/Conclusion: Expression of kinase mTOR protein and its signaling components correlate with grade of fibrosis under chronic alcoholic hepatitis that may be used with prognostic value.

The correlation between liver fibrosis scores and other laboratory parameters in alcoholic hepatitis

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Introduction: Alcoholic hepatitis continues to remain and important health problem in Eastern European countries. Our aim was to study non-invasively the liver fibrosis in patients with alcoholic hepatitis.

Methods: We have studied all the patients who were hospitalised in the internal medicine departments from three cities from Transylvania on a three months period to whom the abdominal ultrasonography revealed a hyperechoegeneous liver. We have studied the prevalence of the alcoholic hepatitis and the correlation between the liver fibrosis scores with other laboratory parameters at the patients with alcoholic hepatitis. The liver fibrosis was noninvasively assessed by the Forns index and the APRI score.

Results: Among the 436 patients with hyperechoegeneous liver, 59 (13.5%) had alcoholic hepatitis. Other aetiologies of the chronic hepatitis were: 52% NAFDL, 15% NASH, 10% viral C hepatitis, 4% drugs induced hepatitis, 3% viral B hepatitis, 2% autoimmune hepatitis, 0.45% liver cancer. At the patients with alcoholic hepatitis, the average Forns index was 4.9566 and the average APRI score was 0.2951 (predictor for significant fibrosis). There is an inverse linear correlation between the number of platelets and the Forns index ($r = -0.544$) and between the number of platelets and the APRI score ($r = -0.402$). There is a direct linear correlation between the Forns score of liver fibrosis and the total bilirubin level ($r = 0.524$), gamma glutamyl-transpeptidase (GGT) ($r = 0.502$) and alkaline phosphatase ($r = 0.433$). An inverse linear correlation was found between the Forns index of liver fibrosis and the levels of cholesterol ($r = -0.572$) and triglycerides ($r = -0.278$). The APRI score correlated with the level of transaminses, but the Forns index did not.

Discussion/Conclusion: The liver fibrosis, non-invasively assessed, is significant in patients with alcoholic hepatitis. There is a direct linear correlation between the liver fibrosis scores and the levels of bilirubin, GGT and alkaline phosphatase. There is an inverse correlation between the liver fibrosis scores and the number of platelets, the levels of cholesterol and triglycerides in patients with alcoholic hepatitis.

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Recognition of alcohol misuse and impact on admissions in a district general hospital

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Introduction: The 2005 UK census suggests that 38% of men and 18% of women drink an average of > 20 g of alcohol per day. The National Plan for Liver Services in the UK documents the increasing problem of alcohol related diseases and recommends that all UK hospitals should have alcohol services on site.

Methods: We assessed the burden of alcohol related admissions by examining 1) a random sample of case notes and 2) ICD-10 codes for all 28944 admissions over a one year period.

Results: Notes were reviewed for 246 patients (143 medicine, 37 surgery, 66 orthopaedics: 111 male, 135 female). An alcohol history (simple yes/no) was documented in 120 (49%). Any more thorough alcohol history (eg occasional, binge or units) was documented in only 52 (21%). The primary diagnosis was reported as directly alcohol related in 12 (5%). Less overt diagnoses associated with alcohol were either not recognised or reported, none being documented in medical notes. The mean Alcohol Attributable Fraction for admission diagnoses was 8.0%, or 12.4% when including all those with a fall/fracture as per ICD classification. Of 28944 admissions, 569 patients (2%) were coded as having an alcohol related diagnosis as a contributor to admission.

Discussion/Conclusion: Alcohol associated illness is a common cause of hospital admission (Alcohol Attributable Fraction 8–12%) but recognition and documentation of alcohol use is poor. Better recognition, implementation of brief intervention strategies and/or referral to alcohol services may reduce the predicted future harmful impact of alcohol on personal and public health.

Genetic polymorphisms of CYP2E1 and alcohol liver cirrhosis

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Introduction: Genetic polymorphism of enzymes involved in alcohol metabolism plays a relevant role in etiopathogenesis of alcohol liver disease. The aim of this study was to find in the Polish population the CYP2E1 alleles and genotypes, which are likely to be responsible for higher susceptibility to alcohol dependence and alcohol liver cirrhosis and to examine if this genotype differentiates age, at which the patients have become alcohol dependent.

Methods: The CYP2E1 alleles and genotypes frequency were examined in 176 alcohol dependants: 94 with alcohol liver cirrhosis, 82 without damage to gastrointestinal organs, and 95 healthy volunteers who do not drink alcohol – as a control group. Genotyping of the CYP2E1 was performed using PCR-RELP methods on white cell DNA.

Results: In the examined population the frequency of CYP2E1*c2 allele was 2.45% and CYP2E1*c1 – 97.55%. CYP2E1*c1/*c1 genotype occurred in 95.10%, CYP2E1*c1/*c2 in 4.90% of examined subjects. Nobody had CYP2E1*c2/*c2 genotype.
CYP2E1*c1 allele and CYP2E1*c1/*c2 genotype were statistically more frequent among the alcohol dependants (with alcohol liver cirrhosis, 82 without damage to gastrointestinal organs) than in the controls. Differences in the CYP2E1 allele and genotype distribution between alcohol dependants groups were not significant. Average age of the patients with CYP2E1*c1/*c2 genotype who became alcohol dependent was 26.19 years and in patients with CYP2E1*c1/*c1 genotype was 37.60 years ($p < 0,001$).

Discussion/Conclusion: Our studies suggest that the presence of c2 allele promotes alcohol dependence and alcohol liver cirrhosis amongst Poles. The persons with CYP2E1*c1/*c2 genotypes became alcohol dependent at a considerably younger age than the subjects with CYP2E1*c1/*c1 genotypes.

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Dynamic changes in liver stiffness measured by transient elastography during acute alcoholic hepatitis

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Introduction: Liver tissue alterations other than fibrosis (such as hepatic necroinflammation) may have an impact on liver stiffness measurement. The aim of this study was therefore to evaluate the dynamic changes in liver stiffness measurement (LSM) during the course of acute alcoholic hepatitis (AAH).

Methods: We prospectively recruited consecutive patients with acute alcoholic hepatitis. In each patient, aminotransferase, bilirubin (BIL), the international normalized ratio (INR) and LSM were performed on the same study day, at admission and then every 2 days, until the peak levels of all parameters were identified. Subsequently, these parameters were measured every 1 week until they had normalized.

Results: In all patients, the degree of liver stiffness at the time of the peak increase in aminotransferases exceeded the cutoff values proposed for the prediction of significant fibrosis or cirrhosis. A progressive significant reduction in liver stiffness values was observed ($p < 0.01$) in the follow-up period in parallel with the reduction of aminotransferase levels ($p < 0.01$). Moreover, a statistically significant, positive correlation between aminotransferases and LSM was found ($r = 0.63$, $p < 0.05$ and $r = 0.61$, $p < 0.05$ for alanine aminotransferase and aspartate aminotransferase, respectively). INR and BIL were significantly associated with peak LSMs ($p < 0.05$).

Discussion/Conclusion: LSMs changed dynamically during the course of AAH. LSM should be assessed after normalization of ALT levels in order to accurately assess the degree of fibrosis.

Study of portal and systemic levels of nitric oxide, endothelin-1 and procollagen III peptide in chronic liver disease in Egypt

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Introduction: Egypt has one of the highest incidence of liver diseases in the world with prevalence of schistosomiasis. NO diffuses into cytosol of adjacent vascular smooth muscle cells play a role in the pathogenesis of vasodilation. Endothelial cells also produce the most potent vasoconstrictor agent endothelin (ET-1).

Aim: Evaluation of nitric oxide and endothelin-1 and procollagen III peptide in patients with chronic liver disease and portal hypertension in both systemic and portal blood samples together with the histopathological scoring of liver biopsies.

Subjects: The control-group: 15 subjects free from any liver disease. The patient-group: 30 patients with chronic liver disease and schistosomal portal hypertension.

Methods: Clinical examination, abdominal ultrasonography, measurement of portal venous pressure and histopathological examination of liver biopsy. Laboratory investigations included evaluation of total nitric oxide (NO), endothelin-1 (Et-1) and type III procollagen (PIIINP) in both portal and systemic blood. In addition prothrombin, serum alanine, aspartate aminotransferase (AST, ALT), γ-glutamyl aminotransferase (γGT) activities, serum bilirubin, albumin, serodetection of hepatitis B surface antigen (HBsAg) and hepatitis B core antibody and (anti-HCVAb).

Conclusion:
- NO and ET-1 levels in both systemic and portal blood of SHF patients were significantly higher than in the control group.
- NO is a potent vasodilator.
- ET-1 increase may be a compensatory mechanism to antagonize the vasodilatory effect of NO.
- Child class B subgroup had higher NO and ET-1 than class A.
- NO and ET-1 levels did not differ between anti HCV positive and negative SHF patients.

References:

1. Angelica M, Renganathan E , Gandin C. Chronic liver disease in Alexandria Governorate, Egypt: Contribution of schistosomiasis and hepatitis virus infections. Comment in Hepatology. 2000; 32: 172–4.

2. Waked IA, Saleh SN, Moustafa MS. High prevalence of hepatitis C in Egyptian patients with chronic liver disease. Gut. 1995; 37: 105–7.

3. Moeti A, Zaki S. Discovery of missed link between Schistosomiasis and HCV infection. Arab J Gastroenterol. 2000; 2: 289–95.

4. Arthur MJP. Matrix degradation in liver: A role in injury and repair Hepatology. 1997; 26: 1069–71.

5. Plebani M, Burlina A. Biochemical markers of hepatic fibrosis. Clin Biochem. 1991; 24 (3): 219–39.

6. Okabe K. Hepatic fibrosis and its serum markers. Jpn J Clin Pathol. 1992; 40 (12): 1258–64.

7. Don C Rockey. Vascular mediators in the injured liver. Hepatology. 2003; 37 (1).

8. Stamler JS, Single DI, Loscalze S. Biochemisry of NO and its redox-activated forms. Science. 1992; 258: 1898–1902.

9. Sakura T, Goto K. Endothelins: Vascular actions and clinical implications. Drugs. 1993; 46: 795–804.

10. Kamath PS, Tyce GM, Miller VM. Endothelin-1 modulates intrahepatic resistance in a rat model of noncirrhotic portal hypertension. Hepatology. 1999; 30: 401–7.

11. Moller S, Bendtsen F, Heniksen J. Splanchnic and systemic hemodynamic derangement in decompensated cirrhosis. Can J Gastroenterol. 2001; 15: 94–106.

12. Uemura K, Kawaguchi T, Sodeyama T. Antibody to hepatitis C virus in patients with chronic schistosomiasis. Ann Trop Med Parasitol. 1992; 86: 257–62.

Adiponectin: A differential marker between steatosis and steatohepatitis

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

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

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

Results:

1. Plasma adiponectin levels were significantly lower in NAFLD patients than control gp (6.15 ± 1.39 ng/ml vs 12.03 ± 3.46 ng/ml).
2. Adiponectin was significantly lower in NASH than NAFLD (1.80 0 ± 0.96 ng/ml vs 6.15 ± 1.39 ng/ml).
3. Leptin level was significantly higher in NAFLD than NASH gp (69.50 ± 18.70 ng/ml vs 43.20 ± 6.93 ng/ml).
4. Adiponectin ROC curve showed an AUROC curve in NAFLD gp (0.945 p = 0.049) while inNASH was (0.995 p = 0.007).
5. TNF-α & IL-6 was significantly higher in NASH than NAFLD gp (79.25 ± 13.89 pg/ml vs 41.25 ± 17.53 pg/ml) and (110.20 ± 55.34 pg/ml vs 43.85 ± 16.13).
6. Plasma adiponectin level in NAFLD gp was inversely correlated with T.G (r = -0.368 p = 0.111) GOT (r = –0.037 p = 0.878) & GPT (r = –0.022 p = 0.926) while it was $^{+}$ve correlated in NASH gp with Cholesterol (r = 0.317 p = 0.174) & T.G(r = 0.042 p = 0.861).

Conclusion: This data support a role for low circulating adiponectin in the pathogenisis of NAFLD and hypoadiponectinemia found to be a feature of NASH. Adiponectin found to be a non-invasive differential marker between NAFLD & NASH.

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Prehepatic portal hypertension in adults

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The aim of the investigation: Study pathogenesis and elaborate management and prophylaxis of variceal bleeding (VB) in adult patients with prehepatic portal hypertension (PPH) without chronic myeloproliferative disorder (CMPD).

Materials and methods: We studied polymorphism of genes responsible for blood coagulation in 81 adult patients (46 men and 35 women, median age = 44.4 years) in a period from 2005 to 2010 years. Polymerase chain reaction was used to detect thrombophilia. The absence of CMPD was confirmed by bone marrow biopsy. In 62 patients VB was diagnosed during their life (24 of them had acute VB at the moment of hospitalization). The remaining 19 patients did not have bleedings, but they had large (diameter > 5 mm) oesophageal and gastric varices and “red markers” at endoscopy. Acute VB or high risk of hemorrhage were the indications for operation or endoscopic treatment.

Results: All examined patients (100%) had thrombophilia presented as combination of mutations in genes of different coagulating factors. In 62 of them (76.5%) there was a combination of more than 4 mutations at the same time (maximum 9). The most frequent polymorphisms were detected in genes responsible for development of hyperhomocysteinemia (methylenesynthase-reductase – in 30 [66.7%], methylen-tetrahydrofolatreductase – in 20 [44.4%] and other), integrin α-2 – in 26 (57.8%), plasminogen activator inhibitor – in 25 (55.6%), platelet glycoprotein 1B – in 21 (46.7%), fibrinogen – in 20 (44.4%) and other. Comparatively rare mutations were revealed in genes of coagulating factor VII – in 7 (15.6%), coagulating factor V (Leiden) – in 2 (4.4%) and factor II (prothrombin) – in 2 (4.4%). Detection of thrombo-philia as a thrombotic risk factor allowed starting early anticoagulation therapy that prevented development of portal thrombosis during intra- and postoperative periods. Surgical treatment was performed in 69 patients (85.2%): 14 selective portocaval shunts (20.3%) and 55 gastric devascularisations (79.5%) in cases when shunting was impossible due to total thrombosis of the portal system. In addition, very frequent presence of large gastric varices prevented us from radical endoscopic treatment in these patients. Endoscopic band ligation (EBL) was performed in 12 patients (14.8%). There was no postoperative lethality. During long-term follow-up (from 1 to 5 years) 5 patients (6.2%) had VB so endoscopic treatment was performed (EBL or sclerotherapy).

Conclusion: Our data showed that presence of thrombophilia was one of the leading prothrombotic factors for extrahepatic portal vein thrombosis in adult patients with PPH in absence of CMPD. Early detection of thrombophilia allows to prescript pathogenetic adequate prolonged anticoagulation therapy in these patients, including pre- and postoperative period.

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The data mining approach optimizes the interpretation of biochemical tests in Wilson disease

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Introduction: The first line of diagnosis of WD consists of laboratory tests: Serum ceruloplasmin concentration, total and free serum copper concentration, 24h urinary copper excretion (available, inexpensive and no time-consuming). The aim of the study was to optimize interpretation of results of WD diagnostic test with approach of advanced data mining techniques.

Methods: 62 WD patients were included to study group. They had serum ceruloplasmin concentration [mg/dl], total and free serum copper concentrations [ug/dl], 24h urinary copper excretion [ug/24h] measured. The reference group (RG) was 69 patients with liver injury different than WD. The results of tests were analyzed with Weka environment with application of data mining algorithms.

Results: DTNB algorithm (patients with urine copper over 49.17 OR patients with ceruloplasmin less than 15.5 AND urine copper over 9.55 are classified as WD) produced optimal sensivity – 0.98 and can be used of screening of WD.
SMO algorithm (with line classificator

0.5239* (standardized) ceruloplasmin
+1.6479* (standardized) total serum copper
-2.0764* (standardized) urine copper
-0.315* (standardized) free serum copper
+0.3945; result > 0 classified as WD)

produces optimal specifity – 1 and PPV – 1, enables to properly recognize 53 WD patients (sensivity 0.85, NPV – 0.88). Algorithms used each after other provide proper diagnosis in 85% of WD patients and decrease the number of patients requiring further expensive, invasive diagnostic tests to 16 (9 WD and 7 CG).

Discussion/Conclusion: The first line diagnostic tests supported by data mining approach seem to be very efficient in screening and diagnosis of patients with Wilson disease.

Noninvasive scoring systems in nonalcoholic fatty liver disease may reduce the need for liver biopsy

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Introduction: The diagnosis of fatty liver disease depends mainly on the liver biopsy. To avoid this invasive method, many noninvasive tests were introduced.
Our aim was to assess the diagnostic accuracy of two noninvasive tests for the detection of steatohepatitis

Methods: We recorded in a prospective manner demographic, biochemical and histological features of 98 patients with nonalcoholic fatty liver disease based on morphological criteria (according to Kleiner score). Three categories of histological findings were assessed: No Nash (NASH Activity Score [NAS] = 1–2), borderline (NAS = 3–4), and NASH (NAS = 5–8). As noninvasive tests, we assessed the diagnostic value of BAAT score (based on body mass index [BMI], ALT, age and triglycerides) and Fatty Liver Index (FLI), (triglycerides, BMI, GGT, waist circumference) in detecting patients with steatohepatitis.

Results: From 98 patients having NAFLD, 57 had histological features of NASH, 19 were borderline and 20 did not have NASH. The mean age was of 46.18 ± 11.57, and 72.4% were males. The value of BAAT score and of FLI in predicting the presence of steatohepatitis is presented in table 1.

	BAAT score						**FLI**					
	ROC	Se (%)	Sp (%)	PPV (%)	NPV (%)	p	ROC	Se (%)	Sp (%)	PPV (%)	NPV (%)	p
NoNASN vs. BL+NASH	0.80	60	84.6	97.08	59.9	0.01	0.67	60	74.4	97.1	59.9	0.05
NoNASH+BL vs. NASH	0.63	41	86.4	82.01	49.1	0.01	0.58	53.8	66.1	79	21	ns
NoNASH vs. BL	0.80	100	52	64.1	100	0.01	0.70	60	83.3	75.5	70.8	0.01
NoNASH vs. NASH	0.80	60	86.4	92.8	42.2	0.00	0.67	60	72.9	86.7	38.1	0.01
BL vs. NASH	0.53	52.6	64.4	83	30	ns	0.50	36.8	78	84	28	ns

Table 1: The effectiveness of BAAT and FLI for identifying the presence of steato-hepatitis.

Discussion/Conclusion: Both BAAT score and FLI performed well in identifying patients with significant steatohepatitis and therefore, using these scores could decrease the need for liver biopsy in these patients.

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Clinical and laboratory characteristics in patients with portal thrombosis and prehepatic portal hypertension

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Background: The prehepatic portal hypertension (PPH) due to portal thrombosis was believed to be a rare condition (about 5–20% of all cases of portal hypertension). During the last 7 years we observed more than 139 patients with portal thrombosis who developed PPH.

The aim of the investigation: To study clinical, laboratory signs of PPH and genetic polymorphism of hemocoagulation factors in patients with portal thrombosis.

Materials and methods: We studied 139 patients (mediana of age 43,4 years) with portal thrombosis confirmed by Doppler sonography. The period from the first manifestation of portal hypertension (splenomegaly, esophageal varices) to examination in our Center varied from 2 to 420 months (mediana = 46 months). 58 patients had blood picture compatible with diagnosis of chronic myeloproliferative disorders (MPD). Other patients had PPH due to hereditary or acquired thrombophilia. Basic laboratory and clinical examinations were made. Also we studied laboratory parameters concerning the hemostatic status and thrombophilia markers.

Results: The main clinical signs were the following: splenomegaly (100%), hepatomegaly (46%), varices (100%), bleedings from esophageal and gastric varices (74%), transient ascites (18%). The majority of patients had normal albumin, bilirubin and transaminase levels. Cholestatic markers were increased in more than half of the patients (alkaline phosphatase – in 52% of patients, glutamintranspeptidase – in 51% of patients). Regarding the hemostatic status, we observed the decrease of prothrombin level (mediana = 71). Blood and plasma viscosity did not differ from those in the control group. Index density of erythrocytes was elevated in 54% of patients, rate of erythrocyte aggregation was increased in 75% of patients. All investigated patients (100%) had a trombophilia presented as combination of mutations in genes of different coagulating factors. 106 of them (76 %) had a combination of more than 4 mutations at the same time (max. 9). The most frequent polymorphisms were in genes responsible for development of hyperhomocysteinemia (methylenesynthase-reductase – 90 [65%]), plasminogen activator inhibitor – 75 (54%), fibrinogen – 58 (42%) and other. Factor V (Leiden) in 7 (5%), factor VII - 21 (15%), factor II – 6 (4.5%).

Conclusion: Cholestatic markers, microhemorrheological disturbances and thrombophilia are the distinctive clinical and laboratory features of PPH in patients with portal thrombosis. We found no significant differences in these parameters in patients with portal thrombosis associated with MPD and without MPD.

Red distribution width (RDW) and ferritin as prognostic factors in acute pancreatitis

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Introduction: Red blood cells distribution width (RDW) is a standard parameter of complete blood count. The usefulness of determining the value of RDW-examined in many pathological inflammatory and non- inflammatory conditions such as inflammatory bowel diseases, myocardial infarction, heart failure, renal failure, but not in acute pancreatitis (AP). Ferritin is an acute phase reactant and thus may be increased in patients with inflammation, like AP, and also in liver disease, chronic infection, autoimmune disorders, and some types of cancer. Ferritin is not typically used to detect or monitor these conditions. The aim of this study was to determine correlation of RDW and ferritin with the clinically used predictors of severity of acute pancreatitis such complex scoring systems (Ranson and APACHE II).

Methods: Eight hundred and eighteen patients (52% male and 48% female) in a 10- year period (2000–2009) were enrolled in this study. RDW and ferritin values were obtained on an automated hematology analyzer (Olympus AU 640, Tokyo, Japan). Statistical methods used Pearson's correlation test.

Results: RDW showed moderate correlation to Ranson ($r = 0.216$, $p = 0.0001$) and moderate correlation to APACHE II ($r = 0.180$, $p = 0.0001$) as a markers for predicting severity of AP. There was neither significant correlation between ferritin and Ranson ($r = -0.0281$, $p = 0.7481$), either Appache II scores ($r = 0.0398$, $p = 0.6484$).

Discussion/Conclusion: To our knowledge, our study demonstrates for the first time that RDW is inexpensive, easily obtained, and powerful prognostic marker for AP, but warrants further confirming studies in larger series of patients.

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Perspectives of using of c-kit-positive Langerhans islet cells in elaboration of new therapeutic and diagnostic approaches for pancreas diseases

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Diabetes and chronic pancreatitis still remain poorly treated despite modern developments in medicine. Stem cell therapy may be a solution for these diseases and stem cell marker c-kit can be molecular target in elaboration of some theraputical techniques.

Aim: The aim of our work was to identify c-kit expressing cells in human pancreas.

Materials and Methods: Histological sections of human embryonic, fetal (5–28 weeks of gestation), postnatal and adult pancreas were stained immuno-histochemically with antibodies against c-kit, insulin and glucagon.

Results: First c-kit-positive cells expressing glucagon emerge in pancreas on 8.5 weeks of gestation as single cells in ducts. Later these cells can be seen in Langerhans islets. From 11.5 weeks of gestation c-kit-positive cells of Langerhans islets start to synthesize both insulin and glucagon. C-kit-positive progenitor cells, co-expressing insulin and glucagon remain in postnatal pancreas. So, c-kit-positive cells are common progenitors for α- and β-cells of Langerhans islets and remain in postnatal pancreas.

Conclusion: Localization of c-kit on cell membrane gives an opportunity for isolation of pancreatic progenitor cells, research and development of new methods for treatment of pancreas diseases. Adults can be donors of pancreatic progenitor cells. Besides c-kit can be used as diagnostic tool for identification of activation of Langerhans islet’s stem cell compartment during pancreas regeneration also.

The importance of Ursofalk® in the complex therapy of patients with alcoholic steatohepatitis

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An increase of cases of alcoholic steatohepatitis has been noted recently. Because of this the development of new tactic schemes of treatment is actual.

Aim: To define the worth and the effectivity of *Ursofalk*® in complex therapy of patients with alcoholic steatohepatitis.

Materials and methods: Thirty patients with alcoholic steatohepatitis have been under observation. The diagnosis was made relying on the clinical data, anamnesis, functional hepatic test and sonography of the organs of the abdomen. The patients were divided into two groups. The first group – 18 patients that together with the ordinary medicines received *Ursofalk*® 750 mg per day. The second group – 12 patients received regular treatment. The therapy lasted 6 months.

Results: The first group – The syndrome of bowel dyspepsia, pain in the right hypocondrium has disappeared at all the patients, complete restoration of service-ability- at all the patients, the decrease till normal of the size of liver – at 15 patients (83,3%), normal levels of ALT (0,37 ± 0,15 mcmol/h.l) have been shown at 16 patients (88,9%), γ-GT (48,3 ± 0,7 IU) – 16 (88,9%) patients, Bilirubin (16,5 ± 1,3 mcmol/l) at all the patients (100%). The changes at the second group are analogical but they are less expressed in terms of quantity. The syndrome of bowel dyspepsia, pain in the right hypocondrium has disappeared at 10 patients (83,3%), complete restoration of serviceability – at 10 patients (83,3%), the decrease till normal of the size of liver – at 6 patients (50%), normal levels of ALT at 8 patients (66,7%), γ-GT – at 9 (75%) patients, Bilirubin – at 10 patients (83,3%).

Conclusion: The use of *Ursofalk*® in therapeutically dozes, in the complex therapy of patients with alcoholic steatohepatitis leads to achievement of positive results during shorter periods of time and for a greater number of patients than the use of therapeutically schemes of treatment. This is due to the expressed hepatoprotective, choleretic effect of *Ursofalk*® and suppression of synthesis of cholesterol in liver, its absorption in intestine and decrease of contain of cholesterol in liver.

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Acute alcohol-induced liver steatosis in mice: Protective effects of chicoric acid

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Increased intestinal permeability and bacterial overgrowth subsequently leading to increasing portal lipopolysaccharide (LPS) levels, an activation of hepatic Kupffer cells and induction of tumour necrosis factor (TNF) α have been found to be key factors in the development of acute and chronic alcohol-induced liver damage. Recent studies have suggested that chicoric acid, a dicaffeoyl ester found in chicory but also in Echinacea pupurea and other plants, may have anti-oxidant and anti-inflammatory effects. In the present study the protective effects of chicoric acids given orally (4 mg/kg b.w.) on acute alcohol-induced liver steatosis were assessed in a mouse model. Acute alcohol ingestion caused a significant increase in hepatic lipid accumulation. Treatment with chicoric acid significantly attenuated the effect alcohol on hepatic lipid accumulation. The protective effect of chicoric acid was associated with a suppression of the alcohol-induced formation of 4-hydroxynonenale adducts and induction of MyD88 in livers of mice. Taken together, these data support the hypothesis that chicoric acid may protect the liver from acute alcohol-induced steatosis through interfering with the TLR-4 signaling cascade and the formation of reactive oxygen species.

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The study of the efficacy UDCA (ursodeoxycholic acid) therapy in alcoholic steatohepatitis

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Introduction: UDCA improves biochemical tests in a number of cholestatic disorders and may also have a beneficial effect on disease activity in alcoholic steatohepatitis irrespective of whether or not cholestasis is present.

Aim of study: Our study sets as an objective to investigate UDCA effect in alcoholic steatohepatitis.

Patients and methods: 116 patients (77 males, 39 females), mean age 56 ± 6 years with biopsy proven steatohepatitis were included in this study. All patients had biochemical evidence of impaired liver function with bilirubin > 25 μmol/l and/or serum alkaline phosphatase > 150 IU/l at entry to the study. Patients were randomized: 66 patients to receive UDCA (15 mg/kg/day) and 50 patients to receive placebo for 26 weeks. Paired Student's t-test was used to assess changes in the liver function tests during the observation period.

Results and discussion: Treatment with UDCA for 26 weeks resulted in a significant decrease of total bilirubin ($p < 0,01$), gamma-glutamyl transpeptidase ($p < 0,05$) compared with placebo treatment. The improvement in the liver biochemistry was evident after 12 weeks of treatment with UDCA. One patient was withdrawn from the study because of development of diarrhaea but UDCA had no adverse effect on full blood count or platelets.

Conclusions: UDCA improves biochemical tests in a number of cholestatic disorders and may also have a beneficial effect on disease activity in alcoholic steatohepatitis especially if cholestasis is present. UDCA had a good tolerance for the patients in these cases.

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Perspectives of different populations of stem/progenitor cells in treatment of alcoholic hepatitis

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There are many investigations of the possibility of stem cells therapy of chronic liver disease. The **aim** of our experimental and clinical studies was to compare perspectives of using of different populations of stem/progenitor cells in treatment damaged liver, particularly, alcoholic hepatitis/cirrhosis.

The results of clinical trials of auto-transplantation of hematopoietic stem cells (HSC) from peripheral blood have shown its safety and effectiveness. Thus, 1–3 months after a single injection of HSC the clinical, biochemical and morphological indices are improved in patients with alcoholic hepatitis/cirrhosis. However, the effect of treatment remains only a few months.

Alternatively, we explored the capability of the another progenitor cells to differentiate into hepatocytes: human HSC human umbilical cord blood; rat bone marrow mesenchymal stem cells (MSC); rat hepatic stellate cells (SC) – potential regional liver stem cells. Umbilical HSC survive only in co-culture with SC and they do not differentiate into hepatocytes in vitro. HSC transplanted to rats after partial hepatectomy migrate into the liver and differentiate into hepatocytes, cholangiocytes and sinusoidal cells like our clinical research results. We found out that SC and MSC in medium of SC can differentiate into hepatocytes in vitro.

We conclude these cells have greater hepatocyte potential and can be more perspective for alcoholic liver diseases treatment.

Evaluation of CD34 expression in liver in alcoholic liver cirrhosis after autologous stem cell transplantation

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Introduction: Fibrosis is the main cause of organ failure in chronic liver damage. One of significant mechanisms of liver fibrosis is a capilliarization of sinusoids which reflects progression of fibrosis and the ability to reverse development. CD34 (a marker of endothelial and hematopoietic stem cells) is absent in the endothelium of sinusoids in normal liver and appears in these cells only in case of capilliarization. One of the novel treatment options in liver diseases is using of autologous stem cells.

The purpose of the study was the evaluation of CD34 expression in liver of patients with alcoholic liver cirrhosis before autologous stem cell transplantation and 3 and 12 months after the procedure.

Methods: Our research was performed as a part of the Program «Development of Cell Medicine in Tatarstan» on the base of Republican Clinical Hospital. The study was performed on liver biopsies of 10 patients with alcoholic liver cirrhosis (Child-Pugh class A). Peripheral blood stem cells were mobilized by granulocyte colony-stimulating factor (G-CSF) and injected into celiac trunk of patients. Liver biopsies were taken before cell transplantation, 3 and 12 months after the procedure.
Formalin-fixed, paraffin-embedded liver biopsy specimens were stained immuno-histochemically with antibodies against CD34.

Results: Before transplantation the number of CD34-positive cells in liver biopsies very much especially in the portal tracts and in the infiltrates around the portal tracts. Also we observed CD34-positive cells in porto-portal and porto-central septa and isolated cells in liver parenchyma.
After three months after transplantation the number of CD34 positive cells markedly decreased but they also were located mainly around the portal tracts. 12 months the number of CD34-positive cells again increased but does not reach the primary level. The number of cells correlated with the severity of infiltration.

Discussion/Conclusion: Our data suggest that autologous intra-celiac transplantation of hematopoietic stem cells in patients with alcohol liver cirrhosis is an effective procedure as transplanted autologous mobilized peripheral blood stem cells can restore endothelial sinusoids. But this procedure should be repeated in a year.

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Autologous stem cells transplantation as novel therapy for alcoholic liver disease

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Introduction: Unfortunately most of chronic liver diseases including alcoholic liver cirrhosis need better treatment as all the present treatment options aren't very effective.

Methods: We attempted to use autologous stem cell transplantation in patients with compensated alcoholic liver cirrhosis (Child-Pugh class A). Results of therapy were assessed clinically, biochemically and immunohistochemically. Liver biopsies were obtained before and at different terms after transplantation and were stained immunohistochemically with antibodies to A-smooth muscular actin, CD34, bcl-2 (antiapoptotic protein) and PCNA.

Results: In primary liver biopsies we observed different levels of liver injury resulting in hepatic stellate cells transdifferentiation (A-SMA-positive cells), capillarisation of liver sinusoids (CD34-positive cells), increased expression of Bcl-2 and proliferation of hepatocytes and single sinusoidal cells. Our results show that autologous stem cells transplantation in patients with alcoholic liver cirrhosis is safe and effective in terms of not only clinical and biochemical but also histological improvement. We showed that transdifferentiation of hepatic stellate cells into myofibroblasts assessed by A-SMA and capillarization of sinusoiuds assessed by CD34 expression diminishes in three months after transplantation comparing with the primary level. Expression of Bcl-2 and number of proliferating hepatocytes (PCNA) decreased as well as the result of liver tissue improvement. Further evaluation one year after transplantation revealed reversal of abovementioned positive changes although they didn't get to primary levels.

Discussion/Conclusion: So our data shows that efficacy of the autologous stem cells transplantation lasts at least 12 months, however, after one year patients probably need transplantation to be repeated.

Corticosteroids + pentoxifylline versus corticosteroids alone in the treatment of severe alcoholic hepatitis (SAH): A retrospective study

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Introduction: Severe Alcoholic Hepatitis (SAH) is a major cause of mortality and morbidity. Corticosteroids are recommended in patients with SAH[1,2], i.e. *Maddrey's discriminant function[3] (MDF) > 32, or *Glasgow Alcoholic Hepatitis Score[3] (GAHS) > 9. Pentoxifylline has been used with corticosteroids in patients with SAH. However there have been no studies to assess the efficacy of the combined treatment with pentoxifylline and corticosteroids.

Aims and methods: We compared the short-term effect of Corticosteroids alone (Group A) versus Corticosteroids with Pentoxifylline (Group B), in patients with SAH in two UK District General Hospitals between April 2007 and April 2009. The hospital's databases were searched for patients with SAH.
Inclusion criteria: Documented clinical diagnosis of SAH on discharge summaries & negative viral hepatitis serology and negative sepsis screen.
Exclusion criteria: The patients with chronic liver disease and multi organ failure requiring ITU admission were excluded.
57 patients were identified. The clinical notes were used to analyse the effects of these two treatments. The primary end point was *Lille score[2] of < 0.45 on day 7.

*MDF, MELD & GAHS scoring systems are used to decide whether to initiate corticosteroid therapy, whereas Lille score is use to decide whether to stop the use of corticosteroid after 7 days or complete 30 days course.

Results: The 57 patients were identified: 35 men and 22 women. Mean age: 43 years (range 22–62). All patients were previously well and presented with a short history of jaundice against the background of alcohol abuse. Group A – 30 patients, received 30 mg Prednisolone PO OD. Group B – 27 patients, received Pentoxifylline 400 mg TDS in addition to 30 mg Prednisolone. 9 Patients in group A and 10 patients in group B had treatment stopped because of failure to respond (Lille score > 0.45 on day 7). 21/30 patients (70%) in group A and 17/27 patients (62%) in group B improved (Lille score < 0.45 on day 7 of treatment). Chi squared equals 0.079 with 1 degree of freedom. The two-tailed P value equals 0.778.

Discussion: Although the patients studied had a normal CLD screen, none of them had a liver biopsy to confirm the diagnosis. All the patients received supportive treatment alongside the specific therapy. Patients who were septic or required intensive care admission for renal failure or other organ failure excluded from the study.

Conclusion: There was no major difference noticed in the response of the two groups studied, as assessed by Lille score. It was therefore concluded that the combination treatment with Corticosteroids and Pentoxifylline is not advantageous over Corticosteroids alone in the treatment of SAH. The efficacy of the combined treatment with pentoxifylline and corticosteroids may warrant a further randomized controlled trial.

References:

1. Mathurin P, Mendenhall CL, Carithers RL. Corticosteroids improve short-term survival in patients with severe alcoholic hepatitis (AH): individual data analysis of the last three randomized placebo controlled double blind trials of cortico-steroids in severe AH. J Hepatol. 2002; 36: 480–7.

2. Michael R, Lucey MR, Mathurin P, Morgan TR, Timothy R, Medical Progress: Alcoholic Hepatitis. N Engl J Med. 2009; 360: 2758–69.

3. Tilg H, Kaser A. Predicting mortality by the Glasgow alcoholic hepatitis score: the long awaited progress? Gut. 2005; 54: 1057–9.

Influence of zinc supplementation in patients with alcoholic liver cirrhosis

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Introduction: Zinc deficiency is common in cirrhosis, especially in alcoholic patients. Aim of the study: Evaluation of 12 months zinc supplementation on clinical status, biochemical and immunological parameters in patients with alcoholic liver cirrhosis.

Methods: The study was carried out on 23 cases of active alcoholic liver cirrhosis. Plasma and urinary zinc concentration were determined before and after oral administration of zinc sulphate (3 x 200 mg/day). Biochemical parameters were compared to a control group (12 subjects). Clinical status, psychometric tests (for hepatic encephalopathy), biological and immunological tests were repeated every two months during the therapy.

Results: Plasma zinc levels were reduced in all cirrhotic patients and returned to normal after oral zinc therapy. Psychometric tests improved in patients with mild or latent hepatic encephalopathy, as well as liver function tests after zinc supplementation; we observed a tendency of “recovering” of immune disorders: Increase of T-lymphocytes on the account of CD8 subset. No significant changes were observed in the control group.

Discussion/Conclusion: Our results argue for the positive effects of zinc supplementation in alcoholic liver cirrhosis.

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Management of the variceal bleeding in patients with chronic myeloproliferative diseaeses

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Background: In certain patients prehepatic portal hypertension (PPH) is caused by chronic myeloproliferative diseases (CMPD). These disorders not only lead to the development of the pathological process but also influence on the features and dynamics of the process.

The aim of the investigation: Develop the surgical approach to the management and prophylaxis of variceal bleeding in patients with CMPD.

Materials and methods: We examined 74 patients with CMPD and portal hypertension (PH) in a course of 15 years. Treatment with cytostatic agents was previously given to 38 of them. In 36 patients MPD was diagnosed for the first time. Variceal bleeding occurred in 57 patients. And 17 patients had a “high risk of bleeding” picture at endoscopy. The distinctive features in these patients were a large size of varices and their extension to a major portion of oesophagus and to cardial, fundal parts and corpus of stomach. These features prevented us from radical endoscopic treatment.

Results: Indication for routine surgical treatment (41 patients) was either variceal rebleeding or high risk of its occurrence. The following operations were performed: selective portocaval shunting (SPS) in 5 patients (12.2%) and gastric devascularisations in 33 patients (80.4%), including 5 in combination with splenectomy. Isolated splenectomy due to segmental portal hypertension was performed in 3 patients. Such a low percent of SPS performed was due to high frequency of total thrombosis of splenoportal axis. Urgent operations included 14 gastric devascularisations. In the postoperative period 2 patients have died (16.7%). The causes of death were hemorrhagic shock and DIC-syndrome. During the long-term follow-up (from 1 to 15 years) 9 patients died (6 – of CMPD progression, 2 – of rebleeding episode, 1 – of myocardial infarction). At present 60 patients are alive. 19 of them (27.5%) had rebleeding episode. Endoscopic control was performed 3 months after surgery: detection of 3-d degree gastric varices was an indication for repeated operation (3 patients); in patients with oesophageal 3-d degree varices endoscopic band ligation was performed. After repeated interventions no rebleeding was detected.

Conclusion: Thus, the following features of patients with PH and CMPD were revealed: massive extension of the varices to the major part of oesophagus, involvement of cardia, fundus and even body of stomach; total thrombosis of the portal venous system. Explicit advantage of the routine surgery with both preoperative preparation and postoperative treatment provided with specific cytostatic and anticoagulant therapy. Specific treatment must be prescribed individually and depends on clinical course of a chronic leukosis. Therefore, such patients should be managed in collaboration with hematologists.

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Study of proliferative activity of hepatocytes after autotrans-plantation of hematopoietic stem cells in patients with alcoholic liver cirrhosis

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Introduction: Liver cirrhosis is a problem that requires new therapeutical approaches because the existing treatment is not efficient enough. One of the most promising directions at present is elaboration of stem cell therapy methods. The aim of our work was to study cell proliferation in alcohol liver hepatitis/cirrhosis after autotransplantation of hematopoietic stem cells (HSC).

Methods: The study was performed on liver biopsies of 9 patients with alcoholic liver cirrhosis. Biopsies were taken before autotransplantation of HSC mobilized by granulocyte colony-stimulating factor into celiac trunk, 1, 3 and 12 months after the procedure. Liver biopsy sections were stained immunohistochemically with antibodies against PCNA (Proliferating Cell Nuclear Antigen).

Results: Before transplantation PCNA was expressed in 30% of hepatocytes which were usually localized in periportal zones and portal tract infiltration areas. Three months after transplantation 5% of hepatocytes were PCNA-positive and were localized in periportal zones. At the same time patients had significant clinical, biochemical improvements and decrease of histological activity of hepatitis, which indicates efficiency of treatment and therefore decrease of tension of regenerative process. 12 months after procedure we found elevation of hepatocytes proliferation up to starting levels.

Discussion/Conclusion: We suggest that single autotransplantation of HSC in patients with alcohol liver cirrhosis is a safe procedure and has a positive theraputical effect which remains up to 6–12 months. Autotransplantation of HSC enhances regeneration and has to be repeated once each 6 months.

Expression of Bcl-2 in liver biopsies of patients with alcoholic cirrhosis after stem cell transplantation

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Introduction: The number of patients suffering from chronic hepatitis, including alcoholic hepatitis, is growing every year. Very often these patients end up with cirrhosis. Treatment of cirrhosis is very comlicated and not always effective, that is why it is necessary to look for new therapeutic approaches. The aim of our work was to study the effects of autologous stem cell transplantation on livers of patients with alcoholic cirrhosis.

Methods: The study was performed on liver biopsies of 9 patients with alcoholic liver cirrhosis. Biopsies were taken before the injection of autologous peripheral blood stem cells mobilized by granulocyte colony-stimulating factor (G-CSF) into celiac trunk, 3 and 12 months after the procedure. Formalin-fixed, paraffin-embedded liver biopsy preparations were stained immunohistochemically with antibodies against Bcl-2, the anti-apoptotic protein, one of the stem cells markers.

Results: In the biopsies that were taken before the transplantation of peripheral blood stem cells we have seen many Bcl-2 positive cells in the portal tracts. Three months after transplantation the number of positivly stained cells significantly decreased. Nevertheless, twelve months after transplantation the amount of cells expressing anti-apoptotic protein grew up again, but didn’t reach the level of first biopsies.

Discussion/Conclusion: Transplanted autologous mobilized peripheral blood stem cells can reduce liver fibrosis, but this effect is not stable enough and it is necessary to repeat the procedure. We also suppose that stem cell transplantation can reduce the risk of hepatocarcinoma development.

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Profiling of liver triglycerides in *cftr*+/+ and *cftr*-/- mice: Accumulation of specific molecular species in steatotic liver of susceptible genetic strains

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Introduction: Steatosis is a common feature of liver disease in patients with cystic fibrosis. The present study was designed to determine the relative impact of *cftr* status, genetic background and diet on the accumulation of lipids that causes steatosis.

Methods: cftr^{tm1Unc} knockout mice *(cftr-/-)* and their control littermates *(cftr+/+)* were bred on a mixed or congenic C57BL/6j genetic background, and at weaning, were fed either a high fat diet infant formula (Peptamen®, Nestlé) or a low fat diet, *i. e.* standard chow together with an osmotic laxative, polyethylene glycol (PEG 4000), to prevent intestinal obstruction. Livers were collected from all the animals at the age of 3 months (n = 133), and processed for histology and lipid profiling. Lipids were extracted from liver specimens and analyzed by ESI-tandem mass spectrometry (TQ API 3000) for profiling of the molecular species of the distinct phospholipids, cholesterol esters and triglycerides.

Results: Histological analysis showed steatosis in cftr-/- (9/12; 75%) and cftr+/+ (18/24; 75%) mice with a mixed C57BL/6j genetic background and in congenic cftr-/- (1/12; 8%) and cftr+/+ (4/18; 22%) mice that were fed Peptamen®. No steatosis was seen in mice fed standard chow whatever the genotype and the genetic background (0/44), and in congenic cftr-/- (0/24) mice fed peptamen. Based on the variability of triglyceride profiles in Principal Component Analysis, mice were clustered into distinct subgroups as a function of diet, of genotype and of genetic background. No segregation according to *cftr* genotype or gender was found. Molecular species derived from *de novo* lipogenesis (saturated and monounsaturated) and arachidonylcontaining triglycerides were identified as potential specific biomarkers.

Discussion/Conclusion: The genetic background and diet are determinant in the accumulation of arachidonyl-containing triglycerides and steatogenesis in the liver, whereas cftr genotype *per se* has little or no influence.

Involvement of magnesium deficiency in the appearance of type 2 diabetes mellitus in patients with acute pancreatitis

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Introduction: Magnesium deficiency has been associated with insulin resistance and increased risk for type 2 diabetes in adults. The purpose of this study was to evaluate the involvement of magnesium deficiency in the appearance of type 2 diabetes mellitus in patients with acute pancreatitis.

Methods: This study comprised 173 patients diagnosed with acute pancreatitis and hospitalised between January 2007–January 2010 in The IVth Medical Clinic of University of Medicine and Pharmacy Victor Babes Timisoara. We measured in all patients fasting plasma glucose, fasting plasma insulin and serum magnesium. The patients were monitorised each year after the following parameters: fasting glucose and fasting insulin levels, serum and urinary magnesium.

Results: We observed that the levels of fasting serum insulin were decreased in 43% of the patients and the levels of fasting glucose were increased in 58% of them. 72% of them needed insulin administration during the hospitalisation. It was observed a presence of Mg deficiency (expressed as a decrease of plasma Mg) in 61% patients. In the second year of follow-up, we observed that 28% of the patients were diagnosed with type 2 diabetes mellitus and in the third year 27% of the remaining patients were diagnosed also with type 2 diabetes mellitus.

Discussion/Conclusion: The study indicated that the patients with acute pancreatitis have an increased tendency in developing type 2 diabetes mellitus. Magnesium supplementation or increased intake of magnesium-rich foods may be important tools in the prevention of type 2 diabetes in patients with acute pancreatitis.

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Utilization of medical ozone in patients of advanced age with chronic pancreatitis

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The chronic pancreatitis is a chronic inflammatory disease characterized by fibrosis with exocrine destruction and after a long period by destruction of the endocrine tissue of the pancreas. The chronic pancreatitis (CP) refers to the most important problems of the clinical gastroenterology.

The order of the study: To appreciate the effectiveness of the use of ozone therapy in the complex therapy of chronic pancreatitis.

The results of the research: Under observation were 30 persons (men) suffering from CP, the average age was 66.5 ± 6.5 years, the duration of the disease – 18.2 ± 4.2 years. In the clinical picture of all the patients were revealed the pain syndrome in the epigastrium and in the left hypocondrium, as well as nausea, multiple vomitings without relief, flatulence, garguiments and repeated mushy stool. Along with this, the average level in the group of α-amylase constituted 108.4 ± 7.4 u/l.
Depending upon the type of the performed therapy, all the patients were divided in 2 groups. The groups were comparable on all the parameters. The first group consisted of 15 patients (the main group) who were treated with ozone along with traditional therapy. The second group (control group) consisted of 15 patients who received traditional therapy.
After the applied therapy was established reduction of pain syndrome in 10 (66.67%) patients of the first group had already occurred in the first day of treatment, in the second group – only in 4 patients (26.67%). And it should be noted that in 6 (40.00%) patients of Group 1, the pain syndrome was arrested already in the process of the first procedure of ozone therapy (intravenous ODF), and in the rest 4 patients the pain syndrome liquidated during the next 2 hours. Along with this, the remaining 5 patients of Group 1 showed a significant decrease in the intensity of pain. During 3 days, in all patients of Group 1, the pain syndrome has been removed, whereas in group 2, this was achieved only in 9 (60.00%) patients. During 7 days in 15 patients (100%) patients of Group 1 were completely eliminated the symptoms of dyspepsia. In patients of group 2 this was achieved only in 9 (60.00%) patients.
In parallel, it should be noted that the ongoing treatment favorably acted and on the level of α-amylase. Thus, in group1, in all the patients the normalization of this index was pronounced at the 4th day of treatment, whereas in group 2, even at the 7-th day of treatment, in 4 (26.67%) patients, the index remained higher.

Thus, the results of the performed research permit to make the conclusion on the feasibility and necessity of including ozone therapy in complex treatment during the exacerbation of chronic pancreatitis.

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