Chronic Hepatitis: Metabolic, Cholestatic, Viral and Autoimmune

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Abstracts
Poster Abstracts
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CHRONIC HEPATITIS: METABOLIC, CHOLESTATIC, VIRAL AND AUTOIMMUNE

Freiburg (Germany)
October 10–11, 2006

Scientific Organization:
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Session I

Metabolic and toxic hepatitis
NASH: From bench to bedside

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of chronic liver damage, ranging from steatosis (on the most clinically benign end of the spectrum) to cirrhosis (on the opposite extreme where most liver-related morbidity and mortality occur). Non-alcoholic steatohepatitis (NASH) is a lesion of intermediate severity that is characterized by overt hepatocyte injury and death, as well as steatosis. NASH-related liver injury often incites a fibrotic response and sometimes culminates in cirrhosis. Some of these cirrhotic patients go on to develop hepatocellular carcinoma.

It is unclear why liver disease progresses to cirrhosis in some, but not other, individuals with NAFLD. In general, the ultimate severity of liver damage is dictated by the extent to which repair mechanisms can reconstitute healthy liver tissue following injury. Hence, treatments for NAFLD aim to eliminate or reduce injury while bolstering repair. Development of successful treatments for NAFLD will be facilitated by understanding the mechanisms that mediate liver injury and repair in this disease.

Mechanisms of liver Injury in NAFLD

Hepatocyte steatosis and liver injury

Studies of experimental animals and human subjects with NAFLD suggest that liver injury in NAFLD results from hepatocyte accumulation of free fatty acids, as well as various cellular stress responses that develop as fatty cells attempt to dispose of the excess lipids. Increased hepatocyte accumulation of triglyceride is the hallmark of NAFLD. However, the mechanisms driving triglyceride accumulation differ among individuals. These differences in steatosis pathogenesis, in turn, prompt heterogeneous compensatory responses and exert different outcomes on hepatocyte viability. This concept is well illustrated by different rodent models of NAFLD.

Pathogenesis and consequences of steatosis differs among various animal models of NAFLD

Feeding otherwise healthy mice or rats high caloric diets enriched with fat and/or sucrose induces hepatic steatosis, analogous to that which accompanies habitual overeating in humans. Hepatocytes respond to the increased load of FFA by up-regulating several mechanisms to limit cellular exposure to potentially toxic FFA. These include increasing rates of TG synthesis, up-regulating FA oxidation by mitochondria, microsomes and peroxisomes, and enhancing excretion of lipoproteins. In addition to increased delivery of FFA to liver, de novo lipogenesis is a significant contributor to increased hepatocyte FFA exposure in mice, rats and humans with leptin deficiency or leptin resistance. Again, hepatocytes respond to this challenge by up-regulating TG synthesis, FA oxidation and lipoprotein export. Unlike the aforementioned models in which hepatic steatosis results primarily from increased FFA “input” into hepatocytes, methionine choline deficient (MCD) diets promote hepatic steatosis mainly by inhibiting two mechanisms that normally dispose
of FFA. Chronic consumption of such diets depletes hepatocyte carnitine pools, thereby restricting the efficiency of mitochondrial FA oxidation. At the same time, MCD diets induce hepatocyte choline content and this restricts lipoprotein excretion. In these regards, MCD diets mimic human diseases, such as congenital enzymopathies that limit mitochondrial beta oxidation of FA, or abetalipoproteinemia, which inhibits hepatocyte lipoprotein export. To compensate for decreased rates of FFA disposal in these disorders, hepatocytes increase their rate of TG synthesis to store FA in a relatively inert lipid depot, while dramatically increasing non-mitochondrial mechanisms for FA oxidation, such as occurs via microsomal and peroxisomal pathways.

**Impaired fatty acid detoxification increases risk for hepatotoxicity related to fatty acid exposure**

Situations that impair FFA detoxification in the context of an increased FFA challenge generally result in the most severe liver injury. For example, like most leptin-resistant obese diabetic humans, leptin-resistant, obese, diabetic db/db mice have relatively mild steatosis-related liver injury. However, these mice develop NASH with fibrosis when fed MCD diets that compromise lipid disposal mechanisms. Blocking TG synthesis with DGAT2 ASO in db/db mice on MCD diets removes one of their remaining "safe" mechanisms for lipid disposal, further exacerbating NASH and fibrosis. Thus, increased hepatic exposure to FA is generally well tolerated when compensatory lipid disposal mechanisms can be induced, but the same lipid challenge becomes progressively hepatotoxic as lipid disposal mechanisms are lost.

The specific factors that injure fatty hepatocytes are likely to be multiple, and probably vary somewhat according to the principle cause(s) of fat accumulation. In addition to direct cellular toxicity from FFA-induced lipid peroxidation, certain FA (e.g., linoleic acid) may affect global changes in liver gene-expression by functioning as ligands for nuclear hormone receptors (e.g., HNF4-alpha). FA oxidation also generates metabolic by-products, such as reactive oxygen species (ROS) and NAD(P)H that exert their own effects on hepatocytes by acting as signaling molecules and metabolic cofactors. By eliciting modifications in DNA, mitochondria, and other structural components, ROS may also be directly cytotoxic.

In an effort to constrain noxious consequences of ROS and NADPH accumulation, hepatocytes induce compensatory changes in mitochondrial respiration and up-regulate various antioxidant and anti-apoptotic defenses. These adaptive maneuvers, themselves, however, introduce other vulnerabilities by depleting antioxidant reserves and/or limiting the efficiency of ATP synthesis. Thus, even "successful" adaptation to steatosis-imposed "stress" increases hepatocyte vulnerability to cell death. Conversely, failure to sufficiently up-regulate survival responses results in immediate lethality.

These concepts are supported by studies of animals and humans with NAFLD. In many animal models of hepatic steatosis, various antioxidant and anti-apoptotic defenses have been up-regulated sufficiently to maintain hepatocyte apoptosis at relatively low (albeit increased) levels. However, these adaptations enhance vulnerability to secondary insults that overwhelm the residual capacities for antioxidant defense or ATP synthesis, such as ischemia reperfusion or lipopolysaccharide exposure. Hepatic injury tends to be greater in models that limit
antioxidant or ATP synthesis, while promoting hepatic steatosis, such as feeding MCD diets. A similar situation occurs when individuals with fatty livers are exposed to transient hepatic ischemia, or when patients with inherited trans-sulfuration pathway defects (e.g., homocysteinemia) that increase basal levels of oxidative and ER stress, become obese and impose additional demands on these systems.

**Adipocytokines and liver injury in NAFLD**

Adipose tissue is a rich source of several soluble factors that promote hepatic steatosis. Lately, attention has focused on adipocytokines in the pathogenesis of NAFLD. Studies in several animal models of NAFLD (e.g., ob/ob mice, HFD fed mice, Agouti mice exposed to LPS) suggest that unbalanced production of pro-inflammatory cytokines (especially, TNF-alpha) relative to anti-inflammatory factors (e.g., adiponectin) plays a pivotal role in the pathogenesis of NAFLD. In such models, various treatments that directly inhibit TNF-alpha activity (e.g., anti-TNF antibodies, genetic disruption of TNF-alpha or TNF receptor (TNFR)-1, pentoxifylline, TZD) or that increase activity of adiponectin or its downstream target, AMP kinase, (e.g., supplemental adiponectin, metformin) improve steatosis and steatohepatitis.

Since TNF-alpha and adiponectin are mutually antagonists, any treatment that inhibits TNF-alpha enhances adiponectin activity. Conversely, anything that boosts adiponectin's actions blocks TNF-alpha's effects. At the cellular level, TNF-alpha and adiponectin exert opposing effects on hepatic lipid homeostasis. Adiponectin inhibits FA uptake and promotes FA oxidation and lipid export from hepatocytes by inducing the phosphorylation of AMP kinase, a master regulator of energy homeostasis and lipid intermediary metabolism. TNF-alpha inhibits adiponectin expression, as well as AMPK activation, thereby blocking adiponectin activity. Hence, when TNF-alpha activity predominates, hepatocytes accumulate FA and become subject to all of the outcomes discussed previously. Conversely, hepatocyte FA exposure and its consequences are limited when adiponectin activity predominates.

Adiposity generally increases serum levels of various adipose-derived factors, including both TNF-alpha and adiponectin, although serum levels of TNF-alpha tend to be higher, and adiponectin levels tend to be lower, in individuals with more advanced liver injury (i.e., NASH) than in those with less severe liver injury (i.e., steatosis). However, neither adipocytokine is a useful biomarker for NAFLD because serum TNF-alpha and adiponectin levels vary considerably amongst individuals with any given level of liver injury. Advanced NAFLD also occurs in lipodystrophic individuals who have severely reduced adipose depots.

One explanation for these inconsistencies may be inherent differences in adipocytokine production by various adipose depots. In general, visceral adiposity (which persists in lipodystrophy and is most strongly associated with obesity-related diseases) results in greater relative exposure to proinflammatory factors (e.g., TNF-alpha). In addition, there may be non-adipose tissue sources of adipocytokines. Such sources include resident liver cells, themselves. Adipocytic hepatic stellate cells produce both resistin and adiponectin and HSC-derived resistin evokes production of cytokines, including TNF-alpha, IL-1beta, and IL-6, by neighboring hepatocytes. Variations in tissue adipocytokine production might immediately impact paracrine signaling that regulates hepatocyte intermediary...
metabolism and viability without causing major alterations in serum levels of adipocytokines that are derived predominately from body fat depots. Hence, important contributions from locally-produced adipocytokines might help to explain the imperfect correlation between absolute levels of various serum adipocytokines and the severity of liver injury in individuals with NAFLD.

**Insulin resistance and NAFLD**

In addition to modulating lipid homeostasis, various adipocytokines also regulate carbohydrate metabolism by influencing cellular sensitivity to insulin. NAFLD is strongly associated with insulin resistance. Indeed, clinical data link the severity of NAFLD-related liver damage with the severity of insulin resistance. Hepatic fibrosis and liver-related mortality tend to be most marked in NAFLD patients with overt type 2 diabetes. Despite the apparent robustness of this clinical correlation, it has been difficult to understand whether the association between NAFLD and diabetes primarily reflects the negative outcomes of inhibiting insulin actions in the liver itself, as opposed to extra-hepatic tissues. If hepatic (rather than systemic) insulin resistance is, in fact, the driving force for NAFLD, this might explain why systemic insulin resistance is not a pre-requisite for hepatic steatosis. On the other hand, it does not clarify why many humans and experimental animals with NAFLD exhibit both increased rates of hepatocyte fatty acids and triglyceride synthesis (suggestive of enhanced insulin activity), and hyperinsulinemia and excessive hepatic glucose output (typical of insulin resistance). The latter findings suggest that differential inhibition of various insulin-regulated signaling pathways within hepatocytes might be necessary for NAFLD pathogenesis.

The confusing role of insulin resistance in NAFLD pathogenesis/progression has not been resolved by studies of animal models of NAFLD. Indeed, such work only underscores the complexity of this relationship. Similar to many obese humans, leptin-deficient (ob/ob) and leptin-resistant (db/db) murine models of NAFLD exhibit hyperinsulinemia, hyperglycemia and evidence of both systemic and hepatic insulin resistance. Treatment with insulin sensitizing agents, such as TZDs, has been beneficial, albeit inconsistently. On the other hand, MCD diet-fed mice develop NASH and progressive hepatic fibrosis despite diet-induced decreases in serum insulin and glucose, suggestive of enhanced systemic sensitivity to insulin. Nevertheless, insulin sensitizers, such as TZDs, also seem to improve MCD diet-induced liver disease. Recent evidence of reduced insulin receptor- and IRS-2 tyrosine phosphorylation in the livers of MCD diet-fed mice supports the possibility that these animals have hepatic insulin resistance. On the other hand, findings of severe steatohepatitis, progressive fibrosis and eventual hepatocellular carcinoma in mice with hepatocyte-specific activation of AKT, a prototypical downstream target of insulin signaling, suggest that certain insulin-initiated signals might actually promote (rather than prevent) liver damage. At this point, no unifying hypothesis has emerged to reconcile these seemingly contradictory results.

It is conceivable that part of the confusion derives from efforts to equate type 2 diabetes with extremely severe insulin resistance. Recent data from the Diabetes Prevention Program demonstrated that amongst insulin resistant individuals, hyposecretion of insulin was a better predictor of subsequent type 2 diabetes than the initial severity of insulin resistance per se. This finding led the DPP investigators to speculate that early hyposecretion of insulin identifies a subset of insulin resistant
subjects with a reduced capacity for pancreatic beta cell hyperplasia. Such subjects would have a reduced ability to compensate for insulin resistance and thus, develop overt hyperglycemia earlier than more hyperinsulinemic individuals. This insight raises the intriguing possibility that advanced NAFLD (which strongly correlates with type 2 diabetes) might result from an inadequate hyperplastic response to liver injury. The latter is a particularly attractive concept given that liver regeneration is necessary to restore the architecture of damaged livers. Thus, progressive liver damage results when regeneration cannot keep pace with injury.

**Mechanisms of Inhibited Liver Regeneration in NAFLD**

**Replicative senescence in mature hepatocytes**
In healthy livers, acute loss of mature hepatocytes promptly triggers a compensatory proliferative response in surviving mature hepatocytes, permitting efficient reconstitution of liver mass. However, many viable hepatocytes in chronically damaged livers have sustained sufficient oxidative damage to induce replicative senescence. Thus, in steatotic livers, most mature hepatocytes have reduced proliferative capacity and cannot replicate to replace dying hepatocytes. Liver atrophy is circumvented by engaging hepatic progenitors in the regenerative response. Thus, unlike regeneration of healthy livers, repair of chronically damaged livers is dependent upon the differentiation of liver progenitors, a process that is less efficient than replication of mature hepatocytes.

**Differentiation of liver epithelial progenitors, stellate cell activation and fibrosis**
Relatively little is known about the mechanisms that regulate the responses of liver progenitor populations to liver injury. However, emerging evidence suggests that paracrine signaling between liver mesenchymal cells, such as hepatic stellate cells (HSC), and liver epithelial cells figures prominently in this process. Liver injury is known to reduce the lipid content of adipocytic HSC and to promote their transformation into myofibroblastic cells that drive the hepatic accumulation of extracellular matrix. Multiple factors participate in HSC activation during liver injury, including the release of factors, such as PDGF and TGF beta, from aggregating platelets and dying hepatocytes, respectively. Phagocytosis of apoptotic hepatocytes also contributes to HSC activation.

Myofibroblastic HSC, in turn, down-regulate their production of factors, including resistin, adiponectin, IL-6, and hepatocyte growth factor, that transduce various viability signals in neighboring hepatocytes. Hence, one of the earliest consequences of HSC activation is a reduction of paracrine factors that normally maintain the survival and proliferative capacity of mature hepatocytes. Furthermore, other factors produced by myofibroblastic HSC, such as plasminogen activator inhibitor (PAI)-1 and tissue inhibitors of metalloproteinases (TIMPs), inhibit localized release of matrix-associated HGF, further reducing hepatocyte access to this mitogen. HSC-derived factors, such as leptin and angiotensin, also promote proliferation and survival of myofibroblastic HSC that generate large amounts of type 1 collagen. Thus, the accumulation of myofibroblastic HSC, long known to signify fibrogenesis, also marks a situation in which damaged mature liver epithelia is undergoing relative "debridment".
Fortunately, myofibroblastic HSC produce other factors, such as stromal derived factor (SDF)-1 and Sonic hedgehog (Shh), that promote the accumulation of hepatic epithelial progenitors in damaged livers. Paracrine signaling between myofibroblastic HSC and immature hepatic epithelial cells also enhances growth of the latter cells, thereby encouraging "re-epithelialization" of damaged livers from hepatic progenitor reserves. Ultimately, the restoration and maintenance of healthy mature liver epithelia probably requires the re-emergence of adipocytic HSC to provide a steady supply of factors that assure optimal viability of adult hepatocytes. This helps to explain why the numbers of myofibroblastic HSC in steatotic livers distinguish individuals who are likely to exhibit NASH and fibrosis on a subsequent liver biopsy from those whose liver disease will not progress over time. State another way, accumulation of myofibroblastic HSC heralds remodeling of the hepatic architecture and implies that scarring is likely because wound healing is incomplete.

Much research focuses on delineating the mechanisms that modulate this process, with the intent of re-directing wound healing responses to optimize epithelial repair while minimizing fibrosis. Interestingly, adiponectin (a product of adipocytic HSC and adipose tissue) appears to inhibit activation of adipocytic HSC to myofibroblastic cells. Thus, various treatments that enhance adiponectin activity offer the promise of reducing hepatic steatosis, limiting hepatocyte death, and preventing hepatic fibrosis. Based on the earlier discussion, therapies that inhibit HSC activating factors, including TGF beta, PAI-1 and angiotensin, or that enhance HGF activity, are also attractive therapeutic candidates in NAFLD.

**Progenitor accumulation and hepatocarcinogenesis**

Sustained efforts to regenerate damaged livers may promote hepatocarcinogenesis. By promoting the accumulation of hepatic progenitors within a microenvironment of ongoing oxidative stress, the risk that some of these cells might develop stochastic mutations that promote their neoplastic transformation increases. Hence, ideal cancer prevention approaches remove the stimulus for progenitor accumulation by minimizing liver injury and expediting liver repair.

**Summary**

The hallmark of NAFLD is triglyceride deposition within hepatocytes. Studies of experimental animals and human subjects with NAFLD suggest that liver injury in NAFLD results from hepatic accumulation of free fatty acids, as well as various cellular stress responses that develop as fatty hepatocytes attempt to dispose of the excess lipids. At least in part, inter- and intra-individual variability in the outcome of hepatic steatosis probably reflect differences in the mechanisms that drive triglyceride accumulation, which, in turn, evoke heterogeneous compensatory responses that exert different effects on hepatocyte viability. Death of mature hepatocytes triggers hepatic regeneration to restore lost hepatocyte mass and function. Ultimately, the net amount of liver damage that accrues is determined by the liver's regenerative capacity. Because hepatic steatosis induces chronic oxidative and metabolic stress, it increases oxidative damage and limits energy reserves in mature hepatocytes, thereby reducing their proliferative capacity. This gradually increases the requirement for hepatic progenitors in the repair process. Emerging evidence suggests that paracrine signaling between dying mature hepatocytes,
hepatic stellate cells and epithelial progenitors is a major factor that modulates regeneration of chronically injured, fatty livers. Various adipocytokines that are produced both by adipose tissue and hepatic stellate cells regulate this process. Transient increases in myofibroblastic stellate cells probably enhance repair by mobilizing fresh crops of hepatic progenitors to replace damaged mature hepatocytes, while sustained accumulation of myofibroblastic (as opposed to adipocytic) stellate cells perpetuates hepatic injury and increases liver fibrosis. Rather than reconstituting normal liver architecture, the latter increases the risk for cirrhosis and hepatocellular carcinoma. Therefore, in order to develop treatments that will optimize recovery from NAFLD, it is important to clarify the mechanisms that regulate liver repair, as well as those that initiate liver injury.
Alcoholic hepatitis: Pathophysiology and treatment

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Alcoholic steatohepatitis (ASH) reveals a specific clinical and histomorphological feature within the broad spectrum of alcoholic liver disease (ALD). ASH is best characterized by histomorphology caused by chronic heavy alcohol consumption. Histomorphology is indistinguishable from that observed in non-alcoholic steatohepatitis (NASH). It includes macro- and microvesicular steatosis, lobular hepatitis, ductular proliferation, perivenal fibrosis and Mallory bodies. Although almost 100% of chronic alcohol consumers develop fatty liver, only 10–35% of them develop ASH. Similar as in NASH, a two-hit hypothesis may explain the pathogenesis of ASH. Firstly, fatty liver occurs which is a prerequisite for the second hit since it sensitizes the liver against various noxae. These include particularly the action of gut-born endotoxins and the induction of cytochrome P4502E1 (CYP2E1). The intensity of the second hit may vary, depending on individual factors and, therefore, the clinical and pathomorphological feature of ASH may also vary.

Various mechanisms contribute to the production of alcoholic fatty liver including the oxidation of ethanol to acetaldehyde and the simultaneous generation of reducing equivalents in the form of NADH. NADH overfloods the liver and leads to a shift in the hepatic redox state favouring free fatty acid and triglyceride synthesis and inhibition of mitochondrial β-oxidation. Additionally, alcohol increases the release of free fatty acids from fat tissue and its shift to the liver. Also gut-derived chylomicrons enter the liver. Subsequently, acetaldehyde injures hepatic mitochondria and microtubules resulting in a decreased oxidation of NADH and an inhibition of the secretion of very low density lipoproteins (VLDL) from the liver to the serum. Alcohol also affects PPARγ and SREBP-1c via AMP kinase. All these mechanisms work n concert resulting in fatty liver. Fatty liver is extremely sensitive to lipid peroxidation which can be initiated by reactive oxygen species (ROS). Most ROS in ALD are produced via an induction of CYP2E1 which differs individually. ROS may oxidize proteins and results in lipid peroxidation. Lipid peroxidation products such as 4-hydroxynonenal may bind to DNA forming exocyclic etheno DNA adducts. In animal experiments, the inhibition of CYP 2E1 reduces alcohol-induced liver injury significantly. In addition, gut-born endotoxins bind to hepatic Kupffer cells resulting in a secretion of various cytokines including interleukin-1, -4, -6, -8 and -10, TGF-β and especially TNF-α. TNF-α binds to its receptor and mediates proliferation, necrosis and apoptosis. It is important to know that TNF-α can also be increased via NFkB.

In experimental animals therapy with antibiotics to decrease intestinal bacterial flora, elimination of Kupffer cells by gadolinium chloride, administration of antibodies against TNF-α and the use of TNF-α receptor knock-out mice, all reduces alcohol-induced liver disease.

Therapeutic strategies in ASH include abstinence, immune suppression by steroids, reduction of the action of TNF-α either by pentoxiphyllin or TNF-α antibodies as well as nutritional therapy with hyperalimentation and substitution of vitamins and trace elements. With respect to steroid therapy, a more recent metaanalysis reported a benefit of 30 mg prednisolone in severe ASH with a Maddrey index over 32. This treatment resulted in a significant reduction of short-term and 1-year mortality which was abolished after the second year. Especially patients with jaundice and encephalopathy may benefit from this treatment and most importantly if serum
bilirubin does not decrease after 6 days of treatment this should be stopped. Patients with upper GI bleeding and infections do not benefit and there is an increased risk for sepsis. During the last years, pilot studies using TNF-α antibodies have been used with promising results. However, a most recent double-blind placebo-controlled trial with high doses of TNF-α antibodies (3 times 10 mg/kg bw) and application of prednisolone resulted in an increased mortality in the treatment group due to sepsis and infections. Certainly, this study has to be criticized because of the extremely high dose of TNF-α antibodies. Finally, pentoxiphyllin has been used in one placebo-controlled trial. It inhibits TNF-α secretion and mortality, especially the occurrence of hepatorenal syndrome, was significantly reduced in the treatment group.
Drug-induced hepatitis

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Drug induced liver injury (DILI) is the most common cause of acute liver failure (ALF) in the U.S. and Europe, can mimic any form of acute or chronic liver disease and is the most common cause of pre- and post-marketing withdrawals. Although some examples are predictable, most are unpredictable or idiosyncratic and fall into two-categories; allergic (concomitant features such as rash, eosinophilia, 1–4 week latency and rapid positive rechallenge) and presumed non-allergic (no signs of allergy, variable latency up to one year and often negative rechallenge). The bulk of cases of DILI present as hepatitis, cholestasis and a mixed picture. The hepatitis cases are at great risk for ALF (Hy’s Law) whereas cholestatic cases resolve very slowly and can progress to ductopenia. Causality assessment relies on excluding plausible alternative causes for liver injury as well as assessing latency, de-challenge, known risk factors, and previous experience with the drug. The pathogenesis of DILI remains ill-defined although genetic analysis as well as “omic” technologies offer promise for unraveling the mechanisms in the near future. One important characteristic of both allergic and non-allergic idiosyncratic DILI is the production of reactive drug metabolites as a prerequisite. Reactive metabolites can covalently bind to protein and when coupled to the co-stimulatory effects of cell stress or necrosis or concomitant illness can lead to an adaptive immune response (danger hypothesis). Non-allergic DILI has been most extensively assessed in the acetaminophen (APAP) model. APAP is converted to the reactive metabolite, NAPQI, which is preferentially detoxified by GSH. When GSH is nearly fully depleted (especially in mitochondria), NAPQI covalently binds to proteins, and profound mitochondrial GSH depletion unleashes oxidative stress. Thus, upstream factors which control NAPQI exposure (production via CYP2E1 and removal via GSH and GSH S-transferase) are key prerequisites for toxicity. However, the progression to injury is determined by hepatocellular stress-induced protective and injurious responses to redox sensitive transcription factors and signal transduction. For example, GSH depletion activates Nrf-2, a transcription factor which increases expression of genes which control detoxification. At the same time GSH depletion/oxidative stress activate stress kinases, such as c-jun-N-terminal kinase (JNK), in a sustained fashion. JNK targets mitochondria as an additional hit and inhibition of JNK prevents APAP toxicity without altering covalent binding or GSH depletion. This illustrates that the stress of toxic metabolites is not necessarily directly lethal but activates built-in death programs. The initial hepatocellular injury from APAP then triggers an innate immune response; immunodepletion of NK/NKT cells, IFNγ KO mice, Fas and FasL deficient mice, and neutrophil depleted mice are all partially protected against APAP without altering upstream metabolism, GSH depletion or covalent binding. Conversely, IL-10 and IL-6 KO mice are more susceptible to APAP. These finding illustrate the potential for the influence of genetic and environmental variations in stress response or the innate immune response in determining the susceptibility to drug hepatotoxicity.
Session II

Cholestatic hepatitis
Liver disease in pregnancy: Diagnosis and treatment

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Abnormalities in liver tests occur in 5% of pregnancies and jaundice in 0.1% with causes ranging from self-limiting to rapidly fatal (1). There exist liver disorders pregnancy-related, diseases occurring coincidentally, and preexisting chronic liver disease. Liver diseases unique to pregnancy occur at characteristic time. Hyperemesis gravidarum (HG) begins in the first trimester, intrahepatic cholestasis of pregnancy (ICP) in the second or third trimester, preeclampsia, HELLP syndrome and acute fatty liver of pregnancy (AFLP) usually in the third trimester. HG is characterized by intractable nausea and vomiting resolving by week 20 of gestation. Up to 50% patients have liver enzymes abnormalities. Complications are secondary to repeated vomiting. Fetal outcomes are favourable. Treatment is symptomatic though hospitalization is occasionally necessary for rehydration and nutritional support (2). ICP is the most common pregnancy-related liver disorder with highest incidence in Chile, Scandinavia, and Baltic states. ICP is characterized by pruritus associated with increase in aminotransferases and serum bile acids. Maternal prognosis is benign. In contrast, ICP may have serious consequences for the fetus. The cause of ICP is still under discussion. There is increasing evidence that genetically determined dysfunction in the canalicual ABC transporters bile salt export pump (BSEP, ABCB11) and multidrug resistance protein 3 (MDR3, ABCB4) might be risk factors for development of ICP (3). The treatment of choice is ursodeoxycholic acid, which improved maternal and fetal morbidity in several clinical trials and observational studies (4). Preeclampsia affects 5–7% of all women during pregnancy. Abnormalities in liver tests occur in 20 to 30% of patients (5). HELLP syndrome develops in 0.1–0.6% of all pregnancies. As many as 30% of patients with HELLP are diagnosed postpartum. Although it is considered a complication of preeclampsia, only 4–12% of women with severe preeclampsia experience the HELLP (6). It is characterized by microangiopathic hemolytic anemia, hepatic dysfunction, and thrombocytopenia. Complications include DIC, abruptio placenta, acute renal failure, hepatic hemorrhage, rupture, hepatic infarction. The definitive treatment is delivery (7). The incidence of AFLP is 1: 10,000–15,000 pregnancies. Recently, an association between AFLP and a deficiency of the enzyme long-chain 3-hydroxyacyl-CoA dehydrogenase was suggested (8). The clinical spectrum of AFLP is broad, ranging from asymptomatic elevations in aminotransferases to hepatic failure with profound coagulopathy, hepatic coma, hypoglycaemia (9). With early delivery and advances in supportive management maternal mortality is now 10–18% and fetal mortality 9–23% (10).
References:


Primary biliary cirrhosis (PBC) is an autoimmune disease of unknown etiology, often associated with other autoimmune conditions. Controlled studies have so far provided conflicting data on risk factors and comorbidity rates in PBC. We enrolled patients with PBC (n = 1032) from 23 tertiary referral centers for liver diseases in the United States and random digit dialed controls (n = 1041) matched for sex, age, race, and geographical location. Patients and controls were administered a modified version of the US National Health and Nutrition Examination Study (NHANES III) questionnaire by trained personnel to evaluate associations between PBC and social, demographic, personal and family medical histories, lifestyle, and reproductive factors and the rates of comorbidity in affected individuals. Data indicate that having a first-degree relative with PBC (adjusted odds ratio [AOR] 10.736; 95% confidence interval 4.227–27.268), history of urinary tract infections (AOR 1.511, 95% CI 1.192–1.915), past smoking (AOR 1.569, 95% CI 1.292–1.905), or use of hormone replacement therapies (AOR 1.548, 95% CI 1.273–1.882) were significantly associated with increased risk of PBC. The frequent use of nail polish slightly increased the risk of having PBC. Other autoimmune diseases were found in 32% of cases and 13% of controls (P < 0.0001). In conclusion, environmental factors, possibly including infectious agents through urinary tract infections or chemicals contained in cigarette smoke, may induce PBC in genetically susceptible individuals. Exogenous estrogens may also contribute to explain the female predominance of the disease. These data should be contrasted with the concordance of PBC in identical twins.

There is growing evidence that the interplay of genetic susceptibility and environmental factors leads to primary biliary cirrhosis (PBC). In particular, family members of an infected individual have up to a 100-fold higher risk of developing PBC. Although concordant rates for identical twins in other autoimmune diseases range between 25% and 50%, there are no such data on PBC. Accordingly, we evaluated the concordance of PBC in a genetically defined population of twin sets and evaluated the clinical characteristics between concordant subjects. We identified 16 pairs of twins within a 1400-family cohort followed up by several centers worldwide, evaluated the diagnosis of PBC in all individuals, and determined the zygosity of sets reported as identical by the analysis of 2 highly variable HLA class II regions and 5 short tandem repeats. Eight of 16 sets of twins were monozygotic. In 5 of 8 monozygotic twin sets, both individuals had PBC (pairwise concordance rate, 0.63). Among the dizygotic twins (n = 8), no set was found to be concordant for PBC. Interestingly, the age of onset of disease was similar in 4 of 5 concordant sets of monozygotic pairs; however, there were differences in natural history and disease severity. The concordance rate of PBC in identical twins is among the highest reported in autoimmunity. However, discordant pairs were identified. The data show not only the role of genetics but also emphasize that either epigenetic factors and/or environment play a critical role.
Interface hepatitis in PBC: Prognostic marker and therapeutic target

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The initial injury in PBC affects medium-size bile ducts. The inflammatory infiltrate is intimately related to the bile ducts and confined within the portal tracts. The subsequent progression of the disease is classically thought to be mainly the result of cholestasis, i.e., bile retention in the vicinity of the damaged bile ducts leading to a “biliary” interface hepatitis with piecemeal necrosis, ductular proliferation associated with loose fibrous tissue infiltrated by a mixture of inflammatory cells. In our experience, this type of interface hepatitis is nowadays extremely rare, probably due to the systematic use of ursodeoxycholic in the treatment of PBC. In contrast a moderate to severe interface hepatitis characterized by a lymphocytic piecemeal necrosis similar to that observed in autoimmune hepatitis is frequent in liver biopsy specimens of stages 2–4. The biochemical expression and impact on prognosis of this histological lesion will be reviewed. The differential effects of therapies on this crucial step in the progression of liver disease in PBC will be discussed.

References:
Session III

Viral hepatitis (Part 1)
Role of innate immunity for outcome of viral hepatitis

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Innate immunity serves as a first line of defense against pathogens and/or cancer cells during the initial phase of responses before adaptive immunity is sufficiently induced. Dendritic cells (DCs) are known to be the most potent cellular lineages to serve as sentinels between innate and adaptive responses. The ability of DCs to activate adaptive immune responses is dependent on the state of their maturation. DC maturation is controlled by various kinds of innate cytokines as well as Toll-like receptors (TLRs), which constitute recognition for invading pathogens as pathogen-associated molecular patterns. Natural killer (NK) cells are another lineage of innate immune cells that can distinguish tumor cells from healthy cells via various types of NK receptors. Upon activation NK cells directly kill tumor cells and produce a variety of cytokines. Recent study establishes interaction between DC and NK cell as an important determinant to direct adaptive immunity. Activated NK cells induce DC maturation either depending on cell-to-cell contact or soluble factors. On the other hand DC upon stimulation can activate NK cells. We recently found DC activates NK cells via a MICA-NKG2D system upon type 1 interferon stimulation. Thus, DCs and NK cells are equipped with complementary sets of receptors that allow the recognition of various pathogenic agents and transformed cells and establish the coordination of innate and adaptive responses by their crosstalk.

To clarify the significance of DCs in hepatitis C virus (HCV) infection, we examined DC subsets. Blood DC consist of myeloid DC (MDC) and plasmacytoid DC (PDC) subsets and play distinct roles in the regulation of anti-virus immune response. We compared the numbers and functions of MDC and PDC between HCV-infected patients and age-matched uninfected donors. Absolute numbers of MDC, PDC and DC progenitors in the periphery were significantly lower in chronic hepatitis patients than in control subjects. MDC and PDC from the patients were impaired in the stimulation of allogeneic CD4 T-cells and in the production of IL-12 p70 and TNFα. Thus, MDC and PDC in HCV-infected patients were reduced in number and impaired in their ability to promote Th1 polarization.

The function of NK cells is regulated by a fine balance of inhibitory and activating signals, which are mediated by a diverse array of cell-surface NK receptors.

We previously found that hepatoma cells, but not non-transformed cells, frequently expressed ligands for the NKG2D activating receptor, such as MICA/B and ULBPs. Hepatoma cells were capable of activating NK cells which appeared to be dependent on NKG2D-mediated signals. Hepatoma cells constitutively expressed HLA-E, a ligand for the NKG2A inhibitory receptor. Interestingly, NKG2A was highly expressed in patients with chronic hepatitis C. This caused lower responsiveness of NK cells towards hepatoma cells in these patients than healthy donors. Furthermore, we found that, in patients with liver cirrhosis and hepatocellular carcinoma, soluble forms of MICA/B were frequently detected in circulation, leading to internalization of NKG2D expression on NK cells. Thus, the aberrant expression of NK inhibitory and activating receptors negatively regulates NK cell functions towards transformed hepatocytes and may be involved in initiation and progression of hepatocellular carcinoma.
In HCV infection, various alterations were found on phenotypes and/or function of innate immune cells including DCs and NK cells. These alterations may play a role in persistent infection of this virus as well as high incidence of hepatocarcinoma.

References:


Pathobiology of HBV infection

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Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the major etiological factors of chronic liver diseases, including cirrhosis and hepatocellular carcinoma (HCC). Chronically infected HBV carriers count up more than 350 million people worldwide harboring the risk of HCC development. Due to lack of fully effective and cost-effective antiviral treatments, the number of persistent HBV carrier is still increasing. Recent studies suggested that occult HBV infection in HCV-related patients increases the risk of chronic liver diseases. Pathogenesis and carcinogenesis accompanied by HBV infection occur over years of chronic infection and associate with hepatocyte death and regeneration, however, their mechanisms are partly understood yet.

Among the proteins encoded by the HBV genome, X protein (HBx), the 17-kDa regulatory protein, has been shown as a most potential factor that correlates to liver carcinogenesis. Expression of HBx induces transformation of some cell types and liver cancer in some transgenic mice. However, HBx transgenes exhibit no obvious pathology in the artificial genetic construct without HBx promoter, although these non-pathogenic transgenes cause an increased susceptibility to chemical carcinogenesis.

HBx exhibits multiple cellular functions through interaction with transcription factors and signaling cascades proliferative to the cells. Although HBx itself is unable to bind DNA directly, HBx is a potent transcriptional co-activator through multiple cis-acting elements, including AP-1, AP-2, ATF/CREB, NF-kB, NF-AT, c-myc, and C/EBP. HBx is also shown to mediate several signal transduction cascades, such as RAS/MAPK-, JNK-, NF-kB- and Src-dependent pathways. These functions interacting with transcription factors and signaling cascades may contribute in proliferation control of the cells.

On the other hand, we and other groups have recently shown that HBx facilitated cell death induction by associating with the mitochondria. The association of HBx with mitochondria causes loss of mitochondrial membrane potential and subsequently causes cell death. Thus, HBx has both proliferative and cell death-promoting activities under each respective conditions.

In this study, we firstly analyzed a cytopathic function of HBx localized on mitochondria by the protein transduction method. Recombinant HBx (rHBx) and its mutants, an internal deletion mutant, XΔ(5-67), and a C-terminus truncation mutant, X(1-67), were successfully transduced into cells in a concentration-dependent manner. Surprisingly, we found that rHBx and its mutants tested here all crossed the cell membrane and allowed us to analyze their direct effects on cells. Using the protein transduction protocol, we overcame the problem generated by DNA transfection such as over-expression and low efficiency of introduction.
rHBx and mutant XΔ(5-67), which associated with mitochondria, inhibited cell proliferation and decreased the cellular ATP level. On the other hand, mutant X(1-67), which was unable to associate with mitochondria, exhibited no effect on cell growth. Generation of reactive oxygen species (ROS) was stimulated after incubation with rHBx or XΔ(5-67), but not with X(1-67).

Subsequently, mutagenic effect of rHBx or XΔ(5-67) was examined using the Big Blue rat cell line method, in which lambda phage shuttle vector containing the cII gene was integrated exogenously. We studied the frequency and spectrum of mutation on these cells by protein transduction. We first found that rHBx has an intrinsic function to increase mutation frequency in the cell culture system more than 4-times as compared with that of control in the absence of rHBx. Increased mutation frequency by rHBx was counteracted by addition of ROS scavenger, Tiron or N-acetylcysteine (NAC) to the culture medium, indicating the ROS generation enhances mutation frequency. Data, thereby, strongly suggest that HBx has a cytopathic function to cause DNA mutation via ROS generation and a mutagenic role of HBx in the necro-inflammatory symptoms of HBV chronic hepatitis is also suggested.

References:


Individualized treatment of chronic hepatitis C

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Patients infected with HCV genotype 1 are treated with a pegylated interferon plus ribavirin for 48 weeks, while HCV-2 or 3 infected patients are treated for 24 weeks. Using HCV genotype and fibrosis stage together with baseline viremia and the initial virologic response to therapy enables further individualization of treatment duration. Non-cirrhotic patients with HCV-1 infection, a baseline viremia < 600,000 IU/mL and a rapid virologic response (< 50 IU/mL at week 4) can achieve a sustained virologic response rate of almost 90% with only 24 weeks of combination therapy. HCV-2 and HCV-3 infected patients with low baseline viremia can be treated for less than 24 weeks without compromising sustained virologic response rates. Longer treatment duration up to 72 weeks appears reasonable for HCV-1 infected patients with serum HCV RNA levels of 50–6000 IU/ml at week 12 of therapy.

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* Data derive from small trials, results of the ACCELERATE study should be awaited
Session IV

Viral hepatitis (Part 2)
The goal of hepatitis C virus (HCV) therapy is permanent viral eradication. This requires the use of drug combinations with multiple modes of action. The main target of HCV therapy is steady-state HCV replication kinetics, which can be disrupted by drugs that inhibit virus production (antiviral molecules), inhibit de novo cell infection, and/or accelerate the clearance of infected cells. Interferon-α and ribavirin combine all these mechanisms of action when used together, yet fail to clear HCV from a significant number of patients. New therapeutic approaches are needed. The next generation of anti-HCV therapeutic agents will fall into four main categories. (i) New interferons and interferon inducers will combine various modes of action in a more potent way than current molecules. Albumin-linked interferon appears promising in combination with ribavirin, and gene-shuffled interferon alpha has been recently pegylated and will enter clinical experimentation soon. Oral interferon inducers act through unclear mechanisms. They could add to the effect of interferon and ribavirin but the preliminary results need confirmation. (ii) Alternatives to ribavirin will have the same efficacy target without significant side effects. Currently, the only alternative to ribavirin is viramidine, a prodrug of ribavirin that is preferentially transformed into ribavirin in the liver. The results of a recent phase III trial have been disappointing in comparison with ribavirin. (iii) Specific HCV inhibitors target key HCV enzymes. Inhibitors of the HCV RNA-dependent RNA polymerase and of the HCV NS3 protease have been developed and shown to modestly to potently inhibit viral replication when administered to chronically infected patients. Selection of resistant variants appears as a major problem with these drugs, an issue that will need to be dealt with by means of combination therapies. (iv) Immune therapies may target de novo infection of hepatocytes, by means of monoclonal or polyclonal anti-HCV antibodies. Therapeutic vaccines are developed to stimulate the anti-HCV cellular responses and accelerate infected cell clearance. Ideally, these new treatments will increase the rate of sustained viral eradication and improve tolerability and acceptability. Drug combinations will be tailored to the individual patient, based on baseline parameters and viral kinetics during therapy.
Session V

Autoimmune hepatitis
Update on autoimmune hepatitis

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Autoimmune hepatitis (AIH) is a remarkably well treatable chronic liver disease (1). In view of a potentially excellent prognosis with the induction of disease remission, which is more favorable than that of any other chronic inflammatory liver disease, the timely diagnosis and an efficient therapy represent a continuous challenge for clinical hepatologists. Untreated, the prognosis of active AIH is dismal with 5 and 10 year survival rates between 50 and 10% and a well recognized therapeutic effect exemplified by the last placebo-controlled treatment trial reported in 1980 (2, 3).

In comparison to chronic hepatitis C (HCV) and B (HBV) AIH is frequently reported to represent a rare disease. However, based on limited epidemiological data it is believed to account for between 50 to 200 cases per million in Western countries, and is diagnosed in as much as 20% of all cases of chronic hepatitis. In contrast to chronic HCV and HBV infection the diagnosis of AIH is reached by exclusion rather than by a single sensitive and specific diagnostic parameter which considerably confounds the possibility of an accurate assessment of prevalence. A serological clue pointing to AIH are autoantibodies and hypergammaglobulinemia (i.e. immunoglobulin G). However, serum autoantibodies in AIH are characterized by heterogeneity reflected by the detection of antinuclear (ANA), anti-smooth muscle (SMA), anti-liver kidney microsomal (LKM), anti-soluble liver/pancreas antigen (SLA/LP) autoantibodies, and other more rare reactivities, which form the basis for serological subclassification but do not carry a significant relevance for differences in therapeutic strategy (4). With the exception of SLA/LP none of these autoantibodies are very specific for AIH. They occur in other conditions including rheumatological diseases (5), drug-allergic reactions (6), virus-associated autoimmunity (7) and genetic autoimmune disease (8). However, from the point of view of clinical presentation serum autoantibodies are also indicative of heterogeneity in AIH (4). LKM-positive and also anti-liver cytosol type 1 (LC1)-positive (9) AIH is more prevalent among younger patients and is characterized by higher inflammatory activity, higher bilirubin and aminotransferase levels, a higher rate of acute presentation and an increased prevalence of cirrhosis at diagnosis (10, 11). In contrast, ANA-positive AIH has been designated a disease of elderly patients by some authors (12) although it is undoubtedly also prevalent in children (10, 11). Viewed from a historical perspective AIH was first described in young jaundiced women by Waldenström (13) and Kunkel (14), and subsequently defined as lupoid hepatitis and later autoimmune hepatitis, and thereby coined as a separate disease entity by Mackay (15). This clinical description of a disease of young women was later expanded to older (male and female) patients and described with a bimodal occurrence (16), which acknowledges patients presenting with AIH not only between 10 and 30 years of age but also later than 40–50 years of age.

The presentation of acute hepatitis, clinical symptoms of jaundice, abdominal pain and malaise have a high likelihood of attracting medical attention and subsequently leading to the diagnosis of AIH (17). More subtle courses of AIH may not lead to clinically relevant signs and may develop unnoticed other than by routine work up for other problems or by screening programs. The question of disease onset in terms of
an initiation of the immune-mediated liver disease versus the clinical consequences that become noticeable after an unknown period of disease progression cannot be easily resolved. Thus, “late onset” AIH may just simply reflect a less severe course of the disease with a slower progression to cirrhosis. While LKM-positive patients display a tendency towards an earlier presentation, both acute and subtle (earlier and late presentation) variants appear to exist in ANA-positive AIH. In practice, the diagnostic dilemma is that AIH is still perceived by many as a disease of younger individuals and that therefore this differential diagnosis is less frequently considered in elderly patients with “cryptogenic” hepatitis or cirrhosis.

Another relevant question resulting from these considerations is the issue of treatment. Standard therapy in AIH consists of steroids alone or in combination with azathioprine. In maintenance therapy azathioprine monotherapy can also be implemented but induction with azathioprine alone is not effective. From a general standpoint most internists will use caution when administering steroids to elderly patients, especially in women in whom osteopenia or diabetes may be present. Recommendations for the treatment of AIH suggest that the steroid side effects be weighted against the potential benefit of therapy, and that consequently not all patients with AIH are good candidates for steroid treatment (1). In the group of older AIH patients budesonide may be an alternative (18, 19) to prednisone medication which awaits study confirmation. Recent pilot studies suggest that budesonide may play a possible role as alternative to steroid medication as first and second line therapy (20, 21) and possibly in combination with azathioprine.

Controversy exists surrounding the benefit of therapy in this group of elderly patients. One series reported 12 patients aged over 65 years out of 54 AIH patients. Cirrhosis had developed after follow up in 26% irrespective of age although the histological grade of AIH activity was more severe in the elderly group. 42% of the patients over 65 did not receive therapy and yet deaths were reported only in the younger group (22). In a different series of 20 patients aged > 65 years, a longer time to the establishment of the diagnosis (8.5 vs. 3.5 months) was reported, patients presented mainly with jaundice and acute onset AIH and showed a response rate to immunosuppression comparable to that of younger patients (23). The authors also noted that the prevalence of the HLA-A1-B8 allotype was less frequent in older patients suggesting a role for immunogenetics. This point was further elaborated by a recent report analyzing 47 patients with ANA-positive AIH 60 years and older, as well as 31 patients 30 years and younger in whom DR4+/DR3- prevalence was 47% (older) versus 13% (younger) patients (24). In older patients steroid responsiveness was better, which is in line with previous findings in the same collective (25). Cirrhosis and extrahepatic immune-mediated syndromes including thyroid and rheumatological disease (47% vs. 26%) were more prevalent in older AIH patients. However, although more treatment failures were observed in younger patients (24% vs. 5%) the rate of remission, sustained remission and relapse was similar. Interestingly, an assessment of age stratified prevalence showed an increase after the age of 40 from 15% to over 20%. From all of these data AIH in elderly patients appears to be characterized by a distinct clinical feature, a distinct immunogenetic profile, favorable response rates and higher rates of cirrhosis present at diagnosis, which contributes to the heterogeneity of AIH. This view is again modified by recent data from the UK. In a cohort of 164 patients including 43 individuals 60 years and older AIH characterized by ANA, SMA and LKM were analyzed. Not surprisingly,
LKM-positive AIH was only found in the younger patients (7%). At first glance no significant differences regarding serum biochemistry, autoantibody titers, time to the establishment of the diagnosis, and mode of presentation were observed. The substratification of patients below and above 40 years of age found that older patients had a higher median histological stage, a comparable median grade but younger patients had more median relapse episodes and a higher median stage at follow-up biopsy. The most distinguishing clinical sign was a higher prevalence of ascites in the older group. However, rates of complete, partial and failed response were similar, and the median number of relapses higher in younger patients, who nevertheless did not lead to differences in liver related deaths in both groups (12% vs. 15%). In comparison to the study of ANA-positive AIH patients from the U.S. (24) the differing findings regarding HLA association are noteworthy. In the U.K. study there was no differential distribution of HLA DR3 and DR4 and this questions the suggested hypothesis of a primary influence of immunogenetics on the observed clinical distinctions. However, both studies agree with respect to most of the clinical findings including the differential prevalence of extrahepatic immune-mediated disease. One can speculate about the reasons for the clinical differences of AIH in older and younger patients. The authors discuss differences in hepatic blood flow but alterations involving the regulation of cellular immunity during aging are also a likely pathophysiological reason (26, 27).

Two additional points require consideration. One is that of hepatocellular carcinoma, which was diagnosed in 10.4% of cirrhotic patients and in 5% of the overall cohort contrasting previous reports. Since viral disease was excluded from the analysis this leaves room for speculation regarding the effects of azathioprine or other external factors that may have escaped the medical work up. The other point is that of fibrosis progression. Despite a rate of complete response of over 90% in both groups there was a clear increase of histological grade in both groups under treatment. Although sampling error may play a role this does suggest that therapeutic monitoring in AIH irrespective of age is a critical issue, which appears to be only incompletely represented by biochemistry and clinical presentation.

What are the practical clinical lessons from these considerations? Older patients present with AIH with an increased prevalence after 40 years of age and require treatment which has a favorable outcome. Whether this represents de novo initiation of AIH or clinical presentation of previously ongoing asymptomatic AIH is scientifically interesting and remains unresolved but should not influence decision making to initiate therapy. Clinical assessment should consider that older patients present with more extrahepatic immune-mediated disease components, which may be related to altered regulation of cellular immunity during ageing and possibly to immunogenetics. However, the main point is a wary clinical work up with an early diagnosis and the prospect of an effective therapeutic intervention even in older AIH patients. In view of the aforementioned considerations for elderly patients but also for younger AIH patients the development of new effective therapeutic strategies is required.
References:


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Overlap syndromes

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At present there is no universally accepted definition of so called overlap syndromes or variants of autoimmune hepatitis (AIH). It is well known that a patient with any autoimmune disease is at high risk to develop a second or even a third one, and overlapping is a frequently observed phenomenon, e.g. glomerulonephritis plus rheumatoid arthritis.

This means, that the overlap syndrome is not a new invention, it is just a new name for an old observation. Although treatment of liver diseases has markedly improved during the last few decades we learned that overlapping of different diseases may present great problems in clinical management. Therefore we have to investigate what overlap syndromes in hepatology are.

AIH frequently overlaps with primary biliary cirrhosis (PBC). Serologically we find ANA, SMA, and histologically interface hepatitis besides typical findings of PBC. Immunogenetically this variant is associated with HLA DR3 of DR4 as seen in AIH. In a second variant, which is called autoimmune cholangitis or AMA-negative PBC histology is that of PBC. In some of these patients HLA risk factors are DR3 or DR4 as in AIH and simultaneously DR8 as in some patients with PBC. Both variants present with increased IgG concentrations. Basic therapy is performed with ursodeoxycholic acid (UDCA), in some studies the combination with glucocorticoids was superior, whereas in others not.

The overlap syndrome AIH-primary sclerosing cholangitis (PSC) is predominantly seen in children. Histology is that of PSC, serologically ANA, SMA, AMA and an increase of IgG have been observed. Optimal clinical management is not known. UDCA constitutes basic therapy, whether one should combine UDCA with glucocorticoids or azathioprine is still under investigation. Treatment with glucocorticoids alone improves laboratory data but histologically the disease progresses.

As long as there is no universally accepted definition of what an overlap syndrome is, AIH may also overlap with chronic viral hepatitis B or C. In Hep C we frequently find interface hepatitis, autoantibodies and increased IgG concentrations. Antiviral treatment in these patients is safe. Three own cases of the AIH/Hep C-overlap syndrome did not respond to interferon and ribavirin treatment, one of them developed severe side effects. This rises the question whether the overlap of Hep C and AIH should not be better treated with a combination of antiviral therapy and a low dose of an immunosuppressant.
Hepatic encephalopathy (HE) is a neuropsychiatric syndrome, which frequently develops in the course of chronic liver disease and which is precipitated by a variety of factors such as bleeding, infections, sedatives and electrolyte disturbances. Current evidence suggests that HE in cirrhotics is the clinical manifestation of a low-grade cerebral edema, which aggravates under the influence of precipitating factors and which can be demonstrated by means of modern magnetic resonance techniques in vivo, such as 1H-MR-spectroscopy and magnetization transfer ratio determinations. More recently, quantitative brain water mapping using the TAPIR (T1 mapping with partial inversion recovery) technique was developed, which showed a correlation between white matter water content and HE severity. This brain edema primarily involves the astrocyte with consequences for glia-neuronal communication. Astrocyte swelling is largely due to astrocytic glutamine accumulation and the generation of oxidative/nitrosative oxidative stress. Oxidative stress and astrocyte swelling are closely interlinked by a positive feed-forward regulation: on the one hand astrocyte swelling induces oxidative stress and on the other oxidative stress triggers astrocyte swelling. This self-amplifying cycle involves the action of NDMA-receptors and Ca\textsuperscript{2+} transients, as well as oxidative stress generation by mitochondria and NADPH oxidase isoenzymes. The cell-physiological consequences of astrocyte swelling/oxidative stress are multiple and affect gene expression, metabolism, transport, covalent protein modifications, taurine loss, signalling and neurotransmitter/receptor processing as well as disturbances of corticostriatal synaptic plasticity in portocaval-shunted rats, which correlate with alterations of animal behaviour in the open field. Oxidative/nitrosative stress under the influence of ammonia and other HE-relevant toxins such as inflammatory cytokines or benzodiazepines triggers protein tyrosine nitration with consequences for protein function. To what extent these modifications translate to HE symptoms, however, remains to be established. Interestingly, in brain slices and astrocytes, ammonia not only induces protein tyrosine nitration involving autocrine vesicular glutamate release and NMDA receptor activation, but also mRNA modification through formation of 8-hydroxy-guanosin in astrocytes and neurons. Such modified mRNA is also transported to the astrocyte periphery and may affect local protein synthesis in areas of astrocytic-neuronal communication. All these sequelae of astrocyte swelling and oxidative/nitrosative stress may explain why multiple neurotransmitter systems are altered in HE. It is not yet clear how these alterations translate to the neurophysiological level. However, studies on magnet-encephalography in cirrhotics provided evidence that HE is characterized by a shift of cerebral oscillatory networks to lower frequencies with more rigid electrical coupling between the motor-cortex and the periphery, explaining the tremor in HE.
Interestingly, cortico-muscular coupling matched the frequencies of thalamocortical electrical coupling, pointing to the possibility that swelling and neurotoxin-sensitive thalamic structures may be involved in triggering the motoric abnormalities in HE.
Session VI

Sequela of chronic hepatitis (Part 1)
Progression of chronic hepatitis: From inflammation to fibrosis

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Progressive liver fibrosis is the main cause leading to organ failure in chronic liver diseases of any etiology. Fibrosis develops with different spatial patterns and as a consequence of different prevalent mechanisms according to the diverse causes of parenchymal damage. Indeed, fibrosis observed as a consequence of chronic viral infection is initially concentrated within and around portal tract, while fibrosis secondary to toxic/metabolic damage is located mainly in the centrolobular areas. In addition, it is increasingly evident that different cell types are involved in the deposition of fibrillar extracellular matrix (ECM) during active hepatic fibrogenesis: hepatic stellate cells are mainly involved when hepatocellular damage is limited or concentrated within the liver lobule, whereas portal myofibroblasts and fibroblasts provide a predominant contribution when the damage is located in the proximity of portal tracts. In further stages of evolution (septal fibrosis) is likely that all ECM-producing cells contributes to fibrogenesis. Recruitment and activation of ECM-producing cells to the site of tissue damage can be due to different major mechanisms: 1. chronic activation of the tissue repair process. In this case, as a consequence of the reiterated damage, accumulation of fibrillar ECM reflects the impossibility of an effective remodeling and regeneration; 2. effect of oxidative stress products, including reactive oxygen intermediates and reactive aldehydes. These products, whose concentration become critical in toxic/metabolic liver injury, are able to induce the synthesis of fibrillar ECM even in the absence of significant hepatocyte damage and inflammation; 3. derangement of normal the epithelial/mesenchymal interaction. This typically occurs in all conditions characterized by cholangiocyte damage/proliferation, where a consensual proliferation of ECM-producing cells and progressive fibrogenesis is commonly observed. A major advancement towards the understanding of the molecular mechanisms of fibrogenesis derives from a consistent number of in vitro studies investigating the biological role of growth factors/cytokines and other soluble factors and their intracellular signaling pathways. The relevance of these factors has been confirmed by studies in animal models and in studies performed in pathological human liver. Along these lines, the elucidation of a consistent number of cellular and molecular mechanisms responsible for the progression of liver fibrosis has provided sound basis for the development of pharmacological strategies able to modulate this important pathophysiological process.
Treatment of portal hypertension and gastrointestinal bleeding

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There are several goals in the treatment of portal hypertension: prevention of portal hypertension, primary prophylaxis of variceal bleeding, treatment of acute bleeding and prevention of rebleeding. While prevention of portal hypertension may be achieved through a variety of measures (interruption of alcohol intake or viral replication) long before the patient exhibits any signs of portal hypertension, i. e. via prevention of ongoing fibrosis, there is no clear evidence that further development of collaterals can be prevented to a larger extent once portal hypertension has occurred.

One the other hand, there are two options to prevent first bleeding in patients who have developed esophageal varices: long-term treatment with a non-selective β-blockers or ligation of varices. Both approaches are equally effective. Most centres still opt for β-blockers due to their lower potential for acute life threatening complications such as heavy bleeding from ligation ulcers.

Acute bleeding is treated by application of vasoactive drugs, endoscopy as early as possible together with ligation or injection sclerotherapy if ligation proves technically difficult. Antibiotic prophylaxis is an integral part of therapy. Failure of initial endoscopic therapy and of a second attempt is best managed by TIPS.

Prevention of rebleeding should be started as soon as possible. Combination of β-blockers and ligation is probably the best treatment but more trials are needed. To what extent assessment of the hemodynamic response to β-blockers (WHVP) can tailor the mode of treatment has not yet been sufficiently examined. Patients in whom endoscopic and pharmacological treatment fails should be treated by TIPS or surgical shunts.

Areas for further research include: pharmacological modulation of intrahepatic resistance or peripheral and splanchnic vasodilation, assessment of the role of WHVP determination for therapeutic strategies, influence of the different therapeutic approaches on kidney and pulmonary function.

Further reading: Portal hypertension IV, proceedings of the fourth Baveno International Consensus workshop (Ed Roberto de Franchis) Blackwell Publishing 2006
Treatment of ascites and spontaneous bacterial peritonitis

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Nearly 50% of patients with compensated cirrhosis develop ascites within 10 years. The development of ascites in cirrhosis is a poor prognostic feature and it has been estimated that half of these patients will die within 3 years without liver transplantation. Thus, evaluation for possible liver transplant should be done when ascites develops. The mainstays of therapy include sodium restriction and diuretic therapy including spironolactone with or without a loop diuretic. For more difficult to manage cases such as those who develop recurrent side effects from, or are refractory to, diuretic therapy, either repeated large volume paracenteses or, in selected cases, a transjugular intrahepatic portosystemic shunt (TIPS) is an option. Spontaneous bacterial peritonitis is a common and severe complication in cirrhotic patients with ascites. Recognition and rapid treatment is imperative. The diagnosis is made by documenting a polymorphonuclear count in ascitic fluid greater than 250/mm³. Antibiotic therapy should consist of an intravenous third-generation cephalosporin initiated empirically after the diagnostic paracentesis and before culture results are available. Albumin should also be given as this reduces the risk of hepatorenal syndrome and improves survival. The prophylactic administration of a quinolone, generally norfloxacin or ciprofloxacin, should be started after resolution of spontaneous bacterial peritonitis to reduce the risk of recurrence.
Session VII

Sequelae of chronic hepatitis (Part 2)
The hepatorenal syndrome: From pathophysiology to treatment

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HRS is a common complication of advanced cirrhosis, characterized by renal failure and major abnormalities in the systemic circulatory function. Renal failure is caused by intense vasoconstriction of the renal circulation. The syndrome is probably the final consequence of an extreme underfilling of the arterial circulation secondary to vasodilatation in the splanchnic vascular bed and a decrease in cardiac output due to central hypovolemia. The diagnosis of HRS is based on the exclusion of other causes of renal failure. The survival of patients with HRS is very short, particularly when there is rapidly progressive renal failure (type 1-HRS). Liver transplantation is the best therapeutic option but its applicability is low. During the past few years effective treatment for HRS, such as vasoconstrictor drugs (vasopressin analogues, µ-adrenergic agonists) associated with i.v. albumin infusion and TIPS, have been introduced. They improve circulatory function, normalize serum creatinine and may improve survival. Sequential treatment with vasoconstrictors plus albumin and TIPS is an attractive therapeutic possibility. Plasma volume expansion with albumin at infection diagnosis in patients with spontaneous bacterial peritonitis and the administration of pentoxiphilline in patients with severe alcoholic hepatitis significantly reduce the development of type-1 HRS.
Hepato-pulmonary syndrome

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The classic triad of chronic liver disease, arterial hypoxemia on room air, and intrapulmonary vascular dilatation characterizes HPS (1, 2). The clinical presentation of HPS includes dyspnea and fatigue (manifestations of hypoxemia at rest, with exertion and during sleep). Physical examination findings associated with HPS are nail bed and lip cyanosis, as well as skin spider angiomas. Screening for hypoxemia can be accomplished by finger pulse oximetry or arterial blood gas analysis. Oximetry saturations < 90% or PaO₂ < 80 mm Hg merit testing for pulmonary vascular dilatations.

Profound hypoxemia (PaO₂ < 50 mm Hg) is not uncommon. Spontaneous resolution of the syndrome with normalization of arterial oxygenation can occur, but is rare. Although hypoxemia is common (40–50%) in the setting of chronic liver disease, the prevalence of HPS is uncommon. Approximately 20–30% of HPS patients may have other comorbidities that cause significant hypoxemia (i.e. ascites, hydrothorax, pneumonia, pulmonary fibrosis and COPD). Approximately 5% of patients referred to the Mayo Clinic liver transplant program have HPS.

Non-invasive methods used to determine the existence of pulmonary vascular dilatations are based upon the concept of either microbubbles or technetium labeled macroaggregated albumen (⁹⁹ᵐTc MAA) traversing dilated pulmonary vessels. The echocardiography test is qualitative and more sensitive in detecting pulmonary vascular dilatation the lung perfusion test. The lung perfusion-brain uptake study gives a quantitative measure of the degree of pulmonary vascular dilatation. Pulmonary angiography as a mean to document (or further characterize) pulmonary vascular dilatation is not necessary in most cases.

The pathophysiology of HPS may be related to circulating mediators that result in excess local effects of nitric oxide and bacterial translocation, upregulated pulmonary endothelium endothelin B receptors causing vasodilatation, and pulmonary capillary proliferation via angiogenesis. Yet no proven medical treatments exist for HPS, other than nasal cannula O₂ supplementation. There is no proven role for TIPS. As the definitive treatment for HPS, orthotopic liver transplantation (cadaveric or living donor) is strongly advised assuming no other major co-morbidities complicate advanced liver disease.

HPS alone has prognostic significance in patients with cirrhosis (3). In addition, a 30–38% mortality within 12 months of OLT and 16% transplant hospitalization mortality have been reported. PaO₂ < 50 mm Hg and ⁹⁹ᵐTc MAA lung perfusion-brain uptake > 20% is associated with higher post-OLT mortality (2). However, complete resolution of HPS can occur even in the setting of severe hypoxemia prior to OLT and the time to resolution (usually many months) is related to the severity of hypoxemia. The 5-year survivals with and without liver transplantation are 76% versus 23%, respectively. PaO₂ < 50 mm Hg with or without OLT is associated with worse survival compared to those with PaO₂ > 50 mm Hg (4).
Current questions of significance:

1) What is the most recent thought about pathophysiology?
2) Should endothelin antagonists be considered as experimental treatment in HPS?
3) Should HPS patients receive increased priority for liver transplantation?
4) Do the lungs of patients with HPS really normalize after liver transplantation?

References:


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Hepatocellular carcinoma

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Liver cancer is a neoplasm with well defined risk factors. Most of the patients diagnosed with hepatocellular carcinoma present an underlying liver disease related to chronic viral infection (due to HCV or HBV infection) that in some areas is associated to aflatoxin intake, excessive alcohol consumption or to metabolic conditions such as hemochromatosis. This offers the opportunity to be successful in primary prevention by avoiding the acquisition of the risk factors that will induce chronic liver damage and ultimately, liver cancer. Vaccination against HBV has shown its efficacy in preventing the dissemination of this agent and also in reducing the incidence of HCC, and hence HCC is the first neoplasm that has been successfully prevented by vaccination. There is no vaccination for HCV and thus, dissemination of HCV infection is solely prevented by avoiding procedures involving potentially contaminated blood (uncontrolled transfusion, needle sharing, risky sex practices). Aflatoxin intake can be diminished by storing food in proper conditions and there are attempts to increase its body clearance through pharmacologic intervention, but there is no proof of efficacy. Screening for hemochromatosis should detect disease at a precirrhotic stage and thus, avoid progression to cirrhosis and HCC. When viral infection is acquired and chronified, it is evident that successful antiviral therapy will prevent chronic inflammation with fibrosis deposition and thus, progression to cirrhosis. In this setting, antiviral therapy should be considered effective in preventing HCC or at least diminish the risk. However, if cirrhosis is already present, the impact of antiviral therapy or just chronic interferon administration is not well established despite several promising data. Ongoing investigations (EPIC and HALT trials) should unequivocally establish if such intervention is effective. However, in some patients antiviral treatment may not be feasible or effective and in them, the sole option to diminish the deaths related to cancer is to achieve early detection and apply radical therapy.

Several studies have shown that the development of hepatocellular carcinoma (HCC) is the main cause of death in patients with cirrhosis. Since the sole option to apply effective therapies such as resection, liver transplantation and percutaneous ablation is to achieve diagnosis at an early stage, it is recommended to incorporate cirrhotic patients who would be treated if diagnosed with HCC into screening plans. These should be based on Ultrasound (US) examination every 6 months. US has adequate sensitivity to detect focal lesions within a cirrhotic liver and the challenge upon detection of a suspicious mass is to establish its benign or malignant nature. Biopsy using fine needles is a useful tool, but several factors reduce its sensitivity. Large tumors may have areas of necrosis and no diagnosis can be established if such an area is punctured. More clinically important however, is the reduced sensitivity in small HCC. These are usually well differentiated and pathology is frequently unable to distinguish between high or low grade dysplasia and HCC. As a whole, a negative biopsy does not rule out HCC diagnosis and this has triggered the development of non-invasive diagnostic criteria. In that sense, it is well known that HCC develops an extensive net of vessels fed by the hepatic artery and this result in a characteristic
appearance on dynamic imaging using either contrast-US, CT or MRI. HCC is recognized as a mass with intense contrast uptake in the arterial phase followed by contrast washout in the delayed venous phase. If this pattern is observed in a mass within a cirrhotic liver the diagnosis of HCC is certain if the tumor exceeds 1 cm in size. The recent AASLD and EASL recommendations suggest that a single positive technique is enough in tumors larger than 2 cm, while coincidental findings with two techniques are required in lesions between 1 and 2 cm. Lesions < 1 cm are almost impossible to be diagnosed by biopsy or imaging techniques and hence, the recommendation is to follow them up until progression or disappearance. Important to stress is that the non-invasive criteria should be restricted to patients with cirrhosis.

The staging system and treatment strategy applied in our group is depicted in Figure 1. Surgical resection should be limited to single asymptomatic HCC in patients with preserved liver function. Selection of Child-Pugh A patients is not efficient enough, as the optimal results are obtained in subjects with normal bilirubin and absence of portal hypertension. Resection is associated with a high recurrence rate (> 70% at 5 years) that can be predicted by pathological characteristics, such as vascular invasion, satellites and poor differentiation degree. Several agents (retinoids, interferon, lipiodol coupled to 131-I, adoptive immunotherapy) have been evaluated, but their preventive value and survival impact need further investigation. The optimal candidates for liver transplantation are patients with early HCC (single < 5 cm or up to 3 nodules < 3 cm). They achieve 70% 5-year survival with a recurrence rate below 15%. Transplantation is not available worldwide and there is a huge shortage of donors that deteriorates the results when analyzed according to intention to treat. Living donor liver transplantation (LDLT) may partially solve this limitation but medium and long-term results are still not available.

Percutaneous ablation (ethanol injection or radiofrequency) under US guidance achieves complete necrosis in 70–80% of solitary tumors ≤ 3 cm. Child-Pugh A patients achieve a 50% 5-year survival rate, which compares with the outcome of surgical patients that do not qualify for an optimal long-term outcome. By contrast, survival benefits of PEI in Child-Pugh B class patients are controversial. More than 50% of the patients are diagnosed at an advanced stage when only palliative options can be applied. Amongst these, only chemoembolization has been shown to provide survival benefits within randomized trials and meta-analytical assessment. Tamoxifen has no positive impact on survival and there are no robust data to support a benefit from options such as systemic chemotherapy, octreotide, internal radiation with I-131, proton beam radiation, antiandrogens, Interferon and immunotherapy.

References:

Table. Diagnostic criteria for HCC

- Cyto-histological criteria.
- Non-invasive criteria (cirrhotic patients)
  Focal lesion > 1– ≤ 2 cm / Two imaging techniques with arterial hypervascularization and venous washout
  Focal lesion > 2 cm / One imaging technique with arterial hypervascularization and venous washout

Figure 1. The BCLC staging and treatment strategy

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Liver transplantation

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Liver transplantation is challenged by a shortage of organs and a prolonged waiting-list time. The large disparity between the number of available cadaver donor organs and recipients awaiting OLT has created an ongoing debate regarding the appropriate selection criteria. Novel surgical techniques, including split cadaveric livers, LDLT, and broadening the donor criteria towards acceptance of marginal donors have been used as strategies in order to expand the donor pool.

HCV has become the leading indication for cadaveric transplantation and LDLT in the United States, accounting for approximately 50% of all cases. Moreover, the number of patients with HCV cirrhosis continues to increase. There is ongoing research aiming to define host or viral factors that predict recurrence, the impact of immunosuppressive regimens, and the appropriate timepoint and dose for antiviral treatment.

Due to the availability of antiviral drugs, the survival of patients undergoing OLT for HBV infection has dramatically improved and has become comparable to or even better than the survival of patients with non-virus-related liver diseases.

Data about the frequency of disease recurrence in cholestatic and autoimmune liver diseases vary widely in the literature, but excellent medium-term and long-term results have been reported.

Recent alcohol abuse is a contraindication to liver transplantation and most centers require 6 months of documented abstinence prior to OLT. Patients transplanted for alcohol-related liver damage experience fewer episodes of acute cellular rejection and chronic ductopenic rejection after liver transplantation than patients transplanted for non-alcoholic liver disease.

Liver transplantation in HCC patients provides excellent outcomes and low recurrence rates applying the Milan criteria. Living donation to transplant recipients with HCC who exceed the Milan criteria has been discussed controversially. CCC represents a contraindication for transplantation, except for highly selected cases with very early stage of disease. Combination with neoadjuvant chemoirradiation may further improve results after OLT.

Due to excellent short-term outcomes after liver transplantation, attention has shifted to reducing long-term complications. Cardiovascular comorbidities due to metabolic complications, such as diabetes mellitus, dyslipidemia, obesity, and arterial hypertension, account for 30–70% of long-term morbidity. Chronic renal insufficiency appears in 50–80% of patients during long-term CNI therapy. Immunosuppressive drugs without renal side-effects, such as MMF and SRL, have been used increasingly as renal-sparing agents. The need for immunosuppression has to be balanced against drug-related side-effects. Possible routes to clinically relevant immune tolerance may include the application of tolerance-inducing immunotherapy, with or without low dose conventional immunosuppressants.
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POSTER ABSTRACTS

Poster Numbers 1 - 225
Transfer factor activation of the natural killer cell in patients with chronic hepatitis C infection

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Infection with hepatitis C virus (HCV) is a leading cause of chronic liver disease worldwide, little is known about how the virus is able to persist or whether this persistence might be because of its ability to alter the early innate response. The major HCV envelope protein E2 has been shown to bind to CD 81 and this leads to restricted cytotoxicity mediated by NK cells. Transfer factor advanced formula is formed of bovine and egg colostrum and has been found to increase NK cell activity by 473% above baseline. Also it contains several growth factors as insulin growth factor, transforming growth factor and epidermal growth factor. It is produced by 4life research company USA.

**Aim:** Assessment of natural killer cell (NK) cell activation by transfer factor in patients with chronic HCV infection whom are not candidate for standard therapy.

**Material:** 30 patients with chronic HCV infection.

**Methods:** (1) History taking and clinical examination. (2) Liver function tests. (3) Viral markers (HBsAg, HCV Ab). HCV RNA by PCR in the serum. (5) Flow cytometric analysis of NK cells in blood sample. (6) Patients were given transfer factor plus capsules 1 x 2/daily before meals for 3 months then reevaluation was done.

**Results:** Significant reduction of ALT from (mean ± sd 79.27 ± 71.47) to (36.4 ± 3.23), AST from (80.73 ± 46.88) to (36.4 ± 3.23). Significant elevation of serum albumin from (3.19 ± 0.7) to (3.59 ± 0.54) and prothrombin activity from (0.63 ± 0.14) to (0.82 ± 0.12). No significant change in serum HCV RNA by PCR nor natural killer cell count by flow cytometry.

**Conclusion:** Transfer factor advanced formula plus is an effective new therapeutic option for patients with chronic HCV infection whom are not candidate for standard therapy.
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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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).

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.

The present work conducted on 45 subject:
The control group 15 subjects free from any liver disease. The patient group 30 patients with chronic liver disease and schistosomal portal hypertension.

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).

- 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.
The influence of HAI and fibrosis grade on the expression of A-smooth muscle actin in liver

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Introduction: Activation of hepatic stellate cells and their transdifferentiation to myofibroblasts is considered to be the key event in liver fibrogenesis. The aim of our work was to evaluate the influence of histology activity index (HAI) and fibrosis grade on the level of A-smooth muscle actin (A-SMA) expression.

Methods: 53 liver biopsies obtained from the patients with chronic viral hepatitis C were examined. 3.3% of patients had mild hepatitis (HAI 1-3), 38.3% - HAI 4-8, 51.7% - moderate activity hepatitis (HAI 9-12), and 6.7% - severe hepatitis (HAI 13-18). All biopsies were stained immunohistochemically with antibodies to A-SMA which is considered to be the marker of activated hepatic stellate cells in human liver.

Results: The severity of fibrosis correlates with HAI: in case of HAI 4-8 most of patients (61.9%) had no signs of fibrosis and only 38.1% of patients revealed slight fibrosis (grade 1) while in the group with HAI 9-12 55.2% of patients had periportal (grade 1) and 27.6% of patients develop moderate fibrosis (grade 2). The level of fibrosis did not correlate with the expression of A-SMA in liver sinusoids. The number of A-SMA-positive cells correlate with HAI: in case of HAI 4-8 in most of biopsies A-SMA-positive cells were found in areas of inflammation (67%) and in sinusoids (67%); in HAI 9-12 myofibroblasts were observed in areas of inflammation almost in 100% of biopsies though in sinusoids they were present only in 47% of samples.

Discussion/Conclusion: We suppose that in severe hepatitis with expressed fibrosis myofibroblasts loose their A-SMA-positive pattern as the cells which are not able to take part in restoring of liver tissue any more.
Investigation of the molecular basis of patient-derived monoclonal antibody interactions with E2 & E3BP lipoyl domains of the human pyruvate dehydrogenase complex (hPDC)

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Primary biliary cirrhosis is an autoimmune liver disease characterised by the presence of autoantibodies mainly against the E2 and EBP subunits of hPDC. PD1 and PD2 are patient derived hybridomas (IgG/λ) secreting hypermutated monoclonal antibodies (mAbs), which interact with a common lipoylation-dependent epitope that is present on both E2 and E3BP lipoyl domains. These mAbs appear to be representative of the PBC-specific polyclonal antibody population as a whole; therefore, the aim of this study was to determine the epitope recognised by these mAbs, which is of interest in terms of the aetiology of the disease. To address this issue three amino acids around the lipoylated lysine residue of the inner lipoyl domain of the human PDC-E2 (ILD-PDC) were mutated to the equivalent residues found in a non-reactive lipoyl domain of Arabidopsis thaliana plastid E2-PDC using site-directed mutagenesis as single, double, and triple mutants. In parallel, the non-reactive lipoyl domain of human 2-oxoglutarate dehydrogenase complex (OGDC-LD) was mutated systematically at several amino acids around lipoylated lysine residue to the equivalent residues found in the reactive lipoyl domains of E2 & E3BP-PDC. These studies have permitted us to determine the key residues involved in PD1 and PD2 recognition and also their influence on the extent of lipoylation. The predominant epitope is located on the C-terminal side of the key lipoyl lysine residue of the lipoyl domain and includes the octanoyl chain of the lipoamide cofactors but not the diothiolane ring. However, modification of lipoamide thiols with bulky substituents causes loss of antibody reactivity, probably as a result of steric hindrance. Current studies are directed towards understanding if there are other types of lipid modification that can induce the autoimmune response.

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Autoimmune hepatitis developed as an outcome of acute viral hepatitis

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Difficulties in evaluation of hepatitis viruses role in development of autoimmune hepatitis (AIH) are partially caused by a small number of documented clinical cases with transformation of viral liver disease to autoimmune.

We observed, for 13 years, young male patient who showed rapid chronization of acute viral hepatitis developed in 5 months after invasive procedure.

Chronic hepatitis in this patient had typical signs of AIH: 8-fold elevation of serum ALT, gamma-globulins - 7.3 g/L, IgG – 6000 mg/dL, SMA – 1:1000, absence of serum HBsAg. Extrahepatic manifestations: generalized involvement of exocrine glands, fibrosing alveolitis, interstitial nephritis with tubular acidosis and renal diabetes insipidus. High doses of prednisolone and azathioprine were effective, with relapses at low doses. Severe adverse events were limiting and making impossible the treatment by adequate doses. The patient had dead.

In liver tissue autopsy showed the massive lymphocyte infiltrates with large quantity of plasmatic cells, lymphoid follicles, piecemeal necrosis, some lobules surrounded by connective tissue, containing cellular infiltrates. In endocrine glands (submandibular and parotic, gastric and duodenal): lymphatic and plasmatic cell infiltration with obstruction of glandular parenchyma. Lymphocyte, macrophage and plasmatic cell infiltration of pulmonary tissue (alveolar septs, sclerosis of some alveoli). Lymphocyte, macrophage and plasmatic cell infiltration of proximal and distal renal tubular stroma. Lymphatic nodes, spleen and bone marrow also showed severe plasmatic and macrophage reactivity. Orsein staining did not show presence of HBsAg in hepatic tissue and in other organs.

**Conclusions:** Role of hepatitis viruses in development of AIH in case of this patient may be confirmed by epidemiological anamnesis and observed chronization of acute viral hepatitis. Identification of viral agent was impossible at that period, but we suppose role of HCV in progression of patient’s disease that could be explained by systemic involvement and presence of lymphoid follicles in hepatic tissue.
Pathomorphogenesis of chronic hepatitis C

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**Introduction**: The histological findings help to estimate the degree of inflammatory reaction in a liver at chronic hepatitis C (CHC). Morphology is not specific, but it has features – triad: lymphoid follicles, periductal sclerosis, a fatty hepatocyte degeneration, prevalence low-activity forms.

**Methods**: Liver biopsies were studied from 71 patients. The histological evaluation was based upon Knodell’s index and the METAVIR fibrosis severity score. HCV antigen (protein NS3) in a liver and kidneys at 5 patients with HCV and HCV+HBV was defined by immunohistochemistry method (Novocastra, Great Britain).

**Results**: At CHC macrophagal or mixed infiltration, swollen liver cells, lymphoid follicles and periductal sclerosis were registered. Moderate piecemeal, bridging, focal necrosis, occupying over 50% of portal tracts were registered. Activity according Knodell’s index at CHC and HCV-cirrhosis was identical, accordingly 8.93 ± 0.34 and 8.23 ± 0.62 points. Morphological activity at HCV correlated with parameters of inflammation, lymphadenitis, ↑ AST, ↓ cholinesterase, and piecemeal and bridging necrosis - ↑ ALT, factor AST/ALT; hepatocyte degeneration and focal necrosis - with hepatomegaly. Liver fibrosis and its degree correlated with duration of disease, complications, alkaline phosphatase, bilirubin, glucose, T-lymphocytes level in blood. Fibrosis was the more, than less the parameters of inflammatory reactions were.

**Conclusion**: The expression of HCV NS3 antigen was revealed as hazel and deep-brown granules in structures of kidneys and liver by immunohistochemistry method.
Clinical course, complications and outcomes of chronic mono/mixed hepatitis B and C at various stages of infectious process

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We study clinical course and outcomes of a chronic viral hepatitis for estimation prognostic criteria.

Methods: For 1994-2005 years 485 patients with chronic hepatitis (CH), cirrhosis of liver (LC) and primary hepatocellular carcinoma (HCC) were observed.

Results: For the 8-years period of monitoring HCV-cirrhosis had formed at 13.04% of patients HCV-hepatitis; HBV-cirrhosis - at 5.7% of patients HBV-hepatitis, HBV+HCV-cirrhosis - at 4.1% of patients with mixed hepatitis. In groups with LC the stage of decompensation developed in 27.1%, more often at HCV-cirrhosis (42.1%) and HBV+HCV–cirrhosis (44.4%).

Complications were registered at all patients with LC. CH was complicated with encephalopathy (45.7%), acute hepatic failure and hepatorenal insufficiency (8.6%), the disseminated intravascular clotting (DIC) (17.1%), immune cytopenia (28.6%). LC was complicated with encephalopathy (45.7%), acute hepatic failure (9.35%), hepatorenal syndrome (4.67%), DIC (7.48%), bleedings from veins of a gullet (12.1%), formation of erosion and ulcers of stomach and duodenum, complicated bleedings (24.8%), an anemia (14%), a thrombosis of system V. portae with cavernous transformation (3.74%), an infectious syndrome (1.87%). Hypersplenism was revealed at 8.4% of patients.

HCC was complicated in 7 of 10 cases with mechanical jaundice at each third patient, acute hepatic failure, bleedings from veins of a gullet, DIC and cachexy at 6 of 10 patients. At 8 from 10 patients with HCC massive ascitic syndrome was diagnosed.

Conclusion: The 8-years survival rate make up: at CH 99.2%, at CHB 99.2% and at CHC 98.3%; at LC of 88.8%, from them at HBV-cirrhosis 85.7%, HCV-cirrhosis 94.7%, HBV+HCV-cirrhosis 83.3%. 10 patients HCC died for 5.7 ± 1.45 months from the moment of diagnostics. The reasons of death were: at CH – hepatic/hepatorenal insufficiency; at LC - bleeding from veins of a gullet, hepatic insufficiency; at HCC - cancer intoxication, cachexia, hepatic insufficiency.
Morphogenesis of chronic mono- and mixed hepatitis B and C

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Introduction: Morphological characteristics are the basis in diagnostics of chronic hepatitis.

Methods: Morphological picture of chronic mono/mixed hepatitis B and C is investigated according to criteria of activity (Knodell’s index) and to stages fibrosis (the METAVIR scale) at 121 of 485 patients (24.9%).

Results: In a liver biopsy at HCV the signs of portal, periportal, perivascular and periductal inflammation revealed. Mainly mixed infiltration prevailed. “Ground-glass cells”, intracellular cholestasis were revealed. Moderate bridging and piecemeal necrosis were revealed mainly at HCV, hepatocyte degeneration, moderate and focal necrosis - at HBV, HBV-cirrhosis and HBV+HCV-cirrhosis. Infiltration of portal tracts was determined as more intensive at CHB+CHC, it was identical at CHB and CHC. The inflammation in a liver according Knodell’s index was significantly higher at CHB in comparison with CHC or CHB+C and was significantly higher at HBV+HCV-cirrhosis in comparison with HBV/or HCV-cirrhosis. Histologic activity was more expressed at CHB in comparison with HBV-cirrhosis whereas CHC and mixed hepatitis B+C on the given parameters did not differ from HCV/mixed cirrhosis accordingly. At CHC mainly mixed infiltration, fatty liver infiltration, lymphoid follicles, periductal sclerosis, fibrosis FII-FIII were registered.

Conclusion: Fibrosis was more expressed at CHC in comparison with CHB, but by the heaviest fibrosis was received at mixed hepatitis in comparison with CHB and CHC. The massive fibrosis and the greater number pseudolobules were defined at HBV+HCV-cirrhosis.
Can non-invasive biochemical markers predict fibrosis in patients with chronic hepatitis C?

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Introduction: Knowledge of the stage of liver fibrosis is essential for prognostication and decisions on antiviral treatment.

Methods: We retrospectively studied 128 patients with chronic HCV who underwent percutaneous liver biopsy and fibrosis was staged using Metavir score. 3 biochemical markers were used to correlate with the disease stage: aspartate aminotransferase, alanine aminotransferase, platelet counts and 2 ratios: aspartate aminotransferase/alanine aminotransferase and aspartate aminotransferase/platelet.

Results: Significant fibrosis (F2-F3) was present in 52.3%. The 3 biochemical markers alone had a reduced positive predictive value (< 70%) and the highest sensitivity among them has alanine aminotransferase (91%). The sensitivity of aspartate aminotransferase/alanine aminotransferase ratio was 94% in comparison with the sensitivity of aspartate aminotransferase/platelets ratio of 85.1%. The accuracy of aspartate aminotransferase/alanine aminotransferase ratio in predicting significant fibrosis is 1.68 compared with 1.18 of aspartate aminotransferase/platelets ratio. Prevalence of severe fibrosis is significantly higher in patients with aspartate aminotransferase/alanine aminotransferase ratio or aspartate aminotransferase/platelets ratio < 0.5 in comparison with those who had aspartate aminotransferase/alanine aminotransferase ratio or aspartate aminotransferase/platelets ratio > 0.5 (p < 0.001).

Conclusion: Our study shows that among these 5 parameters the most sensitive one in predicting significant fibrosis is aspartate aminotransferase/alanine aminotransferase ratio followed by aspartate aminotransferase/platelets ratio.

Key words: non-invasive markers, fibrosis, chronic hepatitis C
Evaluation of clinical course of α₁-antitrypsin deficiency in patients requiring liver transplantation

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Introduction: The aim of the study was to evaluate the clinical presentation and liver tests in two groups of children with α₁-ATD with different prognosis: patients requiring LTx and pts surviving long-term without LTx.

Methods: We studied 72 children admitted to our hospital with cholestasis or chronic hepatitis from infancy who were hetero- (21) and homozygous (PiZZ-51) for α₁-ATD. Among them six children required liver transplantation (group I). Patients homozygous (PiZZ) for α₁-ATD and without liver transplantation were in the group II (45).

Results: All the children treated with liver transplantation (6) were homozygous PiZZ for α₁-ATD. In this group cholestasis disappeared in 3 patients at the age of 9-14 years. Hepatitis was observed in 5 patients at 1 year of age and 4-7 years of age, and in 3 children aged 8-11 years. INR > 1.2 and hypoalbuminemia < 35 mg/dl appeared in all of them from 4 to 10 year of life, ascites - from 5 to 16 years. Small oesophageal varices were observed in 1 patient aged 5 years and in 5 pts aged about 10 years. LTx was performed at the age of 10 to 16 years. In the group II 8 children still suffered from cholestasis at the age of 9-14 years, 9- had hepatitis at the age 9-14 y. We observed temporarily increased INR (1.2-1.5) in 8 pts and slight hypoalbuminemia (30-35 mg/dl) in 9 children.

Discussion/Conclusion:
1. Our centre experience points to relatively good prognosis of ATD in early childhood and even if signs of slight liver insufficiency appear decision on LTx may be delayed
2. Chronic cholestasis and transient liver insufficiency do not seem to be strong predictors of fatal prognosis in children with ATD.
Prevalence and risk factors for alcoholic liver cirrhosis in patients with ethanol-induced chronic pancreatitis

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Introduction: Chronic pancreatitis and liver cirrhosis are major alcohol-related diseases. Environmental and genetic factors make this association to be quite rare.

Purpose: To assess frequency and risk factors for alcoholic liver cirrhosis (ALCi) in patients with alcoholic chronic pancreatitis (ACrP).

Methods: We performed a retrospective record analysis and a subsequent prospective follow-up of 99 patients with ACrP operated between 1991 and 2000. Average duration of ACrP was 15.6 years (7.3 years postoperative follow-up). All patients had preoperative and follow-up functional and imaging assessment of the liver. Intraoperative liver biopsy was performed in those with altered liver tests, and as routine from 1994. To find risk factors for ALCi, we applied a non-linear estimation test for the following factors: age, sex, alcohol intake, cigarette smoking, fat diet, presence of diabetes and calcifications.

Results: At the end of follow-up, 12 patients had ALCi. Mean age was 56.7 years (min 48 – max 64 years). 22 ACrP patients died, 4 of them because of ALCi complications. Routine liver biopsy showed features of alcoholic chronic hepatitis in 7 patients with no altered functional or imaging liver tests. We found that age > 60, > 15 years alcohol consumption, > 200 ml alcohol/day, presence of diabetes and pancreatic calcifications are strong risk factors for ALCi ($p < 0.005$).

Discussion/Conclusion: ALCi has a prevalence of 12.12%, and is a major cause of death in patients with ACrP. Overt ALCi needs at least 15 years of alcohol consumption, but chronic hepatitis was found in patients without altered liver tests, showing similarities with early phase of ACrP.
High expression of agrin in cholangiocarcinoma

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Introduction: Heparan sulphate proteoglycans mediate cell adhesion and control the activities of numerous growth and motility factors. They play a critical role in carcinogenesis and tumour progression. Agrin is a large, multidomain heparan sulphate proteoglycan which is associated with basement membranes of several tissues. Recently, the tissue expression of agrin was described in several organs, including the liver, under physiological conditions. So far, little is known about its role in malignancies, especially in primary liver tumours. We aimed to study the mRNA and protein expression of agrin in cholangiocarcinoma (CC), to understand its role in carcinogenesis.

Methods: The mRNA expression was measured on 32 surgically resected, RNA later fixed samples by real-time PCR method. Agrin immunostaining was carried out on 80 formalin fixed, paraffin-embedded surgical biopsy specimens (35 CC, 35 HCC, 10 normal liver). Western blot analysis on representative CC and HCC samples was also performed.

Results: CC samples showed increased mRNA expression of agrin in comparison to both normal liver and HCC. Intense protein expression of agrin was observed in the tumor-specific basement membrane in well differentiated areas of CCs, which staining was fragmented, decreased or even disappeared in less differentiated fields and sites of infiltration. Immunohistochemistry showed mild positivity around the bile ducts and the blood vessels within the portal area in normal liver, however, no expression within the hepatic lobules was found. High expression of agrin was shown along the neovascular basement membrane in HCCs. Western blot analysis confirmed the presence of increased agrin expression.

Discussion/Conclusion: Our results proved that agrin plays an important role in tumour progression in human CCs.

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Peripheral blood B-cell activation influences pretreatment status and prognosis in active hepatitis C patients

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Introduction: Activation of B-lymphocytes is important part of immune response effectiveness against active hepatitis C infection.

Aim: To evaluate the extent of peripheral blood B-cell activation in pretreatment patients with chronic active hepatitis C and to correlate it to the common markers of hepatitis activity – serum ALT and actual HCV viral load.

Methods: Pretreatment serum ALT and HCV RNA (bDNA assay, Bayer Diagnostics) are assessed for 26 patients with active hepatitis C. Peripheral blood levels of B-lymphocyte populations (CD19 cells, %CD5 in CD19 - FACS, B. Dickinson) are evaluated.

Results: In the group of patients (N = 7) with peripheral blood B-cells activation (more than 15% CD5 positive B-lymphocytes) there is twice higher HCV viral load (mean HCV RNA 278,769.23 IU/ml), associated with mean ALT 218 U/l. Patients (N = 19) with less than 15% CD5-positive B-lymphocytes in peripheral blood show lower but same logarithmic blood level of HCV RNA (mean 139,596.15 IU/ml) and much lesser degree of ALT elevation in serum (mean ALT 83 U/l).

Discussion/Conclusion: A strong tendency of more active chronic hepatitis is found in patients with peripheral blood B-cell activation. Peripheral blood B-lymphocytes activation correlates better with biochemical grade of hepatitis (ALT) than with viral load and possibly with better antiviral response to treatment.
Early viral slope in chronic active hepatitis C treatment as a predictor of sustained treatment response

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Introduction: There is a well known correlation between HCV RNA viral load dynamics in the first few weeks of antiviral treatment and sustained treatment response. The aim of this study is to compare the sustained antiviral treatment response in chronic active hepatitis C patients with undetectable HCV RNA and patients with more than 2 log decrease in positive HCV RNA on the 4th week of antiviral treatment.

Methods: 19 patients with chronic active hepatitis were assessed. All patients have been treated with pegylated interferon and ribavirin for 12 months. Viral kinetics (HCV RNA at 4th week, 6th month and 6 months after end of treatment) are measured by bDNA assay (Bayer Diagnostics). Median, SD and range of sustained response was evaluated in both groups. Mann Whitney 2-tailed significance between groups was evaluated.

Results: Sustained viral response (undetectable HCV RNA six months post treatment) for 4th week HCV RNA negative patients is found in 10 from 13 patients (76.9%). Only 1 between the 6 patients (16.7%) showing 2 log decreased but positive HCV RNA on 4th week of treatment has sustained viral response six months after end of treatment. A statistically significant difference (p 0.101) in the sustained treatment response between the two groups of patients was found.

Discussion/Conclusion: Early viral slope is a good predictor of sustained antiviral treatment response and might be used as a marker for treatment enhancement or prolongation in patients with decreased but positive HCV RNA on treatment.
The assessment of viral hepatitis with vasculitis manifestations

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The viral hepatitis may be complicated by vasculitis manifestations which worsen the evolution been necessary supplementary investigations and therapeutical measures.

**Aim:** To present the assessment in viral B and C hepatitis associated with vasculitis.

**Patients and methods:** The study was made on a group of 25 patients, aged 28-54, who were diagnosed with viral hepatitis and vasculitis, 14 with hepatitis B virus and 11 with hepatitis C virus, observed on a 5 years period. The methods were the clinical exam, biological, immunological, pathological and imaging investigations.

**Results:** The vasculitis complicated the evolution both in patients with chronic hepatitis B and C. It were associated many abnormal immunologic tests (the presence of CIC, hypocomplementemia, hypergammaglobulinemia, cryoglobulinemia). The histopathologic appearance at skin biopsy was the leukocytoclastic vasculitis and necrotizing cutaneous vasculitis with fibrinoid necrosis of the vessel wall and transmural inflammatory reaction. This is correlated with deposition of CIC in vessel wells, the immune dysfunctions, elevated values of liver function tests and the increase of interleukin-1 (IL-1) and tumoral necrosing factor (TNF-α) which are potent inductors for ELAM-1 and VCAM-1 which intensify the leukocytes adhesion at endothelial cells of vessel wall. The increase of IL-1 and TNF-α was on average 15 times. A mixed cryoglobulinemia was present in 8 patients diagnosed with hepatitis C and not in hepatitis B. The vasculitis was diagnosed in the same time with viral hepatitis in 9 patients, after 1-4 years in 16 patients and it was recurrent in 12 patients. The treatment with corticosteroids and immunosuppressive agents was moderately efficacious over long time and with possible enhancement of viral replication, been necessary combination with antiviral therapy. Polyarteritis form of systemic vasculitis was described in 2 patients.

**Conclusions:** In B and C hepatitis, vasculitis is an important complication, observed in the clinical onset or with recurrence in evolution of liver disease. It is associated with the increase of IL-1, TNF-α, perturbation of immune and liver tests, with cryoglobulinemia in hepatitis C and leukocytoclastic vasculitis at biopsy. The increase risk for health and life of hepatitis with vasculitis make necessary the monitoring for both. The treatment is a combination of plasmapheresis, immunosuppressive and antiviral therapy and possible anticytokinic agents.
The utility of neuroelectrophysiological investigations in interferon pre-therapeutical evaluation in patients with chronic viral hepatitis

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The neuroelectrophysiological investigations by visual evoked potentials (VEP) and/or electroencephalography (EEG) in patients with chronic viral hepatitis may record some precocious modifications, determined by hepatic disease or its treatment, especially with interferon. Together with biological, imagistical and biotical hepatic investigations, VEP and EEG, may be useful in the interferon pre therapeutical evaluation, this antiviral therapy, etiologic important, been for a long time treatment and with possible adverse reactions, including those neuropsychics (depression, concentration perturbations, suicide attempts, delirium, epileptic crisis) which are considerable.

Aim: To present the importance of neuroelectrophysiological investigations by VEP and EEG in the assessment of patients with viral chronic hepatitis and in monitoring interferon therapy.

Patients and methods: The study was made on a group of 72 patients with active viral chronic hepatitis and a healthy group with 62 persons, aged 50 ±12 years, 42 women (58%) and 30 men (42%). In viral ethiology were involved B ± D, C, B + C hepatitis viruses. The diagnosis of viral hepatitis was made by clinical and investigation methods (biological, imagistical by ultrasound, endoscopy, x-ray and pathological). At all patients were recorded VEP and EEG.

Results: On observed abnormal aspects of VEP at 37% of patients and of EEG at 33%. The observation of modifications in VEP waves parameters (N75, P100, N135), with elongation of latency and duration, diminution of amplitudes, of abruptness and surfaces, the increase of the differences in answer between eyes and in the some time in EEG recording with appearance of slow waves (δ and θ) in high percentage in some patients with B or C viral chronic hepatitis, there are objective dates for estimation neuropsychic impact.

Conclusions: VEP and EEG permit the appreciation of a neuroelectrophysiological status of chronic hepatic patients and a modified aspect of recordings (in 1/3 patients) can have significance and measure the degree of initially perturbations, before interferon therapy, the risk of neuropsychic secondary reactions of interferon administration, inclusive peginterferon, the impact and possible reversible in time of some drug reactions and in monitorising of antiviral therapy, at initiation, in the time and after treatment.

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Chronic hepatitis associated with Sjögren syndrome

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Immune mediated disorders can be observed in chronic hepatitis, with destruction in liver as target organ, but in the some time at distance, like in salivary and tear glands.

Aim: To describe the chronic hepatitis in association with Sjögren syndrome and risk factors.

Patients and methods: The study was made on 45 patients divided in: a group of 15 patients with chronic hepatitis and Sjögren syndrome, other group of 15 patients with chronic hepatitis without Sjögren syndrome and a group of 15 with Sjögren syndrome without chronic hepatitis.

The methods for diagnosis were: clinical, biological, immunological, bioptic, ultrasound, TC, x-ray and endoscopic investigations. It was determined in the some time sicca tests and antibodies against Helicobacter pylori (H.P.)

Results: The chronic hepatitis was represented by: viral hepatitis C (20 patients), viral hepatitis B (19 patients) and autoimmune hepatitis (6 patients). Sjögren syndrome was associated in chronic hepatitis like an extrahepatic manifestation. It was present before hepatic specific features at 5 patients and after diagnosed of liver disease at 9.

H.P. infection is able to elicit a local and systemic immune response against bacterial antigens; the homology between microbial and human Heat Shock Proteins suggests that the immune response may play a role in pathogenesis of autoimmune disorders. The antibodies G and M against H.P. were present frequently in patients with Sjögren syndrome and chronic hepatitis (12 patients).

The TNF-α, IL-1, IL-6 were increased in Sjögren syndrome and chronic hepatitis. The Sjögren syndrome was described in 9 patients with viral hepatitis C, in 4 with viral hepatitis B and 2 patients with autoimmune hepatitis.

Conclusions:
1. Sjögren syndrome associated with chronic hepatitis is an autoimmune manifestation.
2. It was observed in viral and autoimmune hepatitis, but most frequent in C viral hepatitis.
3. The risk factors were the autoimmune perturbations of active chronic hepatitis, the increase of immunoglobulins G, M and against H.P., CD4 cells and cytokines (TNF-α, IL-1, IL-6).

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The clinical and biological characteristics of chronic C hepatitis with vasculitis syndromes

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Introduction: To describe clinical, biological, and immunological features of patients with vasculitis syndromes.

Methods: A retrospective study on 83 patients with chronic C hepatitis.

Results: Of the 83 chronic C hepatitis patients, only 20 patients (14 women, 6 men; mean age 43 ± 9 years) had clinical manifestations of cryoglobulinemic vasculitis: purpura (80%), urticaria (5%), livedo reticularis (5%), cutaneous ulcers (40%), artralgias (50%), myalgias (20%), symmetrical distal sensory polyneuropathies (50%), proteinuria (25%), and renal insufficiency (20%), diffuse abdominal pain (25%), elevated ALT (55%), rheumatoid factor (75%), low C3 serum (90%), cryoglobulinemia (100%), and hyperglobulinemia (20%).

Three patients (1 man, 2 women; mean age 52 ± 12 years) had clinical manifestations of polyarteritis nodosa: fever (100%), weight lost (100%), arthritis (66%), multifocal sensitivomotor mononeuropathies (66%), malignant hypertension (66%), abdominal pain (66%), anemia (33%), elevated C reactive protein (100%), and increased erytrocyte sedimentation rate (100%).

Discussion/Conclusion: HCV infection may be associated with cryoglobulinemia vasculitis and polyarteritis nodosa. Because of the different therapeutical strategies, cryoglobulinemia vasculitis should be distinguished from polyarteritis nodosa in HCV infection patients.
Genetic variability of hepatitis B virus isolates in Poland

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Introduction: There is very limited knowledge about the genetic variability of HBV strains circulating in the population of Polish chronically infected HBV patients. The aim of this study was to analyse the phylogenetic relatedness and polymorphism in some functional domains of HBV genome among chronically infected patients from northern Poland.

Methods: Fifty-one serum samples were included to analysis of HBV genomes. The sequences of the rt polymerase/S and preC/BCP regions of those isolates were analysed by direct DNA sequencing of PCR fragments and compared to genome sequences of different variants of HBV from GenBank database. Genetic relatedness of Polish genotypes to known reference strains was estimated by phylogenetic analysis.

Results: A phylogenetic tree of 41 analysed genotype A isolates as well as 8 genotype D strains was constructed showing relationship to know reference strains. Two isolates, initially classified as genotype F turned to be related to genotype H, newly described genotype deriving from genotype F, a very rare genotype in Europe.

Discussion/Conclusion: HBV genotypes’ distribution pattern in Poland and phylogenetic relatedness seems to be different from our Eastern neighbours. Regarding the clear difference in the A to D genotypes ratio between Poland and Eastern Europe, it would be interesting also to explore this phenomenon as the potential epidemiological marker of HBV infection spreading across the region of Eastern and Central Europe. Moreover, relatively high prevalence of genotype H in northern Poland has to be further investigated and detailed phylogenetic relatedness, based on the whole genome comparison to reference strains, performed.

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The role of vanilloid sensitive neurons in the immune-mediated model of hepatic tissue injury

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Introduction: Vanilloid sensitive neurons endings have a close contact with the hepatic stellate cells (HSC), which are responsible for adjusting blood supply to the liver and for modulation of the hepatic immune-mediated mechanisms. Substance P and other tachykinins are known to stimulate neurogenic inflammation by releasing TNF-α and IFN-γ in a variety of experimental models of hepatic injury. However in our previous experiments we proved that CGRP has a potent protective action upon hepatic injury.

Methods: We performed the experiments on Wistar rats weighing 200-220 under pentobarbital anesthesia using a Concanavalin A-induced liver injury model to explore the role of both tachykinins and CGRP in the mediation of hepatic injury. Mean arterial blood pressures (AP), portal blood flow (PBF) and microcirculatory hepatic blood flow (HBF) using laser-Doppler flowmetry and the levels of hepatic tissue injury markers (ALT, AST),were measured. Experimental groups: control group (placebo pre-treated), ConA pre-treated animals, ConA + CGRP receptor antagonist (CGRP 8-37), ConA + NK-1, Con A + NK-2, Con A + NK-3 and ConA + NK-1, NK-2 and NK-3 receptor antagonist. In the additional series of experiments we used capsaicin-denervated and vanilloid receptor-blocked (ruthenium red) rats.

Results: In ConA alone pre-treated animals ALT and AST serum levels raised to 4233 ± 1540 and 2578 ± 980 U/L whilst HBF were reduced by 12% (NS) in comparison to the values in the control group. In CGRP receptor-blocked rats both ALT and AST serum levels were raised by 35% and 21% and HBF was reduced by 27% in comparison to ConA alone pre-treated rats. In capsaicin-denervated and vanilloid receptor-blocked (ruthenium red) ALT levels were increased by 42% and 36% respectively while HBF values were reduced by 32 and 29% respectively (NS versus ConA alone pre-treated rats). In contrast NK-1 receptor blockade decreased ALT and AST serum levels to 3267 ± 789 and 2436 ± 652 U/L without affecting the HBF values in this group. Both NK-1 and NK-3 receptor antagonists failed to influence the circulatory and enzymatic parameters in the ConA-induced hepatic injury.

Discussion/Conclusion: Vanilloid sensitive neurons play a dual role in the ConA-induced model of hepatic injury. Tachykinins released from sensory endings exacerbate hepatic tissue injury acting via stimulation of NK-1 receptors whilst CGRP has a potent protective effect in this experimental model. The protective effect of vanilloid sensitive neurons is strictly correlated with vasodilatatory action of CGRP upon hepatic circulation.
The first results of genotyping hepatitis B virus in Serbia and Montenegro

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Background: Relationship of genotypes of hepatitis B virus (HBV) to clinical outcome, disease progression and response to antiviral therapy still is not well defined. Genotype have differences in biological properties and heterogeneity in global distribution. Genotype D and A are prevalent in Mediterranean area.

Methods and results: Genotyping of virus and HBV DNA done by Real Time PCR methods on ABI Prism 7000 with TaqMan primer in same sample. The first results show that genotype D is dominant in Serbia and Montenegro but there is also genotype A. We analysed 17 patients with HBV infection. Ten of them (58.82%) were with chronic hepatitis B (CHB) and 7 (41.17%) were with acute hepatitis B (AHB). Genotype D was isolated from 16 (94.11%) patients and only one patient had genotype A. All patients with AHB had genotype D high level of aminotransferases (median value of ALT 2370 ui/L), with serious cours of disease. Five patients were treated with lamivudine and two of them were HBsAg-negative after two months of treating. The rest of them had normalisation of ALT level but no HBsAg negativisation during first two months of treating. In second group of patients with CHB nine patients had genotype D and only one had infection with genotype A. HBeAg-negative form of CHB had 6 (60%) patients and 4 patients had HBeAg-positive CHB. All of them were treated with antiviral therapy interferon with/or lamivudine.

Conclusions: In region of Serbia and Montenegro genotype D is dominant but there is and genotype A. We didn't found another genotype of HBV. HBeAg-negative variants of HBV is more frequent then HbeAg-positive CHB in this region.
The etiology of cholestasis in a neonatal intensive care unit

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Introduction: Cholestasis requires always an immediate evaluation. The prognosis and the outcome depend on an early diagnosis, because if cholestasis is not relieved, chronic liver disease may ensue. The purpose of this study is to reveal the aetiology of cholestasis in our NICU.

Methods: We performed a retrospective study on 895 admissions in our NICU between years 2000-2006; from these, 32 cases had cholestasis. The goal of diagnostic evaluation (laboratory and imaging studies) was to identify conditions amenable to therapy.

Results: Nineteen babies had digestive malformations and nine had necrotizing enterocolitis. All above required surgery; five remained with various stomas and three with short bowel. One baby had Alagille syndrome and one had biliary atresia. Ten babies were premature, twenty developed sepsis with gram-negative germs and three had systemic candidosis. All the surgical cases received parenteral nutrition for at least 2 weeks. Eleven babies died from sepsis complications.

Discussion/Conclusion: The differential diagnosis is difficult, especially in a critically ill newborn. Beside genetic condition and biliary malformations there are risk factors predisposing to cholestasis: prematurity, parenteral nutrition, sepsis, lack of enteral feeding and short bowel syndrome. In order to prevent or to alleviate cholestasis we have to: avoid parenteral overfeeding, start enteral feeding early and treat sepsis. In addition, ursodeoxycholic acid is useful in improving symptoms and laboratory parameters.
Three years of following up an autoimmune hepatitis patient

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Introduction: We would like to submit to your attention the case of a 62 years old female, admitted in Infections Diseases Hospital, 3 and a half years ago for elevated serum transaminase levels and cholestasis. Viral aetiology was excluded. We looked for immunological changes and we found ANA+, ASMA+ and antiLKM-.

Needle biopsy of the liver was performed and showed marked activity and extensive fibrosis.

She began treatment with prednisone and azathioprine with improving of biochemical tests (transaminase evolved towards normal level).

In 6 months, after a sudden falling down she was checked under emergency regime, revealing a 600 mg/dl glycaemia.

She was administrated with insulin, then anti-diabetic drugs and finally remained on diet.

The cortico-therapy and azathioprine were stopped.

After that she presented important elevations of transaminase and serum bilirubin; serum iron was also high and at that moment we thought of haemochromatosis which wasn’t re-confirmed later by repeated serum iron levels.

From then on she received chronic treatment with Ursofalk®. Magnetic Resonance showed hepatic cirrhosis. At current moment transaminase and GGT are about 1.5 x normal.

As a special aspect we noticed the fact that the diabetes was induced by cortico-therapy.

Together with the iron levels in serum situation, it suggested the differential diagnosis with haemochromatosis.

We underline the excellent evolution of the patient under the Ursofalk® treatment.

Methods: Case report.

Results: Positive reaction to treatment.

Discussion/Conclusion: Excellent patient evolution under Ursofalk®
Hepatoprotective effect of silymarin and unsaturated fatty acid combination in experimental steatohepatitis

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Introduction: Silymarin is a hepatoprotector widely used in the clinics. The basic mechanism of hepatoprotection by silymarin is scavenging reactive oxygen species and prevention of lipid peroxidation activation that, in turn, prevents liver membrane structure damage. The administration of polyunsaturated fatty acids also improves liver membrane structural and functional properties. We tried a combination of silymarin and fatty acids mixture for a treatment of two types of steatohepatitis induced by either ethanol or orotic acid administration.

Methods: Male Wistar rats weighing 180-220 g were used. Alcoholic steatohepatitis was induced by ethanol (4 g/kg, i.g., 30 days), non-alcoholic steatohepatitis – by orotic acid added (1%) to the high-carbohydrate diet (64% of energy as sucrose) during 21 day. One of the groups with steatosis from each trial was treated with both silymarin (100 mg/kg, i.g.) and fatty acid mixture (50 mg/kg, i.g.) which was administered to rats throughout the experiments, the second group - only with silymarin at the same dose. The fatty acid mixture consisted of linoleic acid (ω-6, 4%), α-linolenic acid (ω-3, 4%), γ-linolenic acid (ω-6, 2%), arachidonic acid (ω-6, 15%), eicosadienoic and eicosatrienoic acids (ω-6, 4%), monoenoic acids (ω-9, 45%) and saturated acids.

Results: The ethanol-treated rats showed liver focal necroses, lymphoid infiltration and microvesicular fatty dystrophy. The activities of serum marker enzymes, ALAT, AsAT and alkaline phosphatase (AP) as well as liver triglyceride content were significantly elevated in ethanol-treated rats. Ethanol increased cytochrome P-450 (CYP450) content and aniline-p-hydroxylase activity in liver microsomes. Both the combination and silymarin alone equally decreased the square of sudanophylic area in liver slides evaluated by computer morphometry. All the compounds decreased the above biochemical parameters to a similar extent except for AP activity where the effect of the combination of silymarin with fatty acids was more pronounced then that of silymarin alone. The combination also more effectively by decreased the CYP-450-related parameters.

In the group treated with orotic acid, liver fatty dystrophy ranging from microvesicular form and accompanied by diffuse lymphocytic infiltration were observed. These rats demonstrated an activation of serum ALAT, AsAT, AP and liver triglycerides content. The CYP450 content and aniline-p-hydroxylase activity in liver microsomes were significantly decreased in this group. Silymarin alone, and especially in combination with fatty acids, significantly decreased the square of sudanophylic infiltration in the liver. Silymarin and its combination with fatty acids normalized serum marker enzyme activities, liver triglyceride content and CYP-450-related parameters where the effect of the combination was more pronounced.

Discussion/Conclusion: The data obtained suggest that applied composition of fatty acids improved the hepatoprotective properties of silymarin and enhanced detoxification properties of the liver.
Endoscopic ligation of esophageal varices for prophylaxis of first variceal bleeding in children and adolescents with portal hypertension

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Introduction: Endoscopic variceal ligation is effective in controlling rebleeding from esophageal varices. The objective of this study was to prospectively evaluate of it’s efficacy in preventing of first haemorrhage in children.

Methods: 57 children with portal hypertension (36 liver cirrhosis, 21 – portal vein thrombosis), aged from 4 to 17 years (M = 10.15 y). The criteria for inclusion were: (1) no previous variceal bleeding; (2) the presence of varices at least grade II and (3) their enlargement after 6 months observation or endoscopic signs of high bleeding risk. Multi-band ligation was applied every 3 months to achieve eradication of all varices.

Results: Six patients’ did not followed the protocol (4 - liver transplant, 2 - lack of compliance). Eradication was achieved in all of the remaining 51 children (89% ITT, 100% PP) after 2.1 ± 0.99 sessions of ligation. Minor bleeding occurred during one procedure. The follow-up after cessation of treatment is 26 ± 16 months. Recurrence of varices occurred in 13 out of 30 children with cirrhosis (43%) after 20.13 ± 10.8 months (20% after 12 months) and in 4 out of 21 with portal thrombosis (19%) after 35.6 ± 17.7 months from eradication.

Conclusion: Endoscopic variceal ligation is safe and effective procedure in children with portal hypertension, diminishing the risk of first bleeding onset.
Expression of CD31 in liver in chronic viral hepatitis

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Introduction: CD31 is known as the marker of endothelial cells. The aim of our work was to study CD31 expression in liver biopsies obtained from patients with chronic liver hepatitis.

Methods: 51 liver biopsies from patients with chronic viral hepatitis B and C were examined. Paraffin liver sections were stained immunohistochemically with antibodies to CD31 (Novocastra, UK).

Results: In all cases of chronic viral hepatitis CD31-positive cells were observed in endothelial cells of portal tract vessels. The number of CD31-positive cells in liver parenchyma correlates with histology activity index (HAI): in case of HAI 1-3 they were not observed in any cases, in case of HAI 4-8 they were found in 42.8%, in case of HAI 9-12 - in 50%, in case of HAI 13-18 expression of CD31 in sinusoids was revealed in 100% of liver biopsies. Distribution of positive cells also seems to be dependent on HAI: in case of HAI 4-8 they were observed predominantly in periportal areas while in case of HAI 9-12 CD31-positive cells were present also in areas of inflammation and in liver sinusoids; in biopsies with HAI 13-18 expression of CD31 in sinusoids was higher than in case of HAI 9-12. Moreover, expression of CD31 in liver sinusoids increased proportionally with the grade of fibrosis.

Discussion/Conclusion: So the number of CD31-positive cells in liver in chronic viral hepatitis depends on histology activity index and severity of fibrosis; latter proves that development of liver fibrosis is accompanied with capillarization of liver sinusoids.
Skin lesions in chronic viral hepatitis – Coincident diseases or extrahepatic manifestations of infection?

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Introduction: Skin lesions as jaundice, spider angiomata, palmar erythema, are common findings with known diagnostic significance in chronic viral hepatitis and cirrhosis. Some other skin lesions can be extrahepatic manifestations of viral hepatitis C (VHC) or B (such as cryoglobulinemic purpura, lichen) or reveal them (as vasculitis). Some other skin lesions are side effects of antiviral treatment, while other are not causally linked with hepatitis, but may be more prevalent in these patients. Prevalence data concerning skin disease in chronic viral hepatitis are controversial.

Methods: We hereby present the partial results of an ongoing prevalence study on skin lesions and disease in chronic viral hepatitis and cirrhosis. Possible correlations with etiology, stage of disease and liver function tests, are also questioned. All patients admitted in the Internal Medicine Department having the mentioned diagnosis, since October 2004, were examined by the same dermatologist.

Results: Until April 2006, we assessed 70 patients, among which 50 were female. Most of the patients were VHC infected (57). We found them to have lichen planus and psoriasis (in 8% each), cryoglobulinemic purpura (in 5%), photosensitivity (in 20%), urticaria (15%), and rarely amyloidosis. Seborrheic lesions, angiomata, infectious skin disease, are also frequent. Prevalence of skin lesions was higher in women. 35% of VHC patients treated with interferon and ribavirin had cutaneous lesions.

Discussion/Conclusion: Prevalence found in our study is similar with the one found in other studies. Skin lesions can reveal important diagnosis clues. VHC infected patients are prone to skin comorbidities.
Prevalence of hepatocarcinoma risk factors in hospitalized patients with cirrhosis

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Introduction: Hepatocarcinoma (HCC) incidence and prevalence is increasing worldwide. Its risk factors were recently reviewed by international experts. Viral hepatitis C infection prevalence in Romania is in the medium range, but continuously increasing. Careful searching and appropriate management of HCC risk factors in hospital and general practice is mandatory for reducing HCC incidence in our country in the next years.

Methods: This retrospective study aims to assess the prevalence of HCC risk factors and their management in patients hospitalised for cirrhosis in 2004 in our hospital. We reviewed the records, the treatment and the recommendations at discharge.

Results: Among the 234 records, the main cause of cirrhosis was viral hepatitis C and B, and also alcoholic liver disease. About 20% of the patients were diagnosed in end-stage disease. Alcohol intake and smoking were not accurately registered. Serum iron was rarely determined. Body mass index was seldom registered. Patients with diabetes had the better follow up. Few patients (less than 1%) were previously treated with Interferon. Treatment was mainly symptomatic.

Discussion/Conclusion: Raising doctors’ and patients’ awareness on HCC risk factors and appropriate measures to control the “modifiable” ones could be beneficial for disease incidence in the following years.
Association of Crohn’s disease with latent pulmonary tuberculosis – Case presentation

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Introduction: Crohn’s disease has an increasing incidence in our country, while tuberculosis is already concerning prevalent, with a very high incidence also. Granuloma, ileocecal localization of digestive lesions, as well as multiorgan involvement is common features of both mentioned diseases. Early and accurate diagnosis of these diseases is the cornerstone for an efficient and safe therapy, and it can still be challenging.

Methods: We discuss a clinical case: a young man with Crohn’s disease, associated with a lung interstitial involvement of unknown etiology.

Results: A 33-years old male was diagnosed nine month ago with ileocecal Crohn’s disease. Under treatment with corticosteroids and aminosalicylates, intestinal perforation occurred. Differential diagnosis of intestinal pathology included other parasitic (anisakiasis) and mycotic (candidiasis) infections. He was repeatedly examined and underwent many diagnostic procedures (clinic examination, plain chest radiography, computed tomography, bronchoscopy, bacteriology and histopathology exam) for the etiology of lung involvement. Pulmonary tuberculosis was finally diagnosed using the QuantiFERON-TB Gold test. The association of a Crohn’s ileocecal disease with latent pulmonary tuberculosis (reactivated during classical immunosuppressive treatment) raised some discussions on diagnosis, follow-up and therapy on this clinical circumstance, which may be not so rare in clinical practice.

Discussion/Conclusion: QuantiFERON-TB Gold test proved to be a sensitive and specific diagnostic tool. If used earlier in this case’s diagnostic approach, it could have radically changed both the management and the outcome.
SPANX/(CTp11) cancer/testis-associated genes family as possible tumor vaccine for liver neoplasia

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Introduction: SPANX/(CTp11) is a recently identified cancer-testis (CT) antigen in multiple myeloma, melanoma and other tumours of unrelated histological origin. We here show that it is expressed at both mRNA and protein levels in neoplastic cells from up to 35% of patients affected by liver cancer. Since the expression of this tumor antigen has been demonstrated to be restricted in normal tissues, it seems to be an excellent target for tumor vaccine of liver cancer.

Methods: Peripheral blood mononuclear cells (PBMCs) were separated from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation. Dendritic cells (DCs) were differentiated from PBMC. Briefly, PBMCs were seeded into 6-well culture plates containing 3 ml of RPMI 1640 and 10% FCS at 5-10 × 10⁶ per well. After 2 hr at 37°C, non-adherent cells were removed and the adherent cells were cultured at 37°C in RPMI 1640 supplemented with 10% FCS, 800 IU/ml GM-CSF and 1000 IU/ml IL-4. After 7 days of culture, DCs were harvested for pulsing with the synthetic peptide and used as antigen-presenting cells for the generation of SPANX/(CTp11)-specific CTLs. Successful generation of DCs was confirmed by flow cytometry analysis.

Results: SPANX (CTp11) has been found to be expressed at both mRNA and protein levels in neoplastic cells from up to 35% of patients affected by liver cancer. It contains functional cytotoxic T cell (CTL), supporting its use as a tumor vaccine. In this study, we determined the ability to generate Sp17-specific HLA-class I restricted CTLs from the peripheral blood of 5 patients, 3 consecutive SPANX (CTp11)+ patients and two SPANX (CTp11)- patients, using a recombinant SPANX (CTp11) protein.

Conclusions: Despite previous chemotherapy and the immunosuppression so often associated with liver cancer, CTL generation was successful in all 3 patients, suggesting the presence in the immune repertoire of SPANX (CTp11)-specific T cells in these patients, irrespectively of the SPANX (CTp11) status of their tumours. These observations provide the basis for a clinical study aimed at inducing a cellular immune response directed at SPANX (CTp11)-positive liver cancer patients.
The evaluation of HFE gene mutations in outpatients with chronic liver disease

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Introduction: The aim of this study was to establish whether HFE gene mutations contribute to chronic, non-viral liver disease.

Methods: During the period 2002-2006, we tested for the three major HFE gene mutations in 65 subsequent new outpatients with chronic liver disease of non-viral etiology eventually classified as either liver cirrhosis, steatofibrosis or steatosis.

Results: The S65C mutation was found in one person in the heterozygous form, the C282Y was recognized in one homo- and three heterozygotes. The H63D mutation was present in the homozygous form in four and in the heterozygous form in 22 subjects. Thus, total count of HFE gene mutations was 31 in 31 patients. Of these 31, alcohol abuse was present in 16. Alcoholic liver disease was found in 31 patients out of 65. History of acute pancreatitis or the presence of chronic pancreatitis was established in 9 subjects with alcoholic liver disease; one of them was a C282Y and seven were H63D heterozygotes. In three subjects in whom autoimmune liver disease was diagnosed no HFE mutations were present, the same as in another three subjects with parallel diabetes

Discussion/Conclusion: The results of this study do not confirm the presumed role of HFE gene mutations in the pathogenesis of alcoholic liver disease. However, our finding of high prevalence of HFE gene mutations in patients with alcoholic pancreatitis warrants further investigation.

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**TGF-β promote alcohol-dependent liver damage by interfering with ADH and MEOS metabolizing systems**

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**Introduction**: Fibrogenesis involves cytokines, acetaldehyde, oxidative stress and complex interactions between liver cells. Hepatotoxic effects of chronic alcohol consumption result from activating ADH and MEOS systems. TGF-β is induced in liver of patients with alcoholic liver disease, suggesting its involvement adverse effects of alcohol, however, a molecular connection was not yet identified. We aim to show a crosstalk between TGF-β signaling and alcohol dependent damage in hepatocytes.

**Methods**: Using DNA microarrays, a TGF-β dependent expression profile was established for hepatocytes. Results were confirmed by RT-PCR and Western blot analysis.

**Results**: Microarray data implicate that TGF-β induces expression of Cyp21A1 in hepatocytes. This finding was confirmed by RT-PCR, showing that TGF-β and alcohol stimulate expression of Cyp2E1 and Cyp21A1 to a similar extent. During culture of mouse hepatocytes, Cyp2E1 expression is down-regulated. Co-stimulation with TGF-β and alcohol did not display enhanced Cyp2E1 expression, suggesting that TGF-β might be a downstream mediator of alcohol effects. Interestingly, while inducing MEOS activity, TGF-β downregulates alcohol dehydrogenase ADH1 expression, which represents the enzyme of the primarily used alcohol metabolizing mechanism. Currently we delineate the TGF-β signalling pathway leading to regulation of Cyp2E1, Cyp21A1 and ADH1 using Smad7 overexpression an siRNA interference.

**Discussion/Conclusion**: TGF-β regulates expression of enzymes involved in alcohol metabolism. Genes encoding enzymes of MEOS are upregulated, whereas ADH1 expression is decreased. These data suggest that TGF-β contributes to alcohol induced liver damage by favouring an oxidative stress producing metabolic route.
Prognostic factors for survival in Child-Pugh class A HCV liver cirrhosis

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Introduction: The presence of esophageal varices has been reported to be a prognostic factor in patients with compensated HCV cirrhosis. We studied the prognostic value.

Methods: The study included 125 patients with Child class A HCV liver cirrhosis, prospectively followed-up over a period of 60 months (range 52-96 months). We evaluated parameters related to liver function, hematology, ultrasonography and endoscopy. The follow-up ended on the date of death or liver transplantation or was censored at the last recorded visit before January 2005. Prophylactic treatment of digestive hemorrhage was performed in 52 pts. Predictive factors for death were evaluated using Cox models (survival) and Fine-Gray models (liver-deaths).

Results: Survival rate at 3 and 5 years were 75.2% and 60% respectively. Death was due to liver disease in 68% of cases (hepatocellular carcinoma 15, hepatic failure 12, digestive hemorrhage 7) and to extrahepatic causes in 16 cases. Portal hypertension defined as evidence of grade 2 or 3 esophageal varices or gastric varices was associated to overall mortality (hazard ratio 2.41; 95% CI: 1.09-5.20; p = 0.02) and to liver-specific mortality (p = 0.02). In addition, liver-specific mortality was related to albuminemia < 3.8 g/L (p = 0.005), platelet count below 85,000/mm³ (p = 0.01) and bilirubinemia (total bilirubin > 1.5 mg/dL, p = 0.02). At multivariate analysis only hypoalbuminemia, platelet count and bilirubinemia had independent value.

Discussion/Conclusion: Portal hypertension has a limited value for assessing the prognosis of patients with Child-Pugh A HCV cirrhosis. Prognosis is accurately predicted by serum albumin, platelet count and bilirubin.
Proliferating cell nuclear antigen (PCNA) immunohistochemical staining in hepatocellular carcinoma: Relationship to histological grade

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Introduction: Proliferating cell nuclear antigen (PCNA), also known as cyclin, is an auxiliary protein of DNA-polymerase delta and is found only in the nuclei of proliferating cells in the late G1 and S phases. Previous researches have demonstrates it is a potentially useful tool for the study of tumor proliferative activity and have correlated it with tumor grade.

Methods: The proliferation activity of hepatocellular carcinoma (HCC) was analyzed by immunohistochemical staining for PCNA using LSAB technique on formalin-fixed, paraffin-embedded tissue sections of 20 surgically resected HCC specimens.

Results: Mean percentage of PCNA-positive nuclei in the HCC tissue was 10.8% in grade I, 26.2% in grade II, 29.2% in grade III, 42.2% in grade IV and showed statistically significant difference (P < 0.01) between each other. There was no statistical difference in the mean PCNA staining rate of various histological types among hepatocellular carcinoma (P > 0.05); PCNA staining of non-neoplastic hepatic tissue adjacent to the tumor was also evaluated and their mean PCNA rate revealed no significant difference (P > 0.05) from grade I HCC. PCNA-positive nuclei were numerous in the tumor thrombi found in portal vein branches, in regions of extracapsular tumor growth and in the inner nodules of tumors with a nodule-in-nodule formation.

Discussion/Conclusion: PCNA positivity was correlated with the histological grade and invasiveness of HCC, suggesting that this antigen may be used as an indicator to predict tumour invasion in patients with HCC. The mean PCNA staining did not differentiate non-neoplastic hepatic diseases that were adjacent to HCC, including chronic active hepatitis, cirrhosis of liver, reactive hepatitis and nuclear dysplasia from grade I HCC.
Iron overload in patients with chronic hepatitis C: A clinico-pathologic study

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Introduction: Recent studies suggest that increased hepatic iron may impair the response to interferon therapy in patients with chronic hepatitis C. The prevalence of iron overload and its relationship with liver histology was evaluated in chronic hepatitis C.

Methods: We reviewed the records and liver biopsies of 125 patients with chronic hepatitis C to determine the prevalence of iron overload and to evaluate whether there is a correlation between serum and hepatic iron concentrations and activity of liver disease. Patients with other causes of liver disease or iron overload were excluded. Necroinflammatory activity and fibrosis were evaluated using Knodell score. Liver biopsy specimens from patients with chronic hepatitis were stained with Perls (Prussian blue). Hepatic iron was assessed using Deugnier grading system.

Results: Increased serum iron and ferritin levels were found in 29% and 43% patients, respectively. Iron deposits stained by Prussian blue were seen in hepatocytes, sinusoidal cell and portal mesenchymal cells. Hepatic iron grades 0, I, II, III and IV were present in 49%, 29%, 20%, 2% and 0% of patients, respectively. A significant correlation was found between hepatic iron grade and serum ferritin (P = 0.0001). There was no correlation between hepatic iron grade and histological activity index or fibrosis score.

Discussion/Conclusion: In summary, we found a high proportion of patients with chronic hepatitis C who had mild to moderate increase in hepatic iron content even when patients with alcoholism and recurrent transfusions were excluded. However, very few patients have severely increased iron load.
Extreme hypercholesterolemia and xanthomatosis after laparoscopic cholecystectomy. Recovery following surgery

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Introduction: Hypercholesterolemia and xanthomatosis are known complications of chronic cholestasis. This is the first report on xanthomatosis and severe hypercholesterolemia caused by common bile duct stenosis following laparoscopic cholecystectomy.

Case report: Laparoscopic cholecystectomy was performed in a 32-year old woman because of numerous tiny gallstones. Bile leakage was observed in the early postoperative period which stopped spontaneously. Six months later itching, progressively increasing serum bilirubin level, cholestasis syndrome, xanthelasma and widespread eruptive xanthomatosis developed in a few months. The cholesterol level was extremely high (92.3 mmol/l) with normal triglyceride level.

The ERCP verified a 2 mm long, hair's-breadth-thin stenosis at the level of cystic duct. Primary biliary cirrhosis and primary sclerosing cholangitis were excluded, no evidence for familial hypercholesterolemia was found.

Choledocho-jejunostomy was performed and the patient became complaint- and symptom-free within two months. All xanthomas disappeared; the extremely high cholesterol level gradually returned to the normal level, all laboratory data became normal. The anticholesterol-antibody level was undetectable at presentation, but later reached the level of the healthy controls.

Transient, cholestasis induced regulatory disturbance of cholesterol metabolism is supposed. Research on genetic background (transporter gene mutations, etc.) is in progress.

Discussion: The presented case is an example of laparoscopic cholecystectomy caused bile-duct stenosis with extrahepatic cholestasis induced xanthomatosis and severe hypercholesterolemia. The bile leakage as early complication of bile duct damage may predict the later developed stenosis. Even severe xanthomatosis and extreme hypercholesterolemia can be totally reversible following elimination of biliary obstacle. Genetic background of altered cholesterol metabolism is supposed.
Autoantibodies against the "tubulointerstitial nephritis antigen-related protein" (TINagRP) in patients with autoimmune liver disease

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Introduction: TINagRP, now also classified as "lipocalin-7", is an extracellular matrix protein and is predominately expressed in smooth muscle cells. It shares a high degree of homology with the "tubulointerstitial-nephritis antigen" (TINag), which is the major autoantigen in patients with glomerulonephritis.

Aim: To establish an assay for detecting autoantibodies against TINagRP and to investigate their prevalence in patients with gastrointestinal diseases.

Methods: An ELISA-based assay using recombinant protein, expressed in HeLa-cells, was developed. TINagRP-specific autoantibodies were determined in 1,400 serum samples obtained from patients with inflammatory and malignant diseases including H. pylori-infection (HP) gastroesophageal reflux disease, autoimmune liver disease (AILD), inflammatory bowel disease, gastric cancer, hepatocellular carcinoma as well as controls (healthy and dyspeptic patients).

Results: The prevalence of TINagRP-specific autoantibodies varied between 8 and 22% among patient groups and controls. The detection rate of 22% in patients with AILD was significantly higher than those determined in other groups (P < 0.03, Chi-Quadrate test). The actual concentration of these autoantibodies in "positive samples" was significantly higher in samples from patients with AILD and HP compared to "positive samples" of all other groups (P < 0.001, Mann Whitney U test).

Discussion/Conclusion: Autoantibodies against TINagRP seem to be ubiquitously present at a low rate in all subjects. Patients with AILD show the highest prevalence of these antibodies. The pathophysiological relevance of these antibodies remains to be clarified.
Budesonide in autoimmune hepatitis

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Introduction: Prednisolone and azathioprine represent the standard treatment of autoimmune hepatitis (AIH). Budesonide (BUD) was reported to be a promising alternative for AIH. Published data on BUD are very limited and controversial.

Aim: To study the efficacy and safety of BUD in patients with AIH.

Methods: Fifty patients (F:M = 34:16; mean age 46 yr, range 18-72 yr) with AIH were treated with BUD (Budenofalk®) 3 mg thrice daily and followed up for at least 24 weeks (range 24 to 177 weeks). Eleven patients presented with an overlap of AIH and a cholestatic liver disease (primary biliary cirrhosis or primary sclerosing cholangitis). Advanced liver fibrosis or cirrhosis (Ishak’s fibrosis scores of 3 to 6) was present in 14 (28%) patients.

Results: Thirty seven (74%) patients achieved a complete and seven patients (14%) a partial biochemical remission giving an overall response rate of 88%. Treatment failure or deterioration of the disease was recorded in six cases. Significant improvement of liver histology was seen in all patients (n = 7) who received a follow-up liver biopsy. Clinically relevant drug-induced side effects were mainly recorded in patients with advanced liver fibrosis.

Discussion/Conclusion: BUD is effective in remission induction in the majority of our presented patients with AIH. Treatment failure and side effects were mainly observed in patients with severe fibrosis.
The assessment of lipid peroxidation and antioxidant mechanisms in children with Wilson’s disease

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Introduction: Copper accumulation in the liver seems to be the basic cause of liver damage in Wilson disease (WD). Still additional factors like free radical injury are suspected to be involved. The aim of the study was to assess lipid peroxidation and basic antioxidant mechanisms in children with WD.

Methods: 29 children with confirmed WD aged 14.76 ± 2.87 years [mean ± SD] were enrolled to the study. The level of lipid peroxidation was measured with serum TBARS concentration [nmol/ml]. Serum vitamin E [µg/ml] and erythrocyte glutathione (GSH) concentrations [µmol/ml] and erythrocyte glutathione peroxidase (GPx) activity [U/gHb] were measured as antioxidant barrier assessment. 66 age-matched children were the control group (CG). The Mann-Whitney U-test was used in statistical analysis.

Results: The children with WD have higher TBARS concentration (0.513; 0.405; 0.66 vs. 0.41; 0.271; 0.527 [median; lower quartile; upper quartile]), lower GSH concentration (690.7; 589.0; 761.1 vs. 768.5; 710.6; 804.6) and lower GPx activity (30.5; 24.9; 34.3 vs. 34.1; 30.8; 38.4) when compared to CG. No differences were found in Vitamin E concentrations between both groups No differences in all these parameters have been found between WD subgroups with normal and elevated AspAT and AIAT activity.

Discussion/Conclusion: WD children present with increased lipid peroxidation and deficiency of GSH and GPx. In conclusion: 1. Lipid peroxidation is increased in WD and may be involved in liver injury. 2. Children with WD have lower erythrocyte GSH concentration and GPx activity than healthy controls. 3. There is no clear relationship between lipid peroxidation and aminotransferases activity.
Application of thymosin-alpha1 in combined treatment of chronic hepatitis C patients who had contraindications to ribavirin usage

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According to consensus decisions of EASL and AASL in therapy of chronic hepatitis C (CHC) patients using of alpha-interferon (IFN) in combination with ribavirin is considered as optimum. In the same time, there are some publications demonstrated that including third component tymosin-alpha1 (zadaxin) in the treatment program can increase efficiency of therapy. Besides ribavirin has negatively influence to red blood steam and contraindicated for using at patients with defects of hemopoesis - different types of anemia including clinically manifested talassemia. In such cases when patients have contraindications to using ribavirin some authors recommended to use a combination of IFN with zadaxin, which has no ability to promote any side effects development.

Having summarized our, saved up for 3 years, clinical experience of supervision of CHC patients has been treated with using zadaxin, we have found possible to give some preliminary recommendations for zadaxin application in CHC patients having contraindications to ribavirin usage.

Combined therapy of CHC patients with direct contraindications to ribavirin usage with IFN and zadaxin allowed to obtained the therapeutic effect completely comparable with effect of traditional combined therapy with IFN and ribavirin. Such effect was registered at patients with CHC caused by HCV belonged to 1b genotype too.
Clinical features of nephrotic syndrome of glomerulonephritis in children with hepatitis virus

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Combes et al. (1971) first have found that the glomerular deposition of HBsAg-containing immune complexes. Since this time many nephrologists are reporting hepatitis virus is playing an important role in the development of glomerulonephritis.

The objectives of this study were to determine clinical features of nephrotic syndrome in children with hepatitis virus.

Patients and method: 60 patients with nephrotic syndrome aged from 3-17 were studied. The 30 (50%) of them have detected hepatitis virus markers. 13 (21.7%) of them Anti-HCV, 10 (16.7%) - HBsAg, 3 (5%) - HBsAg+AntiHDV, 1 (1.7%) - HBsAg+AntiHCV, 1 (1.7%) - HBsAg+HBeAg 1 (1.7%) - HBsAg+HBeAg+AntiHDV, 1 (1.7%) - HBsAg+HBeAg+AntiHCV.

Results: The patients who have hepatitis virus 12 (40%) were treated first time, 18 (60%) were included to study on relapse. Patients with hepatitis virus have more frequent relapse, 4 (77.8%) of patients who were on relapse (p = 0.01), 8 (88.9%) of patients who were treated first time (p = 0.027).

The patients with hepatitis virus associated nephrotic syndrome had symptoms of sickening (p = 0.004), vomiting (p = 0.017), edema (p = 0.006), ascites (p = 0.038), elevated blood pressure (p = 0.05), abnormal face color (p = 0.02) and hepatomegaly (p = 0.009). Ten (33.3%) children with hepatitis virus were diagnosed chronic viral hepatitis (p = 0.028).

After standard treatment in children with hepatitis virus edema was cleared slowly that in children without hepatitis virus (p = 0.02), and they were corticosteroid resistant variant (p = 0.007).

Conclusion: Patients with hepatitis virus had clinical manifestation of nephrotic syndrome obviously; most of them had steroid-resistant variant and high frequent relapse rate. The patients with hepatitis virus were diagnosed chronic viral hepatitis, despite nephrotic syndrome.
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Hepatitis C virus infection and cryoglobulinemia – Correlation with liver histology

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Introduction: Hepatitis C virus is responsible for the majority of nonA-nonB chronic hepatitis. Chronic HCV infection can lead to systemic complications due to mixed cryoglobulinemia. Aim of the study. We wanted to find the influence of cryoglobulinemia on liver histology in patients with chronic viral hepatitis C.

Methods: We took into study 113 patients with chronic hepatitis C, admitted in our clinic between 2003-2005, which were clinically, biochemically and histologically evaluated. We also searched for the presence of serum cryoglobulin in these patients.

Results: 72 patients of our group (63.7%) had detectable cryoglobulin. Most of the patients (65.5%) were asymptomatic, major extrahepatic manifestations were present in 10.6% of the patients. Assessment of liver biopsies, ALT levels and viral load were studied in two patient groups with or without detectable cryoglobulin. No significant differences of ALT values were found between patients with and without cryoglobulinemia (83.3% vs. 85.3%). 37.5% of the patients with cryoglobulinemia and 43.9% of the patients without cryoglobulinemia had a viral load less than 800,000 UI/ml (no significant differences). 62.5% of the patients with cryoglobulinemia and 56.1% of the patients without cryoglobulinemia had a viral load over 800,000 UI/ml (no significant differences). Histological activity and Metavir scores of the patients from either group showed that the patients with cryoglobulinemia had higher degree of fibrosis -F2/F3 Metavir score- (52.8%) compared to those without cryoglobulinemia (39.1%).

Discussion/Conclusion: Patients with cryoglobulinemia and chronic hepatitis C are more likely to have progressive disease than the patients without cryoglobulinemia.
Chronic hepatitis of triple etiology: B hepatic virus, alpha1 antitrypsin deficiency and non-alcoholic steatohepatitis

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Introduction: Highlighting one cause of chronic hepatitis does not exclude the necessity of looking for other kinds of etiology that might have important consequences for the evolution of the disease and the therapy.

Methods: The paper presents the clinical evolution of a patient with chronic hepatitis of triple etiology: B hepatic virus, alpha1 antitrypsin deficiency and non-alcoholic steatohepatitis.

Results: A male patient, aged 51, with type II obesity, was admitted to our clinic for pain in the right upper quadrant of the abdomen, nausea, fatigue. Six months previous to the admission he had had increased values of the aminotranspherases. At the age of 39 he had been diagnosed with lung emphysema and bronchiectasis, when significantly increased values of the seric alpha1 antitrypsin were found. Five years ago he was diagnosed with diabetes mellitus type II (for which he received treatment based on diet) and arterial hypertension. The objective examination indicated hepatomegalia with 2nd degree consistency, without splenomegalia. Laboratory examinations revealed hyperglycemia, hypertriglyceridemia, increased values of the aminotransferases (3 times higher than normal), the existence of Ag HBs and Ag HBe. Ultrasonography confirmed the hepatomegalia, an increased ecogenity of the hepatic tissue and posterior attenuation. The histopathological picture of the hepatic biopsy puncture revealed morphological elements characteristic of the three types of etiology of chronic hepatitis: perportal inflammation, piecemeal necrosis, bridge necrosis, sabled glass cells, macrovesicular steatosis, hepatocitary swelling, rare positive Schiff hepatocitary eozinophile inclusions. Two months after diagnosis the patient died from acute myocardic heart attack.

Discussion/Conclusion: The establishment of a correct diagnosis supposes sometimes an ampel investigation that is useful for a correct treatment of chronic liver disease.
Fatigue shows a high prevalence in liver cirrhosis, especially if based on hepatitis C

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Introduction: Chronic fatigue, together with mental disorders, limitations in quality of life and alleviation of cognition, represents a common problem in chronic diseases. Patients with liver cirrhosis particularly seem to be affected by fatigue, therefore we investigated symptoms of fatigue in a population of patients with liver cirrhosis of different origin.

Methods: 43 patients with Child A - liver cirrhosis and without encephalopathy were enrolled after informed consent. 32 patients with inflammatory bowel disease and 38 healthy patients served as control groups. Visual Analogue Scale (VAS), Fatigue Severity Scale (FSS) and Fatigue Impact Scale (FIS) were used for diagnosis and grading of fatigue. Further questionnaires were used for the estimation of the influence of fatigue on quality of life, cognition and mental well-being.

Results: 51% of patients with liver cirrhosis suffered from fatigue. Interestingly, all patients with Hepatitis-C-based cirrhosis (n = 12) complained about fatigue-related symptoms. Patients with verified fatigue, in contrast to those without fatigue, showed mild to moderate depression and significant limitations in quality of life, independently from the etiology of cirrhosis. Fatigue was similarly prevalent in patients with inflammatory bowel disease, but showed a higher influence on cognition in this patient group. However, the severity of fatigue was independent from the etiology of the chronic disease.

Discussion/Conclusion: Fatigue is prevalent in about half of patients with chronic diseases of the liver and the gut, but patients with hepatitis C all suffered from fatigue. Its extent is independent from the underlying disease, but characteristics differ between patient groups.
Cardio-pulmonary morphologic and ultramicroscopic features in experimental hepatic cirrhosis in rats

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Objective: The objective of this study was to assess the differences in morphological evolution of hepatotoxicity obtained with ethanol or carbon tetrachloride (CCl₄) in rats and to evaluate the pulmonary and cardiovascular lesions associated with hepatic cirrhosis and portal hypertension.

Methods: Chronic liver injury was induced in 40 rats who received CCl₄ (N = 14), ethanol (N = 14) or saline solution (N = 12, control rats) and the morphology was evaluated at 4, 8 and 12 weeks by optically and electronically microscopic examination. For histological assessment, liver, pulmonary, myocardial, aortic and pulmonary artery samples were fixed in 4% formalin and stained with hematoxylin and eosin.

Results: The evolution of hepatic alterations was more rapidly in tetrachloride hepatotoxicity. Cirrhosis was induced after a mean period of 8 weeks in tetrachloride group and 12 weeks in ethanol group. Cardio-pulmonary evaluation showed medial hypertrophy of muscular arteries and plexiform lesions, interstitial fibrosis and parcelar hypertrophy of cardiac muscles.

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The diagnostic value of per-rectal porto-scintigraphy in liver cirrhosis patients

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Introduction: Per-rectal porto-scintigraphy (RPS) is a non-invasive, radio-isotopic method that evaluates the portal hypertension by calculating the shunt index (SI) in the porto-caval collateral vessels. The aim of this study was to evaluate the performances of RPS for the diagnosis of liver cirrhosis.

Methods: 312 patients with a previously confirmed diagnosis [202 - liver cirrhosis (LC); 69 - chronic hepatitis (CH); 17 - steatosis (S) and 24 healthy controls (C)] were studied. All of them underwent RPS (performed according with Shiomi’s original method that uses a 99m pertechnetate solution) and the SI was calculated. Sensitivity, specificity, positive and negative predictive value were calculated to assess the diagnostic value of RPS using non-parametric tests. p value less than 0.05 was considered statistically significant.

Results: The median SI value was 5% in the C and S groups, 6% for CH, and 73.5% in LC group. In the LC group, only 5.44% of the patients had a SI less than 10%, and in 79.22% of them the SI was greater than 30%. The sum of ranks of the SI values increased (p = 0.000) with the progression of the liver damage. In our series, for a SI > 30%, the Se, Sp and PPV values for the diagnosis of LC, were 75%, 100% and 100%. If SI < 5%, the NPV was also 100% for the diagnosis of LC.

Discussion/Conclusion: RPS is a simple, sensitive and non-invasive method, which is extremely accurate for the diagnosis of LC if the SI is greater than 30%.
The role of per-rectal porto-scintigraphy for the assessment of morphological changes in patients with chronic liver diseases

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Introduction: Per-rectal porto-scintigraphy (RPS) is a non-invasive, radio-isotopic method that evaluates the portal hypertension by calculating the shunt index (SI) in the portal-caval collateral vessels. Our aim was to study the relationship between RPS and morphopathological changes in chronic liver diseases (inflammation, necrosis and fibrosis, all responsible for haemodynamic alterations).

Methods: 202 subjects with liver cirrhosis (LC), 69 with chronic hepatitis (CH), 7 with liver steatosis (LS) and 21 healthy controls (HC) were evaluated by RPS. Morphological evaluation by liver biopsy was perform to all patients with CH, LS and HC, as well as to 53 of the patients with LC (for all the other, clinical and biological criteria were used for the diagnosis). The liver biopsies were evaluated using the Knodell-Ishack score. The statistical analysis was done using non-parametric tests. p value less than 0.05 was considered significant.

Results: When evaluated globally, no correlation could be found between SI and the Knodell score or one of its determinants (SI-fibrosis: r = -0.199, p = 0.09; SI-HAI: r = 0.161, p = 0.19). If the analysis was perform separately, the SI value was significantly higher (p = 0.000) in patients with ILC compared with those with CH, LS or HC.

Discussion/Conclusion: SI value is not useful to evaluate the degree of morphological changes in CH patients, but it seems to increase significantly only when cirrhosis stage is reached, so it can be used in diagnosis purposes.
Therapeutic options in diabetes mellitus, associated with liver cirrhosis

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Introduction: Liver cirrhosis’ (LC) presence at diabetics is limiting the hyperglycemia therapy options. Insulin therapy remains almost the only solution. The aim of the study was to appreciate the therapy efficiency, the hypoglycemias incidence and the patients’ compliance, after an insulin administration conventional scheme, vs. the insulin analogues administration.

Methods: There were investigated 42 diabetic patients, diagnosed with LC:
Group A (24 patients): the insulin therapy consisted in administrating two daily doses of premixed insulin 70/30, at 12 h period;
Group B (18 patients): the metabolic balance was realized by administrating prandial doses of Aspart insulin, with ultra-rapid action.
There were followed: pre-prandial and postprandial glycemies; incidence of major hypoglycemic episodes; patients’ compliance, focusing the recommended intake of carbohydrates and the meals rhythm.

Results: Group A findings: hyperglycemia, at 2h postprandial, at 6 patients (25%); hypoglycemia, at 4h postprandial, at 8 patients (34.3%); night hypoglycemia at 5 patients (22.2%); the non-compliance, focusing the free period between the insulin administration and the food intake, was present at 12 patients (50%) and the non-observance of the recommended quantity of carbohydrates was discovered at 10 patients (43.7%).
Group B findings: hyperglycemia, at 2h postprandial, at 2 patients (13.5%); hypoglycemia, at 4h postprandial, at 1 patient (5.5%); night hypoglycemia was absent; all the patients were able to adjust the insulin analogue dose to the food intake.

Discussion/Conclusion:
- The therapy option, in diabetes mellitus associated with LC, is represented by the insulin analogues, which are reducing the hypoglycemic episodes incidence.
- The absence of time between the insulin administration and the food ingestion is increasing the patients’ compliance.
The frequency of abnormal ultrasonographic findings of the liver in the infectious patients – Results of a five-year study by portable ultrasound device

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Introduction: The objective of our study was to establish, through retrospective analysis of sonographic findings obtained by portable ultrasound devices, the frequency of abnormal findings of the liver in non-selected group of infectious patients.

Methods: During five years (January 2000 - December 2004), at the Institute of infectious and tropical diseases in Belgrade, the liver was examined by ultrasonography in 3131 patients (1659 males and 1472 females, mean age 46 ± 18 years, ranging from 4 to 92 years). The examination involved the measurement of craniocaudal diameter of the right lobe, assessment of parenchymal echogenicity and homogeneity, recording of focal changes and evaluation of intrahepatic bile duct condition. Conventional portable ultrasound devices ALOKA-SSD 500 and SSD 1000 (B-mod) with convex 3.5 and 5 MHz tubes were used.

Results: The enlarged right lobe of liver (> 12.0 cm) was found in 769 (28.2%) adult patients. The liver was homogeneous in 2562 (81.8%) of cases, and diffuse non-homogeneous in 569 (18.2%) patients. The parenchyma exhibited normal echogenicity in 1868 (59.6%), and it was hyperechoic in 1224 (39.1%) and hypoechoic in 39 (1.2%) of subjects. The most common focal changes were simple cysts – 159 (5.1%), then hemangiomas – 137 (4.3%) and metastatic tumors – 102 (3.2%). Dilated intrahepatic bile ducts were detected in 134 (4.3%) patients.

Discussion/Conclusion: Development of focal or diffuse hepatic abnormalities was frequent in non-selected population of the infectious patients. The majority of them may be viewed by portable ultrasound devices, what makes these units rather applicable for large clinical-epidemiological population studies.
TGF-β and hepatocyte transdifferentiation in liver fibrogenesis

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Chronic alcohol consumption results in overrepresentation of TGF-β in the liver. TGF-β induces activation of hepatic stellate cells, deposition of extracellular matrix and hepatocyte (HC) apoptosis. Our aim was to delineate how Smad7 affects profibrogenic signaling in different liver cell types. Smad7 ko mice develop more severe fibrosis after CCl₄ intoxication compared to wt animals. We established a mouse model, in which Smad7 expression is controlled by the HC specific C-reactive protein promoter. CCl₄ treatment of animals triggered to overexpress Smad7 in HCs by LPS lack TGF-β signaling, accumulate less collagen and display improved values of damage and fibrosis as compared to controls. HCs in culture spontaneously changed cellular morphology from typical honeycomb to fibroblastic shape. This process was enhanced by TGF-β. As during epithelial to mesenchymal transition (EMT), E-cadherin was downregulated and fibronectin and vimentin were upregulated, while typical HC markers, e.g., albumin and transferrin were unaffected. In a TGF-β dependent expression profiling, regulation of further genes related to fibrogenesis and EMT were identified.

The data indicate that HCs undergo TGF-β dependent EMT like phenotypic changes during culture and blocking TGF-β signaling specifically in this cell type is sufficient to abrogate fibrogenesis.
Interleukin-1beta, tumor necrosis factor-alpha and interferon-gamma in patients with chronic hepatitis C: Relation to erythropoiesis

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Introduction: Chronic hepatitis C is an immunoregulatory disease characterized by elaboration of various cytokines and may be associated with anemia. Therefore, the present study was designed to determine the inter-relationship between serum levels of cytokines [interleukin-1beta (IL-1beta), tumor necrosis factor-alpha (TNF-alpha) and interferon-gamma (IFN-gamma)] and erythropoiesis in this disease.

Methods: To achieve this goal, 27 patients with chronic hepatitis C (13 patients were anemic and 14 patients were non-anemic) and 12 healthy subjects were included in the study. All patients had no history of bleeding. Serum levels of IL-1beta, TNF-alpha and IFN-gamma were measured using immunoenzymatic assay. Parameters of serum iron status (serum iron, transferrin saturation and serum ferritin) were also assessed. Bone marrow (BM) was examined in patients with chronic hepatitis C for cytological study and was stained by Prussian blue to detect iron granules in macrophages.

Results: Serum levels of IL-1beta, TNF-alpha and IFN-gamma were significantly higher in patients with chronic hepatitis C than in control subjects and in patients with than in those without anemia unrelated to serum HCV RNA levels ($P < 0.05$). Bone marrow examination in patients with anemia showed erythroid hyperplasia with late erythroid maturation arrest, a significant reduction in sideroblast percentage and normal BM iron stores. These findings were associated with significant decreases in serum iron levels and transferrin saturation and elevated serum ferritin levels. Serum levels of TNF-alpha (but not of IL-1beta or IFN-gamma) showed significant positive correlations with the late erythroid maturation arrest, serum alanine aminotransferase levels and histopathological grading and staging scores and inverse correlations with serum iron levels, transferrin saturation, sideroblast percentages and hemoglobin concentrations in patients with chronic hepatitis C ($P < 0.05$).

Discussion/Conclusion: Elevation of serum IL-1beta, TNF-alpha and IFN-gamma levels occurs in chronic hepatitis C. Only TNF-alpha seems to play an important role in the suppression of late erythropoiesis, disturbance of iron status and development of anemia in relation to disease activity. This immune-mediated mechanism has to be considered in the management of anemia in patients with chronic hepatitis C.
The evaluation of steatosis in patients with chronic C hepatitis

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Introduction: Steatosis has an increasingly important role in hepatitis C virus infection. Two types of steatosis are described: metabolic (associated to obesity, diabetes, hyperlipoproteinemia, alcohol) and viral, induced directly by HCV genotype3, depending on viral load and viral concentration in the liver.

Aim: Assessment of the importance of steatosis and related risk factors in progression of liver disease.

Methods: 48 retrospective patients with chronic hepatitis C, sex ratio M/W = 36/47, mean age 46.89 ± 12.98 years, were analyzed (clinical exam, biochemical tests, histological exam – Ishak scoring system). 32 patients had obesity, BMI = 35 ± 3.2 kg/m², 21 patients had diabetes and dyslipidemia, 17 patients had alcohol abuse. Steatosis was morphometric described as mild, medium or severe and correlated with ultrasound aspects (3 degrees of attenuation). Patients received antiviral treatment for low and medium viral load.

Results: 64% of patients had steatosis: microvesicular in 13.68%, macrovesicular in 9.34% and mixed steatosis in 48.78% of patients. 39.5% of patients had mild, 33% medium and 26.5% severe steatosis. Average steatosic alterations increased parallel with severity of liver disease (fibrosis), correlation index 0.65. Patients infected with HCV over 40 years old, with obesity, diabetes, dyslipidemia, alcohol abuse, had more severe steatosis (often mixed forms of steatosis). Early viral response and biochemical response at the end of treatment were obtained in 64% of patients, who proved minimal metabolic disorders.

Discussion/Conclusion: Steatosis could be seen as a histological hallmark for possible progression in chronic hepatitis C, considering the relation with fibrosis. Other conditions associated to HCV infection generating metabolic steatosis, could be also important for the prognostic, being associated to failure to antiviral treatment.
Expression of Adiponectin & AdipoR2 during the progression of NASH

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Introduction: To investigate expression of Adiponectin & AdipoR2 in the progression of non-alcoholic steatohepatitis (NASH) resulted from methionine-choline deficient diet (MCD) diets.

Methods: Rats were fed CD and CS diets. They were sacrificed at 3th, 5th, 8th, 12th week respectively. RT-PCR were performed to determine the expression of Adiponectin & AdipoR2.

Results: With development of NASH, the expression of Adiponectin & AdipoR2 decreased significantly between two groups. But no correlation with grade of liver steatosis and inflammation was observed.

Discussion/Conclusion: Adiponectin & AdipoR2 play an important role in the pathogenesis of NASH.
Predictive power of MELD score in patients undergoing TIPS for refractory ascites

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Introduction: Transjugular Intrahepatic Portosystemic Shunt (TIPS) is now well established in the treatment of complications of symptomatic portal hypertension. Child-Pugh classification is used for the stratification of the patients routinely. During the last decade other scoring systems occurred to bring a better prognostic value. MELD (Model for End stage Liver Disease) score, based only on laboratory values is one of them.

The aim of our study was to determine retrospectively the predictive accuracy of MELD score for the early mortality in comparison to Child-Pugh score in patients treated for refractory ascites by TIPS.

Methods: We evaluated 110 patients with liver cirrhosis (61% of patients with alcoholic etiology), who underwent TIPS for refractory ascites in our centre from September 1992 to December 2003. MELD and Child-Pugh score was calculated. ROC analysis for one, three and twelve month was done.

Results: Mean follow up was 23 months. Average MELD score in the whole group was (16). In patients, who died within one month the score before TIPS was 21, three months 20 and one year 18.
Using ROC(AUC) analysis, discriminant power of MELD score was superior to Child Pugh score for one (0.73 vs. 0.63) and three month (0.73 vs. 0.67) mortality. The discriminant power for one year mortality was low in both scores.

Discussion/Conclusion: MELD scoring system is a better tool for prediction of the risk of early mortality in patients with refractory ascites treated by TIPS, than Child Pugh classification. The discriminant power was low in both scores in one year horizon.

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The role of lipid profile on the antiviral therapy in chronic hepatitis C

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Combination therapy with peginterferon (PEG-IFN) and ribavirin (RV) eradicates hepatic C virus (HCV) in 50-80% of patients. The receptor for HCV has been demonstrated to be a low density lipoprotein and therefore serum LDL cholesterol may play a competitive inhibition of HCV binding.

**Aim**: To assess the role of serum lipid profile on the response to antiviral therapy in chronic hepatitis C patients.

**Methods**: 100 patients with biopsy proven chronic hepatitis C were studied. All the patients has undergone serum concentration of total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides and hepatic tests before started antiviral therapy (PEG-IFN and RV). Viral load was determined at baseline, after 3 months of treatment and at 6 months after the end of the one year of treatment.

**Results**: Early viral response (EVR) was documented in 62 patients and sustained viral response in 54. Patients with ESV and SVR were younger (42 ± 6 years) and had higher serum baseline levels of total cholesterol (256 ± 11 mg/dl) and LDL cholesterol (170 ± 9 mg/dl) than non-responders. Multivariate analysis showed that there was a strong association between response to treatment and baseline serum total cholesterol (p < 0.05), LDL - cholesterol (p < 0.001) and viral load (p < 0.001).

**Conclusions**: High baseline values of total cholesterol and LDL cholesterol, are prognostic indicators for a positive response to antiviral therapy in chronic hepatitis C patients.
Expression of MHC class I molecules and tapasin in primary human hepatocellular carcinoma

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Introduction: Major histocompatibility complex (MHC) class I molecules present short peptides, produced in cytosol through proteolytic breakdown of antigens, to cytotoxic T-lymphocytes. The stable assembly of MHC class I molecules with peptides in the endoplasmic reticulum involves several resident chaperones. One of these accessory proteins is called tapasin. It is a transmembrane protein that mediates the binding of MHC class I molecules to the peptide transporter associated with antigen processing. Tapasin also plays a quantitative role in antigen presentation, and deficient tapasin expression appears to be a frequent event in human neoplastic cells of unrelated histological origin. We here investigate the expression of MHC class I molecules and tapasin in primary hepatocellular carcinomas (HCC).

Methods: MHC class I molecules [heavy chain (HC-10) and β2-microglobulin] and tapasin expression were recognized by immunohistochemistry in paraffin-embedded and formalin-fixed specimens taken from patients free of neoplastic liver disease (n = 5), and affected by primary HCC (n = 20).

Results: All the investigated proteins were found in scattered normal hepatocytes and infiltrated T-lymphocytes. Biliary cells were also found immunopositive, while sinusoids express only MHC class I molecules, but not tapasin. Although trabecular HCCs were immunonegative, the pseudoglandular histotype was found lowly immunopositive for all the investigated antigens.

Conclusions: The obtained results demonstrate that liver parenchymal cells moderately express MHC class I molecules and tapasin. This seems linked to the liver complex organismal functions. Interestingly, pseudoglandular tumour express MHC class I molecules and tapasin. However the un-expression of these molecules in some HCCs (i.e. trabecular histotype) stimulate us to further investigate their association with the prognosis of the neoplastic disease, and to explore this peculiar behavior as tumor escape mechanism.
Increased liver mast cell recruitment in patients with chronic C virus-related hepatitis and histologically documented steatosis

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Introduction: Hepatitis C virus (HCV) is still one of the major causes of chronic viral infection worldwide, and hepatic steatosis is a frequent pathological finding in patients with chronic HCV-related diseases. It is unclear whether the steatosis is associated with host factors or the virus itself, although a consistent relationship has been found between steatosis and a necro-inflammatory reaction with the increased secretion of immunoregulators. A primary source of inflammatory mediators are mast cells (MCs) bone marrow-derived cells that are detected in both normal and diseased livers.

Methods: We determined MC density and correlated it with the fibrosis, inflammatory reaction and steatosis observed in the liver biopsies of patients affected by HCV with (n = 23) or without steatosis (n = 23). All of the histological features were assessed using a computer-aided image analysis system.

Results: There was a statistically significant difference in MC density between the HCV-infected patients with and without steatosis, with the lower mean value being detected in those without (p < 0.02). Furthermore, a non-statistically significant difference in fibrosis and inflammation between the two patient groups was found.

Conclusions: This is the first study showing a significant increase in MC density in the tissues of patients with chronic HCV infection and histologically documented steatosis.
Development of intrahepatic bile ducts by detection of cytokeratin 7

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Introduction: In the light of recent advances of medico-biological sciences there are a number of papers covering liver development, including development of bile ducts. There are 2 theories describing development of bile ducts, Hammar’s theory, according to which bile ducts invade into liver through liver hilum, and 2nd, Desmet’s, prevailing one, elucidates bile duct development by transformation of hepatoblasts. Transformational theory based on cytokeratin 19 expression, but cytokeratin 7 – second biliary phenotype marker is completely ignored. The purpose of our research work is: studying development of intrahepatic bile ducts by immunohistochemical detection of cytokeratin 7 during human ontogenesis.

Methods: Human embryos and fetuses were obtained after informed consent from legal voluntary abortions. Embryos and pieces of fetus liver were fixed in formalin and embedded in paraffin using routine procedures.

Results: Our results showed that on 11-12th week first cytokeratin 7 expressing cells are emerging near large vessels, until that no cytokeratin 7 positive cell are detectable. Nearly at the same time hepatic artery enters into liver through liver hilum, shown in our previous results. After that cytokeratin 7 positive cell form groups, on 13.5 week they make tubular structures. Later cytokeratin 7 positive cells and ducts are also seen in portal tracts in the periphery of the liver.

Discussion/Conclusion: Our results supplies Hammar’s theory and suggest re-evaluation of transformational theory.
Extrahepatic manifestations and therapeutic options at patients with autoimmune hepatitis

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Introduction: The aim of our study was to estimate the frequency of extrahepatic manifestations (CAD) and to evaluate the clinical and biological response of treatment.

Methods: We studied 88 patients (62 females/26 males, mean ages 43.2 years) with autoimmune hepatitis (AIH). The diagnosis of AIH was based on international criteria, including biochemical tests, autoantibodies and liver tissue morphology.

Results: AIH type I was present in 52 patients and type II in 36 patients. CAD was present at 32 patients with AIH type I and at 24 patients with type II. The incidence of CAD in AIH type I was: autoimmune thyroiditis (15.62%), rheumatoid arthritis (18.75%), type I diabetes mellitus (37.5%), Sjogren’s syndrome (9.37%), vitiligo (12.5%), polyarteritis nodosa (6.26%). At AIH type II was observed: thyroiditis (20.84%), rheumatoid arthritis (16.67%), I type diabetes mellitus (33.33%), vitiligo (8.33%), vasculitis (12.5%), glomerulonephritis (8.33%). Type I diabetes was diagnosed before AIH type I in 4 cases and in 8 cases it developed after diagnosis was established. In AIH type II all the cases of diabetes was diagnosed after AIH. Most patients with AIH type I were treated by only corticoids (40 mg/day with a reduction of 10 mg every 5 days) and 23 patients follow up associated treatment: corticoids and azathioprine. After diabetes was diagnosed the steroid doses were lowered and subsequently stopped. After 12 months, 42 patients had normal liver biochemistry, 6 had partial response and 4 no response. In AIH type II, 32 patients had therapeutic success and 4 had partial response.

Discussion/Conclusion: The frequency of extrahepatic manifestations in AIH was relatively high. Azathiprine and prednisone associated therapy was effective and safe.
A statistical analysis of risk factors in non-alcoholic steatohepatitis

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Introduction: The aim of our study was to evaluate de causes of non-alcoholic steatohepatitis (NASH).

Methods: A retrospective analysis of 111 patients from Emergency Clinical Hospital Craiova with steatohepatitis was conducted between June 2003 and January 2005. We excluded 43 patients with viral or alcoholic etiology. Only cases with non-alcoholic steatohepatitis (NASH) were studied. The diagnosis of liver steatosis was based on the correlation of histologic and clinical findings. Routine ultrasound was performed using a 3.5 MHz transductor. Statistical analysis using Wilcoxon and Kruskal-Wallis test with p < 0.05 was considered statistically significant.

Results: We studied 68 patients (47 males and 21 females) with NASH. The mean age was 41.6 ± 2.5 years. The causes of NASH were: type II diabetes mellitus in 17 cases, obesity (grd II – 5 cases, III – 8 cases, IV – 13 cases), insulin resistance syndrome – 4 cases, drugs – 2 cases. Lipid profile was abnormal in 19 cases: 10 cases with hypercholesterolemia, 3 cases with hypertriglyceridemia and 6 with both. All patients had hepatomegaly and only 4 had splenomegaly. A number of 26 patients had elevated serum aminotransferases, but 42 had normal level. We found a statistically significant linear correlation between BMI and the severity of steatosis (p = 0.007). Also, we identified a strong relationship between durate of diabetes evolution and severity of steatosis (p < 0.0001). We could not establish a correlation between the values of serum aminotransferases and others parameters, but multivariate analysis showed that the BMI > 28 kg/qm and elevation of serum ALT were associated with steatosis grade.

Discussion/Conclusion: The patients with NASH had a high incidence of normal functional liver tests. The hepatic biopsy remains the most accurately criteria of diagnosis in NASH.
Therapeutic possibilities to the patients with overlap syndrome (autoimmune hepatitis + primary biliary cirrhosis)

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Introduction: The study had as a purpose the analysis of the way of answering of the overlap syndrome patients to various therapeutic schemes.

Methods: In the present study was included a number of 48 patients of overlap syndrome autoimmune hepatitis + primary biliary cirrhosis (AIH + PBC) an whom were determined the specific antibodies and were performed hepatic biopsies (20 cases).

Results: The used therapeutic schemes are: prednisone 30 mg/day - 6 months, then 15 mg/day - 6 months; prednisone + ursodeoxycholic acid 15 mg/kg/day; ursodeoxycholic acid 15 mg/day - 6 months; azathioprine 50 mg/kg/day - ursodeoxycholic acid 15 mg/kg/day.
The evolution was followed through the determination of transaminases, serum level of atc. and gamma globulins.
On the patients comprised in the study the usage of only prednisone was not enough. The usage in monotherapy of the ursodeoxycholic acid to the patients in which the cholestatic syndrome prevailed has masked the autoimmune symptomatology, the disease progressing.

Discussion/Conclusion: We consider that the usage in associated therapy of immunosuppressors with ursodeoxycholic acid to be attempted to these patients. It still remains the question: for how log?
The treatment in patients with cirrhosis decompensated an spontaneous bacterial peritonitis

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Introduction: The study had as purpose the identification of an antibiotic efficient therapy to the patients with cirrhosis and spontaneous bacterial peritonitis.

Methods: In the present study were admitted 30 patients with hepatic cirrhosis and spontaneous bacterial peritonitis. The diagnosis was clinical suspected and biochemical confirmed (patient known cirrhosis, fever, local abdominal pain and tenderness, leukocytosis, ascitic polymorph count excess 250 cells mm³).

Many therapeutic schemes were used: amoxicilinum + acid clavulanic 2.2 g/day – 7 days; then 2 g/day – 14 days; imipenemum + cilastatinum (Tienam®) 1000 mg/day – 7 days; ciprofloxacinum 200 mg/day i.v. and 200 mg/day oral 7 days, cefoperazonum (Medocef®) 4 g/day - 7 days then 2 g/day - 7 days.

Results: In 15 of the patients the liquid culture was positive: 7 cases with E. coli; 3 cases with Klebsiella; 1 case with Enterococcus; 4 polymicrobian cases. In all other cases the culture were negative.

The evolution was favourable in 18 patients; 4 patients have developed renal insufficiency and the other 8 have presented the aggravation of the general state.

From 18 patients with favourable evolution, 4 have presented a new episode during a year. To prevent recurrent spontaneous bacterial peritonitis we used rifampicinum (Normix®) 15 mg/kg/day - 2 months or norfloxacinum 400 mg/day - 2 months.

Discussion/Conclusion: The best clinical and biochemical evolution was present to the patients with cephalosporins treatment.
Modifications of the glycemic equilibrium in the patients with liver cirrhosis

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Introduction: Liver cirrhosis is often accompanied by a disturbed carbohydrate metabolism similar to type 2 diabetes.

Methods: 430 patients with liver cirrhosis were included in this prospective study. Cirrhosis was confirmed by clinical, biochemical tests and histology (210 patients). To investigate disturbed carbohydrate metabolism in relation the Child Pugh score, chronic liver failure and hepatic encephalopathy.
To clarify this disturbed we measured, fasting blood glucose concentration, oral glucose tolerance test (OGTT), fasting serum insulin concentration and often oral glucose, the Quantitative Insulin Sensitive Cheek Index (QUICKI) and Insulin Resistance Index.

Results: The prevalence of diabetes mellitus was 19% to the subjects study with liver cirrhosis (p < 0.001).
In liver cirrhosis the prevalence of diabetes mellitus was significantly high in HCV related cirrhosis (18.86%) and alcoholic cirrhosis (15.66%, p < 0.001). Diabetes mellitus was more frequently occurred in Child’s C (30.4%) then in Child B (13.86%) and Child A (7.81%), p < 0.001, p (Chi 2) < 0.0001, V de Cramer 0.277.
The level of plasmatic insulin was clearly high in the cirrhotic subjects with diabetes mellitus (p < 0.0001).
The indice of resistance and the sensibility indice were corelated to the functional Child class (p < 0.001).
The PLSD Fisher analyses has shown the significant difference for the indice of glucose extraction according to the functional Child class (significance level 5%).

Discussion/Conclusion: The risk of diabetes mellitus increases with the progression of liver disease, and HVC infection and alcohol are additional risk factor for diabetes mellitus.
In our opinion, insulin remains the best chose of treatment in the advanced cirrhotic disease leading to overt diabetes mellitus.
Epidemiological study regarding patients with B and C chronic viral hepatitis, candidates for antiviral treatment

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Introduction: The diagnosis of HBV and HCV infection represents a difficult problem due to the pattern of the disease, mostly asymptomatic. The aim of the study is to compare a batch of patients with HBV, vs. HCV infection, which fulfilled the standard pathological and virological criteria for antiviral treatment (RNA-HCV detectable, DNA-HBV over 100.000 UI/ml and HAI Knodell score over 5).

Methods: We included 256 consecutive patients who performed liver biopsy in our department for viral hepatitis. 4 of them had B+C and 2 - B+D chronic hepatitis and were excluded from the batch. Of the remaining 250 subjects, 12 (4.8%) had HBV infection and 238 (95.2%) HCV infection.

Results: We compared the batch with HBV infection vs. HCV infection, obtaining the following results B/C: mean age 33.5/49.9 years (p < 0.0001 - extremely significant), men 58.3%/35.28% (p < 0.001 - significant), women 41.7%/64.72%, mean Knodell score: HAI – 9.09/10.45 (p = not significant) and F - 0.8/1.53 (p < 0.0001 - extremely significant).
Primary biliary cirrhosis – Effect of ursodeoxycholic acid on the evolution

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Introduction: The purpose of our prospective study was to investigate the long-term effects of ursodeoxycholic acid (UDCA) on the evolution of primary biliary cirrhosis (PBC).

Methods: We included 21 histologically proven PBC subjects (19 F, 2 M) with a mean age of 43.6 years (range 28-68), in various stages of the disease (I-II and III). UDCA was administrated 10mg/kg body weight daily for at least 6 months. We evaluated the subjects clinically and biochemically before and 6 months after starting of the treatment.

Results: 9 (53%) patients reported clinical improvement of the symptoms (weakness and fatigability), 8 (47%) reported reducing of the skin itching. There was a significant improvement in liver biochemistry: γGT (p = 0.015), serum alkaline phosphatase (p = 0.025), ALT (p = 0.05), AST (p = 0.03), bilirubin (p = 0.05). There was no worsening of clinical or biological status and no side effects.

Discussion/Conclusion: In our study UDCA had a favorable effect on the clinical and biochemical evolution (alkaline phosphatase, γGT, bilirubin, aminotransferases) of PBC patients. No side effects or worsening of clinical and biochemical status were encountered during the first 6 months of treatment.
Drug-resistant mutants in children with chronic hepatitis B treated with lamivudine

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Introduction: The aim of the study was to evaluate the genotype of HBV and the emergence of lamivudine-resistant HBV polymerase mutants in children with chronic hepatitis B in the course of the lamivudine therapy.

Methods: 12 children (8 boys – 66.7%, and 4 girls – 33.3%), aged from 6 to 15.0 years (mean 10.2 years) with chronic hepatitis B were included in the study. All patients were treated with lamivudine tablets, 100 mg daily given orally for 12-16 months. All amino acid substitutions within HBV polymerase were detected by PCR amplification and direct sequencing. HBV genotypes and polymerase mutants were determined by comparing the sequences in the overlapping Pol/S genes with published sequences, available in GenBank.

Results: All analyzed children with chronic hepatitis B were infected with genotype A; no change of the type of HBV genome was noted during the lamivudine therapy or after the cessation of the treatment. Positive response for the lamivudine therapy was achieved in 25% children with chronic hepatitis B. The emergence of lamivudine-resistant mutations involving the YMDD motif was detected in 25% patients; all these patients were non-responders to the therapy. Predominant mutations in the YMDD motif were YVDD and YIDD, which were accompanied by other amino acid changes in the viral polymerase. Apart from the mutations in YMDD motif, additional mutation at amino acid position 207 (rtV207V/I/M) was detected.

Discussion/Conclusion: No change of the type of HBV genome was noted during the lamivudine therapy or after the cessation of the treatment. Predominant mutations in the YMDD motif were YVDD and YIDD and they were accompanied by other amino acid changes in the viral polymerase.
Autoimmunity in children with chronic hepatitis C treated with interferon alfa and ribavirin

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Introduction: The role of interferon alpha or the virus itself in the pathogenesis and the risk of autoimmunological disorders in children infected with HCV, still remain unknown.

Methods: Twelve children (mean age 11.8 years) with chronic hepatitis C were treated with interferon alfa and ribavirin for 12 months. Blood count, biochemical tests and indirect immunofluorescent assays for serum autoantibodies (ANA, SMA, LKM-1) were performed monthly during the therapy.

Results: In 4 cases autoantibodies were present in low titers prior to the treatment and they had no prognostic value for the response for the therapy or the risk of autoimmunological disorders. Positive response for the treatment was achieved in 4 cases. In 3 cases indications for discontinuation of the therapy were established: myelosuppression in 1, 1 presented with myocarditis and 1 occurred with features of autoimmunological hepatitis. Furthermore during the therapy with interferon alpha and ribavirin, in 2 children transient elevation of serum titers of antibodies to liver-kidney microsome type 1 (anti-LKM1) (> 1:640) with no abnormalities in gammaglobulin levels, was noted.

Discussion/Conclusion: The presence of autoantibodies in low titres was detected in 30% of children with chronic hepatitis C prior to the therapy and they did not effect the outcome of the therapy: positive response to the treatment or the emergence of autoimmunological disorders, which were rare in the studied group of children.
Quality of life at the chronic hepatitis B

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Introduction: Questions of patients’ self-estimation of the condition are the priority directions at the present stage of development of medicine.

Methods: Parameters of quality of life (QL) using questionnaire WHOQL-100 at 135 patients with a chronic virus hepatitis B (HBV) are investigated. Patients used criteria of standard 24 scales with the numerical score, including physical, psychological, spiritual spheres, a level of independence, social relations, a human environment.

Results: The analysis of test WHOQL-100 at patients HBV revealed decreasing of QL - at calculation of a final total score, and also at a level of other criteria - physical, psychological, spiritual spheres, vitality, a level of independence, social relations, human environment. The final total score at HBV was 75.6 ± 1.88 (p < 0.05) in comparison with the control of 91.7 ± 1.92 points. At the analysis of parameters “General Quality of Life and Health” the most significant was decreasing on scales “F2. Vitality”, “F3. Sleep and rest”, “F4. Positive emotions”, “F10. Daily activities”, “F11. Drug dependence”, “F12. Ability to work”, “F14. Social support”, “F15. Sexual activity”, “F21. Rest and entertainments”.

Conclusion: Dependence between duration HBV and level QL was established: at 2-5 years anamnesis with a total score of 79.8 ± 2.18 points with decreasing of all subspheres parameters At duration HBV more than 8 years decreasing of QL is connected to the expressed restrictions practically on all studied spheres.
Robust stimulation of anti-core CTL activity by AAV/core gene delivery into dendritic cells

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Introduction: Many cases of chronic hepatitis C virus (HCV) infection are resistant to conventional therapies. Such cases might be treated by cell-mediated immunotherapy as cytotoxic T-lymphocytes (CTL) are the main mechanism by which viral infections are cleared. The HCV core gene, being well conserved among HCV types, may be an appropriate antigen for targeting HCV-infected cells. However, two regions within core can stimulate autoimmunity.

Methods: A series of five recombinant adeno-associated virus (rAAV) vectors, carrying the full length (aa1-190) or autoimmune domain-depleted (AIDD) versions of core were used to load dendritic cells (DC) which, in turn, stimulated anti-core CTL.

Results: The AAV vectors were found to be able to transduce 88-95% of DC and the transduced DC displayed higher levels of CD80, CD83, CD86, and CD1a over controls. One vector, AAV/core(49-180)/Neo, with both autoimmune domains deleted from core, stimulated comparable core-positive target killing to the other versions, yet stimulated significantly lower levels of “self” killing of core negative-targets, either of autologous PBMC targets (P = 0.002), or HLA-matched HepG2 liver cancer cells (p = 0.001). The resulting CTL populations displayed higher IFN-expression, higher CD8:CD4 ratios, and lower CD56:CD8 ratios than controls. The rAAV-loading derived CD8+ T-cells had more CD69+ cells and the CD4+ T-populations had fewer CD25+ cells than controls.

Conclusions: AAV/core(49-180)/Neo, containing a dual AIDD core gene, may be particularly useful for clinical treatments as it stimulates lower “self” recognition but stimulates robust anti-core CTL activity.
Pituitary tumor transforming gene 1 is expressed in Kupffer cells, activated T-lymphocytes and a proportion of primary liver cancer cells

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Introduction: Despite the impressive advancement in the cellular and molecular knowledge on the hepatocellular carcinoma (HCC), it still remains one of the most incident neoplasia world-wide. HCC is today universally established as a heterogeneous disease encompassing a variety of pathological entities and a range of clinical behaviours. This acquired general statement is underpinned at the molecular level by a complex array of genetic alterations that affect cellular processes. Here we investigate the expression at the protein level of the oncogene Pituitary Tumor Transforming Gene 1 (PTTG-1) in tumoral and non-tumoral HCV-related hepatitic tissues.

Methods: By use an immunoperoxidase-based staining assay we investigated the presence of PTTG-1 (Zymed Laboratory Inc. South San Francisco, CA), in 2 µm thick liver tissues sections taken from patients affected by HCV-chronic liver disease (n = 20) and primary HCC (n = 20). A co-expression of PTTG-1 and CD68 (Dako, Milan, Italy) was also evaluated by using a double immunohistochemical procedure.

Results: Three cell types were found immunopositive for PTTG-1: a) macrophagic Kupffer cells identified in hepatitic and cirrhotic liver tissues, and those located around the neoplastic lesion; b) a sub-population of T-lymphocytes recognized in inflamed portal areas; and c) a proportion of neoplastic liver cells.

Conclusions: The PTTG-1 is a mitotic checkpoint protein which inhibits sister chromatid separation during mitosis. PTTG-1 was originally isolated and characterized in anterior pituitary tumours, and subsequently found highly expressed in many other cancers of unrelated histological origin. Our findings all suggest a wide expression of PTTG-1 in liver at the protein level being expressed in both tumoral and non-tumoral cells. Furthermore, the co-expression of PTTG-1 and CD68 in Kupffer cells recognized in hepatitic and cirrhotic tissues, and those located around the tumoral lesion suggest PTTG-1 as a new liver macrophagic cytological biomarker.
Immunohistochemical study of dendritic cells in hepatocellular carcinoma

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Introduction: Dendritic cells (DC) are antigen-presenting cell that initiate and regulate immune responses, including antitumor immunity.

Methods: In this study we examined immunohistochemically the distribution of DC subsets (S-100⁺, HLA-DR⁺, CD83⁺) in primary tumor and extratumoral liver tissue of 10 patients with hepatocellular carcinoma (HCC).

Results: S-100⁺ DCs were localized mainly in clusters between tumor nests and at the tumor border with the surrounding liver tissue, while HLA-DR⁺ DCs were more in number and were diffusely distributed in HCC stoma and at the tumor border. The mature CD83⁺ DCs were less in number in tumor tissue (3.74 ± 4.0) than S-100⁺ (9.76 ± 13.7) and HLA-DR⁺ DCs (10.51 ± 8.1, p = 0.008, Wilcoxon Signed rank test). We observed more S-100⁺ and CD83⁺ DCs in the sinusoids and portal tracts of the surrounding liver tissue in HCC than in control liver tissue (p = 0.001). The numbers of S-100⁺ and CD83⁺ DCs in primary tumor and in extratumoral regions were greater in HCC patients with cirrhosis, compared to those without cirrhosis (p = 0.044, for S-100⁺ DCs, Mann-Whitney U test).

Discussion/Conclusion: These results suggest activation of defence immunological mechanisms induced in the host bearing HCC.
C-met-positive mesenchymal cells are possible source of hepatocytes during human liver development and regeneration

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Numerous reports indicative of hepatocytes development from hematopoietic stem cells or mesenchymal cells now. Nevertheless exact location and phenotype of regional liver stem cell is not known yet. C-met is receptor of Hepatocyte Growth Factor which stimulates development and growth of hepatocytes. Aim of our study was investigation of expression of C-met in human liver during its prenatal development and regeneration in chronic hepatitis.

Liver biopsies (n = 68) from patient with chronic hepatitis and 62 embryos and fetuses (4-20 week of gestation) after legal medical abortions and miscarriage were examined immunohistochemically with antibodies to C-met.

In embryonic liver C-met presents in small mesenchymal cells of ventral mesentery and transverse septum (very strong expression), in small hepatocyte-like cells situated in parenchyma between hepatoblasts (strong expression) and within hepatoblasts (weak expression). We suppose that mesenchymal C-met+ cells can be regional progenitor cells and differentiate into hepatoblasts; appearance of this protein in mesenchymal cells is evidence of beginning of their epithelial differentiation. Cholangiocytes didn’t express C-met during pregnancy.

During liver regeneration in case of grave chronic hepatitis we also observed small C-met-positive cells in necrosis and inflammation areas and weak expression of this protein in single hepatocytes. So, if regenerative potential of hepatocytes is depleted, regional mesenchymal stem cells can be activated and they start express C-met, indicated their differentiation into epithelial cells - hepatocytes.
Detectability of autoantibodies (SLA & AMA) according various diagnostic methods

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Introduction: To assess detectability of anti-SLA and AMA in patients with AIH or PBC according indirect immunofluorescence and ELISA.

Methods: Retrospective evaluation results of autoantibodies from 2000 to 2004. Sera were evaluated on rodent liver/kidney/stomach and on HEp2 cells by IIF. Anti-SLA and anti-M2 were tested by ELISA.

Results: 150 sera from 96 patients with AIH and 103 sera from 78 patients with PBC were tested anti-SLA. 31 sera (from 16 patients) in AIH were positive (16%) by ELISA, but in compare to IIF were detectable only in 9 pts. In PBC 5 sera (from 5 pts) were positive (6.4%) by ELISA, but none by IIF. We compared detectability of AMA (76 sera) by both methods. 73 were positive by both, 3 sera were anti-M2 ELISA negative but IIF positive, and 4 sera were positive by ELISA but negative by IIF. Intensity of immunofluorescence did not correlate with values by ELISA. We analysed usefulness combination of IF substrates (rodent and HEp2 cells). From 76 sera in PBC were AMA detectable on both in 75, but HEp2 increased detectability of ANA in 21 sera. Only 1 serum was isolated ANA positive.

Discussion/Conclusion: It is difficult to evaluate anti-SLA on rodent tissues, therefore all suspected sera must be tested by ELISA. We found serologically 6.4% patients with overlap syndrome. Rodent tissues are valuable for the optimal detection AMA, but combination with HEp2 cells increases detectability ANA associated in PBC. In the suspicion of “low-level AMA” we recommend combination of anti-M2 ELISA with lower titration of sera by IIF.
Activation of hepatic stellate cells during fibrosis: Comparison of the CYP2D6 model for autoimmune hepatitis and CCl₄ injection

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Periportal fibrosis is one of the defining parameters of autoimmune hepatitis. Only little is known about the mechanisms involved in the development of fibrosis during this severe human autoimmune disease. We used the virus induced CYP2D6 model system to investigate the activation of hepatic stellate cells (HSC) and the kinetics of fibrosis in comparison with the well established CCl₄-induced fibrosis model. CYP2D6 transgenic mice express the human Cytochrome P450 2D6 under its own promotor in the liver and do not develop liver damage unless infected with an Adenovirus-CYP2D6 vector. We found that in the CYP2D6 model fibrosis, as detected by Sirius Red and anti-Collagen I IgG staining, was mostly subcapsular resulting in the fusion of individual lobules. After 10-12 weeks post-infection, weak periportal fibrosis became apparent. In contrast, in CCl₄-treated mice the kinetics of extracellular matrix deposition were much quicker resulting in periportal fibrosis already after 3-4 week of CCl₄ administration. At later times, fibrosis was much more pronounced in CCl₄-treated mice compared to virus-infected CYP2D6 mice. However, subcapsular fibrosis was not as dominant in CCl₄-treated mice. Activation of HSCs could be detected by staining for α-smooth muscle actin (αSMA) in tissue sections of both CCl₄-treated mice and virus-infected CYP2D6 mice. In addition, isolation of HSCs revealed an enhanced activation status (decreased amount of oil droplets, de novo αSMA expression) in CCl₄-treated mice and virus-infected CYP2D6 mice. Our data indicate that Adenovirus-CYP2D6-infected CYP2D6 mice display subcapsular and periportal fibrosis that correlates with an activation of HSCs similar to CCl₄-induced fibrosis. Thus, the CYP2D6 mouse will be a good model system to further investigate the molecular mechanisms involved in fibrotic events during autoimmune hepatitis.
Endoplasmatic reticulum stress induced by hepatitis C virus following orthotopic liver transplantation

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Introduction: Hepatitis C virus (HCV) induces ER stress and higher expression of ER chaperons. Our aim was to characterize the expression of chaperons in patients after HCV-related orthotopic liver transplantation (OLT) in association with high (> 8.78 x 10^6 copy/ml) and low (< 8.78 x 10^6 copy/ml) copy number of serum HCV. HCV titer over this cut-off point was described to indicate an early recurrence.

Methods: 33 formalin-fixed, paraffin-embedded liver biopsy specimens (10 cases with high and 15 with low HCV titer, respectively, 8 cases with acute rejection) and 8 normal liver samples were analysed by real-time RT-PCR for mRNA expression of XBP1, ATF6, HSP27, GRP94, GRP78, calnexin and calreticulin. Relative quantification was performed using GAPDH as reference gene.

Results: Compared to normal liver, expression of ATF6, GRP94, GRP78, calnexin and calreticulin were significantly (p < 0.05) higher in patients with high (5.8-, 5.4-, 3.0-, 2.9-, 3.1-folds) and low (3.7-, 5.1-, 3.3-, 3.3-, 2.4-folds) HCV titer. Comparison between cases with high and low HCV titer did not reveal significant differences. Expression of XBP1, ATF6, HSP27, GRP94, GRP78, calnexin and calreticulin were significantly upregulated in patients with acute rejection compared to normal liver (7.3-, 13.3-, 3.2-, 24.3-, 22.9-, 16.4-, 11.9-folds).

Discussion/Conclusion: Higher expression of chaperons were detected in patients with acute rejection, high and low HCV titer compared to normal liver. ER stress may occur in patients after OLT in association with HCV recurrence and acute rejection. Upregulation of chaperons might be mediated through the ATF6 signaling pathway.

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The CYP450 mouse model for autoimmune hepatitis: Breaking of self-tolerance in transgenic CYP2D6 and wild-type FVB mice by viral infection

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The etiology of autoimmune hepatitis (AIH) is poorly understood although the major autoantigen, cytochrome P450 2D6 (CYP2D6), has been identified and immunodominant epitopes mapped. Therefore, we generated an animal model for human AIH using the natural autoantigen CYP2D6. We infected transgenic mice expressing human CYP2D6 in the liver (CYP-2D6 mice) with an Adenovirus-CYP2D6 vector (Ad-2D6) to break self-tolerance. Surprisingly, upon infection with Ad-2D6 not only transgenic CYP2D6 mice but also wild-type FVB mice showed several persistent features characteristic for liver damage associated with AIH. These features included massive hepatic fibrosis, ‘fused’ liver lobules, disorganized architecture, cellular infiltrations, elevated serum aminotransferase levels, and focal to confluent necrosis. Further, all Ad-2D6-infected mice (CYP2D6 and FVB) generated high titers of anti-CYP2D6 antibodies. Epitope mapping revealed that such anti-CYP2D6 antibodies predominantly recognized the core peptide sequence AQPPRD (CYP2D6 aa265-270), which is the immunodominant linear epitope recognized by LKM-1 antibodies from AIH patients. In contrast, mice infected with a control Adenovirus expressing green fluorescence protein (Ad-GFP) did neither develop liver damage nor generated anti-CYP2D6 antibodies. Interestingly the kinetics and severity of liver damage as well as antibody formation was enhanced in wild-type FVB mice compared to transgenic CYP2D6 mice. Our data indicate that the autoimmune liver damage was reduced and delayed in transgenic CYP2D6 mice due to a certain degree of tolerance towards human CYP2D6 compared to wild-type FVBs, which do not express the human version of the 2D6 isoenzyme. In wild-type FVB mice, due to the homology of the mouse isoenzymes of the CYP superfamily to human CYP2D6, autoimmune liver damage by Ad-2D6-infection was possibly induced via true ‘molecular mimicry’.
Cirrhosis hepatis in a young adult

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Introduction: Cirrhosis hepatis in juvenile patients can result from infectious, inherited, metabolic diseases, toxic agent exposition and can be cryptogenic. Owing to regeneration capacity of the liver cirrhosis clinically manifests later and only elevated liver enzymes may draw attention to liver injury.

Methods: A 21 years old women, with the history of EBV caused liver injury, from the 16th week of her pregnancy had been observed for a few times because of elevated liver enzymes. The diagnosis was cholestasis of pregnancy. 5 months to the childbirth anasarca, jaundice and ascites developed. The hepatologist initiated detailed out-patient examination. 2 weeks later she was admitted to our department because of deterioration of physical condition. Ultrasonography, laboratory parameters and liver biopsy confirmed cirrhosis hepatis, but immunserology, specific metabolic parameters were negative. Since histology revealed inflammatory activity also and etiology remained unknown the diagnosis was cryptogenic hepatitis and cirrhosis hepatis.

3 weeks later the patient was observed with diarrhoea, severe jaundice, azotaemia, anaemia and was sent forward to Hepatology Department because of progressive liver failure. Acute exacerbation of chronic liver disease contraindicated hyperacute liver transplantation. Despite high dose steroid therapy, haemodialysis, thrombocyte, erythrocyte, ion, fluid, glucose substitution the patient died.

Discussion/Conclusion: By this case the authors would like to emphasize that asymptomatic liver injury of juvenile patients may refer to serious liver disease like cirrhosis hepatis therefore early, detailed examination is needed.
Depression in patients with chronic hepatitis C receiving interferon-alpha plus ribavirin therapy

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Aims: To assess the prevalence and risk factors of depression in interferon (IFN)-treated patients with chronic hepatitis C (CH-C) and to evaluate efficacy and tolerability of antidepressant therapy.

Methods: We studied 91 patients (32 male, 59 female, mean age 37.1) receiving 1-year course of IFN-alpha and ribavirin therapy for CH-C. Exclusion criteria: evidence of cirrhosis, pre-existing psychiatric and severe somatic diseases. All patients underwent psychiatric assessment before starting and monthly throughout the antiviral treatment using the Montgomery-Asberg Depression Rating Scale, the Clinical Global Impressions Scale and the Beck Depression Inventory. Efficacy of treatment with standard doses of tianeptine or mirtazapine were evaluated weekly in 39 patients.

Results: 39 (42.8%) patient developed depression. There were not statistically significant correlations between depression development and age, sex, HCV genotype, viral load, ALT/AST activity, histological activity index, duration of CH-C and doses of IFN alpha. Depression was successfully treated with antidepressant drugs: 80% of patients responded during 12 weeks of therapy. Tests of liver function showed no significant changes. All patients completed the antiviral treatment.

Conclusions: IFN-induced depression is often in CH-C patients. Predictive risk factors of development of depression were not revealed. Monitoring and psychopharmacological management of depression are mandatory in order to maximize antiviral treatment adherence.
Epidemiological study and evaluation of prognostic factors of survival in patients with hepatocellular carcinoma

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Introduction: In Romania more than 85% of HCC are due to cirrhosis caused by HCV or/and HBV infection. The aim of the study was to determine the prevalence of HCC, to compare conventional US, CT scan and MRI in detecting hepatic masses and to evaluate the survival of patients with advanced HCC.

Methods: 135 patients were enrolled between 2000-2005. Conventional US, CT scan and MRI or arteriography were performed routinely.

Results: The rate of carcinogenesis was 10%. HCV represents the main etiological factor, while the association between HCV and positive antiHBc antibodies (HBs antigen negative patients) holds third place after HBV. Hypoechoic lesions correlate with more advanced disease. Patients with less than 3 lesions smaller than 5 cm were treated by percutaneous alcoholisation with similar results (complete necrosis) as chemoembolisation but with fewer accidents (one case – rupture of tumour vs. 3 cases of upper digestive hemorrhage and 2 cases of aggravation of hepatic insufficiency in patients with chemoembolisation). We noticed the presence of portal vein thrombosis in 10 patients.

Discussion/Conclusion: The incidence of HCC is 9.99/100,000 inhabitants for males and 5.29/100,000 for females. The value of GGT is superior to AFP in the detection of HCC. The 4-year survival rate is comparable in patients with percutaneous alcoholisation and with chemoembolisation and is influenced by the number and dimensions of the tumours, portal vein thrombosis and Child class.
The clinical and biological features of patients with chronic hepatic C which have extrahepatic manifestations

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Introduction: To assess the prevalence of extrahepatic manifestations of hepatitis C virus infection and identify the clinical and biological features of their patients.

Methods: We studied 338 patients with chronic hepatitis C (CHC) which were admitted and treated our department. The extrahepatic manifestations assessment were achieved from: anamnesis, clinic examination, dermatological, neurological, and ophtalmic exams, lung X-ray, spirometry, electrocardiogram, abdominal ultrasound exam, haematological, biochemical, and immunological tests.

Results: Between patients with CHC admitted and treated for a 2 years period, only 90 pts. (26.6%) have extrahepatic manifestations. The incidence of these manifestations were: 14.7% (50 pts) with cutaneous manifestations, 20.2% (69 pts) with articular manifestations, 3.5% (12 pts) with respiratory manifestations, 4.43% (15 pts) with cardiovascular determinations, 7.9% (27 pts) with xerostomia, 10.65% (36 pts) with nephrologic determinations, 15.9% (54 pts) with parestesis, 1.7% (6 pts) with thyroid manifestations, 0.8% (3 pts) with ocular manifestations, 16.5% (56 pts) with diabetes mellitus, 8.8% (30 pts) with haematological abnormalities, and 25% (85 pts) with immunological abnormalities.

Discussion/Conclusion: The patients with CHC were developed numerous extrahepatic manifestations, the most frequent being the articular, neurological, nephrological, and metabolic manifestations. These patients associated numerous immunologic abnormalities, the most frequent being the presence of cryoglobulinemia, rheumatoid factors, antinuclear antibodies, and high serum IgG levels.
Focal nodular hyperplasia in children – Own experience

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Introduction: Focal nodular hyperplasia (FNH) is a benign tumor of the liver, often discovered incidentally. It occurs mainly in women between age 25-44. Though it is rarely reported in children the evidence has been growing in the recent years. We present our experience of FNH in pediatric cases.

Methods: 10 children (6 girls 4 boys), aged 6-17 years were hospitalised in Children’s Memorial Health Institute between 2000 and 2005, with provisional diagnosis of FNH.

Results: The final diagnoses in 3 children were based on radiological examination (ultrasonography and computered tomography and/or magnetic resonans imaging) exclusively. In 7 children the diagnosis of FNH had to be confirmed histologically (an operative biopsy was performed in 3 children). There was no history of hormonal treatment before diagnosis FNH in any child, except one young 17 years old woman with polycystic ovaries.

In all children alpha-fetoprotein values were at normal range. In four children mild elevation of liver function tests was observed. Only in two cases FNH was a solitary nodule, in the other cases FNH was multifocal. In two girls the lesions occupied the major part of the right lobe and right hemihepatectomy was performed. One girl underwent arterial embolisation. In all operated girls recurrence of FNH was observed. Currently the follow up ranges from 8 months to 6 years.

Discussion/Conclusion: Establishing the diagnosis of FNH is difficult and sometimes invasive diagnostic evaluation is needed. If FNH is diagnosed correctly, no treatment is needed in asymptomatic patients.
Increased susceptibility to estrogen-induced cholestasis in mice overexpressing the hepatic Abcb11 gene

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Introduction: Estrogen could be involved in intrahepatic cholestasis of pregnancy and oral contraceptive-induced cholestasis, which represent a prominent feature of liver injury characterized by accumulation of bile salts and other toxic bile constituents within hepatocytes and blood. Because only a minority of patients develops cholestasis, we hypothesized that differences in estrogen metabolism could lead to differential inhibition of Abcb11-mediated bile salt transport in predisposed individuals.

Methods: We investigated ovariectomized female wild-type and Abcb11 transgenic (tg) mice with or without disruption of the estrogen receptor α (Erα) gene (i.e., Abcb11.tg and Abcb11.tg/ErαKO) and treated the mice with 17β-estradiol (E2) at 3, 6, 12, 24 µg/day for 14 days. Biliary bile salt outputs were examined by HPLC and bile flow was determined gravimetrically. Expression levels of the genes encoding hepatic bile salt transporters and synthesis enzymes were analyzed by real-time PCR.

Results: There were dose-dependent 5 to 7-fold increases in expression levels of the hepatic Erα, Abcb11, Ntcp, Cyp7a1, and Cyp27 genes in wild-type mice, coupled to significant dose-dependent increases in bile flow and biliary bile salt output from 91 ± 8 to 136 ± 15 µl/min/kg, and 215 ± 54 to 327 ± 82 µmol/h/kg, respectively. The highest dose (24 µg/day) of E2 significantly down-regulated expression levels of the genes for hepatic bile salt transporters and synthesis and reduced bile flow and biliary bile salt outputs to 45 ± 7 µl/min/kg and 125 ± 36 µmol/h/kg, respectively. In contrast, when doses of E2 were ≥ 6 µg/day, Abcb11.tg mice displayed significant decreases in expression levels of the genes regulating hepatic bile salt transport and synthesis by 30 to 50%, coupled to significant reductions in bile flow and biliary bile salt output to 45 ± 7 µl/min/kg and 118 ± 29 µmol/h/kg, respectively, all in a dose-dependent manner. Although being treated with various high (6 to 24 µg/day) doses of E2, Abcb11.tg/ErαKO mice showed essentially stable bile flow (95 ± 10 µl/min/kg) and biliary bile salt output (238 ± 64 µmol/h/kg), as well as constant mRNA expression levels of hepatic bile salt transporters and synthesis enzymes, all of which were comparable to those in wild-type mice treated with 3 µg E2/day.

Discussion/Conclusion: Erα plays a critical role in the biphasic (choleretic vs. cholestatic) effects of E2 on the regulation of hepatic bile salt transport and synthesis, as well as bile formation. The expression levels of Abcb11 partly account for variations in susceptibility to E2-induced cholestasis in mice. These studies point to a better design of individualized estrogen therapy in genetically susceptible patients and to improved prediction of the cholestatic potential of novel drugs.
Clinical significance of HBV genotype analysis in Shanghai

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Introduction: To investigate the clinical significance of HBV genotype analysis in Shanghai.

Methods: From April to December of 2004, the serum of 170 cases were collected, 109 male and 61 female. Mean age 40.2 ± 12.6 years old, from 14-72. The cases were classified as HBV carrier 24; acute hepatitis 14; chronic hepatitis 88; cirrhosis 24; severier hepatitis 18 and hepatic cellular carcinoma 2.Genotype analysis: EIA for serological determination of hepatitis B virus genotypes.

Results: In 170 cases, the main types were C and B, with 61.76% and 34.71% respectively, 3 for D type (1.76%) and 1 for B/C mixed type (0.59%), 2 cases could not be analysis by this method; The morbidity was more likely in age 41-60 for type C, and in 21-40 for type B (P < 0.05); Cirrhosis was more common in type C (19.05%) than that in type B (6.78%), (P < 0.05); In type C group, more subjects’ HBV-DNA were $\geq \log_{10}5$, comparing that in type B group, P < 0.001.

Discussion/Conclusion: The most of subjects with hepatitis B in this study were genotype C or B. The age for morbidity in type C was older than type B. Genotype C was more likely developing to cirrhosis than that in type B. Virus load was much higher in type C than in type B.
Normative values of $^{13}$C-methacetin breath test established in two groups of different age

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Introduction: We investigated if age were a factor influencing the results of a $^{13}$C-methacetin breath test, which in case of a positive answer would imply the necessity of determining separate normative values for people of different age.

Methods: Two groups of healthy volunteers, comprising each 6 men and 6 women, but differing in average age (Y = young, 25.1 ± 0.6 years, MA = middle-aged, 46.0 ± 2.1 years) were examined. The subjects drunk in the morning 200 ml unsweetened black tea into which 75 mg $^{13}$C-methacetin (Euriso-Top S.A., Saint-Aubin, France) was added. Samples of expiratory air for $^{13}$CO$_2$ measurement were collected at 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 40, 50, 60, 75, 90, 105, 120, 150 and 180 min after intake of the substrate.

Results: Between 15 and 150 min the curve the momentary $^{13}$C recovery in breath air was shifted upwards in elderly compared to young subjects. The time to peak of the curve was similar in either group (17.5 ± 1.8 min young vs. 18.2 ± 1.7 min middle-aged). However, the maximum momentary $^{13}$C recovery in breath air was higher in the elder compared to the younger group: 38.55 ± 2.02 %dose/h vs. 34.59 ± 2.67 %dose/h (a difference statistically not significant). In accordance with the pointed-out shift upwards of the curve the momentary $^{13}$C recovery, the cumulative three-hour $^{13}$C recovery was found to be statistically significantly greater in the middle-aged compared to the young healthy subjects (42.20 ± 1.18 %dose vs. 37.57 %dose, p = 0.0011).

Conclusion: The study results imply that the microsomal metabolic efficiency of the liver is not compromised in middle-aged healthy subjects. Nevertheless a better result of the test obtained in the elder compared to the younger group requires elucidation in future research.
Relationship between serum alpha-glutathione S-transferase and aminotransferases in patients with non-alcoholic fatty liver

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Introduction: Serum aminotransferases are routine laboratory indices of hepatocyte injury. Diagnostic significance of aminotransferases in non-alcoholic fatty liver (NAFL) is unclear, as they do not correlate with severity of steatosis and necro-inflammatory reaction. Alpha-glutathione S-transferase (α-GST) is another marker of parenchymal hepatic injury, showing hypothetical advantages over aminotransferases. The aim of the study was determination of relationship between serum α-GST and aminotransferases in patients with non-fibrotic NAFL.

Methods: The blood for measurement of α-GST and aminotransferases was obtained from 9 healthy controls and 71 consecutive patients with hyperechogenic liver without evidence of alcohol abuse, use of drug-induced steatosis, HCV infection, celiac or inflammatory bowel disease. Advanced hepatic fibrosis was excluded by measurement of serum collagen IV and TGF-β1.

Results: In patients with NAFL the mean serum level of α-GST was 11.4 ± 11.0 ng/ml, being significantly higher than in control group (4.46 ± 4.37 ng/ml; p < 0.05). Elevated serum levels of α-GST (+2SD), ALT and AST were found respectively in 19 (26.8%), 38 (53.5%) and 18 (25.4%) patients. Serum α-GST showed no correlation with aminotransferases. The patients with increased α-GST had significantly higher body mass index (31.2 ± 4.27 kg/m² vs. 28.8 ± 4.00 kg/m²; p < 0.05).

Discussion/Conclusion: This study suggests that α-GST and aminotransferases are differently released from fatty liver. The diagnostic significance of α-GST in NAFL should be further investigated in biopsy-based studies.
Severe impairment of autonomic and peripheral sensory nerve function in alcohol-related cirrhosis

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Background: Autonomic neuropathy is associated with high mortality – as proved by follow up studies even in chronic alcoholic liver diseases and in primary biliary cirrhosis – due to silent myocardial infaction and major arrhythmias, cardiorespiratory arrests and other causes not explained yet. Moreover, neuropathy is a frequent cause of disability in patients with chronic liver diseases and early diagnosis is essential for effective treatment.

Patients and methods: Aim of our study was to evaluate autonomic and peripheral sensory nerve function in 12 patients with alcoholic cirrhosis. Controls were 12 healthy subjects. The five standard tests of cardiovascular autonomic function were applied. Peripheral sensory function was studied by the Neurometer® (Neurotron Incorporated, Baltimore, USA). Neuroselective current perception threshold (CPT) was obtained by constant current sine wave transcutaneous nerve stimulation. CPTs for 2000 Hz, 250 Hz and 5 Hz are characteristic for the function of large myelinated, small myelinated and small unmyelinated sensory nerve fibers, respectively. Median and peroneal nerves (digital branches) were studied.

Results: Significant decrease of autonomic function in two tests (Valsalva-ratio and 30/15-ratio, both p < 0.01) and impairment of sensory nerve function indicating hypaesthesia (CPT at 2000 Hz as well as at 5 Hz: p < 0.001 at both median and peroneal nerve testing, CPT at 250 Hz: p < 0.01 for median nerve, p < 0.05 for peroneal nerve) were found in cirrhotic patients compared to controls.

Conclusions: Abnormalities of autonomic function just as widespread impairment of small and large sensory nerve function were found in patients with alcohol-related cirrhosis. Beside assessment of cardiovascular reflexes, evaluation of current perception threshold may be an additional simple non-invasive diagnostic aid in early detection of neuropathy in alcoholic cirrhosis.

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Changes of natural killers cells cytotoxicity at patients with chronic hepatitis C after application of ursodeoxycholic acid

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Taking in consideration data that ursodeoxycholic acid application can reduce level of bile acids which cytotoxically act to NK cells function we have performed assay for determination the ability of the ursodeoxycholic acid used in form of Ursofalk® (UF) to increase functional activity of NK cells at patients with chronic hepatitis C (CHC).

30 patients with CHC were involved in the investigation. In all cases disease was diagnosed with the help of clinical examination and laboratory tests including detection of HCV RNA in the blood. In the start of the investigation we quantitated the cytotoxicity index of NK cells in the blood of all patients. This test was performed radiometrically with application target-cell (K562) labelled H3-uridin.

Before the beginning of combine therapy with interferon and ribavirin all patients perorally took UF: 250 mg 3 times per day for 4 weeks. Then the NK cell cytotoxicity index was repeatedly quantitated at all patients.

The results obtained in the investigation demonstrated that application UF in above mentioned doses and regime was able to increase the NK cells cytotoxicity index (p < 0.05). We considered that such effect of UF to NK cell functional activity was connected with choleretic action of UF and reducing the concentration of bile acids in the blood.
Is subtotal cystopericystectomy a safe procedure to treat hydatid cysts in the liver?

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Hepatic hydatidosis is a disease extended through the whole world. Nowadays the only efficient treatment is surgery. All the proposed techniques can be divided in conservative or radical procedures.

**Introduction:** Objective is to analyze the morbidity and mortality of subtotal cystopericystectomy.

**Methods:** In the last 10 years 69 patients with 81 hydatidic cysts have been operated on. Mean age 40 years (range 8-75). Thirty two cysts had a clear, sterile content. Forty contained bile and 9 were infected. In all cases punction-aspiration of the cyst was performed, injection of 20% hypertonic saline solution or 10% Povidon-jodine, followed by resection of the emergent adventicia, silk suture of the biliar communications and external drainage (12 latex, 57 silicone). Cholangiography was performed in 25 patients, being necessary a choledochotomy in 9 cases. Cholecystectomy was done in 10 cases. All the patients were treated with antibiotics for a period of 3 days minimum.

**Results:** Mortality 1&69 due to traumatic rupture of the cyst in a 75 years old patient. Morbidity: 4 biliar fistulas over 8 days resolved after endoscopic papilotomy, 7 intraabdominal abscesses in patients with latex drainages. In 6 times the abscess were treated by CT guided punction. Culture results were 4 Streptococcus, 3 Staphylococcus aureus. Mean stay 7days.

**Conclusions:**
1. SCP is an easy and safe procedure to treat hepatic hydatid cysts.
2. Close system drainage and antibiotics are necessary to avoid infection of the residual cavity.
Ursodeoxycholic acid in patients with dyslipoproteinemia and non-alcoholic steatohepatitis

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Patients with disturbances of lipid metabolism often show elevation of serum transaminases and gamma-GT, which are interpreted as signs of hepatitis. After exclusion of other causes of hepatic involvement, including alcohol and viruses, with presence of fatty liver signs on abdominal sonography diagnosis of non-alcoholic steatohepatitis (NASH) is usually established.

We observed two groups of patients: first group of 11 patients (9 male, 2 female, mean age 43 ± 7 y.o.) with dyslipoproteinemia 2 and second group of 10 (10 male, mean age 34 ± 6 y.o.) with dyslipoproteinemia 4 (Table 1). In all patients alcohol consumption was excluded, all patients had not serum markers of HBV and HCV (in 2 patients with dyslipoproteinemia 4 serum HGV/GBV RNA was positive). Diabetes mellitus, metabolic and autoimmune liver diseases were also excluded in all cases. Indications for liver biopsy were absent in all cases. All patients were treated by ursodeoxycholic acid (UDCA) with daily dose 15-25 mg/kg. Results are in Table 1. We found also that dyslipoproteinemia correction depended on body mass index (BMI) and is more effective in patients with underlying tendency to obesity.

Conclusions: Treatment of NASH in patients with dyslipoproteinemia of types 2 and 4 results in significant decreasing of serum lipids. Correction of serum lipids elevation with treatment by UDCA is more effective in patients with initially higher body mass index and obesity.
Table 1. Results of NASH treatment and correction of dyslipoproteinemia of types 2b and 4 by UDCA

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Serum values</th>
<th>Initial Levels</th>
<th>2\textsuperscript{nd} week</th>
<th>4\textsuperscript{th} week</th>
<th>2\textsuperscript{nd} month</th>
<th>4\textsuperscript{th} month</th>
<th>6\textsuperscript{th} month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyslipoproteinemia type 2</td>
<td>TG (mean)</td>
<td>187.6</td>
<td>112.4</td>
<td>144.7</td>
<td>109.9</td>
<td>117.5</td>
<td>122.0</td>
</tr>
<tr>
<td>N = 11</td>
<td>CHOL (mean)</td>
<td>313.1</td>
<td>204.1</td>
<td>217.9</td>
<td>237.4</td>
<td>195.9</td>
<td>149.7</td>
</tr>
<tr>
<td></td>
<td>ALT (mean)</td>
<td>56.2</td>
<td>43.2</td>
<td>47.6</td>
<td>34.1</td>
<td>43.9</td>
<td>40.4</td>
</tr>
<tr>
<td></td>
<td>AST (mean)</td>
<td>41.5</td>
<td>23.8</td>
<td>37.9</td>
<td>26.7</td>
<td>29.4</td>
<td>29.9</td>
</tr>
<tr>
<td></td>
<td>GGT (mean)</td>
<td>73.2</td>
<td>34.3</td>
<td>39.8</td>
<td>35.3</td>
<td>41.3</td>
<td>43.1</td>
</tr>
<tr>
<td>Group 2</td>
<td>TG (mean)</td>
<td>221.3</td>
<td>187.4</td>
<td>177.2</td>
<td>192.2</td>
<td>142.1</td>
<td>137.9</td>
</tr>
<tr>
<td>Dyslipoproteinemia type 4</td>
<td>CHOL (mean)</td>
<td>92.4</td>
<td>84.9</td>
<td>73.6</td>
<td>76.5</td>
<td>81.1</td>
<td>71.2</td>
</tr>
<tr>
<td>N = 10</td>
<td>ALT (mean)</td>
<td>74.2</td>
<td>81.3</td>
<td>44.8</td>
<td>35.9</td>
<td>41.2</td>
<td>34.1</td>
</tr>
<tr>
<td></td>
<td>AST (mean)</td>
<td>29.2</td>
<td>31.5</td>
<td>27.4</td>
<td>22.1</td>
<td>24.7</td>
<td>23.3</td>
</tr>
<tr>
<td></td>
<td>GGT (mean)</td>
<td>84.9</td>
<td>51.4</td>
<td>34.9</td>
<td>39.6</td>
<td>37.2</td>
<td>45.6</td>
</tr>
</tbody>
</table>

TG (mean) – triglycerides mg/dL; CHOL – cholesterol, mg/dL; ALT, AST – IU (0-40); GGT – IU (0-49)
Coinfection of HBV, HCV, HIV, antiretroviral treatment and ursodeoxycholic acid

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There are some limitations in management of patients coinfectcd by hepatitis viruses and HIV. Drug abuse history, depression, usage of multiple antiretroviral agents, antibiotics and other sufficient factors limit possibilities of antiviral treatment of active liver disease in these patients.

Female patient, 28, was observed for 7 years with diagnosis of HIV-infection and chronic hepatitis (CH) caused by HBV and HCV, with history of drug abuse and treatment by sedatives. There were not signs of AIDS during all that period of regular observations. Serum ALT and AST levels were controlled effectively for 6 years by ursodeoxycholic acid (UDCA), 750-1000 mg daily. UDCA lower doses leaded to 2-3-fold elevation of serum transaminases. The way of treatment was chosen because of high risk of depression and possibility of suicidal attempts. Liver biopsy showed minimal activity and fibrosis. Serum HCV RNA and HBV DNA were fluctuating independently of UDCA dose.

At the 7th year of observation the first episodes of recurrent pneumonia took place in our patient, condition of AIDS was established (CD4+ 250/ml), HIV viral load was high. Highly-active antiretroviral treatment (HAART) was started, and during first month the complete response was achieved (CD4+ 600/ml), low HIV viral load. CH was inactive. After 4 months of HAART dramatic events in patient's family had caused the "second wave" of AIDS, with 5-fold elevation of ALT, cholestasis and presence of both HCV RNA and HBV DNA in serum. After two weeks of UDCA treatment (1500 mg daily) serum ALT and GGT levels had normalized.

Conclusions: UDCA may be an effective and safe agent for control of liver disease activity in patients with coinfection of HIV and hepatitis viruses.
How reproducible is $^{13}$C-methacetin breath test?

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Introduction: Breath tests with $^{13}$C substrates constitute nowadays an excellent tool to measure the amount of intact liver tissue. In the study we searched for a quantitative parameter which would offer the best reproducibility of a standard $^{13}$C-methacetin breath test.

Methods: Twelve healthy volunteers were recruited (7 F, 5 M, aged 24.9 ± 0.5 years). On three separate days they drank 200 ml unsweetened black tea into which 75 mg $^{13}$C-methacetin (Euriso-Top S.A., Saint-Aubin, France) was added. Samples of expiratory air for $^{13}$CO$_2$ measurement were collected at 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 40, 50, 60, 75, 90, 105, 120, 150 and 180 min after intake of the substrate. Short-term reproducibility was assessed with paired examinations taken 2-4 days (median 2 days) apart, whereas paired examinations separated by 16-23 days (median: 19 days) served for the medium-term reproducibility assessment. The order of intervals between the consecutive sessions (short- or medium-term reproducibility) was randomized.

Results: According to the coefficients of variation for paired examinations (CVp), reproducibility of the time to peak $^{13}$C recovery was poor (38.44% short-term, 40.69% medium-term). Good short-term reproducibility (CVp = 10.48%) of the maximum momentary $^{13}$C recovery deteriorated significantly with increased time interval between repeat examinations (CVp = 19.28%). Noteworthy, excellent reproducibility of the cumulative 3-hour $^{13}$C recovery (area under the curve) appeared to be insusceptible to the length of the time gap separating the examinations (CVp: 8.85% and 5.54% in the case of the short- and medium-term reproducibility, respectively).

Conclusion: Excellent short- and medium-term reproducibility speaks in favour of appointing the cumulative 3-hour $^{13}$C recovery the most reliable measure among parameters commonly used to provide quantitative result of the $^{13}$C-methacetin breath test.
May autonomic neuropathy play a role in the development of hyperdynamic circulation and portal hypertension in liver cirrhosis? (hypothesis)

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Hyperdynamic circulation (HC) is in partly responsible for the maintenance of portal hypertension in patients with chronic liver diseases (1,2) and the vasodilation theory has also implicated HC in the pathogenesis of ascites (3). In the development of HC vasodilation precedes plasma volume expansion and sodium retention. Although a lot of candidates are known, mediators of arterial vasodilation in cirrhosis have not been definitively identified.

It seems possible that a fall of the splanchnic resistance as well as arteriovenous shunting may occur secondary to sympathetic neuropathy and the consequence is an increased flow in the portal venous circulation. Similarly, vasodilation in the pulmonary, dermal and muscle circulation due to sympathetic neuropathy may contribute to the hyperdynamic circulation. On the other hand, a marked resting tachycardia due to parasympathetic neuropathy may be another important pathogenetic factor for elevated cardiac output and HC. Recent data indicate, that the splanchnic circulation is a main site of vasodilation.

Some experiences from diabetic autonomic neuropathy (AN) and also other data seems to support the hypothesis. Reversal of type 1 hepatorenal syndrome has been reported with administration of midodrine and octreotide (4). Treatment with octreotide (5), midodrine, isosorbite-dinitrate and prazosin reverses peripheral vasodilation, decreases portal pressure, while these agents are used also for the treatment of AN (6,7). Persistence of increased hepatic blood-flow and elevated cardiac output after orthotopic liver transplantation may be also a consequence of denervation. Simultaneous evaluation of portal pressure and autonomic function as well as effects of pharmacological intervention may reveal further data on this topic. Obviously, to prove a causal relationship is more difficult.

References:

Mild to moderate autonomic dysfunction and impairment of large myelinated sensory nerve fibre function are the characteristic features of neuropathy in patients with anti-HCV-positive chronic liver disease

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Background: Autonomic neuropathy may be present in chronic alcoholic and non-alcoholic liver diseases (1,2) and is associated with a poor prognosis in these patients (3). In our previous study autonomic and sensory nerve dysfunction was detected in patients with primary biliary cirrhosis (4). Neuropathy has not been evaluated up to now in patients with anti-HCV-positive chronic liver disease – it was aim of our present study.

Patients and methods: Examined were 12 patients with anti-HCV-positive chronic liver disease, controls were 14 healthy subjects. The five standard tests of cardiovascular autonomic function were applied. Peripheral sensory function was studied by the Neurometer (Neurotron, Baltimore, MD, USA). Neuroselective current perception thresholds (CPT) for 2 kHz, 250 Hz. 5 Hz were assessed evaluating large myelinated, small myelinated and small unmyelinated sensory nerve fiber function, respectively. Median and peroneal nerves (digital branches) were studied.

Results: Autonomic neuropathy was observed in 7 patients, heart rate response to deep breathing was abnormal in 3 patients, 30/15 ratio in 1 patients, Valsalva ratio in 2 patients while sustained handgrip test in 5 patients. 4 patients had one, 2 patients had two and 1 patient had three abnormal autonomic function test. Sensory neuropathy could be detected in five patients at peroneal nerve testing; two of them had median nerve damage as well. CPT at 2 kHz was significantly higher in patients compared to controls at median nerve (p < 0.01) as well as at peroneal nerve testing (p < 0.001) indicating hypaesthesia. Four patients had both autonomic and sensory nerve dysfunction while another 4 patients were free of neuropathy.

Conclusion: Early to moderate autonomic abnormalities and impairment of large myelinated sensory nerve fiber function are the main characteristic features of neuropathy in patients with anti-HCV-positive chronic liver disease.

References:


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Neuropathy in chronic liver diseases – Therapeutical implications

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Severe forms of autonomic neuropathy (AN) may be present in chronic liver diseases (1). AN carries a fivefold risk for mortality in patients with diabetes mellitus just as in those with chronic liver diseases (2). Silent myocardial infarction, major arrhythmias, QT-interval prolongation (3) and other causes not explained yet may be responsible for the high mortality rate. Considering the natural history, therapy of neuropathy is of particular importance. A key question is still open: it is not known whether treatment of hepatopathy itself may have a beneficial impact on the progression of neuropathy or not. Among non-alcoholics the most severe forms of neuropathy were found in patients with primary biliary cirrhosis (PBC) (4). It may be of particular interest whether treatment with ursodeoxycholic acid in PBC may alter the natural history of neuropathy or not.

Thiamin deficiency is common in liver diseases, first of all in alcoholic patients and in subjects treated with high doses of diuretics. Absorption of the water-soluble thiamin compounds is poor. Bioavailability of the lipid-soluble derivates benfotiamin is five times higher (5). A benfotiamin – vitamin B combination has been shown to be effective in the treatment of diabetic neuropathy and may be effective for the treatment of neuropathy in liver diseases (6). Glucose autooxidation and enhanced lipid peroxidation leading to increased oxidative stress contribute to the development of neuropathy. The antioxidant alpha-lipoic acid should be considered as another powerful option for the treatment of hepatic neuropathy (7). Magnesium deficiency is frequently found in liver patients. Magnesium supplementation is essential for the effective treatment of neuropathy. Some autonomic symptoms may require specific therapy which will be reviewed on the poster.

References:


(Supported by the National Research Fund OTKA-T 37807 and by the Ministry of Health ETT 228/2003)
Autonomic neuropathy and QT-interval prolongation in alcoholic liver diseases – Possible markers of survival?

Kempler P., Keresztes K., Abonyi M., Hermányi Z., Istenes I., Tóth N., Tyahor J., Putz Z., Balázs B., Tímár C., Kádár É., Szalay F.
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Background: Gastrointestinal motor disturbances and various cardiovascular symptoms represent the main characteristic features of autonomic neuropathy (AN). Beside diabetes mellitus even chronic liver diseases are pathogenetic factors of AN. The poor prognosis of AN has been shown even in patients with chronic liver diseases, but the exact pathomechanism of increased death rate is still poorly understood. Sudden death is not rare in patients with AN and corrected QT-interval (QTcI) prolongation is thought to be important in this respect. A correlation between AN and QTcI prolongation has been observed in patients with diabetes.

Patients and methods: Aim of our study was to evaluate a possible relationship between severity of AN and prolongation of the corrected QT-interval in 165 patients with chronic alcoholic liver diseases. Chronic alcoholics were classified into three groups: 32 patients had no liver disease, 48 had fatty liver and 83 had alcohol-related cirrhosis. Controls were 85 healthy subjects. The five standard tests of cardiovascular autonomic function were applied, QTcI was determined with Bazett’s formula.

Results: 13/32 among patients without liver disease, 39/48 having fatty liver, 76/83 among those with cirrhosis as well as 2/85 of healthy controls had AN. Abnormal QTcI (> 440 ms) was seen significantly more often in patients with AN than in those without AN in all three groups (p < 0.001). The severity of AN was characterised by the number of abnormal reflex indices on patient. Significant linear regression was found between QTcI lengthening and severity of AN in all three groups (p < 0.001).

Conclusions: Our data provide evidence of a relationship between presence and severity of autonomic neuropathy and degree of QTc-interval prolongation in chronic alcoholic liver diseases. QTcI prolongation is associated with an increased risk of ventricular arrhythmias and may be responsible for sudden death. Assessment of cardiovascular reflexes and of the QTc-interval may be a simple additional diagnostic aid to identify individuals with an increased cardiovascular risk among patients with alcoholic liver diseases.

(Supported by the National Research Fund – OTKA 37807 and the Ministry of Health – ETT 228/2003)
Autonomic and sensory neuropathy in primary biliary cirrhosis

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Introduction: Autonomic and sensory neuropathy is a known complication of chronic liver diseases. We aimed to assess the prevalence and risk factors for autonomic and sensory nerve dysfunction in patients with primary biliary cirrhosis (PBC).

Methods: Twenty-four AMA M2 positive female patients with PBC and 20 age matched healthy female subjects were studied. Five standard tests and heart rate variability (HRV) analysis were performed to define autonomic function. Sensory nerve function was characterized by current perception threshold (CPT), measured by the Neurometer.

Results: Fourteen patients (58%) had at least one abnormal cardiovascular reflex test and thirteen (54%) had peripheral sensory neuropathy. Each patient had at least one abnormal parameter of HRV. Lower heart rate response to deep breathing, standing and Valsalva manoeuvre, and more profound decrease of blood pressure after standing was found in PBC patients than in controls. As a novel finding we proved that both time domain and frequency domain parameters of HRV were significantly reduced in PBC patients compared to controls. Lower CPT values indicated hyperaesthesia as a characteristic feature at peroneal nerve (p < 0.01) in the PBC group compared to controls. Correlations of autonomic dysfunction with the severity and duration of cirrhosis were observed.

Discussion/Conclusion: Autonomic and sensory nerve dysfunctions are frequent in PBC. HRV analysis is more sensitive than standard tests for detecting autonomic impairment. Our data suggest that hyperaesthesia is characteristic feature of sensory neuropathy and might contribute to itching in PBC. Autonomic dysfunction is related to the duration and severity of PBC.
The minimal portal cholangitis in patients, monoinfected by hepatitis virus TT (TTV)

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Introduction: TTV is frequently revealed in people, infected by other viruses (HCV, HBV, HGV). The goal of the study was to examine histological and ultrastructural changes in liver at patients with verified TTV-infection in the absence of other virus serologic markers.

Methods: 380 patients with chronic liver diseases were enrolled into clinical examination. ELISA-method was used for detection of virus antibodies in serum. TTV DNA was detected by PCR in 128 patients (33.7%), monoinfection was confirmed at 49 of them. Liver biopsy was carried out at 18 patients with TTV-monoinfection.

Results: The histological examination revealed signs of chronic portal hepatitis (involving 1/3 of portal tracts) at 16 patients. All patients had edema of portal tracts and inflammatory changes in small biliary ducts (BD). The vacuolization of cytoplasm and desquamation of the epithelial cells into the BD lumen was frequently observed. The lymphocytes and apoptotic bodies were constantly revealed among epithelial cells. The ultrastructural study revealed bunches of viral particles in several hepatocytes. The viral particles penetrated into the lumen of fine biliary capillars located between the separate hepatocytes. By this way the virus can proceed into a bile flow within small portal BD with the subsequent invasion into their epithelial cells.

Conclusion: The minimal portal cholangitis may be caused by certain affinity of TTV to the epithelial cells of BD. The lesion of small BD at a TTV-monoinfection can play a trigger role in development of some autoimmune liver diseases.
Hepatoprotective effects of panthenol and carnitine under chronic alcohol intoxication

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Introduction: Earlier it was shown, that carnitine prevents development of ethanol-induced liver steatosis in experimental animals. The mechanism of carnitine action is not clearly understood. Different models of oxidative stress, involving ethanol-induced one, demonstrated that carnitine is capable of protecting the liver from lipid peroxidation. Data were obtained on hypolipidemic effects of panthenol using models of alimentary and hypothalamic obesity and Triton WR-1339-induced hyperlipidemia. The aim of the work was to study the hepatoprotective effects of panthenol, L-carnitine and their combination as well as to evaluate the substance ability to counteract development of oxidative stress manifestations in liver tissue under chronic alcohol intoxication.

Methods: Male Wistar rats treated with ethanol intragastrically (4 g/kg twice a day for 1 month) were used in this experiment. The control animals received water. Since day 20 of intoxication the animals were additionally treated with either L-carnitine (100 mg/kg, i.g.) or panthenol (100 mg/kg, i.g.) or their combination at the same doses.

Results: After chronic alcohol intoxication, blood plasma GGT activity was considerably increased, and tended to decrease both under individual carnitine and panthenol treatments and under their mixture. The administration of panthenol and the panthenol+carnitine decreased the total plasma bilirubin content. The activities of liver alcohol dehydrogenase and aldehyde dehydrogenase did not differ significantly in all the groups studied. The chronic ethanol treatment significantly diminished liver superoxide dismutase (SOD) and catalase activity. The administration of carnitine, panthenol or their combination prevented a SOD and catalase activities reduction. The reduced glutathione level was significantly higher in alcohol-intoxicated rats treated with carnitine or panthenol. The panthenol or carnitine administration decreased sudanophilia in liver histochemical preparations by 40% and 14%, respectively, as opposed to the ethanol-treated group.

Discussion/Conclusion: Thus, the panthenol or carnitine treatment and their combined application during chronic alcohol intoxication activated the enzymatic and non-enzymatic antioxidant systems and prevented some manifestations of ethanol hepatotoxicity. Further research is needed to assess the mechanisms of action of these drugs.
Presence of minimal hepatic encephalopathy in patients with chronic liver disease

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Introduction: Minimal hepatic encephalopathy (mHE) is a neurocognitive disorder secondary to liver failure. Neuropsychological abnormalities are mostly on memory and psychomotor tests. mHE is usually defined as presence of cortical dysfunction on EEG and abnormal finding of at least one neuropsychological test.

Methods: Thirty patients with chronic liver disease (hepatitis B virus [60%], hepatitis C [16.7%] and alcohol [23.3%]) without overt hepatic encephalopathy were tested (19 male, 11 female, mid age 59.9 years, education 12 years). All patients had normal neurological examination and normal mental functioning. Blood ammonia levels and liver biochemical tests were done in order to assess liver function. Patients neuropsychological functions were tested using Number Connection Test Part A (NCT-A), Digit Symbol Test (DST) and EEG. The results of these tests were compared to controls (n = 30) matched to gender, age and education.

Results: All patients were classified as either Child-Pugh score C (53.3%) or B (46.7%). Most of them had normal blood ammonia levels (66.7%). NCT-A and DST scores were significantly lower in the group of patients with chronic liver compared to the control group. Almost all (93.3%) chronic liver disease patients had diffuse cortical dysfunction registered on EEG.

Discussion/Conclusion: Chronic liver diseased patients showed significant impairment in psychomotor and memory functions and cortical dysfunction registered on the EEG. Almost all liver patients in our study group had subclinical hepatic encephalopathy. Gender, age and education did not significantly influence the results of the neuropsychological tests nor did the etiology of chronic liver disease. Blood ammonia levels also did not influence the test scores.
Clinical features, serum IL-6 and IFN-γ levels of patients with hepatoporal sclerosis

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Introduction: Hepatoporal sclerosis (HPS) is a clinical disorder of obscure pathogenesis with a variable clinical profile. The aim of the study was to summarize the clinical features of Turkish patients with HPS and to measure the serum levels of IL-6 and IFN-γ in order to determine the Th cell profile in the pathogenesis.

Methods: The study was conducted on 34 HPS patients (17 male, 17 female; mean age at diagnosis: 27 ± 10 years) and 15 healthy controls. The clinical features of HPS patients including demographics, clinical history, laboratory, and ultrasonography findings were summarized. Serum IL-6 and IFN-γ levels were measured by using commercially available enzyme-linked immunosorbent assay kits.

Results: Gastrointestinal bleeding was the most common dominant presenting symptom. Majority of the patients had preserved liver function tests. Serum triglyceride levels were decreased in 30%. Abdominal ultrasonography revealed well-demarcated bands of increased echogenicity surrounding the portal vein wall and sudden narrowing of the intrahepatic second degree portal vein branches in all cases. Spontaneous shunts and/or collaterals were seen in 13 cases (37%). Extrahepatic portal vein thrombosis were seen in 7 (20%) patients after at least 5 years of disease duration. Serum levels of both IL-6 (median: 3.2 pg/ml) and IFN-γ (median: 7.8 pg/ml) were significantly higher in HPS patients compared to control group (median: 1 pg/ml).

Discussion/conclusion: HPS has variable clinical profile in different geographic areas of the world. Both Th1 and 2 cells may have a role in the regulation of immune response and pathogenesis of the disease.
HBV, HCV and HIV infection among prisoners

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Introduction: Individuals incarcerated in prisons and jails have a disproportionate burden of infectious diseases, including infections with hepatitis B virus (HBV), hepatitis C virus (HCV) and HIV. Several risk behaviours for transmission of these infections occur in prison, including the injection of illicit drugs and tattooing with inadequately disinfected equipment as well as unprotected sexual intercourse. Although there are opportunities for prevention efforts, barriers still exist.

Objective: To study the prevalence of serological markers of HBV, HCV and HIV among a cohort of prisoners in Sofia, Bulgaria.

Methods: Sixteen male prisoners aged 19-45 (mean 30) were enrolled in the present study in 2003. Serological markers of HBV, HCV and HIV were determined by ELISA (Dia-Sorin). LaRoche equipment and diagnostic reagents were used for detection of HCV RNA by PCR.

Results: Four (23.5%) inmates have history of close contact with hepatitis, but none - past history of viral hepatitis. Five (31.5%) were intravenous drug users (IDUs). Eight (50%) prisoners were anti-HCV positive, all viremic as HCV RNA was detected in their sera. Another eight (50%) were found to carry any serological markers for HBV: 6 with resolved and one with current HBV infection. In one person the serological pattern “anti-HBc alone” was established. Four (23.5%) exhibited HBV/HCV coinfection, 3 of them being IDUs. None was anti-HIV positive. All were asymptomatic by the time of the study.

HBV and HCV have equally prevailing rates among the studied prisoners. As none of them has history of past viral hepatitis HBV infection might run asymptomatic course. Thus they hadn’t met the surveillance case definition. Another reason for underreporting of prisoners with acute viral hepatitis could be that they might not access the health system.

Discussion/Conclusion: Our findings suggested that alternative methods of hepatitis case finding among prisoners may be needed to monitor hepatitis incidence in a community. Offering hepatitis B vaccination to this at risk and high incidence population is an important public strategy. At the other hand, identifying HCV-positive inmates is an opportunity for them to access health education, substance abuse treatment and risk reduction.
Serum markers of liver fibrosis. Alpha1-antitrypsin – New kid on the block

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Introduction: Tissue inhibitor of matrix metaloproteinase-1 have shown promise in detecting liver fibrosis and was included in algorithm (ELF) for non-invasive assessment of hepatic morphological changes. Alpha1-antitripsin is the most potent serum proteinase inhibitor.

Aim: To determine the diagnostic utility of alpha1-antitrypsin for assessment of liver fibrosis in patients with chronic viral hepatitis.

Methods: Serum levels of alpha1-antitrypsin (measured by immunoturbidimetry) were compared with liver histology (METAVIR) in 71 patients with chronic viral hepatitis – 38 HCV and 33 HBV. Forty seven patients had F2-F4 fibrosis and 7 of them had cirrhosis. The primary outcomes were significant fibrosis /F2-F4/ and cirrhosis. Statistical analyze was done by receiver operating characteristic curves and Mann-Whitney test.

Results: Serum levels of alpha1-antitrypsin were significantly higher (p = 0.002) in the F2-F4 group compared with F0-F1 group. AUROC values of alpha1-antitrypsin for prediction of significant fibrosis and cirrhosis was 0.728 ± 0.07 and 0.773 ± 0.07 respectively.

Discussion/Conclusion: These results suggest that serum levels of proteinase inhibitor alpha1-antitrypsin is a promising biochemical marker of liver fibrosis. Measurement of alpha1-antitrypsin is easily obtainable and with low cost. It could therefore be included in panels of serum markers for estimation of liver fibrosis.
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Two-step treatment of cultured human hepatocytes Chang liver cells with lovastatin

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Introduction: Hepatotoxicity is one of the adverse effects during therapy with statins. However, the cellular mechanisms underlying the statin-induced liver injury are not fully understood.

Methods: We investigated the effect of two-step treatment of lovastatin in human hepatocytes after long-time incubation. The cells were incubated in vitro with 20 µM or 40 µM lovastatin. After 24 hours we administrated the same dose lovastatin in the culture medium.

Results: The cell viability, LDH activity, mitochondrial transmembrane potential and the level of the intracellular calcium were measured at 24 h, 48 h and 72 h. In controls, the LDH activity increased three folds at the 72 h. In the lovastatin treated cells LDH activity increased three folds at the 48 h and at the 72 h LDH leakage was 10 times higher, compared to the control. In accordance to these results the cell viability decreased linearly both in controls and treated cells. The alterations were similar to the LDH activity. An increase in the mitochondrial calcium and in mitochondrial transmembrane potential was found only in lovastatin-treated cells. We didn’t observed dose-dependent alterations in the tested biological parameters.

Discussion/Conclusion: This study shows that lovastatin has several cellular effects that may be related to its toxicity and its metabolism.
Effect of lovastatin on freshly isolated rat hepatocytes

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Introduction: The interactions between lovastatin and hepatocytes are still not well clarified. The hepatotoxicity of lovastatin is already communicated. However, in hypercholesterol animals, lovastatin, such as vitamin E and amlodipine, reduced lipid peroxidation. They are no available experimental data on the combination lovastatin and CCl₄.

Methods: On isolated hepatocytes, the alterations on cell viability, LDH-activity, GSH and TBARS contents were measured. A) The cells were treated with 1 mcM, 3 mcM, 5 mcM and 10 mcM lovastatin. B) The cells were treated with the combination lovastatin and 86 mcM CCl₄. C) The cells from model B were pretreated with 14 mcM amiodarone.

Results: A) Lovastatin promoted hepatotoxicity estimated by decrease in cell viability and GSH concentration by 45% and by 84%, respectively in the highest concentration. LDH-activity increased by 114% and TBARS content by 90%. B) In the combination the toxicity was smaller, compared to CCl₄ alone. Cell viability and GSH-level increased by 29% and by 505%, respectively; LDH – activity decreased by 58% and TBARS content by 57%. C) In hepatocytes, pretreated with amiodarone, the values of the studied parameters were between group A and B.

Discussion/Conclusion: The toxicity of lovastatin is dose-dependent. Probably the "protective" effects (model B) were due to an interaction at the metabolic level between lovastatin and CCl₄. The pretreatment of hepatocytes with amiodarone, inhibited the metabolism of lovastatin and we didn't observed these "protective" effects.
Induction of liver biotransformation activity in patients with diabetes mellitus

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Introduction: Diabetes results from abnormality in the production or the use of insulin and it is associated with a lot of changes in intermediary metabolism. It is not surprising that diabetes mellitus induces marked metabolic changes in liver tissue which would lead to disorders of liver function. Several authors reported on increased activity of liver biotransformation system in animals with experimental diabetes mellitus. The aim of our study was to investigate the activity of liver biotransformation system in patients with diabetes mellitus type I.

Methods: The study group consisted of 27 patients with diabetes mellitus aged 15-44 years. Control group consisted of 46 healthy volunteers. Activity of liver biotransformation was determined using antipyrine test. Glycated hemoglobin was determined as parameter for assessment of compensation of diabetes.

Results: The results of our study showed significantly decreased half-life of antipyrine in patients with diabetes mellitus (controls vs. diabetes: 8.15 hours vs. 6.21 hours), which confirmed induction of liver biotransformation system. There was significant negative correlation between plasma glycated hemoglobin levels and antipyrine half-life ($r = -0.812$, $P < 0.001$).

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Compensated diabetes</th>
<th>Decompensated diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antipyrine half-life</td>
<td>8.15 hours</td>
<td>7.25 hours</td>
<td>4.85 hours</td>
</tr>
<tr>
<td>Glycated hemoglobin</td>
<td>5.2%</td>
<td>7.8%</td>
<td>13.5%</td>
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Discussion/Conclusion: The results of our study support the hypothesis that diabetes mellitus is connected with changes of liver function and diabetic hepatopathy could be one of chronic diabetic complications.
Dynamic evaluation of plasma bile acids and parameters of proteosynthetic liver function in patients before and after liver transplantation

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Introduction: Different complication before and after liver transplantation (infection ischaemia-repefusion injury, hyperacute, acute, or chronic rejection, recurrence of the disease, toxic injury etc.) represent a new metabolic situation for the organism. Damage or necrosis of hepatocytes, increased formation of fibrotic tissue, decrease of excretoric liver function, cholestasis etc. may be assessed by many liver function tests. The study aims at changes in plasma bile acids and serum proteins - parameters of proteosynthetic liver function - and at the importance of their measurement in patients before and after liver transplantation.

Methods: Out of 14 patients, after undergoing liver transplantation, in this study we are presenting dynamic observation of plasma bile acids, serum albumin, prealbumin, transferrin and cholinesterase activity in 7 selected cases before and 12 months after the liver transplantation. Indications for liver transplantation in the whole group of 14 patients were: Liver cirrhosis caused by Primary sclerosing cholangitis (3), Primary biliary cirrhosis (1), Secondary biliary cirrhosis (1), Liver cirrhosis caused by Autoimmune hepatitis (2), Cryptogene liver cirrhosis (2), Liver cirrhosis caused by Chronic viral hepatitis B (1), Haemangioendotelioma (1), Crigler – Najjar syndrome (1), Budd-Chiari syndrome (1), Liver cirrhosis of combined etiology (1). The levels of bile acids were determined enzymatically with commercial diagnostic kit. The levels of albumin, prealbumin and transferrin were measured immunochemically, cholinesterase using spectrophotometric assay.

Results:

Before the liver transplantation, plasma bile acid levels were significantly higher in investigated patients than in healthy controls. The most expressed elevation of bile acid was expressed in patients with Primary sclerosing cholangitis and in one patient with Cryptogene cirrhosis. In remaining patients the level of bile acids was less increased. The levels of prealbumin did not reach the lower border of the normal range in 85.7% of patients. Activity of cholinesterase was under the lower border of the normal range in 100% of patients. Levels of albumin were decreased in 57.1% of patients before the transplantation. Levels of transferrin did not reach the lower border of the normal range in 42.8% of patients.

After the liver transplantation, plasma bile acids levels decreased significantly in transplanted patients, but were higher, than in healthy controls. 85.7% of patients have reached normal levels of prealbumin within 3 months after the transplantation. The remaining 14.3% of patients have reached normal levels after the third month. 57.1% of the patients have reached normal levels of cholinesterase within 3 months after the transplantation, the remaining 42.8% did so after the third month. 71.4% of
patients have reached normal levels of albumin within 3 months after the transplantation, the remaining 28.6% did so after the third month. 71.4% of the patients have reached normal levels of transferrin within 3 months after the transplantation, the remaining 28.6% did so after the third month.

**Discussion/Conclusion:** Increased levels of plasma bile acids were present in our study not only in connection with cholestasis, but may be also considered as a sensitive indicator of impaired liver function. Monitoring of serum protein concentration after liver transplantation can be helpful in diagnostic of complications after liver transplantation and can contribute to the treatment and survival of the transplanted patients.

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Liver steatosis in chronic hepatitis C patients

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Introduction: Histopathological features of hepatocyte steatosis are frequently observed in hepatitis C patients. The aim of the study was to evaluate the frequency of liver steatosis and the relationship between the statement of steatosis and clinical and histopathological progression of chronic hepatitis C.

Methods: We studied 173 patients with chronic hepatitis C. Patients with diabetes mellitus and alcohol abuse were excluded. All patients were subjected to physical examination, ultrasonography of the liver, biochemical and serological tests, and histopathological examination of liver biopsy specimens with the evaluation of fibrosis, histological activity, and steatosis scores.

Results: Among 173 patients liver steatosis was confirmed in 63 (36.4%). We did not find any dependence between liver steatosis and sex, age, hyperlipidemia, obesity, or fibrosis score in the morphological picture of the liver. The obtained results confirmed usefulness of ultrasonography in the diagnostics of liver steatosis. The statistical analysis proved a significant association between the presence of steatosis and ALT, AST, GGT levels and the grade of activity in the morphological picture of the liver.

Discussion/Conclusion: These findings indicate that liver steatosis observed in hepatitis C patients is strongly associated with the histological activity progression and consequently with the progression of liver disease.
End-stage renal disease patients with hepatitis C and the iron disorders

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Introduction: Hepatitis C virus (HCV) infection is the most common cause of chronic liver disease in patients with end-stage renal disease (ESRD). The aim of the study was an assessment of HFE gene mutations C282Y and H63D in patients with chronic hepatitis C and ESRD.

Methods: Among 36 ESRD patients with HCV-Ab(+), HCVRNA was detectable in 28 patients. Liver function biochemical tests, iron metabolism tests and histopathological examination of liver biopsy specimens were performed in the group of 28 ESRD patients and 25 control subjects with chronic hepatitis C and without renal disease. The C282Y and H63D mutations of HFE gene were detected by PCR-RFLP methods.

Results: Aminotransferases and bilirubin levels were significantly lower in the patients with ESRD. Patients with ESRD had less inflammatory activity but increased hepatic iron deposition in histopathological examination of the liver. There were no significant differences in GGT levels, fibrosis stage and steatosis in histopathological examination between patients with ESRD and control group. Distribution of C282Y and H63D mutations in patients with ESRD and control group was similar without statistical significance. We did not find any C282Y HFE gene mutation in both groups, H63D was only in heterozygotes in few cases.

Discussion/Conclusion: The distribution of HFE gene mutations in patients with ESRD is similar to control group and disturbances in iron metabolism in ESRD patients seem do not relate to HFE gene mutations. Moreover, we found that patients with ESRD had less severe hepatitis C than control subjects.
Clinicopathological features of HCV infection and effectiveness of interferon therapy in patients with end-stage renal disease

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**Department of Nephrology, Transplantology and Internal Diseases, Medical University of Gdansk, Poland

**Introduction**: The aim of the study was to characterize chronic hepatitis C in end-stage renal disease (ESRD) patients, to compare hepatitis C-related liver disease between patients with ESRD and control subjects without renal disease and to evaluate the effectiveness of interferon (IFN) therapy in ERDS patients.

**Methods**: Among 36 ESRD patients with HCV antibody positivity, viraemia (HCV RNA) was detectable in 28 patients. Liver function biochemical tests (ALT, AST, GGT, bilirubin) and histopathological examination of liver biopsy specimens were performed in the group of 28 ESRD patients and 36 control subjects with hepatitis C and without renal disease. The group of 18 ERDS patients received therapy with IFN alpha.

**Results**: Aminotransferases and bilirubin levels were significantly lower in the patients with ESRD. Patients with ESRD had less inflammatory activity but increased hepatic iron deposition in histopathological examination. There were no significant differences in GGT levels, fibrosis stage and steatosis in histopathological examination between patients with ESRD and control subjects without renal diseases. In 4 of 18 patients (22%) who started IFN therapy, treatment discontinuation was necessary in 2-4 months of therapy. Seven of 14 patients (50%) who were treated for 48 weeks became HCV RNA negative at the end, and at 6 months after the treatment.

**Discussion/Conclusion**: Patients with ESRD had less severe hepatitis C than control subjects. ESRD patients had less biochemical and histological activity of liver disease. The IFN treatment in ERDS patients was connected with poor tolerance, but virologic efficacy is even better than in general population.
HBV DNA, sFas and sFasL concentration in healthy HBsAg carriers - Three years observation

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**Introduction**: The death of HBV infected hepatocytes is the result of inflammatory necrotic changes and processes of programmed cell death. It seems, that concentration of HBV, Fas and FasL maybe show on progress of disease among healthy HBsAg carriers.

**Methods**: We analyzed 34 healthy HBsAg carriers after 3 years. HBV DNA was extracted using the Gene Elute Mammalian Genomic DNA Miniprep Kit (Sigma, USA). HBV DNA concentration and YMDD mutations were measured by RT-PCR based on TaqMan Universal Master Mix (Applied Biosystems, USA). HBeAg and anti-HBe in serum were detected by MEIA method (ABBOTT, Germany). The concentration of sFas and sFasL in serum was-estimated by ELISA method (Bender MedSystems, Austria).

**Results**: After 3 years the number of carriers with absent HBV DNA increased from 19% to 33%. HBV DNA above $10^5$ copies/ml, was detected in the beginning in 63% of carriers, and after 3 years in 11% of carriers ($p < 0.05$). The sFasL in serum was detected in 56%, after 3 years in 48% of HBsAg carriers. The rate of sFasL presence correlated with high HBV DNA levels ($p < 0.05$). The concentration of sFas after 3 years of observation underwent a statistically significant reduction ($p < 0.01$). Chronic hepatitis B developed in 11% men, whereas 11% eliminated HBeAg, anti-HBe and HBV DNA. YMDD was not detection in any HBsAg carriers.

**Discussion/Conclusion**: High concentration of sFasL in serum suggests the development of chronic hepatitis and no sFasL detection is a very good prognostic factor.
ssDNA as an index of programmed death of hepatocytes in patients with HBV, HCV or HCV and HIV infection

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HBV, HCV and common HCV/HIV infection modify of programmed death of hepatocytes.
In the liver tissue of patients with HBV, HCV and common HCV/HIV infection, were examined of ssDNA concentration, as an index of PD of. Then, were estimated correlation between ssDNA in hepatocytes and levels of HCV-RNA, HBV-DNA, HIV, morphological changes in the liver and number of CD3+, CD4+ and CD8+ cells.

Patients: 153 patients were included into study, among them 48 HBV infection, 19 HIV and HCV coinfected and 86 HCV-infected without HIV infection.

Methods: HBV-DNA and HCV-RNA concentration was measured by RT-PCR method (TaqMan chemistry, Applied Biosystems, USA). HIV-viral load was measured by RT-PCR method (Cobas Ampliticor HIS 1.5, Ultra Sensitive, USA), CD3+, CD4+ and CD8+ T-lymphocyte counts by use of flow cytometry (Becton Dickinson). Apoptosis ssDNA in hepatocytes were measured by monoclonal antibody and ELISA test (CHEMICON, Germany). Morphological state of the liver was defined accepting Scheuers classification.

Results: High concentration of HBV-DNA (HBV-DNA = $10^{7.8}$ copy/ml vs. HBV-DNA = $10^{4.3}$ copy/ml) correlated with from high concentration of ssDNA (1795 mg/mg vs. ssDNA = 1412 mg/mg). Among HBV patients, inflammable activity and intensification of fibrosis in liver tissue were reverse relation to concentration of ssDNA. Concentration of ssDNA were correlated with higher concentration of HCV-RNA and with genotype 3 in compare to genotype 1. Among HCV patients, inflammable activity and intensification of fibrosis in the liver's tissue were not influence on concentration of ssDNA. Concentration of HCV-RNA among HCV/HIV infected patients was statistical lower ($10^{2.9}$ geg/ml) in comparison to infect only HCV ($10^{3.8}$ geg/ml). The patients with HIV and HCV infection were lower concentration of ssDNA (886 µg/mg) in comparison to infect only HCV (ssDNA = 1332 µg/mg).

Conclusions: HBV in comparison to HCV more stimulates programmed death of hepatocytes. HIV by directly or indirectly inhibit of stimulate activity HCV on programmed death of hepatocytes.
Uridine supplementation with NucleomaxX™ antagonizes zalcitabine-induced mitochondrial hepatotoxicity in mice

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Objective: Uridine abrogates the mitochondrial hepatotoxicity of thymidine analogue reverse transcriptase inhibitors in vitro. We evaluated if Mitocnol (NucleomaxX™) a dietary supplement with high uridine bioavailability antagonizes this hepatotoxicity in vivo.

Methods: BalbC mice (7 weeks of age, 9 mice in each group) were fed zalcitabine with or without Mitocnol (0.1 g/kg/d) for 15 weeks. Liver histology and mitochondrial functions were assessed.

Results: One mouse exposed to high dose zalcitabine died at 19 weeks of age. Zalcitabine induced a dose dependent microvesicular steatohepatitis with depleted mitochondrial DNA (mtDNA), reduced cytochrome c oxidase activity (COX/SDH ratio) and elevated reactive oxygen species (Table). TUNEL assay showed increased apoptosis. Mitocnol attenuated all pathology.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver weight (g)</th>
<th>Necroinflammatory score</th>
<th>Steatosis score</th>
<th>Intrahepatic lipids</th>
<th>COX/SDH activity §</th>
<th>mtDNA copies ‡</th>
<th>Malondialdehyde</th>
<th></th>
<th>Superoxide content §</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.68±0.07</td>
<td>0.1±0.3</td>
<td>0±0</td>
<td>0.8±0.5</td>
<td>100±17</td>
<td>658±101</td>
<td>207±78</td>
<td>100±49</td>
<td></td>
</tr>
<tr>
<td>Mitocnol</td>
<td>0.67±0.11</td>
<td>0.2±0.4</td>
<td>0±0</td>
<td>0.7±0.6</td>
<td>104±15</td>
<td>626±104</td>
<td>184±37</td>
<td>101±36</td>
<td></td>
</tr>
<tr>
<td>Zalcitabine (0.36 mg/kg/d)</td>
<td>0.73±0.11</td>
<td>3.6±1.7**</td>
<td>1.1±0.3**</td>
<td>2.3±1.4*</td>
<td>45±15**</td>
<td>522±105*</td>
<td>335±114*</td>
<td>207±53**</td>
<td></td>
</tr>
<tr>
<td>Zalcitabine (0.36 mg/kg/d)+ Mitocnol</td>
<td>0.68±0.07</td>
<td>1.9±0.6**†</td>
<td>0.6±0.5**†</td>
<td>1.4±0.8†</td>
<td>73±24**†</td>
<td>607±91</td>
<td>215±103†</td>
<td>116±54†</td>
<td></td>
</tr>
<tr>
<td>Zalcitabine (13 mg/kg/d)</td>
<td>0.81±0.06**</td>
<td>8.8±1.3**</td>
<td>3.4±0.9**</td>
<td>5.9±1.8**</td>
<td>21±9**</td>
<td>413±48**</td>
<td>446±117**</td>
<td>420±162**</td>
<td></td>
</tr>
<tr>
<td>Zalcitabine (13 mg/kg/d)+ Mitocnol</td>
<td>0.70±0.10†</td>
<td>4.9±1.4**††</td>
<td>2.2±0.4**†</td>
<td>3.0±1.3**‡</td>
<td>52±9**††</td>
<td>543±78**</td>
<td>253±59**</td>
<td>178±36**††</td>
<td></td>
</tr>
</tbody>
</table>
Significance (P < 0.05) vs. controls * and vs. no Mitocnol †). Highly significant (P < 0.001) vs. control ** and vs. no Mitocnol ††. Units: $\$, % of control mean; ‡, copies/hepatocyte; $\parallel$ = μmol/ g tissue; $\infty$, mg lipid/mg tissue.

**Conclusion**: Zalcitabine induces a mitochondrial steatohepatitis. Which can be antagonized with dietary uridine without intrinsic effects.
Tumor necrosis factor-alpha, antimicrobial and antiendotoxin antibodies in chronic hepatitis (CH) and liver cirrhosis (LC) viral origin

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The aim of the investigation was to study the immune response to E. coli and Proteus spp. lypopolysaccharides and Common endotoxin in CH&LC with endogenic intoxication syndrome (EIS) indications.

Methods: 46 CH and 53 LC patients and 50 healthy volunteers were included in the investigation. Tumor necrosis factor-alpha (TNFα), E. coli antibodies (AEC) and Proteus spp. antibodies (APS) and Common antiendotoxin antibodies (CAA) concentrations were assessed by the ELISA method.

Results: TNFα is one of the most universal factors of EIS realization and also its main laboratory marker. The average TNFα level in CH&LC was authentically increased in comparison with the donors (86.4 ± 11.5 IU/ml and 140.3 ± 19.3 IU/ml cons 23.5 ± 6.0 IU/ml). CAA level in the blood of healthy donors was 8.0 ± 0.38 IU/ml, in CH – 7.7 ± 4.5 IU/ml, and in LC – 9.1 ± 5.9 IU/ml. High heterogeneity of CAA level in CH&LC was established (but not in controls). CAA level in 51.8% CH and 46.5% LC patients exceeded the norm for certain (p < 0.05), while 40.8% CH and 44.2% LC patients CAA level was below the norm. TNFα concentration in CH&LC with authentically increased CAA level (> 10 IU/ml) was nearly twice higher then the patients with lower CAA level.

The AEC and APS concentration in CH&LC was significantly increased in comparison with the norm. The direct correlative dependence between TNFα and AEC (r = 0.481) was found while this one didn’t exist between TNFα and APS (r = -0.262).

Conclusions: The antiendotoxic immune response was observed in CH&LC with EIS expressed. The direct correlative dependence between AEK and TNFα concentration was determined that testify the role of E. coli in cytokine cascade activation.
Primary biliary cirrhosis specific antinuclear antibodies can predict disease severity but not outcome

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²Liver Unit, Institut de Malalties Digestives Hospital, Clinic, IDIBAPS, University of Barcelona, Barcelona, Spain
³Inova Diagnostics, San Diego, California, USA

Introduction: Primary biliary cirrhosis (PBC) is characterized by disease-specific anti-mitochondrial antibodies (AMA) directed and antinuclear antibodies (ANA) giving by indirect immunofluorescence (IIFL) a rimlike (RL) pattern targeting gp210 antigen or a multiple nuclear dot (MND) pattern against sp100. In view of recent studies indicating that PBC-specific ANAs may have prognostic significance, we decided to investigate their role in PBC patients followed-up in Catalonia, Spain.

Material/Methods: 170 patients with PBC were initially tested for AMA and ANA by conventional IIFL. AMA was also evaluated by ELISA (Inova Diagnostics). The ANA subtype was evaluated using HEp-2cells and ELISAs detecting anti-gp210 and sp100 antibodies (Inova Diagnostics).

Results: 155 of 170 patients were AMA-positive by IFL and/or anti-M2 ELISA. Anti-RLM was present in 13 (8%) and anti-MND in 41 (24%) patients including 2 double-positive. Anti-gp210 was present in 19 (11%) including 4 RL-positive and anti-sp100 in 34 (20%) patients, including 22 MND-positive. There were no differences in ANA specificities according to AMA status. Compared to those negative, anti-gp210 positive patients had higher baseline and 12-month alkaline phosphatase, baseline bilirubin, and Mayo risk score (p < 0.05 for all). Gp210 positivity did not predict outcome (survival, transplantation, liver-related death) nor histologically defined disease severity. Compared to those negative, patients positive for RLM and/or gp210 (n = 27) had higher baseline and 12-month alkaline phosphatase, baseline bilirubin (p < 0.001), and Mayo risk score (p = 0.067). MND and/or sp100 seropositivity did not predict outcome or disease severity.

Conclusions: Our data validate the ability of PBC-specific ANAs to predict disease severity but not that to presage outcome.
Diagnostic relevance of anti-filamentous actin (F-actin) antibodies in autoimmune hepatitis

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\textsuperscript{1}Academic Liver Unit and Research Laboratory of Internal Medicine, Department of Medicine, Larissa Medical School, University of Thessaly, Larissa, Greece  
\textsuperscript{2}Institute of Liver Studies, King’s College, London School of Medicine at King’s College Hospital, London, UK  
\textsuperscript{3}Department of Immunology, King’s College Hospital, London, UK

Introduction: Filamentous actin (F-actin) has been considered the predominant target of the smooth muscle antibody (SMA)-tubular/glomeruli (T/G) pattern by indirect immunofluorescence (IFL), known also as microfilament (M-F) pattern specifically present in patients with autoimmune hepatitis type 1 (AIH-1). A recent study (Granito et al., J. Clin. Pathol. 2006) has found that anti-filamentous actin antibodies (A-FAA) measured by a new commercially available ELISA, strictly correlates with the SMA T/G pattern in Italian patients with AIH-1.

Aims: To assess the diagnostic relevance, specificity and sensitivity of the new A-FAA ELISA in patients with AIH-1 from Greece.

Material and methods: Autoantibody testing of SMA G/T by IFL on frozen sections of a composite rodent substrate and A-FAA by ELISA (Inova Diagnostics) was performed in 75 patients with AIH-1, 67 with primary biliary cirrhosis (PBC), 31 with primary sclerosing cholangitis (PSC), 70 with chronic hepatitis C virus (HCV) infection, 50 with hepatitis B virus (HBV) infection and 43 healthy controls.

Results: At variance with Granito et al. - and using the same ELISA and modified cut off of 30 AU -, we have found A-FAA seropositivity in a significant proportion of SMA-G/T-seronegative patients with AIH-1 The increased sensitivity of the A-FAA ELISA comes to the cost of its lower specificity since it detects A-FAA in a considerable number of SMA-seronegative pathological controls, especially in patients with primary biliary cirrhosis and chronic hepatitis C (Figure 1). We have also found through inhibition studies that AIH-1 specific SMA-G/T reactivity is not always abolished by F-actin as solid phase competitor.

Conclusions: Our data suggest that F-actin is not the sole target of the AIH-1 specific microfilament reactivity; larger studies are needed to address the specificity and sensitivity of the newly established A-FAA ELISA.
Figure 1

Anti-F-actin abs (RU/ml)

AIH-1 (n=75)
HBV (n=50)
HCV (n=70)
PBC (n=67)
PSC (n=31)
Healthy (n=43)
Novel autoantibody patterns detected by immunofluorescence on HEp-2 cells in patients with chronic hepatitis C virus infection

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²Institute of Liver Studies, King’s College, London School of Medicine at King’s College Hospital, London, UK
³Inova Diagnostics, San Diego, California, USA

Introduction: Autoantibody screening by indirect immunofluorescence (IIFL) on HEp-2 cells of patients with chronic hepatitis C virus (HCV) infections revealed two unreported atypical cytoplasmic patterns characterized by a) numerous granular cytoplasmic speckles extending from around the nucleus to the rest of the cytoplasm resembling that given by anti-mitochondrial antibody (AMA) and b) a crescent staining partially surrounding the nucleus resembling that given by anti-golgi antibodies.

Aims: To investigate the prevalence and fine specificity of these new autoantibodies in chronically infected HCV patients.

Material and methods: Sera from 550 Greek patients with chronic HCV infection were tested for autoantibodies by conventional IIFL on frozen sections of a composite rodent substrate and on HEp-2 cells (Inova Diagnostics).

Results: The AMA-like autoantibody was present in 46/550 (8.4%) Greek patients, median titre 1/320 (1/80-1/1280), persisted over time and belonged to the IgG1/IgG3 subclasses. Of the 46 IIF positive patients, 33 (72%) immunofixed a 47-49 kDa and 13 (28%) a 36-38 kDa cytosolic liver homogenate band. In addition, 17/46 (36%) of the AMA-like positive HCV patients had also a cytoplasmic staining pattern resembling that of anti-Golgi antibodies. This pattern was more clearly visualized when an IgG3 subclass specific antiserum was used as revealing reagent. Identical patterns and similar prevalence were also seen when 380 British HCV infected patients were tested and when we used HEp-2 from two different commercial sources.

Conclusions: Two new autoantibody patterns are described, that are present in some 10% of patients with chronic HCV infection. These patterns should be known to avoid incorrect laboratory diagnosis. The clinical significance of these new autoantibodies should be addressed in future studies.
Congenital extrahepatic biliary atresia as a cause of cholestasis in newborns and infants

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Background: Congenital atresia of extrahepatic bile ducts (CAEBD) is one of most common reason of cholestasis in newborns and infants.

The aim was to analyse clinical presentation, laboratory and imaging investigations and clinical outcome of children with CAEBD.

Materials and methods: 15 children (2 weeks-4 months). Data concerning jaundice onset, faeces colour, manifestation of coagulation disorders coexisting malformations and disorders of other systems were analysed. The following investigations were performed: biochemical tests evaluating the function of the liver and cholestasis (INR, total bilirubin and fractions, bile acids, ALAT, AspAT, GGTP, FALK activities in serum). We also performed tests focusing on hepatotropic infections, metabolic disorders, ultrasound scan of the abdomen and scintigraphy of the bile ducts.

Results: Jaundice, acholic stools and hepatomegaly were present in all children. Concentration of bilirubin and its conjugated fraction and bile acids, GGTP, FALK, ALT were elevated in all children. Active CMV infection was detected in 3. Ultrasonography revealed gallbladder in 7 children, intrahepatic bile ducts were normal in 12 cases. In all cases the HEPIDA-scintigraphy showed no passage of the tracer to the GI tract. Hepatoportoenterostomy was performed in 14 children, 5 of them had liver transplanted.

Conclusions: There is still no one effective and specific diagnostic method in differential diagnosis of CAEBD. This disease must be definitely excluded before other causes of cholestasis is eventually diagnosed. The hepatoportoenterostomy should be considered as the first line treatment in children with CAEBD. Most patients will need liver transplant in the future.
Analysis of HLA alleles polymorphism in Chinese patients with primary biliary cirrhosis

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Introduction: The aims of this study were to investigate the frequencies of human leucocyte antigens (HLA)-A, B and DRB1 alleles in Chinese patients with primary biliary cirrhosis (PBC), as well as to assess the correlation of HLA molecules in clinical and laboratory features.

Methods: Genotyping of HLA-A, B, and DRB1 were performed in 65 well-characterized patients with primary biliary cirrhosis and 431 healthy controls with PCR amplification with sequence-specific primers (PCR-SSP).

Results: The frequency of DRB1*0701 was increased to 29.2% compared with 13.9% in controls ($P_c < 0.05$, OR = 2.55, 95% CI: 1.4-4.6). No association was found with HLA-DRB1*08 which had been reported constantly. The A*2 allele (53.8%) was more frequent in PBC patients group but without statistics significance. The frequencies for the other A, B and DRB1 alleles were similar between patients and healthy controls. There was no difference between patients with and without DRB1*0701 in some clinical and laboratory features.

Discussion/Conclusion: Susceptibility to primary biliary cirrhosis in Chinese individuals is associated with DRB1*0701 allele differing from North America, South America, North Europe and even Japan, but the association is not restricted to any particular subgroup of patients; Valine at position 78 of HLA DRβ1 may play an important role in the pathogenesis of primary biliary cirrhosis.
Different hepatic metal content (Fe, Zn, Cu, Mn, Se) in viral, autoimmunologic and metabolic liver damage

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Introduction: In order to examine the effects of different liver diseases on the hepatic metal metabolism, we determined Fe, Zn, Cu, Mn and Se in 236 tissue samples.

Methods: Fundamental material for the analysis (atomic adsorption spectrometry after wet breaking down) was part (about 20%) of a liver specimen. The liver biopsy has been effected for microscopic examination with different clinical questions.

Results:

<table>
<thead>
<tr>
<th>Type</th>
<th>N</th>
<th>Fe</th>
<th>Zn</th>
<th>Cu</th>
<th>Mn</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic</td>
<td>34</td>
<td>1569</td>
<td>368</td>
<td>40.8</td>
<td>8.89</td>
<td>1.67</td>
</tr>
<tr>
<td>Alcoholic</td>
<td>31</td>
<td>886</td>
<td>242</td>
<td>29.7</td>
<td>5.12</td>
<td>1.91</td>
</tr>
<tr>
<td>PBC/imm</td>
<td>24</td>
<td>889</td>
<td>266</td>
<td>90.2</td>
<td>8.60</td>
<td>2.65</td>
</tr>
<tr>
<td>Hep. B+C</td>
<td>64</td>
<td>1256</td>
<td>384</td>
<td>47.5</td>
<td>7.12</td>
<td>3.34</td>
</tr>
<tr>
<td>Non-specific</td>
<td>83</td>
<td>1715</td>
<td>412</td>
<td>41.9</td>
<td>7.50</td>
<td>2.55</td>
</tr>
</tbody>
</table>

Data in µg/g dry weight of liver tissue

Discussion/Conclusion: Significantly low has been found, as expected, the zinc concentration in the liver tissue of patients with chronic abuse of alcohol. The mangan content is also significantly low in just these group of patients. Conspicuously, but narrow under the significance level, is the low iron concentration, but clearly high copper concentration in the case of (auto)immunologically caused liver diseases. Influences on the hepatic metal metabolism with mild to moderate liver diseases show, in the exceptional case, see above a changing, pointing the way. In case of PBC/autoimmunhepatitis further examinations necessary to find out, what importance has the relatively high copper content in the liver for the pathogenesis of this disease.
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**Side-effects of Interferon-α therapy in children with chronic hepatitis B**

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**Introduction**: To assess the side-effects of Interferon-α (IFN-α) therapy in children with chronic hepatitis B.

**Methods**: One hundred children were treated with 1 million units of recombinant IFN-α given subcutaneously three times a week for 20 weeks. A single dose of IFN-α was lower than 5.0 MU/m² in 80% of children. Side effects were assessed by interviewing the patients and their parents; clinical examinations and laboratory tests performed every month during and every 3 month after the therapy. For all children haemoglobin level, blood cell count, and serum gammaglobulin level were analysed. In 40 children, the presence and titres of autoantibodies (anti-nuclear, anti-smooth muscles, anti-mitochondrial, anti-brush border, anti-reticular, and anti-native DNA) before and after therapy was compared. In 24 patients free thyroxin, triiodothyronine, and thyrotropic hormone level were examined.

**Results**: Ninety-nine children completed the study. In one patient the therapy was discontinued because of repeated febrile seizures. The most frequent side-effects were fever (72%), flu-like symptoms (44%), headaches (40%), loss of appetite (26%), abdominal pain (23%), drowsiness (19%) and irritability (10%). Local discomfort, hair loss, and seizures were rare (2%). Lowering of the mean haemoglobin level, leukocyte, and platelet count was significant, but transient during treatment. No increase in autoantibody titres or significant alterations in thyroid function were observed. The frequency of observed side effects did not differ between groups receiving high or low doses of IFN-α.

**Discussion/Conclusion**: The side-effects of the IFN-α therapy in children such as fever, flu-like symptoms and bone marrow suppression are common, but transient and mild.
Pentoxiphylline promotes resolution of thioacetamide-induced liver fibrosis

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Introduction: Liver fibrosis is a spontaneously reversible process occurring predominantly after withdrawal of fibrogenic agents. Reversibility of liver fibrosis is dependent on ethiological factors, process duration and fibrosis topography. The data on reversibility of thioacetamide (TAA)-induced fibrosis are controversial but recently we demonstrated spontaneous reversibility of this kind of liver fibrosis in repeated experiments. The aim of this study was to evaluate possible antifibrotic effectiveness of pentoxiphylline (PTX), an inhibitor of the principal profibrogenic cytokine, TNFα.

Methods: Male Wistar rats, 230-240 g, received TAA (200 mg/kg, i.p.) during 12 weeks, 2 times a week. After TAA withdrawal, the TAA-treated groups were daily administered i.g. with two doses of PTX (10 and 20 mg/kg, i.g.) or with saline (placebo group) for the subsequent 8 weeks. The severity of liver fibrosis was assessed by a morphometric evaluation of liver slides stained with Azan-Mallory, hydroxyproline (Hyp) determination, and serum TNFα content using ELISA technique.

Results: The 3-month TAA treatment induced micronodular liver fibrosis with expressed deposition of collagen fibers. The square of liver connective tissue stained by Azan-Mallory increased in these rats nearly 7-fold, relative liver Hyp content – more than 2.5-fold and serum TNFα content – more than 2-fold. The TAA withdrawal led to moderate fibrosis regression as suggested the decrease of liver connective tissue area, whereas Hyp content in the liver and serum TNFα concentration did not change in rats deprived of TAA. Both doses of PTX gently decreased the accumulation of connective tissue in the liver whereas only highest dose of PTX (20 mg/kg) significantly decreased liver Hyp and serum TNFα contents.

Discussion/Conclusion: We can conclude that PTX, especially at a dose 20 mg/kg, promotes regression of TAA-induced liver fibrosis. This study suggests the antifibrotic potency of PTX which should be used in patients to prevent liver fibrosis progression.
Electrohepatography a new diagnostic modality?

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²Medex Screen Ltd, Arad, Israel

Introduction: Electrohepatography is the non-invasive transcutaneous detection of liver abnormalities by the measurement of changes in the electrical impedance of defined dermal zones, believed to represent viscera. This method is based on neuroreflexology, a branch of complementary medicine. This study addressed two questions: first, can Electrohepatography detect liver disease, and second, can it measure the severity of a known liver disease.

Methods: Part 1: a retrospective, comparative study, 112 patients suffering from known liver diseases diagnosed according to common clinical practice and 86 healthy controls were referred to one of two blinded MEDEX TEST (US patent 10/210,223) operators. Part 2: 62 treatment naive patients with Hepatitis C were referred to one of three blinded MEDEX TEST operators. The grading of liver necro-inflammation in biopsies (0-4 according to the Batts & Ludwig scale) was compared to the Electrohepatography grading (also on a scale of 0-4).

Results: Electrohepatography identified a liver disease with a positive predictive value of 0.88 and a negative predictive value of 0.77, Sensitivity 80.4%, Specificity 86%, and Accuracy 83.2%. Electrohepatography grading matched the histological grading in 80.6% of the patients. In 11.3% there was a discrepancy of one point and in 8.1% there was a discrepancy of more than one point on the severity score.

Discussion/Conclusion: Electrohepatography, which is non-invasive, painless, rapid, portable, simple to operate and radiation-free, may serve, if confirmed by further studies, as a screening modality for liver diseases, and can also quantify necroinflammatory activity with accuracy comparable to liver biopsy.
Non-alcoholic fatty liver disease in the Philippines: Comparable with other nations?

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Introduction: There has been paucity of data with of non-alcoholic fatty liver disease (NAFLD) in the Philippines. Thus a chart review was done of all NAFLD cases seen at the Philippine General Hospital from January 1999 to December 2004 to note the characteristic features of patients.

Methods: From a total of 1102 patients, 134 NAFLD patients diagnosed based on clinical, ultrasonographic and/or histopathologic findings were accepted for review. Patients with secondary NAFLD were excluded. Chart review was done to note demographics, co-morbid illnesses, physical characteristics, hepatomegaly, AST/ALT levels, albumin, lipid levels, alkaline phosphatase, prothrombin time, and partial thromboplastin time of included subjects. Obtained data was analyzed using SPSS statistical software.

Results: Twelve percent of the study population were diagnosed to have NAFLD. 17 patients had histopathological results with 14 having early stage NAFLD and 3 having non-alcoholic steatohepatitis. These 3 patients all had characteristics of the metabolic syndrome (type 2 diabetes, BMI > 30, hyperlipidemia, and hypertension). Of the 134 patients included, 71% were female and 29% male. Mean patient age was 42.176 years. 60% patients were obese, 56% had hepatomegaly, and 69% were diabetic. AST levels were elevated in 45% of subjects and ALT levels in 64%.

Discussion/Conclusion: The female sex, obesity, elevated liver enzymes, and diabetes, are characteristic features of our NAFLD patients which is comparable to previous data from other countries.
Metformin in the treatment of non-alcoholic fatty liver disease

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Introduction: Twenty percent of the world’s population have non-alcoholic fatty liver disease, about 10% of which progress to non-alcoholic steatohepatitis, cirrhosis and even HCCA. Insulin sensitizing agents such as metformin is a theoretically sound option for this disease. We aim to evaluate effectiveness of metformin compared to diet in improving liver function tests, insulin levels, glucose levels and BMI in patients with NAFLD.

Methods: Cochrane, EMBASE and MEDLINE search was done to look for relevant articles using free-text terms and MESH words. After clinical appraisal of each of the included studies, third party software was used to synthesize the results (RevMan 4.2).

Results: The search yielded a total study population of 116. ALT significantly decreased with Metformin plus diet treatment (n = 116, OR point estimate = 15.78, 95% CI: 5.37, 26.19). There was also note of a significant decrease in insulin levels in the metformin plus diet group (n = 116, OR point estimate = 2.4, 95% CI: 0.88, 3.92). Blood glucose levels showed a trend toward improvement but was not significant (n = 116, OR point estimate = 3.68, 95% CI: -1.68, 9.05).

Discussion/Conclusion: This study seems to show benefit in the use of Metformin for patients with NAFLD in decreasing ALT and insulin levels. More RCT’s with larger population sizes and endpoints including histopathology have to be done to validate the results of this meta-analysis.
Ursodeoxycholic acid application for the prevention of different types side-effects of chemotherapy at non-Hodgkin’s lymphoma patients

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Ability of ursodeoxycholic acid in form of Ursofalk® (UF) to prevent or reduce hepatotoxic and gastrointestinal side-effects of combined chemotherapy (CT) at patients with non-Hodgkin’s lymphoma was investigated. CT performed with traditional CHOP programme. Before CT patients had no any signs of liver pathology.

20 patients undergone CT perorally took UF (250 mg 3 times per day). Taking UF was started 3 days before CT and continued 18 days. Other 20 patients underwent CT only (control group). All patients were biochemically tested two times: before and after CT. Biochemical testing included: determination of bilirubin level in the blood serum and quantitation of activity of liver enzymes. Moreover, before and after CT we quantitated NK cells in the blood, its cytotoxic activity (CA) and levels of several cytokines in the blood serums of all patients.

Results of the observation demonstrated that frequency of biochemically registered hepatotoxic side-effects (increasing of bilirubin level and activities of liver enzymes) at patients who took UF were substantively less than at patients in control group (p < 0.01). After CT patients treated with UF (in comparing with patients in control group) had increased level of alpha-interferon and decreased levels of gamma-interferon, interleukins 1 and 6.
Haematological modifications at patients with chronic hepatitis C, treated with peg-interferon and ribavirin

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¹Department of Infectious Diseases, Timisoara, Romania
²Department of Pulmonology, Timisoara, Romania

Introduction: The diagnosis of haematological modifications at patients under treatment with peg-interferon (PEG-IFN) and ribavirin (RBV) assures this therapy’s efficiency.

Methods: The authors have studied a group of 30 patients with chronic hepatitis C diagnosed and monitored in the Clinic of Infectious Diseases - Timisoara. The selection criteria for the beginning of the antiviral therapy: biochemical (ALT over 1.5 times), virusological (Ab anti HCV positive, ARN-HCV quantitative positive) and morphological (Knodell score, fibrosis F3, indifferent of the necroinflammatory index; fibrosis F0/F1 with the necroinflammatory index over 6; fibrosis F4 without clinical, ultrasonographic or endoscopic signs of portal hypertension, without leucopenia, anemia or thrombocytopenia) and less than 65 year old. All the patients have followed a treatment with PEG-IFN-alpha-2a (Pegasys® - Roche, 180 mcg/ml/week) and RBV (Copegus® - Roche, 1-1.2 g/daily, depending on the corporal weight), for 48 weeks.

Results: The biological tests have demonstrated: thrombocytopenia (under 100,000/mm³) at 13 patients (43.33%), which imposed the decrease of the PEG-IFN dosis; the decrease of haemoglobinosis under 10 g% at 12 patients (40.00%) with the daily decrease of the RBV dosis and the decrease of leukocytes, followed by neutropenia at 11 patients (36.66%). There were no cases of interruption of the combined antiviral therapy at the studied group of patients. The registered clinical manifestations (epistaxis, dizziness, tegumentary bacterial superinfections) were correlated with the aforementioned biological modifications.

Discussion/Conclusion: The thorough and correct clinical-biological monitorization of the patients with chronic hepatitis C, treated with PEG-IFN and RIB, permits the early diagnostication and correction of the haematological modification, assuring the efficiency of this therapy.
Plasma glutathione and glutathione S-transferases T1/M1 genetic polymorphisms in hepatitis C viral infection

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Introduction: Hepatitis C virus is a major cause of viral hepatitis. The infection is characterized by a systemic oxidative stress resulting from chronic inflammation, iron overload and liver damage. The glutathione S-transferases (GST) are responsible for metabolization of several hydrophilic compounds (xenobiotics, aldehydes, hydroperoxides) by conjugation with glutathione. Human GSTM1 and T1 exhibit genetic polymorphisms resulting from gene deletion, which may be correlated with antioxidant activity variation.

The present study evaluates the association between GSTT1/M1 polymorphisms and oxidative stress involvement in chronic hepatitis C (CHC) infection and their implications to antiviral response in 255 patients treated with pegylated interferon alpha and ribavirin.

Methods: GSTT1/M1 genetic distribution in CHC (n = 89) and control group (n = 300) was assayed by Multiplex-PCR. Plasma glutathione (tGSH) and SH groups (tSH) were evaluated by spectrofluorimetric and spectrophotometric methods. Biochemical values of GGT and ferritin were also determined.

Results: CHC individuals presented higher frequency of GSTT1 non-null genotype (89.9%) than control group (57.7%, p = 0.000; OR = 6.525, CI [3.157-13.489]) and lower tGSH levels (173.6 ± 75.9 vs. 408.9 ± 129.8 µM; p = 0.000). There was no correlation between GSTT1 genotype and tGSH, but patients with GSTT1 non-null genotype had lower tSH levels (p = 0.001). GSH depletion is not associated with virus genotype (p = 0.181).

Increased levels of serum ferritin correlate with lower tGSH levels (p = 0.033) and with increased GGT activities (p < 0.01). Patients with GSTT1 non-null genotype have a trend to show less response to treatment (p = 0.064). Non-responders patients presented lower GSH levels than sustained responders.

Discussion/Conclusion: GSTT1 polymorphism, GSH levels and serum ferritin may be considered as risk factors for a sustained antiviral response.
Evaluation of Ursofalk®’s effect in prevention of the gallstones in the patients with chronic hepatitis C virus infection who receive antiviral treatment

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Introduction: Were observed that the administration for a long time (≥ 48 weeks) at the “gold standard” (interferon-alpha and ribavirin) in the chronic hepatitis C virus (HCV) infection was associated with a high rate of the appearance of the gallstones.

The aim of the study was to evaluate the effect of Ursofalk® administration in prevention of the gallstones for the period of “gold standard” treatment.

Patients and methods: We studied 52 patients with HCV infection without gallstones who take “gold standard” for 48 weeks. 25 patients received “gold standard”, but in other group 27 patients were administrated “gold standard” with Ursofalk® (750 mg/daily). We were made abdominal ultrasound in both groups for the examination of the gall initial and after 48 weeks of the treatment.

Results: After 48 weeks of the administration of the “gold standard” without Ursofalk® gallstones were discovered in 6 patients (24%), but in the group with associate treatment the gallstones were discovered in 1 patient (3.7%). The difference of appearance of gallstones between groups is statistical significantly (p < 0.001).

Conclusion: The antiviral treatment for a long time of the HCV infection is associated with appearance of gallstones and Ursofalk® administration can significant reduce the appearance of gallstones for the period of the antiviral treatment.
The assessment of non-alcoholic fatty liver disease in the Department of Infectious Diseases patients

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Introduction: Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are relatively new described chronic liver disease (CLD), which occurred generally in Western countries. The aim was the assessment of NASH and NAFLD prevalence in the hepatology ward of Infectious Diseases Department.

Methods: 25 patients (3 female, 22 male), mean age 46 years (range 21-60) were admitted to Department of Infectious Diseases Medical University of Gdansk in 2004/2005 because of NAFLD suspicion. The initial diagnosis was established based on patients' anamneses, results of liver function tests, disturbances of iron, carbohydrates and lipids metabolism, and result of abdominal US imagining. In all cases autoimmune and viral hepatitis, other metabolic and toxic disorders were excluded. A diagnosis was confirmed by liver biopsy.

Results: A body mass index (BMI) was larger than 30 kg/m² in 18/25 cases and ranged 27-29 in the reminder patients. The ALT activity in 15/25 cases was 41-100 IU/l, in 7/25 was 101-200 IU/l and only in 3/25 individuals was normal. 20/25 patients were diagnosed the impair glucose tolerance and diabetes. In 17/25 cases hypercholesterolaemia and in 8/25 cases hyperglyceridaemia were found. Disturbances of iron metabolism were found in 15/25 individuals. In 16/25 liver specimens a NASH signs, in 9/25 hepatocytes steatosis were found. Concomitant fibrosis in 3/9 cases was present. Arterial hypertension was discovered in 15/25 studied cases.

Discussion/Conclusion: A NAFLD should be taken into consideration of CLD diagnosis, especially in obese patients. The liver biopsy in these patients seems to be essential in NASH and NAFLD diagnosis.
Evolution and prognostic significance of ascites in patients with liver cirrhosis

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**Introduction:** End-stage liver disease is characterized by the appearance of refractory ascites (RA), spontaneous bacterial peritonitis (SBP), hepatorenal syndrome (HRS), dilutional hyponatremia (DH), complications that influence the survival of cirrhotic patients with ascites and require a standardised evaluation and therapy.

The aim of the study is to identify prognostic factors for survival and to determine the adequate and safe therapy that improves the evolution of RA, SBP, HRS or DH.

**Methods:** 213 cirrhotic patients (mean age 57.3 ± 10.5 years) were followed over a period of 48 months, at 3-6 month intervals. The mean Child score was 11.5 ± 4.09.

**Results:** 20% patients developed DH; we restricted water ingestion when serum Na decreased < 130 mEq/l and interrupted diuretics when serum Na decreased < 125 mEq/l. The incidence of RA was 30%; in 8 patients TIPS was performed with good results. SBP was diagnosed in 13% patients and treated with Cefotaxime ± Nolicin, Maxipime ± Nolicin, Ciprinol, Terlipressin and Albumin infusion, with better resolution and lower recurrence in cases in which Terlipressin, Albumin infusion and antibiotics were associated. The rate of developing HRS was 7%.

**Discussion/Conclusion:** The survival rate was influenced by Child-Pugh score, serum creatinine, serum Na and the presence of HRS. In our study the most efficient therapy for RA was represented by TIPS (p < 0.001) compared to repeated paracentesis, while for SBP the association of Terlipressin to antibiotherapy provided the best results.
Prospective study of the natural evolution and prognostic factors of compensated liver cirrhosis

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Aim of the study: To establish the prognostic factors in compensated cirrhosis.

Material and methods: Between 2000-2004 the authors followed-up 137 patients with liver cirrhosis of viral and non-viral etiology, who at the moment of entry in the study presented compensated liver cirrhosis (Child-Pugh score < 7). Patients were evaluated every 3 months and severity was established using the MELD score and the Child-Pugh class. The following parameters were evaluated: serum albumin, total bilirubin, INR, total cholesterol, fibrinogen and liver enzymes. The mortality rate and the influence of different parameters were analyzed using Epi-Info statistical analysis and the Kaplan-Meier survival curves. The following levels of statistical significance were established: p > 0.05 – statistically non-significant, p < 0.01 statistically significant.

Results: The etiology of liver cirrhosis for the 137 patients was: HCV = 22.63%, HBV = 28.47%, HCV+alcohol = 6.56%, HBV+alcohol = 5.1%, alcohol = 23.35%, HBV+HCV = 9.5%, PBC = 0.73%, Wilson disease = 0.73%, HBV+HDV = 2.9%. Mean follow-up period was 18 months. During the follow-up period 13.86% of patients dies, 3.65% benefitted from liver transplantation, 13.14% were lost to follow-up and 69.35% developed non-fatal complications. The parameters that correlated with development of complications and with mortality were albumin (p < 0.01), creatinin (p < 0.01), total bilirubin (p < 0.01), INR (p < 0.01) and cholesterol (p < 0.01). HBV etiology of liver cirrhosis also correlated with the development of complications and death.

Conclusions: In our study group mortality and morbidity correlated with the biological parameters used in the MELD score. According to their power of correlation with morbidity these were total bilirubin, INR, creatinin and albumin. We also noticed that HBV etiology and the level of serum cholesterol influence the survival rate and an earlier development of complications.
Can the number of thrombocytes indicate the progression of C chronic hepatitis to hepatic cirrhosis?

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Introduction: It is known that a number of thrombocytes below 140,000/mm³ correlates to hepatic fibrosis and that the latter becomes more severe as chronic hepatitis evolves into cirrhosis. We attempted to find whether the number of thrombocytes might be relevant as a marker of C chronic hepatitis’s progression into hepatic cirrhosis.

Methods: We studied all the 170 patients with C virus chronic hepatopathy who were admitted in the Medical II Clinic of the County Clinical Hospital Sibiu from January 2002 to December 2005. We examined the value of the ultrasonographic parameters of the portal hypertension in the relation to the number of thrombocytes (< or > 140,000/mm³). We compared the values at the patients with chronic hepatitis (150 patients) with those found in the patients with hepatic cirrhosis (20 patients). We analysed the results statistically using the t Student and the Pearson tests.

Results: The mean age of the patients was 54.41 ± 11.28 years. 63.52% were female and 34.48% were male. The long spleen axis and portal vene diameter in the hepatic hill were significantly higher at the patients with the number of thrombocytes below 140,000/mm³ (13.06 cm, 12.2 mm, respectively) as compared to those with the number of thrombocytes above 140,000/mm³ (11.05 cm, 11.36 mm, respectively) (p < 0.01, p < 0.001, respectively). At the patients with C chronic hepatitis, the long axis of the spleen was significantly higher at those with less than 140,000 thrombocytes/mm³ as compared to those with higher values (12.4 cm as compared to 11.45 cm) (p < 0.01, r = -0.35). At these patients, the portal vene diameter did not vary significantly in relation to the value of the thrombocytes. At the patients with hepatic cirrhosis the ultrasonographic parameters of the portal hypertension had significantly higher values in relation to those with chronic hepatitis (p < 0.001, p < 0.001, respectively), and the number of thrombocytes was significantly smaller (p < 0.001).

Discussion/Conclusion: The number of thrombocytes below 140,000/mm³ at patients with C chronic hepatitis may constitute a sign of the evolution towards hepatic cirrhosis. It correlates with the long axis of the spleen.
Characteristics of alcoholic hepatitis

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Introduction: Alcoholic hepatitis is frequent and is a matter of public health. We proposed ourselves to study the characteristics of alcoholic hepatitis in a group of hospitalized patients.

Methods: We have studied all the 165 patients with chronic hepatitis who were admitted to the Medical II Clinic of the County Clinical Hospital Sibiu from 1 January to 31 December 2005. They were divided into two groups, depending on the etiology of the disease. The first group included patients with alcoholic hepatitis and the second one included patients with hepatitis of different etiology. In the two groups we compared the age, the gender, the biochemical syndromes of hepatocytolysis, bilioexcretory and of liver insufficiency, the number of thrombocytes, the relation between aspartate aminotransferase (ASAT) and the thrombocytes, the echogenicity and the posterior alleviation of the ultrasounds in the liver, the long axis of the spleen, the portal vene diameter in the hill. The results were analysed statistically using the t Student and the Pearson tests.

Results: At the patients with alcoholic hepatitis, ASAT was significantly higher as compared to the patients in the second group (87,654 ± 79.37 compared to 62.41 ± 41.36) (p < 0.01). Total bilirubin values and the cholestasis enzymes (gamma-glutamyl transferase and seric alkaline phosphatase) were significantly higher in the first group (2.2 ± 4.82 mg/dl, 279.51 ± 333.19 U/l, 142.79 ± 89.15 U/l respectively) as compared to the second group (1.15 ± 1.07 mg/dl, 132.24 ± 231.41 U/l, 107.78 ± 82.53 U/l respectively) (p < 0.05, p < 0.01, p < 0.05 respectively). The ASAT/thrombocytes relation did not change significantly between the two groups. There were no statistically significant differences between the two groups concerning the ultrasonographic parameters.

Discussion/Conclusion: The patients with alcoholic hepatitis have hepatic cholestasis and important ASAT increased values on a more frequent basis than those with hepatitis of a different etiology, therefore their therapy should be adapted.
Prevalence of anti-HEV in patients with viral and autoimmune hepatitis

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Introduction: The aim of the study was to determine the prevalence of antibodies to hepatitis E virus (anti-HEV IgG) in “health” population and patients with liver pathology and Shegren’s disease.

Methods: Anti-HEV were detected in blood sera by EIA with test-systems “Diagnostic preparations” (Russia) and “Abbott” (USA). Blood samples were obtained from different population groups in Moscow: blood donors (n = 163); pregnant women (n = 82); patients with autoimmune hepatitis (n = 44); with chronic hepatitis B and C (n = 51); with liver cirrhosis and HCC (n = 27) and Shegren's disease (n = 12).

Results: The prevalence of anti-HEV among blood donors and pregnant women was 0.6% and 1.2%, respectively. The received data are close to results of detection anti-HEV in the population in the industrial advanced countries of Europe and America, i.e. non-endemic world regions in relation to hepatitis E. Frequency of anti-HEV detection among patients was much higher and has made for autoimmune hepatitis; chronic hepatitis B and C; liver cirrhosis and HCC – 20.4%; 5.1% and 11.1%, respectively. In 12 patients with Shegren's disease anti-HEV were revealed in 2 cases.

Discussion/Conclusion: Despite low prevalence of anti-HEV in "healthy" population of Moscow, their high frequency is established in patients with liver diseases which are not characteristic for HEV infections.
Negative influence of the chronic hepatitis C viral infection on the breast cancer prognosis

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It is known that hepatitis B viral infection in patients with breast cancer (BC) is able to influence negatively the survival rates of these patients. Therefore we studied the character of chronic hepatitis C virus (HCV) infection influence on BC prognosis.

In our investigation we compared 3-, 4- and 5-year survival values in two groups of patients with a third clinical stage of BC. The first one consisted of 39 women with persistence of RNA HCV in the blood and the second group was represented by 53 women without RNA HCV and anti-HCV in the blood. Both groups of patients were similarly treated according to the program "Preoperative radiotherapy + radical mastectomy + postoperative polychemotherapy". A comparison was carried out due to the rates of 4- and 5-years survival rates in two groups of BC patients.

The 4-year survival rate in BC HCV-positive patients was found to be lower than in persons without viremia (p < 0.1). The 5-year survival rate among HCV-positive BC patients also turned out to be low (p < 0.05). It is supposed that persistent HCV viremia may be regarded as an unfavorable prognostic factor in cases of this malignant tumor. This effect could be the result of metabolic and immunologic alterations caused by persistent infection. In the same time it was probably connected with hypereostrogenemia developed as a result of hepatic dysfunction.
Effect of combination therapy in HCV-related glomerulopathy

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Background: Mixed cryoglobulinemia (MC) and glomerulonephritis are most important extrahepatic manifestations of chronic hepatitis C virus (HCV) infection. In HCV-infected patients with MC, renal involvement worsens the overall prognosis because of a high incidence of infection or cardiovascular disease. The relationship between MC and HCV infection has prompted the use of antiviral therapy.

Materials and methods: Seventeen patients with associated HCV infection glomerulonephritis were studied. All the patients were examined clinical, for serum cryoglobulins, HCV, HBV markers, morphologi (renal biopsies in 10 patients). 7 patients had cryoglobulinemic mesangio proliferative glomerulonephritis (MPGN).

Results: After primary treatment with immunosuppressants, plasmaexchange, antiviral therapy with standard or pegylated interferon alfa and ribavirin was introducted in 8 patients. These patients were compared with 9 patients who had not received antiviral treatment. Mean duration of antiviral treatment was 12 months, with a follow-up of at least 6 months after treatment withdrawal. HCV RNA clearance (sustained virological response) was achieved in 3 of 8 patients, AST, ALT were normalized, 24-h proteinuria significantly decreased (from median 4 g to 1.25 g), serum albumin increased (from median 2.2 to 3.5 g/dl), lower viral titres and complement concentrations returned to normal levels. Conversely, no changes in serum cryoglobulins levels were found in non-responders or controls. Immunosuppressants were successfully used for treatment of the HCV-infected patients with cryoglobulinemia and progressive renal failure caused by MPGN. Unfortunately, HCV RNA levels were increased in these patients and adverse events of immunosuppressive drugs (nephrotoxicity, arterial hypertension, dyslipidaemia, diabetes mellitus) are connected with poor outcome.

Conclusion: This study confirm the relationship between HCV infection and glomerulonephritis, especially MPGN, and use of a combination of interferon and ribavirin in the treatment of selected cases of HCV-related glomerulopathy.
Study of predictive factors concerning the risk of renal complications of cirrhosis ascites

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Introduction: Mortality in hepatic cirrhosis is influenced by the development of further complications. The most frequent and serious complications in the course of hepatic cirrhosis are due to spontaneous bacterial peritonitis and hepatorenal syndrome.

Aim of study: Our study intends to select specific predictive parameters for assessing the risk of renal dysfunction development.

Material and methods: We studied a group of 387 patients with hepatic cirrhosis with ascites followed over a 5 years period. Global statistics evaluations showed a majority of male patients (73.5%) compared to the female patients (26.5%); mean age was 57.07 ± 7.56 years). The research protocol contained a clinical, biological and a complete imagistic evaluation of the liver and portal system, the ascitic fluid analysis and especially tests for the evaluation of the systemic haemodynamic changes and of the renal function (Holter monitoring of medium blood pressure and cardiac frequency, urea and creatinin quantification, seric and urinar ionogram, creatinin clearance and water diuresis).

Results and discussions: During the study period of 5 years, a number of 41 patients, meaning 10.8%, developed hepatorenal syndrome. Statistically the predictive parameters helpful for early diagnosis of the hepatorenal syndrome were: creatinin clearance, water diuresis, protein’s level of the ascitic fluid, medium blood pressure and urinar sodium. The evaluation of these parameters has to be done in dynamic, following a good schedule.

Conclusion: Hepatic cirrhosis evolution is marked by many various complication that can change the prognosis. Haemodynamic systemic parameters and those evaluating the renal function are the most efficient in assessing the risk of renal complication within the cirrhotic patients and is the only way to indicate full security for hepatic transplant.
The study of the efficacy UDCA (ursodeoxycholic acid) therapy in alcoholic liver disease

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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 liver disease irrespective of whether or not cholestasis is present.

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

Patients and methods: 26 patients (17 males, 9 females), mean age 59 ± 6 years with biopsy proven alcoholic cirrhosis 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: 16 patients to receive UDCA (15 mg/kg/day) and 10 patients to receive placebo for 8 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 8 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 4 weeks of treatment with UDCA. One patient was withdrawn from the study because of development of diarrhoea 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 liver disease especially if cholestasis is present. UDCA had a good tolerance for the patients in these cases.
Protective properties of selenium derivatives in chronic hepatitis

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Introduction: Selenium (Se) deficiency is a factor that provokes hepatitis to take a chronic course and causes aggravation of the disease. It has been shown lately that cirrhosis outcome of chronic hepatitis (ChH) can be prevented by Se-containing substances (SeS). The goal of the present work was to compare the hepatoprotective properties of Se-methionine (SeM), dimethylidipropyrosolylselenide (DMPS), and Se-pyrane (SeP, 9-phenyl-octadihydroseleulium xanthene) in ChH models.

Methods: The experiments were carried out on 40 Wistar CRL: (WI)WUBR female albino adult rats subdivided into 5 groups and treated (2-5 groups) with CCl₄ (1.0 ml/kg twice a week over a month). SeS was administered (3-5 groups, intragastrically, 250 µgSe/kg body weight) for 2 weeks starting from day 14. Blood (erythrocytes and plasma) and liver were assayed for oxidative stress (OS) indices, glutathione (G) system and glutathione peroxidases (GP) (H₂O₂-, t-BOOH-metabolizing). ChH was monitored by transaminases, glutamyl transpeptidase, lactate dehydrogenase and OS.

Results: ChH was characterized by hyperfermentemia and increased blood plasma OS products. The Se-M administration did not abolish the hyperfermentemia and, partially, blood plasma OS product accumulation, whereas DMPS, and particularly, SeP were more effective. All the SeS stimulated GP(t-BOOH) activity and only SeP activity of erythrocyte GP(H₂O₂). The SeM and DMPS administrations elevated blood plasma GP(H₂O₂) activity while SeM raised GP(t-BOOH) activity. DMPS raised erythrocyte G-SH level. The liver of animals with ChH demonstrated increased GSH and GSH+2GSSG levels, glutathione reductase (GR), GPs and GSH/GSSG ratio remaining unchanged. The SeM administration increased this ratio, with GSH, total G being normalized and GR activity decreased. The DMPS effect was manifested by a maximum elevation (over 2.6-fold) of GSH/GSSG as well as of GSH and total G with decreased GR activity. A similar effect was seen after the SeP administration. Only this compound stimulated tissue antioxidant activity.

Discussion/Conclusion: The xenobiotic SeS (DMPS and SeP) had a certain protective activity in ChH, modulating the glutathione system. This effect on the G redox state was maximum (alongside with SeM). DMPS and SeP may act as Se-cysteine precursors and direct antioxidants.
Unusual association of chronic discoid lupus with autoimmune hepatitis

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Introduction: Autoimmune hepatitis could be induced by various triggers, including viruses, drugs, as well as aberrant autoreactivity and genetic susceptibility. The association of other autoimmune diseases is well established, commonly thyroiditis, type I diabetes, rheumatoid arthritis or systemic lupus erythematosus.

Methods: We report the case of a 52 years old patient with history of chronic discoid lupus for 5 years whom presented for arthralgias, overwhelming fatigue, upper right abdominal discomfort, nausea. The patient suffered from discoid lupus, with erythematous papules and plaques with thick and adherent scaling, follicular plugging and scarring with central atrophy, in the absence of any systemic manifestation at repeated evaluations. He showed no clinical signs of renal, neurological or pulmonary involvement.

Results: Laboratory evaluation showed anemia, leukopenia, thrombocytopenia and increased liver enzymes three times the reference range. The patient was currently under no anti-inflammatory medication or hepatotoxic drugs. The erythrocyte sedimentation rate was increased, with positive C-reactive protein test, with no other serological prove of systemic lupus, such as anti-dsDNA, anti-Sm, anti-Ro antibodies, or hypocomplementemia. Viral hepatitis B and C were excluded, as well as infection with Epstein-Barr virus. High titers of ANA and anti-smooth muscle antibodies were found, as well as increased levels of serum gamma globulines. Liver biopsy was performed, confirming the pattern of autoimmune hepatitis.

Discussion/Conclusion: Our case presented diagnosis difficulties, taking into consideration that autoimmune hepatitis is a well defined clinical entity, commonly associated with systemic lupus, but rarely described in association with chronic discoid lupus.
Dermatological manifestations in B and C chronic viral hepatitis

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Introduction: Dermatological manifestations in B and C chronic viral hepatitis can occur as a consequence of the delayed-type hypersensitivity to an antigenic stimulus (HBsAg and VHC) with the forming of circulated immune complexes and their accretion on the vascular level with the consecutive lesion of the latter. Cellular mediated immunospecific injury and immunological decrease that occurs in B and C chronic viral hepatitis can also produce the occurrence of cutaneous manifestations.

Material and methods: This study has been carried out on a group of 78 patients admitted in the Medical Clinic I, from October 2004 to December 2005, 27 (34.6%) patients with B chronic hepatitis (CHB) and 51 (65.38%) patients with C chronic hepatitis (CHC).

In order to establish the occurred dermatological manifestations, the following investigations were made: dermatological clinical examination, biological examinations, skin biopsy, histopathological examination.

Results: Dermatological manifestations were reported at 25 (32.05%) patients in the group: 12 (48%) patients were diagnosed with CHB and 13 (52%) patients were diagnosed with CHC.

We detected: inflammatory papular erythematous squamous dermatitis, such as lichen planus at 3 patients with CHB and 2 patients CHC and psoriasis at 2 patients with CHB and one patient with CHC.

Allergic cutaneous manifestations such as prurigo were detected at 2 patients with CHB and at 2 patients with CHC; vasculitis like urticaria occurred at one patient with CHB and 2 patients with CHC. The cutaneous viroses such as herpes simplex were identified at 2 patients CHB, while zona zoster appeared at one patient CHB. Basal cell carcinomas were detected at 2 patients CHC and amyloidosis at one patient with CHC.

Specifically, at the patients with CHC we reported: cryoglobulinemic vasculitis at 2 patients, porphyria cutanea tarda at one patient and at the patients with CHB we identified eritem nodosus.

Most of the cutaneous manifestations, especially those like vasculitis, evolved favourably when we started the antiviral therapy. The prognosis of these skin manifestations would have been unfavourable without the antiviral treatment.

Conclusion: The cutaneous manifestations can accompany B or C chronic hepatitis (they preceed the diagnosis, can occur simultaneously or intermittently during the evolution), they can emphasize some complications, or can reveal manifestations or commorbiditities, especially autoimmune type.
Antibiotic prophylaxis after esophageal variceal bleeding: Why does it really matter?

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Aim: The aim of our study was to determine the usefulness of antibiotic profilaxis in esophageal variceal bleeding (EVB).

Patients and methods: 70 patients presenting with EVB, 62 M/8 F, mean age 44.5 yrs. admitted into the Intensive Unit Care. All patients had endoscopic variceal sclerotherapy. The patients divided into two groups. GROUP A - 35 patients treated prophylactically with intravenous ciprofloxacin 200 mg every 12 hours during five days, while control group - GROUP B - 35 patients, didn't treated with prophylactic antibiotics. The effect variables were: duration of stay in ICU, bacterial infection, number of rebleeding and mortality in first 6 days. The age and the Child's Classification between two groups were comparable.

Results: Hospital stay in ICU was in Group A 4.8 days and in group B 8.5 days (P < 0.001). Antibiotic prophylaxis decreased infections. The incidence of bacterial infections was significantly lower in the treatment Group A 2/35, than the control Group B 11/35 (P < 0.001). The number of patients who rebleed during the first 6 days in group A was 4 (12%) and in group B was 9 (26%) (P < 0.001). Hospital mortality in group A was 9% and in group B was 21%.
For data analysis Chi-Square test, Friedman test and T-test were used.

Conclusion: In bleeding cirrhotic patients bacterial infection is associated with failure to control bleeding as well as mortality. Antibiotic prophylaxis is an integral part of the treatment of EBV and must be started as soon as possible.
Viral etiology of liver cirrhosis in Turkmenistan

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**Goal:** Assessment of the viral etiology in patients with liver cirrhosis in Turkmenistan, including hepatitis B virus (HBV) and hepatitis C virus (HCV) infection.

**Materials and methods:** The study cohort included 136 patients with liver cirrhosis. Among them 78 (57.4%) were female and 58 (42.6%) were males. The patients’ age ranged from 17 to 78 years (average age 45.7 ± 8.7 years). The diagnosis of liver cirrhosis was based on the histopathological examination of percutaneous liver biopsies.

**Results:** Among the 136 patients with liver cirrhosis 105 patients (77.2%) had chronic viral hepatitis: 40 patients (29.4%) had chronic HBV infection, 36 (26.5%) had chronic HCV infection and 29 (21.3%) had coinfections (HCV and HBC or HBV and HDV or HBV, HCV and HDV).

Among patients with chronic hepatitis B, C and/or D, 4 had liver cirrhosis Child-Pugh A, 26 had Child-Pugh B and 75 had Child-Pugh C.

Among the 31 patients with non-viral etiologies (22.85), 12 had liver cirrhosis Child-Pugh B and 19 had liver cirrhosis Child-Pugh C.

**Conclusions:** Our data indicate that in Turkmenistan the most frequent cause of liver cirrhosis is chronic HBV or HCV infection (77.2%). Further, chronic viral hepatitis has a more severe natural course than non-viral hepatitis and is associated with more advanced stages of liver cirrhosis.
Thrombocytopenia in infection with HCV. Case report

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Introduction: Platelet abnormalities are common in liver disease. Every cause of thrombocytopenia have a different therapeutic strategy.

Methods: A 47-year-old man in November 2002 developed fatigue, gingival bleeding, central right upper quadrant abdominal pain. After physical examination and paraclinical investigations the diagnosis was chronic infection with HCV. Liver biopsy was not performed because patient had persistent thrombocytopenia. Three years later was performed splenectomy with intraoperative liver biopsy. Histological lesions was cirrhosis with necroinflammatory activity (Knodell score 8) and fibrosis 4. The viral load was moderate (452,000 UI/ml) and esophageal varices wasn’t found out to the endoscopic. The diagnosis was cirrhosis Child A with hepatitis C virus. Because the correction of thrombocytopenia wasn’t obtained after splenectomy it was necessary more investigations to find out the etiology. Immunological tests (antibodies to platelet) was negative, CT scan of abdomen revealed accessory spleen but only 1.5 cm diameter and bone marrow biopsy revealed dysmegakaryocytepoiesis.

Results: We were considered that thrombocytopenia was on directly relationship with viral infection (medular effect) and an antiviral regime with pegylated interferon alfa-2a 90-135 µg/week and ribavirin 1000 mg/day was initiated with weekly hematological control. After 12 weeks the early virological response (EVR) wasn’t obtained and patient was considered “non-responder”.

Discussion/Conclusion: In infection with HCV, thrombocytopenia may be both peripheral cause (splenomegaly with hypersplenism or immunological mechanism) and central cause (haematopoiesis inhibition by HCV).
The minimum necessary hepatic bioptic fragment for evaluation of the fibrosis in chronic hepatitis C

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Introduction: It is not established a minimum necessary size of biopsy fragment to evaluate fibrosis. The error which limits the performances of biopsies is the variability of the distribution of fibrosis.

Methods: On 20 surgical prelevations of hepatic tissue from patients with chronic HCV hepatitis with dimensions 19 x 24 mm, the area of fibrosis was determined by morphometry. Each image was decomposed in elementary images, corresponding to biopsies of 2.5 mm long. These images were associated in variable number obtaining 12550 virtual biopsies of 2.5-100 mm long on which, the area of fibrosis was measured and compared with the area of reference. The area of fibrosis is converted in Metavir stage.

Results: The dispersion of the dimension of the area of fibrosis is semnificative. One cannot establish a minimal size of biopsy beyond which the dispersion becomes negligible.
We can remark the following discordance between the stage of fibrosis (Metavir) established on biopsy and the stage Metavir of reference; 32% for fragments of 1.5 cm (15 mm), 23% for fragments of 2.5 cm (25 mm) and 18% for biopsies of 3.5 cm (35 mm).

Discussion/Conclusion: The error-risk in appreciation of the area stage of fibrosis persists in spite of dimension of biopsy but the error diminished with growing of the biopsy fragment. A fragment of 3.5 cm would be preferable having an error of 18%. 
Rare complication of autoimmune cirrhosis: Invasive infection pulmonary and cerebral with Aspergillus fumigatus: Case report

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Introduction: Pulmonary aspergillosis is a rare disease seen in patients with pulmonary preexistent diseases or immunosuppressed and has 3 clinical aspects: hypersensitivity syndromes, saprophyte non-invasive disease, and invasive disease.

Methods: The authors present a case of invasive aspergillosis (pulmonary and cerebral localization) in an immunodepressed child. The diagnosis was established on clinical data with rapid evolution of pulmonary lesions and cerebral dissemination and confirmed by imagistic methods (pulmonary radiographs, pulmonary and cerebral CT-scan) and bacteriologic data (culture positive for Aspergillus fumigatus in broncho-alveolar secretions).

Results: One patient aged 17 years, known with autoimmune cirrhosis, splenectomy and corticosteroid treatment was admitted for bilateral pneumonia with rapid unfavorable evolution. The pneumonia developed in 7 days many pulmonary abscesses, extensive pulmonary necrosis, piopneumothorax without regression under antibiotherapy. In bronchopulmonary secretions was isolated Aspergillus fumigatus. After 2 weeks of evolution the patient presented right motor deficiency and right facial paresis, and cerebral CT-scan examination relieved many cerebral and cerebellum abscesses suggestive for cerebral aspergillosis. Patient received antifungal therapy with Voriconazol with favorable evolution of pulmonary and cerebral lesions, but with the worsening of the hepatic disease as side effect. The patient died by variceal bleeding after 2 months.

Discussion/Conclusion: Invasive aspergillosis is a severe opportunist infection with very rapid evolution in immunodepressed patient. The diagnosis and treatment are extremely difficult in these conditions.
Assessment of correlation between histopathologic changes of gastric mucosa according to Whitehead’s classification and extent of liver damage according to Knodell’s scale in patients with chronic hepatopathy

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Aim of the study: Patients with chronic liver damage often suffer from functional disturbances and pathologic changes in the stomach, including ulceration, erosions and chronic gastritis. It was attempted to establish whether there is a correlation between histopathologic abnormalities of the gastric mucosa and the extent of histopathologic changes in liver bioptates, i.e. inflammatory activity and fibrosis (Knodell's index) in patients with chronic hepatopathy.

Material/Methods: The study was carried out in 4 groups of patients: group I – 10 subjects with autoimmune hepatitis (AIH), group II – 9 patients with chronic toxic liver damage, group III – 11 patients with chronic hepatitis caused by HBV, and group IV – 36 patients with chronic hepatitis caused by HCV. ALL the patients underwent gastroscopy with collection of gastric mucosa bioptates (from the antrum and the gastric body) as well as liver biopsy.

Results: The most frequent gastroscopy finding in all the studied groups were signs of gastritis: in group I – 90%, in group II – 78%, in group III – 64% and in group IV – 99%. Gastric mucosa histopathology assessed according to Whitehead’s classification most frequently led to the diagnosis of gastritis chronica profunda (group I – 80%, group II 78%, group III – 73%, group IV – 58%). No correlation was found between inflammatory activity in the liver assessed according to Knodell’s scale and the extent of changes in histopathology of the gastric mucosa (p = 0.1). A negative correlation which however does not reach significance level (p = 0.054), is observed between the extent of fibrosis in liver bioptates and histopathologic abnormalities in the gastric mucous membrane.

Conclusion: No statistically significant correlation between the extent of gastric mucosa damage and severity of inflammatory lesions and hepatic fibrosis was found.
Assessment of gastric secretory function by 24-hour pH-metry in patients with hepatic cirrhosis due to HBV infection, before and after non-shunt type Sugiura procedure

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Aim of the study: Advanced chronic hepatopathies are very often accompanied by pathologic changes in the stomach as well as functional disturbances affecting the upper gastrointestinal tract. They include ulceration, erosions and congestive (portal) gastropathy, reported with increasing frequency. Some of these patients undergo surgical treatment to prevent recurrent esophageal variceal bleeding. Because of considerable differences in gastric hydrochloric acid secretion, its assessment was attempted in patients with hepatic cirrhosis and portal hypertension before (group II) and after (group III) non-shunt type surgical procedure.

Material/Methods: The study was carried out in two groups of patients, consisting of 11 patients each, and a control group of 15 healthy volunteers. Subjects over 65 years of age, with concurrent chronic conditions, history of surgical procedures (except for Sugiura procedure) and treated with drugs affecting gastric secretion were excluded from the study. All the subjects qualified for the study underwent pH-metry with a DL70 pH-meter equipped with a glass electrode. Median pH (Me) and mean pH values (arithmetic mean, AM) from 24 h were used analysis.

Results: The following results were obtained – in the control group Me 1.7, AM 1.97; in group II Me 2.04, AM 2.53. In the group of patients after Sugiura procedure Me was 2.83 and AM 3.12. No statistically significant differences in gastric secretion were found between the studied groups in statistical analysis using Cochran, Cox and Student-t tests.

Conclusion: There are no statistically significant differences in gastric secretion determined by 24-hour pH-metry in patients with hepatic cirrhosis due to HBV infection and portal hypertension before and after non-shunt type Sugiura procedure.
Acute cholestatic hepatitis syndrome caused by ecstasy

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Introduction: Use of psychoactive drugs, including ecstasy (3,4-methylenedioxy-methamphetamin, MDMA), become more frequent recently. In background of acute liver diseases, we always have to think of the effects of toxic agents, drugs.

Case report: A 33-year-old man had clinical signs of fatigue, vomiting, deep jaundice (se bi: 691, di bi: 465 µmol/l). Elevated transaminase levels (GOT: 212, GPT: 573, GGT: 206 U/l), dark urine, light-coloured stool referred to hepatitis syndrome with cholestatis. The negative serologic markers (anti-HAV, HBsAg, anti-HCV, EBV, IgM) and the lack of auto-antibodies (ANA, AMA, SMA, ANCA) did not prove viral or autoimmune causes. Wilson-disease and hemochromatosis were excluded by mutation analysis. Ultrasound and computer tomography showed moderate hepatomegaly, but no other pathological signs. ERCP did not prove obstruction of biliary flow.

At the first visit, ingestion of toxic agents were denied by the patient, but after the examinations he was asked repeatedly, and finally he admitted of taking two tablets of ecstasy pills, three weeks before his first complaints.

The result of histological examination from fine needle liver biopsy verified the suspicion of toxic liver failure.

The patient's status improved slowly. He did not recieved any medicine. The bilirubin and transaminase enzymes decreased to normal level after five months.

Conclusion: This case draws our attention, that even two ecstasy tablets can cause severe acute hepatitis syndrome. In case of liver diseases, we always have to ask about drug ingestion in the anamnesis, with such an empathy, that we can win the patient's co-operation and confidence.
Immunologic involvements in chronic viral hepatitis

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Introduction: Chronic hepatitis is a hepatic inflammatory process persisting over 6 months. In some cases is suggested autoimmune mechanism by the finding of antinuclear and anti-smooth muscle antibody in serum and by general involvement.

Methods: This study takes in observation 401 patients, admitted in hospital between 1996-2005 with chronic viral hepatitis (298 HBV, 13 HCV, 82 HDV, 9 viral association) aged 9 months-18 years. The patients were divided in 2 groups: patients with viral hepatitis treated with antiviral therapy: interferon, lamivudine or association (314 children) and patients without antiviral therapy (88 patients). Some patients developed immunological disturbances (elevation of ESR, hypergammaglobulinemia, autoimmune anemia or trombocitopenia, presence of autoantibodies).

Results: We tried to establish a connection between the autoimmune involvement and the antiviral therapy, the evolution of the disease and the treatment response. The autoimmune markers were find in 28 patients (9.24%) with chronic viral hepatitis treated with antiviral treatment and only in 1 patient (1.13%) without treatment. In order of frequency the autoimmune disturbance were rheumatoid factor, elevation of IgG and gamma globulin’s and less common antinuclear antibodies, anti-smooth muscle antibodies. In patients with autoimmune markers we observed clinical manifestation: anemia, arthritis, rash.

Discussion/Conclusion: The most important question is: antiviral therapy is responsible for the autoimmune phenomena or this is secondary to chronic viral infection?
Clinical, biological, histopathological and ultrastructural changes in liver and spleen samples during evolution of hepatic cirrhosis

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In the evolution of liver cirrhosis, hypersplenism is a frequent clinical syndrome characterized by splenomegaly and the associated peripheral destruction of blood cells. Splenectomy is a commonly used indication for patients with portal hypertension and hypersplenism in cirrhosis.

Aim of the study: To evaluate the evolution of clinical and biological markers and to correlate with morphologic and ultrastructural characteristics of hepatic and spleen bioptic samples.

Methods: We describe 30 consecutive cases of splenectomy associated with intraoperative liver biopsy in patients with cirrhosis Child's degree A (12 patients) and B (18 patients). We evaluated the degree of splenomegaly, the peripheral blood cells counts before and after the treatment, the associated disabilities and the dynamic evolution of hepatic function's parameters. The hemodynamic status of spleno-portal system was performed ecographically before the intervention. The morphologic and ultrastructural findings in hepatic and spleen samples was evaluated in all cases.

Results: the most common indications for surgery were anemia (69%) and symptomatic splenomegaly (31%). Splenectomy was performed 2.5 years range after the initial diagnosis. The spleen size was significantly higher in cirrhotic patients and correlated inversely with the platelet and erythrocyte counts and splenic macrophages observed had an active phagocytic state. The clinic status of patients was greatly improved after splenectomy.

The morphologic characteristic lesions (blood congestion, steatosis, fibrosis and cirrhosis) were comparatively evaluated in optic and electronic microscopy.

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Seven-year experiences with interferon and ribavirin treatment for chronic hepatitis C in Hungary

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Introduction: In Hungary over the past 7 years more 2400 patients with chronic hepatitis C have been referred for interferon (IFN) and ribavirin (RBV) treatment at 21 hepatology centers. Authors give an account of their experiences.

Methods: A total of 2444 patients with chronic hepatitis C have been investigated. The sustained efficacy of therapy was evaluated in 1455 HCV genotype 1 infected patients. Treatment protocols have changed during this 7-year period. First, 3 x 3-5 MU/week standard IFNα plus RBV 800-1200 mg/day, later PEG-IFNα-2a or -2b (180 mcg/week or 1.5 mcg/kg/week) plus RBV for 6-12 months was administered. Beyond the retrospective analysis of these HCV cases above, a prospective, controlled clinical trial has also been performed and evaluated in 69 patients with chronic hepatitis C treated with PEG-IFNα-2a plus RBV.

Results: Standard IFN+RBV therapy resulted in an overall 22.6% sustained virological response (SVR), PEG-IFN+RBV led to 31% SVR at intent-to-treat analysis (ITTA) and 44% SVR at per-protocol analysis (PPA). The prospective study with PEG-IFNα-2a plus RBV resulted in 37% SVR at ITTA and 48% at PPA. Incidence of serious adverse effects was 10.4%. Duration of IFN treatment, gender, age, presence of fibrosis or cirrhosis, BMI and adherence (“80/80/80”) were the predictors of therapeutic efficacy.

Discussion/Conclusion: Our results - from a Central East European country - are in accordance with the findings reported from the Western world, suggesting continuous advances in the treatment of chronic hepatitis C. Novel, more effective antiviral drugs are still awaited to further improve the clinical outcomes of these patients.
Pegylated-IFN plus ribavirin therapy downregulates serum fibrosis markers independently of virological response

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Introduction: In the outcome of chronic hepatitis C virus infection the progression of hepatic fibrosis is essential. PEG-IFN treatment is supposed to inhibit fibrogenesis, so we compared changes in non-invasive fibrosis markers in patients with chronic HCV hepatitis having different responses to antiviral therapy.

Methods: Plasma levels of TGF-beta1, hyaluronic acid (HA) were determined by ELISA, procollagen-III-peptide (P-III-P) by RIA in 49 patients with chronic hepatitis C before and at 1, 3, 6 month of antiviral treatment and 12 month thereafter. Twenty-two patients became responders (R), 27 were non-responders (NR). Thirty healthy controls were enrolled. Correlation between TGF-beta1, HA, P-III-P levels and histological activity and fibrosis score was evaluated.

Results: Pretreatment TGF-beta1 (R: 14 pg/ml NR: 14,4 pg/ml), HA (R: 154 ng/ml, NR: 149 ng/ml) and P-III-P levels (R: 1,5 U/ml, NR: 1,4 U/ml) were increased in both responders and non-responders compared to controls (TGF-beta1: 9 pg/ml, HA:19 ng/ml, P-III-P: 0,6 U/ml). HA levels correlated with fibrosis score, TGF-beta1 with histological activity index. PEG-IFN treatment decreased both TGF-beta1 and HA levels, not only in responders but also in non-responders. Fibrosis markers were downregulated even 6 months after treatment (TGF-beta1: R: 8 pg/ml, NR: 5,8 pg/ml, HA:R: 35 ng/ml, NR: 66 ng/ml). No correlation was found between fibrosis markers and HCV RNA.

Discussion/Conclusion: PEG-IFN plus Ribavirin treatment decreased TGF-beta1 and hyaluronic acid levels independently of virological response and this was detectable even 6 months after antiviral treatment. These data suggest that antiviral treatment may have potential beneficial antifibrotic effect even in virological non-responders.
Transforming growth factor-beta1 downregulates NKG2D killer activator receptor expression on cytotoxic cells in patients with chronic HCV hepatitis

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Introduction: Impaired natural killer (NK) cell activity may contribute to viral persistence in HCV infection. Recent studies demonstrated that in tumors regulatory T cells (Treg) via secreting TGFbeta1 downregulate NKG2D killer activator receptor (KAR) and responsible for poor NK cytotoxicity. Since in chronic hepatitis C plasma TGFbeta1 level is increased, we analyzed the expression of NKG2D on NK and T cells and its correlation with the percentage of Treg cells and TGFbeta1 levels.

Methods: The percentage of peripheral CD4+CD25high+ Treg cells, NKG2D+ NK and T cells were determined by FACS, plasma TGFbeta1 levels by ELISA. Forty-three patients with chronic hepatitis C, 10 sustained virological responders (SVR) and 15 healthy controls were enrolled.

Results: In chronic HCV hepatitis the NKG2D KAR expression was significantly downregulated both on NK (7.9 vs. 20.9%) and on T cells (18 vs. 26.3%) compared to controls. Impaired expression of NKG2D was associated with increased proportion of CD4+CD25high+ Treg cells (4.6 vs. 3.1%) and increased TGFbeta1 levels (15 vs. 9 pg/ml) compared to controls. TGFbeta1 levels inversely correlated with NKG2D expression on NK cells. In SVR group the percentage of Treg cells, TGFbeta1 levels and NKG2D expression were comparable to controls.

Discussion/Conclusion: Our data suggest that TGFbeta1 -secreted by regulatory T cells- may be responsible for impaired NK cell function via down-regulating NKG2D killer activating receptor in chronic HCV hepatitis. Thus, TGFbeta1 antagonism or soluble NKG2D ligands may provide the basis of a novel antiviral or even cancer immunotherapy to improve the function of NK and T cells.
TNFalpha production of LPS-stimulated peripheral blood monocytes predict virological response in patients with chronic HCV hepatitis

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Introduction: Identifying host immunological factors predicting treatment response in chronic hepatitis C might be important in understanding successful immune response and also in new drug development. Since beside host adaptive immunity, innate immune response is also important in clearance of viral infection, we compared Th1/Th2 cytokine production of peripheral T-lymphocytes and monocytes in patients with chronic hepatitis C having different responses to antiviral therapy.

Methods: Forty patients with chronic HCV hepatitis before and at 3, 6 months of PEG-IFN+ribavirin treatment, 16 sustained virological responders, 20 controls was studied. IL-4, IL-6, IL-10, IFNgamma, TNFalpha, IL-2 production of LPS stimulated CD14+ monocytes, PMA+ionomycin stimulated T-lymphocytes were determined by FACS-CBA assay.

Results: Pretreatment IFNgamma production of the T cells was significantly decreased in chronic hepatitis C - both in non-responders (96.8 ng/ml) and responders (78 ng/ml) - compared to healthy controls (206 ng/ml). IL-10 production of lymphocytes was higher in non-responders compared to responders and showed positive correlation with HCV RNA levels. Monocyte TNFalpha (3.5 ng/ml vs. 0.85 ng/ml) and IL-6 (60.7 ng/ml vs. 23.9 ng/ml) production were significantly (p < 0.001) higher in responders than in non-responders. TNFalpha production of monocytes was predictive for virological response (AUC: 0.948). PEG-IFN and ribavirin treatment increased T-cell IL-2, IFNgamma expression and downregulated monocyte IL-6, TNFalpha production in responders.

Discussion/Conclusion: Pretreatment capacity of the monocytes to produce TNFalpha may be highly predictive for virological response in patients with chronic hepatitis C. Our data suggest that in responders the observed increased TNFalpha production by monocytes - as a vigorous innate immune response - may counteract impaired Th1 cytokine production of the T cells and contribute to the efficacy of IFN therapy.
αvβ6 integrin: A novel antifibrotic target in liver fibrosis

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Introduction: αvβ6 is a rare integrin expressed on certain epithelial cells. It is highly upregulated during morphogenesis and tumorigenesis. αvβ6 is bind and activate latent TGFβ. Mice lacking αvβ6 display resistance to induction of pulmonary fibrosis. Therefore, we aimed to study hepatic expression of αvβ6 and to assess whether its inhibition ameliorates rat biliary fibrosis.

Methods: Liver fibrosis was induced by bile duct ligation (BDL). The highly specific non-peptidic αvβ6 antagonist EMD527040 was given at 20 and 60 mg/kg/day by i.p. Hepatic collagen was determined as hydroxyproline. Liver morphology and histology were assessed by hemotoxylin/eosin and sirus red staining. Gene expression was measured by quantitative RT-PCR.

Results: In secondary biliary fibrosis the expression of αvβ6 integrin in rat liver was 100-fold upregulated compare to non-fibrotic controls. Proliferating bile ducts strongly expressed αvβ6. Treatment of BDL animals with EMD527040 resulted in reduction of total hepatic collagen accumulation by 40% at the high dose of antagonist compared to saline treated controls. ALT, ALP, AST, GGT, and bilirubin were significantly improved at both concentrations of EMD527040. Transcripts for procollagen-α1, α-SMA, TGF-β1, TGF-β2, TIMP-1, CTGF and the integrin β6 chain were significantly downregulated, while fibrolytic MMP-3 mRNA was increased 2-fold. Histological evaluation of treated groups revealed striking a decrease of proliferating bile ducts and the collagenous matrix around them.

Discussion/Conclusion: The integrin αvβ6 is upregulated in bile ductules of rats with secondary biliary fibrosis. Fibrosis progression was retarded significantly by inhibition of the αvβ6 integrin. Therefore, oral αvβ6 inhibitors are attractive drugs to inhibit progression of biliary fibrosis.
Evaluation of dependence between esophageal varices size and the direction and quickness of blood flow - measured by Doppler ultrasonography - in liver and portal veins among patients with liver cirrhosis

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**Background**: Liver cirrhosis is a last stage of liver hepatopathies. One of most often consequences of liver cirrhosis is portal hypertension, the signs of which are esophageal varices, portal hypertensive gastropathy and jejunopathy. Doppler ultrasonography is a not invasive method which enables evaluate hemodynamic disturbances in liver and portal veins.

Is there any dependence between the size of esophageal varices and the direction and quickness of blood flow in liver and portal veins in patients with liver cirrhosis?

**Material and methods**: The examined group of 48 patients with liver cirrhosis were performed endoscopy. The patients with esophageal varices observed in endoscopy underwent Doppler ultrasonography.

Basing on liver cirrhosis etiology patients were divided to 5 groups. Posthepatitic cirrhosis due to HCV infection was diagnosed in 24 patients, posthepatitic HBV cirrhosis was observed in 11 persons. Both HCV and HBV posthepatitic cirrhosis was found in 3 patients. Toxic liver disease was diagnosed in 7 patients, CBP in 2 persons and AIH in one.

Esophageal varices size was estimated with Paquet classification. In Doppler ultrasonography the quickness and direction of blood flow in liver and portal veins were determinated.

**Results**: Esophageal varices were diagnosed in 36 of 48 examined patients (75%). All patients with esophageal varices were performed Doppler ultrasonography. In 12/36 patients (33.3%) the hemodynamic disturbances in portal vein were observed. In 19/36 persons (52%) pathological slow blood flow was observed in liver veins.

**Conclusions**:
- The direction and quickness changes in blood flow in liver veins occur early and are observed in patients with smaller esophageal varices.
- The blood flow disturbances in portal vein occur mainly in patients with big esophageal varices.
Severe episodes of acute rejection in teenager after the combined peginterferon and ribavirin treatment of recurrent hepatitis C after liver transplantation

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Introduction: The recurrence of hepatitis C viral infection after the liver allograft transplantations is common. Recent observations in adult allograft recipients showed that in several patients the disease is more aggressive and leads to graft cirrhosis in the first few years. There is very little evidence in children.

Results: Case report: 15 years old girl transplanted at the age of 10 years for biliary atresia with chronic C viral infection. Her primary immunosuppression therapy consisted of cyclosporine, prednisone and azathioprine which was discontinued after 6 month. Because of histological recurrence of chronic viral infection she was treated with ribavirin for 9 month without the clearance of HCV RNA. Four years after liver transplantation the prednisone was discontinued and she was on cyclosporine monotherapy. Further biopsy revealed the increasing activity of inflammation and fibrosis and she was qualified to combined peginterferon and ribavirin treatment. After two month of therapy due to severe anemia the ribavirin was discontinued. Four month later she had rapid elevation of liver enzymes levels and liver biopsy showed acute rejection. She was treated with steroid pulses and the mycophenolate mofetil was introduced. Despite the discontinuation of peginterferon she was yet twice treated with steroid pulses and was switched for tacrolimus and later for sirolimus because of deterioration of renal function. Control biopsy showed moderate inflammation (G3) and fibrosis (S3).

Discussion/Conclusion: During the combined therapy of peginterferon and ribavirin some patients may develop the acute rejection with necessity of not only the discontinuation of treatment but also enhance of immunosupression.
Cutaneous side effects of antiviral therapy

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Background: The antiviral therapy of B and C chronic hepatitis may induce side effects, cutaneous manifestations included. Their management leads to decisions regarding the continuation of antiviral therapy, depending on the severity of the lesions.

Material and methods: We studied 25 patients admitted to our Medical Clinic from October 2004 to December 2005. 8 (32%) were diagnosed with B chronic hepatitis. 1 received Interferon, 4 lamivudine and 3 interferon and lamivudine. 17 (68%) patients with C chronic hepatitis received PegINF and ribavirin. Until the onset of the antiviral therapy the patients showed no cutaneous manifestations.

Results: Cutaneous side effects appeared in 7 (28%) patients who underwent antiviral therapy. The largest number appeared under therapy with PegINF combined with ribavirin: 2 patients had hypersensitivity reactions (papulo-vesical eczema). They appeared at the beginning of the antiviral therapy on the extension surfaces of the arms and face, and disappeared after specific dermatological treatment. During therapy we also reported xerodermia in 2 cases (after 2-3 months), alopecia (1 female patient after 8 months). They disappeared after therapy. A patient was diagnosed with seborrheic eczema after 4 months of treatment and another one with pustulation acne after five months (they disappeared under dermatological treatment). We could not clearly distinguish between cutaneous side effects owing to therapy with PegINF and those resulting from therapy with Ribavirin. One female patient with B chronic hepatitis presented alopecia and xerosis after 9 months of interferon.

Conclusions: Antiviral therapy is generally well tolerated. The cutaneous side effects were mild or moderate. It was not necessary to alter or interrupt the antiviral therapy.
The study of the incidence of hepatocellular carcinoma in virus C hepatic infection

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Introduction: The infection with virus C is one of the main causes of chronic hepatic pathology having a large structural heterogeneity and a high hepatic tropism. The aim of the study was to appreciate the incidence of hepatocellular carcinoma in 150 patients with hepatic disease of viral C etiology.

Methods: The study was effectuated in Medical Clinic II of Craiova Emergency Hospital between 2002 and 2005. The study was effectuated on 150 patients with chronic liver disease and followed in dynamic.

Results: From 150 cases of chronic liver disease, 104 were diagnosed with chronic hepatitis and 46 cases with liver cirrhosis. From the 104 with chronic hepatitis, 74 had viral C etiology and 30 had viral C+B etiology. From the 46 cases with liver cirrhosis, 30 had viral C etiology and 16 had viral C+B etiology. It has been noted during the study a worse evolution in a lot of 30 cases with chronic hepatitis C+B: 5 cases to liver cirrhosis (LC), 1 case to hepatocellular carcinoma (HCC). From the 30 cases with LC, 12 were diagnosed with HCC in association with 2 cases of fast evolution from chronic hepatitis C+B to liver cirrhosis and HCC. It has been diagnosed 14 cases of HCC: 12 cases from the lot of viral C liver cirrhosis and 2 cases from the lot of liver cirrhosis C+B. It was recognized a high alcohol consumption at 5 patients.

Discussion/Conclusion: The middle age of apparition of HCC was 50-55 years of age. The incidence was higher in men, with a rapport male/female of 3/1. The annual incidence of HCC was 3% in patients with viral C liver cirrhosis. It was not excluded the alcohol aggression in some of them.
The non-organ-specific autoantibodies signification in immune intrahepatic cholestasis diagnosis

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Introduction: To establish the non-organ-specific autoantibodies importance in ethiological diagnosis of immune intrahepatic cholestasis.

Methods: We accomplished a retrospective study in patients with intrahepatic of immune intrahepatic cholestasis.

Results: Sixty-four of the 3710 patients (18%) were diagnosed to have intrahepatic cholestasis. The typical manifestations for immune intrahepatic cholestasis were met at 34 patients (9.16%). Anamnesis excluded exposure to drugs, toxic agents, and alcohol intake. The demographics and biochemical parameters of these patients were: male/female ratio = 6/28, mean age 41 ± 12 years, mean serum levels of total bilirubin 3.2 ± 1.6 mg%, direct bilirubin 2.1 ± 0.9 mg%, alkaline phosphatase 246 ± 131 u/l, gamma-glutamyltransferase 189 ± 64 u/l, alanine aminotransferase 176 ± 64 u/l, aspartate aminotransferase 157 ± 59 u/l, protrombine time 16.2 ± 4 sec, gamma-globulins 2.6 ± 0.6g%, IgG 442 ± 221 ui/ml, IgM 320 ± 108 ui/ml, IgA 229 ± 98 ui/ml, CIC 89 ± 34 uF, ESR 32 ± 14 mm/h. The incidence of autoimmune markers were: ANA (23p; 67.6%), ASMA (20p; 58.8%), AMA (3p; 8.8%), p-ANCA (2p; 5.5%), rheumatoid factor (6p; 17.6%), and viral markers anti-VHC (8p; 23.5%).

Discussion/Conclusion: The type 1- autoimmune hepatitis and its overlap syndromes represented main causes of immune intrahepatic cholestasis. The AMA, and p-ANCA presence and disproportionate increase of the serum alkaline phosphatase level or concomitent viral infection, at patients with classical features of autoimmune hepatitis, represented the cases with overlap syndromes. The accurately appreciate of intrahepatic cholestasis causes will prevent the empirical treatment use.
The study of clinical and etiological forms in the cholestasis induced by medication

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Introduction: The cholestatisis is induced by three mechanisms: alteration of biliary secretion by hepatocytes (hepatocellular cholestatis), obstruction of intrahepatic biliary canals and obstruction of extrahepatic biliary canals. The aim of the study was the etiological appreciation of cholestasis induced by medication and of clinical forms (chronic or acute) in relation with the drug in use.

Methods: The study was effectuated in Medical Clinic II of Craiova Emergency Hospital between 2002 and 2005. The followed parameters were: clinical parameters (icterus, pruritus, hepatomegaly, xanthomas, splenomegaly, masses discoloration, bones affection and biological parameters (bilirubin, alkaline phosphatase (FAL), gammaglutamyltranspeptidase, alanine aminotransferase (ALT)).

Results: We have studied 44 cases with cholestasis: hepatocellular cholestatis, 6 cases (4 cases with sexual hormones treatment; 2 cases with anabolic steroids), 16 cases with cholestatic hepatitis treated with phenotiasines, 4 cases treated with chlorpromamine and 4 cases treated with carbamazepine (probably also by ductal affection); antithyroid carbimazol - 2 cases, antidepressants - 2 cases, hypotensor - nifedipinum - 2 cases, antimicrobian - amoxicilinum and clavulanic acid 1 case (probably also canalicular affection); acute canalicular colestasis due to allopurinol - 1 case; barbiturics - 2 cases.

Chronic cholestasis is appreciated on cases where medication induces a prolonged cholestasis over 6 months with a minor clinical evolution (icterus disappears and hepatic test abnormalities persists in patient treated with sulpiride - 1 case) or biliary cirrhosis clinic form in patients treated with phenitoin - 2 cases.

Cholestasis was encouraged in 1 patient after disulphone administration and in other patients with cithostatics administration for colorectal carcinoma.

Discussion/Conclusion: Medication can induce cholestasis because the affection of biliary hepatocellular secretion by canalicular or interlobular ductal obstruction and by extrahepatic obstruction (sclerosant cholangitis).

Pure hepatic cholestasis is observed more often in sexual steroids hormones and anabolic steroids administration.

Ductal cholestasis can be acute with a limited evolution and chronic with the decrease of the ducts and sometimes evolution to liver cirrhosis.
Histopathological profile and viral load in chronic hepatitis C in patients with normal or elevated ALT

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Introduction: A significant proportion of patients with detectable antibodies to hepatitis C virus have normal serum alanine transaminase levels. Our aim was to study the histopathological and viral load differences between patients with and without high levels of ALT.

Methods: We took into study 113 patients with chronic hepatitis C. In these patients we checked the transaminase levels and viral load, and we also performed the liver biopsy and the histology was evaluated using the Metavir score.

Results: 18 patients (15.9%) had normal values of ALT and 95 (84.1%) had high levels of ALT. The mean elevated ALT value was 99.9 U/l ± 38. In cases of normal ALT, Metavir histological activity was as follows: 3 patients (16.6%) had A1 score, 9 (50%) had A2 and 6 (33.3%) had A3 and the fibrosis scores were: 12 patients (67%) had F0/F1 fibrosis, and 6 (33.3%) had F2/F3 fibrosis. In case of high ALT patients Metavir activity scores were: A1 in 18 patients (18.94%), A2 in 51 (53.68%), A3 in 26 (27.3%) and the fibrosis was: 47 patients (49.5%) had F0/F1, and 48 patients (50.5%) had F2/F3 fibrosis. The viral load over 800,000 UI/ml was found in 50% of patients with normal ALT and in 61.1% of patients with high ALT.

Discussion/Conclusion: Results showed that patients with normal levels of ALT have higher activity scores but lower fibrosis as measured by Metavir, and patients with high levels of ALT have lower activity scores but higher fibrosis, and higher viral load.
Acute fatty liver of pregnancy

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Introduction: Acute fatty liver of pregnancy (AFLP) is an uncommon, potentially fatal disorder, which may result from dysfunction in the beta-oxidation of mitochondrial fatty acids potentiated by the increased peri-partum level of estrogens. The liver biopsy is helpful for positive diagnosis and show microvesicular steatosis, with normal liver architecture.

Methods: In this article we discussed the case of an 18-years old woman with twin pregnancy, spontaneous labor of two male births, who presented after four hours from delivery convulsion due to the severe hypoglycaemia. During the evolution the patient had jaundice, oliguria, fever and gastrointestinal bleeding.

Results: Serum laboratory results included severe hypoglycaemia resistant to perfusion with glucose 20%, (9 mg%, 17 mg%, 22 mg%), thrombocytopenia (90000/mm³), elevation of serum transaminases (GOT - 267 U/l, GPT - 132 U/l), bilirubin (TB - 11.87 mg%, DB - 7.25 mg%), urea (83 mg%), and creatinin (2.34 mg%), prolongation of serum prothrombin time (17”), post haemorrhagic anemia (Hb - 5.03 g%). The fibrin degradation products were absent, and Brahms PCT-Q = 20 ng/ml. Hepatitis B and C markers were negative. Liver ultrasounds and CT shows marked parenchymal reflectivity consistent with fatty infiltration of the liver. Percutaneous liver biopsy (made after correction of coagulopathy) show microvesicular steatosis with characteristic ultra structural changes consistent with AFLP.

Discussion/Conclusion: The patient require supportive care for hepatic-renal failure, correction of hypoglycaemia, coagulopathies, sepsis, fluid resuscitation, parenteral nutrition. Delay in diagnosis and treatment can leads to significant maternal mortality due to the complication: haemorrhage, sepsis, disseminated intravascular coagulopathy, multi-organ failure. In this case AFLP have a good prognosis, the patient recovered normal liver function after two weeks post-partum.
Effects of insulin resistance and hepatic steatosis on the efficacy of antiviral therapy in patient with chronic hepatitis C

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Introduction: Steatosis is a common histological feature of chronic hepatitis C (CHC). Suspected molecular underlying mechanisms include interactions between the viral core protein and intracellular lipid metabolism pathways as well as induction of insulin resistance. The aim of this study were to evaluated the impact of fatty liver and insulin resistance on the virologic response rates to anti-hepatitis C virus therapy.

Methods: 55 non-diabetic patients with CHC received peginterferon alpha-2a plus ribavirin. The body mass index (BMI), and homeostasis model assessment of insulin resistance (HOMA-IR) were calculated. Fatty liver was detected by liver biopsy.

Results: 28 patients out of 55 had sustained virological response (SVR). In particular 10 out of 15 patients with no steatosis had SVR compared to 18 out of 26 patients with mild steatosis and to 5 out of 14 patients with moderate/severe steatosis. Among 24 CHC patients with insulin resistance, 7 had SVR vs 22 patients with SVR out of 31 CHC patients without insulin resistance.

Increased age, gamma glutamyltransferase, alanine aminotransferase, BMI and HOMA-score were correlated with moderate/severe steatosis and treatment failure.

Discussion/Conclusion: HOMA-IR was independent factor for predicting effect of antiviral therapy. Hepatic steatosis and its impact on response to therapy are related to metabolic syndrome. Thus, the management of metabolic syndrome in patients with chronic hepatitis C may be important for improving the efficacy of antiviral therapy.
The course and outcomes of chronic hepatitis in children

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Introduction: Virus hepatitis B and C (HB and HC) in children continues to represent a public health problem due to enhanced hepatitis B and C infections and frequent development of chronic forms of the disease. The aim of the study was to characterize and compare clinical course and outcomes of CHB and CHC in children.

Methods: We observed 123 children with CHB and CHC (65 boys and 58 girls) aged from 1 year to 14 years, CHB occurring in 74 of the cases (with cirrhosis outcomes in 12 and liver cirrhosis in 5 patients) and CHC – in 49 patients. CH occurred more frequently in children aging 11-14 years (36.6%) and 7-10 years (24.7%). CH developed a year after acute hepatitis in 27.5%, after 2 years in 16.7% and within later periods in other children.

Results: In half the patients, CHB developed after acute hepatitis B, some patients contacted their parents, HBsAg carriers, other children were subjected to numerous injections or blood transfusion. Children with pronouncedly active CHB (n = 18) had considerably enhanced ALAT activity, hepatomegaly (in 88.9%) and splenomegaly (in 38.9%). In all the patients, the liver ultrasound examination showed diffuse liver parenchyma induration, 8 children had microfocal granulation and 2 patients – fibrosis foci. In patients with minimum activity of CHB (n = 39) enlarged livers were found in 92.3% of cases with a diffuse change of liver parenchyma. In all the patients with cirrhosis outcome varying intoxication, considerably increased transaminases, reduction of total protein, hypoalbuminemia, γ-globulinemia and increased prothrombin index were observed, whereas the hemorrhagic syndrome as petechia induration and nose bleedings was in 16.2% and skin dryness and pruritus – in 88.6% of the children. CHC infection in half the patients took a latent course without a pronounced clinical picture and was characterized by hepatomegaly, splenomegaly and extrahepatic signs, 34.4% of the patients had arthralgia, anemia, hemorrhagic vasculitis, telangiectasias, cholecystitis and cholecystocholangitis. Biochemical CHC activity was high in 4.1% of the patients, moderate in 25.9%, low in 56.3% and was lacking in 13.7%. Heterogeneity of liver echostructure, enhanced echodensity, thickening and induration of vessel walls, bile ducts and the gallbladder, and liver fibrosis were detected at later stages of the disease. Extrahepatic injuries are the specific features of CHC in children and are distinguished mainly by the biliferous system injury and to a lesser extent by vascular system injury.

Discussion/Conclusion: We believe premature discharge from hospital of patients with acute hepatitis, whose clinico-biochemical indices have not become normal, as well as violation of regimen and diet to rank first among the risk factors and causes of the chronic process. Most of children suffering from CH had an adverse premorbid background (frequent acute rhinoviral infections, asthmatic bronchitis, angina, chronic infection foci, surgical operations).
Hepatoprotective effects of amino acid derivatives and lipoic acid on ethanol- and carbon tetrachloride-induced chronic hepatitis in rats

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Introduction: Chronic alcohol intoxication in rats does not typically provoke liver fibrosis and cirrhosis observed in alcoholic liver injury in humans. To simulate chronic toxic hepatitis in rats, we used a combination of alcohol intoxication with carbon tetrachloride (CCl₄) administration. CCl₄ was shown to aggravate severity of ethanol-induced liver injury, provoking fibrosis combined with steatohepatitis. Since free radical process activation plays an important role in the damaging effect of ethanol and CCl₄, we studied the action of glutamine, N-acetylcysteine, ethanolamine and lipoic acid affecting ethanol and acetaldehyde metabolism and possessing antioxidant properties on the hepatotoxicity induced by ethanol and CCl₄.

Methods: Wistar male rats were treated with ethanol (4 g/kg, daily, 4 weeks) and CCl₄ (0.1 ml/100 g body weight, i.g. 2 times a week, Group 1). Some experimental animals were additionally treated daily with either N-acetylcysteine (100 mg/kg, i.g., Group 2), or glutamine (340 mg/kg, i.g., Group 3), or lipoic acid (40 mg/kg, i.g., Group 4), or ethanolamine (100 mg/kg, i.g., Group 5) during 4 weeks.

Results: The chronic ethanol and CCl₄ intoxication elevated serum ALT activity and decreased the activities of liver aldehyde dehydrogenase, catalase and microsomal ethanol-oxidizing system. The morphologic studies revealed bridge like porto-portal and porto-central necroses, mid- and microdrop fatty degeneration, balloon dystrophy, porto-portal connective tissue septa and micronodular cirrhosis. All the preparations studied prevented increase in blood plasma ALT activity, development of liver fatty degeneration and elevation of glutathione reductase activity. Glutamine prevented reduction in the activities of liver catalase and aldehyde dehydrogenase, whereas lipoic acid and ethanolamine – that in catalase. Glutamine raised the activity of liver glutathione peroxidase.

Discussion/Conclusion: Thus glutamine, ethanolamine, N-acetylcysteine and lipoic acid were shown to be effective in preventing development of liver metabolic impairments and fatty degeneration in chronic ethanol and CCl₄ intoxication, possibly by the activation of the enzymatic antioxidant defence system. These data support the pharmacological potential of the above substances in the management of alcoholic liver injury.
The role of hepatoprotectory therapy in toxic hepatitis due to unspecified ulcer colitis

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Introduction: The frequency of the occurrence of ulcer colitis is consistent throughout the world, according to investigation performed by the center of Coloproctology of the Republic of the Uzbekistan, ulcer colitis occurs in 4.7% of admitted patients who have diseases of the large intestine. Unspecified ulcer colitis, as a pathologic condition may produce toxins and could be influence in to the internal organs. As the disease progress, alterations to the internal organs occur and may have unalterable effects throughout the progression of the disease and intoxication of the organism especially to the liver. The main aims our study was assay effect of hepatoprotectory therapy in the toxic hepatitis.

Methods: We have analyzed the results of the treatment of 235 patients with varied severity of unspecified ulcer colitis. We noted mild forms (in 59-25.1%), moderate forms (in 95-40.5%) and severe forms (in 59-25.1%) of the ulcer colitis. Our investigation demonstrated the ability of the internal organs to function is dependent in direct-proportion to the severity of the disease and the extent of damage to the large intestine. In severe cases of ulcered colitis we noted the tendency of toxic hepatitis to develop into cirrhosis of the liver. This was confirmed not only during the stages of research, but also during the operation. In mild and moderate forms of ulcered colitis we prescribed detoxication therapy and remedies for the improvement of the liver’s function. When we administered treatment for the to protect the liver’s function, we used “Hepatofalk planta” for this cases. For mild and moderate forms of ulcered colitis, we used “Hepatofalk planta” for proghylaxy and for severe forms we prescribed it for a protracted period after the operation.

Results: Examination of clinical-biochemical analyses of the patients who had ulcer colitis revealed a decline in electrolytes, decline in the level of the general protein, increase of bilyrubin (indirect faction) and hyperkoagulation. Instrumental methods of research of the condition of internal organs, particularly ultrasound scan of the liver, showed an increase in the liver’s size, sharp compression of the parenchyma, and stagnation processes in the gall-excretive channel.

Conclusion: We attribute forehand diagnosis of ulcer damage to the large intestine with the prevention of chronic hepatitis transitioning into cirrhosis of the liver.
Risk factors for rebleeding after the first variceal hemorrhage

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Background: Variceal bleeding is a dramatic medical emergency with an increased risk of rebleeding.

Aim: to investigate the possible risk factors of rebleeding.

Material and methods: 102 patients with variceal bleeding. We evaluated age, sex, etiology, Child-Pugh class and variceal grade.

Results and discussion: 39 patients (38.23%) rebleded 53.8% (21) males and 46.15% (18) females with the mean age 57.45 ± 10.47. In the second group we have 63 (61.76%) patients who did not rebleeded 57.14% (36) males and 42.5% (27) females with the mean age 58.39 ± 12.38. In B virus cirrhosis rebleeded 45.8%, in C virus 25%, in B+C 60%, in alcoholics 18.1 in B+alcohol 91.6% in C+alcohol 77.77% In Child-Pugh A class = 34 patients (33.3%) 7 (20.58) rebleeded and 27 (79.41%) did not rebleeded, in Child B = 40 patients (39.21%): 15 (37.5%) rebleeded and 25 (62.5%) did not rebleeded, in Child C = 25 patients (24.50%):17 (68%) rebleeded and 8 (32%) did not rebleeded. Comparing Child-Pugh A and B was no significant risk Odds ratio = 2.3143 P = 0.1829 (95% CI = -3.323-37.163) NS (95% CI = 0.8105-6.6082) NS. Class A versus C: significant rebleeding risk for C class. Chi square test P = 0.0007 (95% CI = 24.637-70.203) ES Odds ratio = 8.1964 (95% CI = 2.5134-26.7292). Class B vs C: significant relative risk for C class. Chi square test P = 0.0325 (95% CI = 6.85-54.15) S Odds ratio = 3.5417 (95% CI = 1.2315-10.1857).

33% from the patients with grade II esophageal varices rebleeded comparing with grade III 47.16% P = 0.6164 (95% CI = -17.677-45.337) NS. Odds ratio = 1.7857 (95% CI = 0.7724-4.1286) NS.

Conclusions: The association between alcohol and viruses biviral infection Child-Pugh C were significant for the risk of rebleeding.
Arterial hypertension at the chronic virus hepatitis

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Introduction: Arterial hypertension syndrome (AH) is considered as extrahepatic manifestations of HBV/HCV-induced chronic hepatitis or as manifestation virus-induced periarteritis nodosa, or as a syndrome at chronic glomerulonephritis.

Methods: 485 patients with chronic viral hepatitis B/C were observed, using complex data.

Results: According to our data AH was diagnosed at one third patients (32.7%) with chronic mono and mixed viral hepatitis and cirrhoses of a liver B and C; 1.44% of cases was complicated by transient ischemic attack of brain. For the first time AH was diagnosed at 53.5% of patients with chronic liver disease during physical examination, and 46.5% - for 5.43 ± 1.07 years prior to clinical manifestation of chronic hepatitis and cirrhoses of a liver. Most frequently AH revealed at chronic HCV infection, at CHC and HCV cirrhosis (38.3% and in 57.9%). At HBV infection and HCC was registered correlation between mainly I-II stage of AH, at HCV infection - II-III stage. AH correlated: at CHB and HBV cirrhosis with antibody to cardiolipin, HBsAb, HBeAb; at CHC and HCV cirrhosis with levels of HCV Ab IgG, NS3, NS4; at CHB+CHC and HBV+HCV cirrhosis with antibody to cardiolipin, HCV Ab IgM.

Discussion: According to data it is possible to assume, that in genesis of development AH at chronic mono/mixed HBV/HCV infection the affection of arterial vessels with antigenes of viruses, and also immune and autoimmune mechanisms are involved.
Hepatitis C virus protein expression induces apoptosis in HepG2 cells

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Introduction: The mechanisms of hepatocyte death and the events that lead to a high rate of chronic liver disease in patients infected with hepatitis C virus are not known.

Methods: Was established a HCV replication system in HepG2 cell culture and utilized this model to address the effect of HCV proteins on HepG2 cell growth and viability. After transfection of HepG2 cells with full-length RNA, a truncated RNA, or an antisense RNA, cell proliferation and cell viability were analyzed by thymidine uptake and the trypan blue exclusion method, respectively.

Results: Full-length RNA transfected HepG2 cells showed a decrease in cell proliferation and viability compared to cells transfected with HCV truncated RNA and antisense RNA control. A subset of cells expressing HCV proteins underwent apoptosis as documented by morphological studies, ultrastructural analysis, cell cycle analysis by flow cytometry, terminal transferase enzyme mediated end labeling of DNA, and DNA laddering.

Discussion/Conclusion: This study suggests that expression of HCV proteins can lead to cell death by apoptosis, which may be an important event in the pathogenesis of chronic hepatitis C virus infection in humans.
Liver cirrhosis followed by diabetes mellitus: Etiopathogenetic and therapeutic aspects

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Aim: The aim of this study was to evidence particular etiopathogenetic and therapeutic features of liver cirrhosis (LC) associated with diabetes mellitus (DM) over a period of evolution of 3 years.

Material and methods: We prospectively investigated annually for 3 years (2002-2004):
- 87 patients with LC, 28 of them also with DM (of which 9 treated with insulin), after LC (36 ± 9 months).
- 30 controls, without DM or liver diseases, by: Oral glucose tolerance test (OGTT), Child functional class, viral markers and alcohol consumption, immunoreactive insulin - IRI-Kit RK, 400M Isotope.

Results: Marked cholestatic tendency was noted in association with DM metabolic imbalance (increased mean DB and AP).
Fasting IRI was increased in LC (29.10 ± 1.36 vs. 15.6 ± 0.35 µU/ml; p < 0.05), and maintained over 3 years of evolution (33.2 ± 1.16 µU/ml).
Hyperglycemia, hyperinsulinemia, and decreased insulinogenic index were found in LC, and maintained during 3 years of evolution (mean glucose 154 ± 0.52; mean IRI: 49 ± 3.54 µU/ml).
Child classes and diabetic complications remained unchanged in LC and DM treated by insulin (9 cases).

Conclusions: Cirrhotic patients presented a more marked cholestatic tendency during the periods of DM metabolic imbalance over a period of 3 years.
Insulinoresistance was more pronounced in cirrhotics with DM and increased during an evolution of 3 years.
Insulin therapy improves the short-term (3 years) evolution of LC preventing complications.
Arsenic-induced apoptosis of the hepatocytes is mediated by mitochondrial pathways

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Background and aims: Exposure to arsenic has been reported to cause liver cell injury. The predominant mode of liver cell death by arsenicosis is apoptosis, the mechanism of which is still unknown. The present study was designed to understand the possible mechanism of arsenic induced apoptosis of hepatocytes.

Methods: Murine model exhibiting liver injury by treating sodium arsenite was used. In this animal model, arsenic induced apoptosis of the hepatocytes were studied. Further in-vitro studies with primary cultured hepatocytes were carried out to study arsenic induced reactive oxygen species (ROS) generation, mitochondrial permeability transition, translocation of pro-apoptotic protein and apoptosis by fluorescence microscopy, confocal microscopy and fluorescence activated cell sorting analysis.

Results: Oxidative stress is the early event in the development of arsenic induced apoptosis in the liver as evident by in-vivo as well as in-vitro studies. Increased ROS altered mitochondrial permeability transition, resulting in translocation of pro-apoptotic proteins from the mitochondria to cytosol and increased caspase 9 and finally caspase 3 activities. Blocking the opening MPT pores by cyclosporine A inhibited increased ROS generation, cytochrome c translocation and apoptosis of the hepatocytes by arsenic.

Conclusion: Arsenic-induced apoptosis of the hepatocytes is mediated by mitochondrial pathways. The overproduction of ROS, driven by arsenic biotransformation, triggers the mechanism.
Formation and secretion of bile is connected with functioning of hepatocyte lysosomes (Fickert et al., 2005), this process has not been studied enough and models of experimental cholestasis are useful for understanding of mechanism of this process. The aim: to evaluate lysosomal enzymes activity in bile of mice during development of intrahepatic cholestasis. Male CBA and CBA/C57BL were used in the experiments. Intrahepatic cholestasis model was reproduced with help of combined administration of lysosomotropic agent Triton WR 1339 (1 mg/1 g of body weight, i.p.), which increased cholesterol synthesis (precursor of bile acids) in liver cells and hepatotropic poison CCL4 in dose 50 mg/kg 2 hours after Triton WR 1339. Activity β-D-galactosidase and chitotriosidase in murine bile and serum were determined by fluorometric methods according to Barrett (1981) and Guo et al. (1995) correspondently. Triton WR 1339 administration was shown to increase significantly cholesterol synthesis, so toxic hepatitis development occurred with intrahepatic cholestasis. In this group we registered the sharp increase of serum ALT activity, normalized 7 days after. In intact mice similar level of β-D-galactosidase was noted in bile and serum whereas in cholestasis group bile β-D-galactosidase activity was increased 15th times more comparatively to serum. In cholestasis model we did not manage to reveal any difference between serum and bile chitotriosidase activity. We can conclude that dramatical increase of β-D-galactosidase in bile is informative index of intrahepatic cholestasis development in mice. Our data support the hypothesis about secretion of lysosomal enzymes from hepatocytes (and not from non-parenchymal cells).
Sustained virological response in the antiviral therapy of chronic hepatitis C – Is there a predictive value of interferon-induced depression?

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Introduction: The goal of our study was to determine a putative contribution of cytokine-induced depression to a predictive model of sustained virological response (SVR) in chronic hepatitis C. The analysis comprised a control for known predictors of SVR (virus genotype and gender).

Methods: In a longitudinal study, we included a sample of 101 therapy-naïve HCV outpatients. They received a combination treatment with pegylated interferon alfa-2b and ribavirin. Neuropsychiatric side effects were monitored prospectively (Hospital Anxiety and Depression Scale, DSM-IV criteria for major depression). The primary end point with respect to SVR was failure to detect HCV by PCR 24 weeks after the antiviral therapy.

Results: SVR rate was 72.3%. Classification data (DSM-IV criteria; HADS cutoff) and the extent of interferon-induced depressive symptoms were not significantly linked to virus eradication. Consideration of other potentially confounding variables did not increase the predictive value of depression for therapy outcome. Virus genotype (P = 0.045) and gender (P = 0.016) contributed significantly to a predictive logistic regression model. However, mean (P = 0.811) and maximum (P = 0.744) increases in HADS depression were no significant predictors of SVR.

Discussion/Conclusion: There is no evidence for a significant association between depression and the efficacy of antiviral treatment in chronic hepatitis C. Patients experiencing no or minor neuropsychiatric side effects do not have to worry about a "lower chance" of successful therapy. Interferon-induced depressive symptoms are still important to be monitored and treated if necessary. They cannot, however, be used to predict the individual’s therapy success.
Septicemia: A rare adverse event of antiviral treatment - Case report

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Introduction: Although some adverse reaction (like fatigue, myalgia, headache, psychiatric and hematological reactions) are quite common among patients with antiviral treatment for chronic C hepatitis, bacterial infections and especially septicemia are rare, but serious adverse events.

Methods: Case report: We present the case of a 47 years old man with chronic C hepatitis since 2001. Starting from April 2004 he received antiviral treatment with 180 µg PEG-interferon alpha-2a plus 1200 mg ribavirin. At the beginning of treatment the patient had: leukocytes = 5500/ml, granulocytes = 2300/ml, haemoglobin = 12.1 g/100 ml, platelets = 127000/ml, ESR = 10/17 mm. After nine months of treatment he had: leukocytes = 3100/ml, granulocytes = 1350/ml, haemoglobin = 11.7 g/100 ml, platelets = 109000/ml, ESR = 9/15 mm. In the tenth month of treatment he was admitted in our clinic for high fever (39 deg. C), chills, headache and important asthenia. Clinical examination revealed: impaired clinical state, fever (39.2 deg. C), pale skin, profuse sweating and multiple, painful furuncles with emerging pus in the occipital aria. The biology showed: leukocytes = 6900/ml, granulocytes = 4500/ml, haemoglobin = 12.3 g/100 ml, platelets = 106000/ml, ESR = 31/64 mm.

Results: From the multiple hemocultures and also from the furuncles pus was isolated the same bacteria: white non-hemolythic Staphylococcus. After eight days of antibiotic treatment (2 g/day of ceftriaxone plus 240 mg/day of gentamicin) the patient went well with no other adverse event.

Discussion/Conclusion: This case reveals that antiviral-induced immuno-suppression may lead to life-threatening infections with low-pathogenic bacteria and also with relatively poor inflammatory reaction despite the “noisy” clinical presentation.
Chronic hepatitis C infection affects immunological resistance in stomach cancer patients

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Introduction: It is known that HCV infection may be accompanied by depression of some immunological parameters in healthy person and patients with malignancies (breast cancer). We have investigated the ability of this infection to cause depression of some cellular (activity of neutrophiles and NK) and humoral (interferon level) characteristics of non-specific immune-mediated resistance (NIR) in patients with stomach cancer (SC).

Methods: The investigation was carried out in 48 SC patients, 25 of them - with persistent HCV-RNA in blood sera. All of these patients were HBsAg-negative without any symptoms of hepatitis. The blood specimens were immunologically tested for quantization of phagocytic activity of neutrophils, cytotoxic activity of NK and concentration of alpha-interferon (IFN-α) in blood serum. The results obtained in HCV-infected and HCV-free SC patients were compared.

Results: The comparison demonstrated the absence of statistically sufficient difference between neutrophils activity in HCV-infected and non-infected SC patients. At the same time the mean index of cytotoxic activity of NK in the HCV-positive patients was lower, than this parameter in non-infected patients: the difference was statistically sufficient (p < 0.05). The similar results were registered in the IFN-α level in these two groups of SC patients (p < 0.05).

Discussion/Conclusion: HCV infection in SC patients depresses NIR by means of decreasing neutrophil activity and NK and IFN-α production.
Cytokine levels in serum of chronic hepatitis C patients during combined treatment with interferon-alpha with thymosin-alpha1

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Introduction: The aim of the study was to determine the main tendencies in cytokine level changes in peripheral blood after complete course of combined therapy HCV-infected patients with interferon-alpha2 (IFNα) and thymosin-alpha1 (Tα1).

Methods: 20 patients with chronic hepatitis C (CHC) were enrolled in the investigation. In all cases CHC was diagnosed with the help of clinical, biochemical and serologic methods including detection of HCV RNA in the blood. All patients underwent therapy with alpha-interferon (18 MU per week for 6 months) and Tα1 (1.6 mg twice per week for 6 months). Sustained virological response (SVR) to combined therapy was registered in 14 patients. All patients were tested before and after combined therapy with ELISA kits for quantization of concentration of IL-1, IL-2, IL-6 and TNF-α in blood serum.

Results: Before combined therapy mean levels of cytokines were follow: IL-1 – 1.3 pg/ml, IL-2 – 77.4 pg/ml, IL-6 – 21.5 pg/ml and TNF-α – 28.2 pg/ml. After treatment the changes in the cytokines levels were detected, but the difference between IL-1 and IL-2 levels was not statistically significant. At the same time in 14 patients with SVR mean level of IL-6 became significantly higher (p < 0.05) and TNF-α level significantly lower (p < 0.01) in comparison with their levels before treatment.

Discussion/Conclusion: Thus, the serum levels of IL-6 and TNF-α in patients with CHC after combined therapy Tα1 became significantly changes at patients with SVR due to combined treatment.
**Fibroscan® in chronic hepatitis C: Can modify the assessment and the follow-up of treated patients?**

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**Introduction**: Liver biopsy is the current “gold standard” to evaluate liver fibrosis but is an invasive procedure.

**Aim**: to evaluate the hepatic elastography for the assessment and monitoring of chronic HCV patients.

**Methods**: 14 controls and 114 chronic hepatitis C patients were studied, 45 untreated and 69 treated with Peginterferon + ribavirin (SVR - 30; Relapsers (RR) - 7; NR - 32). Histological activity index (HAI - Knodell/Peter Scheuer): 27 patients F3/4; 75 patients F0/2. Liver stiffness was measured using Fibroscan® (Echosens, France), 14 controls = 4.5 Kpa (3.30-5.69).

**Results**: A correlation was found between liver stiffness and HAI (p < 0.003): F0,1 – 4.92 kPa (4.21-6.17) vs. F2,3,4 – 6.81 kPa (5.14-15.95), p = 0.006; F0,1,2 - 5.90 kPa (4.85-7.25) vs. F3/F4 – 12.55 kPa (9.10-16.24), p = 0.001. Median liver stiffness for detection of cirrhosis was 13.75 KPa (11.44-27.05), p = 0.001. Using the cut-off 12 kPa: sensitivity 64%, specificity 98%, positive predictive value 98% (VPP) and negative predictive value 73%. A correlation of liver stiffness was seen with patient age (p = 0.009), cholesterol (p = 0.04), triglycerides (p = 0.04), HOMA (p < 0.03) and GGT (p = 0.006). Median liver stiffness in SVR - 5.13 kPa (4.27-6.05), lower then in NR - 8.02 kPa (5.78-22.06), p < 0.0005 and similar to the controls - 4.5 Kpa (3.30-5.69).

**Conclusion**: Fibroscan® is able to differentiate the stages of fibrosis and correlates with other parameters of progressive fibrosis. In sustained virological response values were similar to the controls. Fibroscan® may become a useful and reliable method for the non-invasive follow-up of treated HCV patients.
Efficacy of puncture-drainage echinococctomy for recurrent, residual echinococcosis of the liver and residual cavities after echinococccctomy

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A purpose of the work was to study an efficacy of puncture-drainage method of treatment combined with medicinal therapy for recurrent and residual hepatic echinococcosis and residual cavities after echinococccctomy.

One hundred and twenty-one patients with recurrent and residual hepatic echinococcosis, of them 53 males and 68 females, aged from 17 to 60 have been examined. Their anamnesis included operations for hepatic echinococcosis: once – 73, twice – 28, thrice – 15. All the patients were examined besides common clinical methods of investigations for tests for changes in a number of eosinophiles, an analysis of cysts’ content for presence of echinococcus elements. Before operations the patients obtained albendazol (alben, zentel) at a dose of 10 mg/kg or mebendazol (vermox, varmizol) at a dose 50 mg/kg (2 courses in 15 days with an interval 15 days). Puncture and drainage of hepatic cysts were accomplished in stages. After puncture and drainage the patients received else 2 courses of medicinal therapy.

Analysis of results achieved exhibited that drained cysts were obliterated in all the cases. No cavity was found in 3 months. In 36 cases a cyst was considered as a residual cavity, because all analyses for echinococcosis were negative.

Results obtained confirmed an efficacy of puncture-drainage method of treatment combined with medicinal therapy for recurrent, residual hepatic echinococcosis and residual cavities after conventional methods of surgical treatment of hepatic echinococcosis.
Liver steatosis in relation to iron overload and \textit{HFE} gene mutations

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\textbf{Aim}: Analysis of correlation between liver steatosis and disorders of iron metabolism in association with \textit{HFE} gene mutations.

\textbf{Patients and methods}: 104 patients (91 men, 13 women, mean age 46 ± 12 years) routinely diagnosed because of liver disease with biochemical parameters of iron overload were included to the study. In all cases the histopathological examination of the liver biopsy specimen was performed with assessment of inflammation activity, fibrosis, iron deposits and steatosis. 58 patients were infected with HCV, 5 with HBV. In 37 patients diabetes and in 29 hyperlipidemia were diagnosed. 35 patients were suspected for hereditary hemochromatosis. PCR and RFLP methods were used to detect C282Y and H63D mutations of \textit{HFE} gene.

\textbf{Results}: Histopathological steatosis was diagnosed in 59/104 patients. It was detected statistically more frequently in patients with arterial hypertension and hyperlipidemia. Occurrence of hepatocyte iron deposits in 68/104 patients did not correlate with intensity of steatosis. Degree of liver steatosis was a negative predictor of HCV infection and activity of inflammation in the study group. C282Y mutation was detected in 16 patients with confirmed histopathological steatosis without statistical significance in comparison to patients with no steatosis. Prevalence of H63D mutation was significantly more frequent in patients without marks of liver steatosis (20/45 vs. 17/59). In the subgroup of patients suspected for hereditary hemochromatosis (n = 35) liver steatosis was described with significant correlation in 23 cases and 12 patients appeared to be homozygotes C282Y and 3 - homozygotes H63D.

\textbf{Conclusions}: Liver steatosis may be associated with metabolic syndrome and iron overload but its correlation with \textit{HFE} gene mutations needs further detailed studies.
Clinical significance of occult HBV infection in Bulgarian patients with chronic hepatitis C

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Introduction: A number of recent studies reported increased prevalence of occult HBV infection among patients infected with hepatitis C virus, which is not entirely explained by the prevalence of HBV in the general population. The clinical significance of occult HBV infection remains the source of continued controversy.

Aims: To evaluate the prevalence and clinical significance of occult HBV infection in patients with chronic hepatitis C (CHC)

Methods: Serum samples form 228 Bulgarian patients with CHC, all of them HBsAg-negative were retrospectively analyzed for HBV DNA. Samples were collected since 02.2000 till 01.2006 and were stored frozen at -70°C. For the purposes of detecting HBV DNA we performed real-time PCR analysis, using commercially available COBAS® TaqMan HBV Test, Roche with sensitivity 6 IU/ml. HCV RNA were measured qualitatively and quantitatively. Percutaneous liver biopsies were performed in 200 patients and analyzed with METAVIR. 180 patients with CHC were treated with INF-α/ribavirin or/and Pegylated INF-α/ribavirin according accepted regiments. In addition, 120 HBsAg-negative healthy adults served as controls.

Results: Occult HBV infection, proved by the presence of HBV DNA in serum of HBsAg-negative patient, was detected in 40 (17.5%) with CHC. 32 of them were anti-HBcAg positive and 8 were anti-HBcAg negative. All 40 patients had low HBV DNA load ranging from 6 to 1000 IU/ml. 96 patients with CHC were treated with conventional INF-α and ribavirin (70 - naïve, 18 - non-responders to INF-α monotherapy, 8 - relapsers to INF-α monotherapy) and 84 patients were treated with pegylated INF-α and ribavirin (52 - naïve, 30 - non-responders to conventional INF-α and ribavirin, 2 - relapsers to conventional INF-α and ribavirin) according to accepted regiments. No significant difference were found in AST levels, HCV RNA viral load, inflammatory grade and in sustained responses rates between patients with and without occult HBV. However, the estimated fibrosis grade were higher in patients with occult HBV infection. Only 5 (4%) HBsAg-negative healthy adults had positive HBV DNA in their sera.

Conclusion: Occult HBV infection is more frequently found in patients with CHC (17.5%) than in healthy adults. It is associated with advanced fibrosis and may favor or accelerate the evolution of CHC to cirrhosis.
Symptomatic and asymptomatic primary biliary cirrhosis in Lithuania

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Introduction: The frequency regarding asymptomatic PBC cases are controversial, moreover in Lithuania the problem of PBC is still not evaluated.

The aim of this study was to analyze the clinical, biochemical, immunological and histological data of PBC patients (pts) and to evaluate the patterns of disease presentation and histological features.

Patients and methods: The retrospective record-review study of 131 pts who were examined and followed in the Center of Hepatology, Gastroenterology and Dietetics of Vilnius University Hospital was carried out. Pts were divided into 2 groups: symptomatic and asymptomatic according to the first presentation. Asymptomatic pts didn’t have any specific symptom or sign of liver disease at disease presentation (itching, jaundice, hepatomegaly, ascites, edema, variceal bleeding and portosystemic encephalopathy).

Results: Most of the pts were women 124 (94.6%) older than 50 yrs with the late stages (III-IV) of PBC. Men were significantly older, than women (p < 0.05) and had a shorter duration from disease presentation to diagnosis (4.0 ± 0.4 vs 1.4 ± 0.4 yrs; p < 0.0001).

29.8% of pts had asymptomatic disease presentation and were older than symptomatic ones (p < 0.0357). No statistical difference in the prevalence of early and late stages of PBC in asymptomatic PBC pts was found. The predominant “liver related” symptom at disease presentation was pruritis in 80 (61.1%) of pts and in 38.9% of them it occurred as the first symptom of the disease. In 14.5% and 12.2% of pts disease presented by abnormal liver tests and fatigue, in 11.5% by abdominal pain and discomfort. Almost in one third (29.8%) of PBC pts disease was diagnosed accidentally or after finding of abnormal biochemical liver tests when examined because of unspecific symptoms and signs or on routine check up. Asymptomatic pts were presented with significantly lower prevalence of jaundice, skin signs, weight loss, lower ALP activity, but higher frequency of sicca syndrome. Cholestatic liver enzymes were increased and exceeded UNL 6.1 and 12.1 times. ALP was significantly higher in symptomatic than in asymptomatic pts (p = 0.0164). AMA-M2 was found in 98.7% of pts (titer 1:80-1:800) as the only marker in 50.4%, simultaneously with ANA in 34.7%.

Histological inflammatory bile ducts lesions were presented in all PBC pts, while signs of cholestasis (except copper accumulation in 89.7% of pts) were lower. The mean values of HAI was 6.9 ± 0.3 with the prevalence of mild histological activity (HAI 4-8) in 54.5% of pts and significantly higher values in stages II, III and IV than in stage I, but without difference in symptomatic and asymptomatic pts.
In conclusion, our data showed just one third of PBC pts in Lithuania having initially asymptomatic PBC course, with some differences of clinical signs and symptoms as compared with initially symptomatic pts.
Peginterferon α-2a monotherapy in the treatment of chronic hepatitis C in patients on maintenance hemodialysis

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**Introduction**: Combined antiviral therapy with pegylated interferons and ribavirin is a current standard of chronic hepatitis C therapy. Administration of ribavirin in patients on maintenance hemodialysis is associated with severe hemolytic anemia even at low doses. Our aim was to verify the efficacy and tolerability of peginterferon α-2a monotherapy in hemodialysed patients with chronic hepatitis C.

**Methods**: Seven male patients, average age 47.6 years (range 28-64) with chronic renal failure (5 with renal graft failure, 2 before enlistment for first kidney transplantation) were evaluated. All patients were infected with genotype 1b with low pretreatment viremia (< 8 x 10⁵ IU/ml) and had normal ALT. Liver biopsy was performed in 6/7 patients, showing signs of chronic hepatitis with mild inflammation (grade 6-7/18) and fibrosis stage 1-4/6 according to Ishak. Liver biopsy was not performed in one patient due to concomitant polycystic disease. All patients received peginterferon α-2a 180 µg once a week for 48 weeks with follow-up period of 24 weeks.

**Results**: 6/7 patients had undetectable HCV-RNA after 12 weeks and at the end of treatment. Overall, 5/7 (71%) patients achieved sustained virological response, one relapsed and one was non-responder. Although the treatment was stopped in 2 patients with early virological response (week 15 and 26) due to erythropoetin-resistant anemia, they achieved sustained virological response. No other serious side effects were observed.

**Discussion/Conclusion**: In patients with chronic hepatitis C on maintenance hemodialysis, monotherapy with peginterferon α-2a can be an efficacious and safe treatment modality.
Peginterferon α-2b and ribavirin in the treatment of chronic hepatitis C in the Czech Republic: A multicenter experience

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Introduction: Combination antiviral treatment represents the current therapeutical standard for chronic hepatitis C. We evaluated the treatment efficacy in patients treated during last three years with peginterferon α-2b and ribavirin.

Methods: A retrospective study was performed on 51 male and 49 female patients, of whom 53 were treatment naïve, 27 relapsers and 20 non-responders to previous interferon-α treatment. Genotype 1 was reported in 70/75 subjects with known genotype. Initial dose of peginterferon α-2b was 1.0–1.5 µg/kg/week and ribavirin 800–1200 mg/day. Patients with genotype 1 were treated for 48 weeks, those lacking early virological response for 12-24 weeks, patients with genotype 2/3 only for 24 weeks.

Results: The sustained virological response (SVR) was achieved in 53 patients. Treatment naïve patients achieved the highest SVR (34/53, 64.2%). SVR rate in relapsers or non-responders was 14/27 (51.9%) and 5/20 (25%), respectively. As SVR rate for genotype 1 was similar to that in patients with unknown genotype (25/65 (53.8%) and 14/25 (56.4%)), respectively, it is likely that genotype 1 was prevalent in the latter group. SVR rate in patients with genotype 2/3 was 4/5 (80%).

Discussion/Conclusion: Despite the predominance of genotype 1 in our patients, the efficacy of treatment with peginterferon α-2b and ribavirine was similar to the results of other studies reported up to date.
Detection of *Helicobacter* species DNA in liver of Polish patients with chronic liver diseases by PCR-DGGE and sequence analysis

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**Introduction:** DNA of number of *Helicobacter* species, which have been isolated from the stomach, intestinal tract and liver of a variety of animals, was also detected in human bile and liver samples. The aim of this study was to determine the possible presence of *Helicobacter* species in the liver tissue samples of patients with chronic liver diseases of different aetiology.

**Methods:** 97 patients (46 females, 51 males), aged 18-66 years (mean 41 ± 1) were admitted to the Hospital for Infectious Diseases in Gdansk, Poland because of a chronic liver disease. Liver biopsy specimens were examined for the presence of *Helicobacter* species by a genus-specific PCR assay. PCR products of positive samples were subsequently characterized by denaturing gradient gel electrophoresis (DGGE) and DNA-sequencing.

**Results:** Using Helicobacter genus-specific PCR assay, *Helicobacter* DNA was detected in 69/97 (68%) of liver tissue samples. Among them, 45/70 (55%) positive samples were detected in patients chronically infected with hepatotropic viruses (HBV or HCV), 9/14 (64%) in patients with toxic liver damages and 9/13 (85%) in patients with autoimmune liver diseases. No correlation was found between the frequency of *Helicobacter* PCR-positive results and aetiology of liver diseases and presence of signs of liver inflammation.

**Discussion/Conclusion:** The presence of *Helicobacter* species DNA in liver tissue may suggest possible role of *Helicobacter* infection in human chronic liver diseases, but further study are underway in order to elucidate the probable relation of different *Helicobacters* to chronic inflammation of human liver.
The predictive value of per-rectal porto-scintigraphy in liver cirrhosis patients

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Introduction: Per-rectal porto-scintigraphy (RPS) is a non-invasive, radio-isotopic method that evaluates the portal hypertension by calculating the shunt index (SI) in the porto-caval collateral vessels. The aim of this study was to evaluate the prediction value of the SI calculated by RPS in patients with liver cirrhosis (LC), by studying the correlation between SI and Child-Pugh-Turcotte (CPT) stages and its determinants.

Methods: 308 subjects with LC were evaluated by RPS. The CPT stage was calculated for each patient according to standards. Albumin, Bilirubin and Prothrombine Time levels were taking into account, as well the presence and grade of ascites and hepatic encephalopathy. The statistical analysis was done using non-parametric tests. p value less than 0.05 was considered significant.

Results: The albumin and prothrombine time values were negatively, but well correlated with SI (r = -0.69, p = 0.000, respectively r = -0.6, p = 0.000). Bilirubin levels were also well correlated (positively) with SI (r = 0.58, p = 0.000). Regarding the correlation between SI and ascites and encephalopathy no correlation could be observed. When the CPT stages were taking into account, a strong correlation could be found with SI value, globally and between stages as well (A-B, p = 0.000; B-C, p = 0.0026; A-C, p = 0.000).

Discussion/Conclusion: SI increases as the LC is progressing, so it may be an useful and sensitive tool to complete the data already known and to evaluate the prognosis.
The role of per-rectal porto-scintigraphy in the prediction of variceal haemorrhage in liver cirrhosis patients

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Introduction: Variceal bleeding is the main cause of death in liver cirrhosis patients and it can be predicted invasively performing oesophagoscopy. Per-rectal porto-scintigraphy (RPS) is a non-invasive, radio-isotopic method that evaluates the portal hypertension by calculating the shunt index (SI) in the porto-caval collateral vessels. The aim of this study was to evaluate the role of RPS for the assessment of variceal haemorrhagic risk.

Methods: 110 patients with liver cirrhosis (LC) were evaluated by oesophagoscopy for the presence of oesophageal varices (OV). If present, they were classified as low or high grade. They also underwent RPS and the SI was calculated. The occurrence of variceal bleeding (VB) was also noticed in the patient’s history or in the follow up period. The statistical analysis was done using non-parametric tests. p value less than 0.05 was considered significant.

Results: 39 patients (29.09%) had low grade OV, 60 (54.54) high grade and in 18 (16.36%) the dimension of OV could not be specified. The SI was significantly higher in the high grade OV groups compared with the low grade one (p = 0.000). The VB could be observed in 62 cases (56.36%). In this group the SI was significantly higher compared with patients with no VB history (p = 0.000) and it was also higher than 70%.

Discussion/Conclusion: The SI calculated by RPS is positively correlated with the presence and the grade of oesophageal varices in liver cirrhosis patients. A SI value greater than 70% is highly predictive for variceal bleeding in these patients.
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Improvement of quantitative liver function tests (QTLF) during and after antiviral therapy of chronic hepatitis C

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Introduction: Antiviral treatment of chronic hepatitis C is an extensive therapy with severe side effects. But data on the influence on liver function is still rare. Therefore we investigated if and to what extend quantitative testing of liver function (QTLF) change under the combination therapy with interferon/pegylated-interferon and ribavirin (INF/PEG-INF/RBV).

Methods: Data of 50 patients with chronic hepatitis C treated with interferon/ribavirin or peg-interferon/ribavirin was investigated. Quantitative testing of liver function, including aminopyrine breath test (ABT), galactose elimination capacity (GEC), sorbitol clearance (SCI) and indocyanin green clearance (ICG) was performed before, 12 and 48 weeks after initiation and 24 weeks after completion of the therapy.

Results: After 12 weeks of antiviral treatment 36 patients showed normal transaminases and were negative for HCV-RNA (= Responders). Initially parameters of microsomal and cytosolic liver function (ABT, GEC) were reduced and returned to normal values in therapy responders after 12 weeks of antiviral treatment. These parameters continued to be in the normal range until week 48 after initiation of therapy. In contrast to this fast response to antiviral therapy parameters of liver perfusion (SCI, ICG) did not change significantly after 12 weeks of treatment but were in the normal range at the end of treatment (48 weeks). In responders with SVR (24 week after completion of therapy) all QTLF were in the normal range at the end of the study, whereas non-responders showed no significant improvement of the liver function.

Discussion/Conclusion: Our findings show that after the eradication of the hepatitis C virus the normalization of the microsomal and cytosolic liver function is a fast effect whereas the normalization of liver perfusion tests takes longer and might be depended on time consuming remodeling processes.
Cholestasis, inflammatory bile duct damage and resulting biliary fibrosis in the DDC model of chemical induced bile duct injury

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Introduction: The molecular mechanism leading to fibrosis and cirrhosis in cholestatic diseases are poorly understood. Therefore, we aimed to determine the mechanisms of cholestasis and biliary fibrosis in 3,5-Diethoxycarbonyl-1,4-Dihydrocollidine-treated mice.

Methods: Male Swiss albino mice were fed a 0.1% DDC supplemented diet for 1, 4 and 8 weeks. Liver morphology, ultrastructure, immunostaining for CD11b, VCAM, CK19, Sirius red staining, hydroxyproline content, bile flow and composition, hepatic mRNA for key inflammation and fibrosis genes (TNF-α, TGF-β, iNOS, collagen1a2), mRNA and protein expression as well as tissue localization (immunofluorescence microscopy) of key hepatobiliary transporters (Ntcp, Oatp1&4, Mrp2-4, Bsep, Mdr1a,b&2) and in situ hybridization for TNF-α were compared between control diet-fed and DDC-fed mice.

Results: DCC feeding significantly induced expression of VCAM, TNF-α, TGF-β and iNOS and caused pericholangitis (significantly increased number of CD11b-positive cells), ductular proliferation and proliferation of periductal myofibroblasts. Biliary fibrosis developed over time with increased hydroxyproline content and induction of procollagen 1a2 and TIMP1 mRNA. After 4 weeks, we frequently observed intraductal porphyrin plugs leading to biliary obstruction. Bile flow and glutathione excretion significantly decreased over time whereas phospholipid, cholesterol and bile acid excretion remained unchanged. Expression of Ntcp, Oatp and Mrp2 was significantly reduced, Bsep expression was preserved and Mrp3 and Mrp4 expression was induced.

Discussion/Conclusion: Cholestasis in DDC-fed mice is related to (i) the development of a reactive phenotype of cholangiocytes, (ii) pericholangitis and resulting periductal fibrosis, (iii) downregulation of Mrp2 leading to impaired glutathione excretion, and finally, (iv) bile duct obstruction via porphyrine plaques.
Chronic hepatitis C: A retrospective study of 225 patients

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Introduction: Chronic hepatitis C is a global health problem.

Methods: In a retrospective study of 225 anti-HCV positive cases of chronic hepatitis C in the years 1993-2003, 83 (37%) patients received blood transfusion prior to the year 1992, and 39 (17%) were i.v. drug addicts. The authors evaluated anamnestic data on viral hepatitis B and C, alcohol intake, blood transfusion, i.v. drug addiction, internal diseases and surgical procedures. Histology of the liver had been performed in 129 (57%) patients. Serology was carried out by 3rd generation ELISA methods, HCV RNA was detected by the polymerase chain reaction method.

Results: In case history acute or chronic hepatitis C was presented in 8.4% of the patients only, alcohol was consumed by 45%, the most frequent diseases were obesity, cholelithiasis, and hypertension, and of surgical intervention it was gall bladder surgery, accidents, resection of the stomach and intestines. Drug addicts were significantly younger than subjects with blood transfusion (p < 0.001). In almost 40% of patients liver cirrhosis was present. Cirrhotic patients had significantly higher mean activities of AST than ALT, and lower thrombocyte counts (both p < 0.01) than patients without cirrhosis. HCV RNA was positive at least in 2/3 of the patients. HCV co-infection with hepatitis B virus was revealed in 18.7% of whom 1/3 were drug addicts. Hepatocellular carcinoma was found in 9 patients (4%), all of them suffering cirrhosis. Antiviral therapy was administered in 23% of the patients. Only one male healed spontaneously following acute hepatitis C (0.44%).

Discussion/Conclusion: The results reveal that 90% of the patients were detected as chronic viral hepatitis C. In about 40% of patients liver cirrhosis was present and cirrhotics had significantly higher AST than ALT levels, and lower thrombocytes count. At least 2/3 of patients were viremic and in 19% of patients HCV and HBV coinfection was detected.
Doppler spectral examination of the hepatic artery to the chronic liver diseases patients. Pilot study

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**Introduction**: Measurements of the resistivity (RI) and pulsatility index (PI) to the chronic liver diseases patients (without ultrasound signs of portal hypertensive) for those establishment modifications, as a precocious sigh for hepatitis fibrosis.

**Methods**: We studied 15 hospitalized patients from 3rd Medical Clinic, diagnosed with chronic hepatitis, hepatic steato-fibrosis, hepatic cirrhosis, having 35-62 years old. All the patients were ultrasound examined with performant devices, having Color Flow mode and Pulsate Doppler mode. We identified hepatic artery and we used Doppler pulsate mode for measuring the RI from hepatic artery ([Vmax-Vmin]/Vmax).

**Results**: At 10 of the examined patients we identified an increase of RI on the hepatic artery with values between 0.72 to 0.78. 5 were patients with normal RI values (0.58-0.68). None of the studied patients had suggestive ultrasound signs of hepatic cirrhosis or portal hypertensive. We performed hepatic puncture-biopsy to all the patients that had changes of the RI (8 of them presented different levels of hepatic fibrosis).

**Discussion/Conclusion**: We found that there is a correlation between the increase of RI over 0.72 and the development of hepatic fibrosis. We consider that the changes of the acoustic impedance index on hepatic artery, at the patients with chronic hepatitis is a precocious, suggestive sign for hepatic fibrosis that appears before the portal hypertensive.
Is the spontaneous HBeAg/anti-HBe seroconversion in children with chronic hepatitis B infection associated with the disease remission?

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Introduction: Hepatitis Be antigen clearance and production of anti-HBe antibodies is usually associated with clinical, biochemical and histological improvement. The aim of this study was to analyze whether spontaneous HBeAg/anti-HBe seroconversion in children persistently infected with hepatitis B correlated with the remission of the disease. We have also analyzed the frequency of disease progression after primary remission.

Patients and methods: Fifty children aged 4-18 years with documented chronic hepatitis B infection after spontaneous HBeAg clearance were included in the study. Patients were followed up for 1 to 10 years (mean 7.5 years). The following parameters were analyzed: serum ALT and bilirubin concentrations, serum HBV DNA levels, liver USG and histology.

Results: Eighteen patients (36%) had undetectable HBV DNA in the serum. The complete disease remission was observed in 10 (55.5%) of 18 children without HBV DNA. The hyperbilirubinemia was found in other 4 patients (22.2%) from this group and the results of USG analysis was improper in remaining 4 children (22.2%). One child out of 18 (5.5%) had an evidence of cirrhosis. Liver histology revealed that the majority of children (41, 93%) had minimal or mild inflammation, whereas the remaining 3 showed high inflammation activity. HBV DNA was found in 32 patients (64%) and the viremia levels ranged from 2 x 10² to > 10⁵ copies/ml.

Discussion/Conclusion: The spontaneous HBeAg/anti-HBe seroconversion not always leads to the disease remission. Furthermore, patients after seroconversion should be considered as a possible source of infection and they should be followed-up by a specialist.
The effects of \(N\)-acetylcysteine on bile-duct ligation model induced liver fibrosis in rats

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Introduction: Stellate cells are activated by free-radicals, and synthesize collagen. \(N\)-acetylcysteine (NAC), a thiol-molecule, is a precursor of reduced glutathione and a potent scavenger of hydroxyl-radicals. NAC has recently been shown to have antifibrotic effects in tissues. We aimed to test the effects of NAC on bile-duct ligation (BDL) induced liver damage in rats.

Methods: Forty-seven Wistar male rats were divided into 5-groups. Group-1: \((n = 10)\) bile-duct ligation (BDL) + NAC, group-2: \((n = 10)\) BDL + placebo, group-3: \((n = 10)\) sham-operated + NAC, group-4: \((n = 10)\) sham-operated + placebo, group-5: \((n = 7)\) control group. NAC (50 µmol/kg/day) and NaCl (placebo) single doses were administered intraperitoneally for 28 days. Liver enzymes and free radical tests were studied with commercial kits. Total liver collagen was determined by the method of Lopez de Leon and Rojkind. Liver slides were stained by Haemotoxylene/Eosin and Masson-Tricrom\Gomory reticulum staining.

Results: AST and alkaline phosphatase levels in group-1 were higher than group-2 and were lower than control groups (all – \(p < 0.001\)). Malondialdehide, luminol and glutathione levels in group-1 were lower than group-2 (\(p = 0.011, p = 0.002\) and \(p < 0.001\)) and higher than control groups (all – \(p < 0.01\)). NAC had no effect on ALT, gammaglutamyl transferase, bilirubins, albumin and lucigenin levels. Liver collagen levels were higher in BDL groups (\(p < 0.001\)), however NAC had no effect on collagen levels of the groups. In histopathological examination, group-1 and group-2 showed stage-3 fibrosis, while all the control groups were normal.

Discussion/Conclusion: Daily intraperitoneal NAC administration improved biochemical parameters (AST, alkaline phosphatase) and oxidative stress parameters (Malondialdehide, luminol, glutathione) in BDL model. NAC was found effective on cholestasis induced hepatotoxicity. A longer-term study is needed to evaluate a possible antifibrotic effect.
Role of ursodeoxycholic acid in prevention of methotrexate induced liver toxicity

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Introduction: Methotrexate (MTX) is a frequently used cytotoxic drug with immunosuppressive properties. Hepatic toxicity caused by MTX importantly restricts its clinical use. Increased oxidative stress and inhibition of antioxidative system, mostly by reduced glutathione (GSH) levels, has been noticed recently. In this study, it has been aimed to search the role of oxidative stress on hepatic toxicity of MTX and the protective effect of ursodeoxycholic acid (UDCA) on this toxicity.

Methods: Wistar type rats (n = 32) were divided into 4 groups; group-1 (n = 8) as MTX + UDCA, group-2 (n = 8) as MTX + serum physiologic (SP), group-3 (n = 8) as SP + UDCA, group-4 (n = 8) as SP + SP. For the 1st and 3rd groups 50 mg/kg UDCA was administered orally; for the 2nd and 4th groups SP was administered orally and continued for the next 6 days. On the second day of study, for the 1st and 2nd groups single intraperitoneal MTX with 20 mg/kg dose and for the 3rd and 4th groups similar dose of SP intraperitoneally was administered. On the 6th day, serum samples were examined for ALT, ALP, GGT and tissue ROM (luminal, lucigenin, lipid peroxygenation product malondialdehyde (MDA) and antioxidant GSH levels.

Results: In the 2nd group serum ALT, ALP, GGT and tissue ROM (luminal, lucigenin and MDA) levels were higher but GSH levels were lower. In histopathologic examination of liver tissues, in MTX + SP group hepatocellular necrosis was clearly more evident than the MTX + UDCA group.

Discussion/Conclusion: Increased ROM and reduced GSH in MTX + SP group suggested that oxidative stress may play a role in pathogenesis of MTX hepatotoxicity. The ROM and GSH levels of MTX + UDCA group were not different than control group. In the histopathological evaluation, hepatocellular necrosis was lower in MTX + UDCA group than MTX + SP group which suggest the protective role of UDCA against MTX induced hepatic toxicity.
Evaluation of the efficiency of Ursofalk®’s association in the antiviral treatment in chronic hepatitis C virus with cryoglobulinemia

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Introduction: Chronic hepatitis C virus (HCV) infection is associated with a variety of clinically important extrahepatic abnormalities. Were observed that cryoglobulinemia worsens the evolution of HCV and more rapid development of liver cirrhosis.

The aim of our study was to evaluate the effect of the Ursofalk® administration in addition to antiviral treatment in HCV infection with cryoglobulinemia.

Patients and methods: We studied 41 patients with HCV infection and cryoglobulinemia. Antiviral treatment for 48 weeks with Interferon-alpha (3 million units, 3 times weekly) was given to 19 patients and the same treatment, but plus Ursofalk® (750 mg/daily) was administrated in 22 patients. We have evaluated the clinical, serologic, and biochemical response to treatment in both groups.

Results: Patients treated with Interferon-alpha plus Ursofalk® vs. only Interferon-alpha administration significantly decreased aminotransferase levels (p < 0.05) and marked improvement of manifestations of cryoglobulinemia. Cryoglobulins disappeared from the serum in 77.3% patients who were treated with Interferon-alpha plus Ursofalk® vs. only Interferon-alpha (52.6%, p < 0.01). Interferon-alpha plus Ursofalk® eradicated HCV RNA in 54.5% vs. only Interferon-alpha (47.4%, p < 0.05).

Conclusion: Our study indicates that the administration of Ursofalk® improves the antiviral effect in patients with HCV infection and cryoglobulinemia and should be indicate not only in cholestatic forms, but also in patients with autoimmune forms.
Correlations between the parameters of central, hepatic, peripheral haemodynamics and biochemical parameters in patients with chronic hepatitis

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Introduction: The changes of the central, peripheral and hepatic haemodynamics are found not only in cirrhotic patients, but also in patients with chronic hepatitis.

The aim of our study was to identify some correlation between the central, hepatic, peripheral haemodynamics, the tolerance of physical exertion and biochemical parameters in patients with chronic hepatitis.

Methods: We investigated the haemodynamics, the tolerance of physical exertion and biochemical parameters in 116 patients with chronic hepatitis. The following aspects were studied: 1) correlation between parameters of hepatic haemodynamic and parameters of central and peripheral haemodynamics; 2) correlation between biochemical and haemodynamic parameters; 3) correlation between biochemical and haemodynamic parameters and the tolerance of physical exertion.

Results: We found the follow statistic correlations:
- the increasing of the diameter of portal vein and the portal blood flow correlate with the increasing of the left ventricle telesystolic and telediastolic volume;
- the increasing of the diameter of portal vein and the portal blood flow correlate with the decreasing of albuminemia and the increasing of gammaglobulinemia and bilirubinemia;
- the decreasing of the tolerance of physical exertion correlate with the increasing of the diameter of portal vein, portal blood flow, left ventricle telesystolic and telediastolic volume, gammaglobulinemia, bilirubinemia and with decreasing of albuminemia.

Conclusions: The parameters of hepatic haemodynamics correlate with the parameters of central haemodynamics and biochemical parameters in patients with chronic hepatitis.
Paraoxonase activity in sera of patients with intrahepatic cholestasis of pregnancy

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Introduction: Paraoxonase (PON) is a serum enzyme entirely complexed to HDLs, whose primary physiological role is to protect LDLs from oxidative modifications. The liver plays a key role in the synthesis of serum PON. The aim of present study was to investigate the changes of PON activity in patients with intrahepatic cholestasis of pregnancy (ICP), a reversible form of cholestasis in late pregnancy.

Methods: The study population consisted of 49 patients with verified ICP. Control group consisted of 19 pregnant women without any complication of pregnancy and without liver disease. The determination of PON activity was analyzed with paraoxon as substrate.

Results: The results of our study showed significantly decreased levels of PON in patients with ICP in comparison to control group (99 ± 14 U/l vs. 185 ± 25 U/l). After dividing of patients with ICP according to serum bile acids levels in groups with mild and severe form, the patients with severe form had significantly lower PON activity than patients with mild form (52 ± 15 U/l vs. 110 ± 12 U/l). There was negative correlation between PON activity and total bile acids level.

Discussion/Conclusion: Decreased PON activity in patients with ICP was related to the degree of hepatic dysfunction and addition of PON activity to the current battery of liver tests may improve the evaluation of liver dysfunction in these patients.
Serum magnesium levels in patients with alcoholic and non-alcoholic fatty liver

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Introduction: Magnesium is currently a subject of major interest in medicine. A growing body of evidence links altered status of magnesium metabolism to many pathophysiological states. In the present study we investigated serum magnesium levels in patients with liver steatosis. We compared magnesemia in patients with non-alcoholic and alcoholic fatty liver to estimate if alcoholism is the only cause of magnesium disorders.

Methods: The study group consisted of 44 patients with liver steatosis (non-alcoholic – 25 patients, aged 35-64 years, alcoholic – 19 patients, aged 36-57 years). Patients were administered by essential phospholipids – two capsules Essentiale forte were administered 3 times daily for 3 months. Total serum magnesium was determined by atomic absorption spectrometry.

Results: Results of our study showed hypomagnesemia in sera of patients with alcoholic (0.67 ± 0.10 vs. 1.02 ± 0.11 mmol/l) and non-alcoholic hepatic steatosis (0.65 ± 0.14 vs. 1.02 ± 0.11 mmol/l) before therapy. After therapy with essential phospholipids was hypomagnesemia normalized in both studied groups.

Discussion/Conclusion: Decreased magnesium levels found also in patients with non-alcoholic fatty liver suggest that alcoholism cannot be alone the cause of hypomagnesemia and that there must be another factors which participate on the development of hypomagnesemia in these patients. Hypomagnesemia is not only a laboratory symptom of fatty liver but due to its connection with increased oxidative stress could be a risk factor in the progression of fatty liver to steatohepatitis.
The effect of anti-TNF-alpha therapy on parameters of liver proteosynthetic function

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Introduction: Tumor necrosis factor alpha (TNF) is a multifunctional cytokine playing a key role in apoptosis and cell survival as well as in inflammation and immunity. Several studies have shown that TNF plays a role in viral hepatitis, alcoholic hepatitis, non-alcoholic fatty liver disease and fulminant hepatic failure. In viral hepatitis, elevated levels of plasma TNF and TNF-receptors are frequently observed.

The aim of study: to evaluate the effect of anti-TNF-alpha therapy on liver function in patients with Crohns disease, which is known to have increased levels of TNF. The liver function was monitored with help of parameters of liver proteosynthetic function.

Methods: 44 blood samples of patients with Crohns disease were analyzed. Patients were treated with a single infusion of infliximab. Blood specimens were collected before and 4 weeks after infusion of infliximab. Albumin, prealbumin and transferrin were determined immunochemically, cholinesterase with spectrophotometric assay.

Results: The results of study showed significant improvement of plasma levels of liver proteosynthetic parameters: prealbumin 289 mg/l vs. 220 mg/l, albumin 39.6 g/l vs. 35.1 g/l, transferrin 2671 mg/l vs. 2378 mg/l and cholinesterase 3754 U/l vs. 3250 U/l after anti-TNF therapy.

Discussion/Conclusion: The improvement of liver function parameters after anti-TNF alpha therapy supports the hypothesis of the role of TNF in the pathogenesis of liver damage in various hepatopathies.
Ceruloplasmin specific activity in patients with alcoholic liver steatosis after therapy with essential phospholipids

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Introduction: Copper is an essential trace element. In the blood plasma copper is transported in the form of ceruloplasmin. Ceruloplasmin is not only simple transport protein but it is a multifunctional protein with various other physiological functions. At least a part of these functions is connected with ceruloplasmin’s enzymatic activity. The aim of our study was to investigate the effect of administration of essential phospholipids on the metabolism of copper and ceruloplasmin in patients with alcoholic liver steatosis.

Methods: Patients suffering from alcoholic liver steatosis were enrolled in the study. Two capsules of Essentiale® forte (Rhone-Poulenc Rohrer) were administered 3 times daily for 3 months. The serum levels of copper, immunoreactive ceruloplasmin and oxidase activity of ceruloplasmin were determined.

Results: The therapy with essential phospholipids had no significant effect on copper level. The difference in ceruloplasmin levels between patients with liver steatosis and healthy controls was also not significant, but the specific activity of ceruloplasmin in patients was significantly decreased in comparison to controls (0.64 vs. 0.82, P < 0.001). The specific activity of ceruloplasmin was after therapy moderately increased in comparison to level before therapy (about 18% higher after therapy).

Discussion/Conclusion: The decreased specific activity of ceruloplasmin in spite of normal levels of copper and ceruloplasmin in patients with alcoholic liver steatosis suggested some problems in copper metabolism in these patients.
Serum cholinesterase activity and proteosynthetic function of liver in patients with diabetes mellitus

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Introduction: Diabetes is associated with a lot of changes in intermediary metabolism and several authors reported on higher frequency of liver diseases in patients with diabetes. Aim of the present study was to establish the changes of blood serum cholinesterase, prealbumin and albumin, parameters which are accepted as an index of liver proteosynthetic function, in patients with diabetes mellitus.

Methods: The study group consisted of 207 patients with diabetes mellitus (83 patients with type I and 124 patients with type II diabetes mellitus). Control group consisted of 179 healthy subjects. The activity of cholinesterase was assayed by the kinetic method, concentrations of prealbumin and albumin were determined immunochemically.

Results: Activity of serum cholinesterase was significantly higher in group of patients with diabetes mellitus than in control group (65.05 vs. 73.33 µcat/l). The concentration of prealbumin was lower in blood serum of patients with diabetes than in controls (308.10 vs. 285.85 mg/l). Serum levels of albumin were not different in both studied groups. After dividing of patients according to type of diabetes, 80% of abnormal values of cholinesterase and prealbumin were present in patients with type II diabetes.

Discussion/Conclusion: The character of laboratory changes – increased activity of cholinesterase, decreased concentration of prealbumin and normal levels of albumin – suggests development of liver steatosis in these patients. The most of pathological findings were in patients with diabetes mellitus type II.
Kawasaki disease: A cause of cholestasis syndrome

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Introduction: Kawasaki disease and cholestasis syndrome are rare in children. The aim of the study was to search for cholestasis syndrome in Kawasaki disease.

Methods: We retrospectively reviewed medical records of patients admitted to tertiary University Hospital during 1998-2004. Medical records of all patients with Kawasaki disease were reviewed.

Results: We found 21 patients with Kawasaki disease. Four of them had Kawasaki disease with cholestasis syndrome. The data of these patients are presented in the table. Itching started from the first days of the disease and prolonged for 1-4 wk. The course of the disease was benign in all patients nevertheless they were treated or not with i/v immunoglobulin.

<table>
<thead>
<tr>
<th>Data</th>
<th>1-M</th>
<th>2-F</th>
<th>3-M</th>
<th>4-M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>5.4</td>
<td>8.2</td>
<td>14.7</td>
<td>12.10</td>
</tr>
<tr>
<td>Jaundice/Itching</td>
<td>+/-</td>
<td>-/+</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ALT (U/L) 7-day</td>
<td>206</td>
<td>355</td>
<td>297</td>
<td>25</td>
</tr>
<tr>
<td>21-day</td>
<td>-</td>
<td>-</td>
<td>325</td>
<td>-</td>
</tr>
<tr>
<td>AST (U/L) 7-day</td>
<td>94</td>
<td>120</td>
<td>96</td>
<td>21</td>
</tr>
<tr>
<td>21-day</td>
<td>-</td>
<td>-</td>
<td>218</td>
<td>-</td>
</tr>
<tr>
<td>GGT (U/L) 7-day</td>
<td>123</td>
<td>421</td>
<td>-</td>
<td>109</td>
</tr>
<tr>
<td>21-day</td>
<td>-</td>
<td>445</td>
<td>479</td>
<td>-</td>
</tr>
<tr>
<td>ALP (U/L) 7-day</td>
<td>116</td>
<td>438</td>
<td>904</td>
<td>810</td>
</tr>
<tr>
<td>21-day</td>
<td>-</td>
<td>534</td>
<td>2305</td>
<td>-</td>
</tr>
<tr>
<td>Bilirubin/DBi (µmol/L)</td>
<td>28/11.8</td>
<td>-</td>
<td>51.4/35</td>
<td>24/8.7</td>
</tr>
<tr>
<td>Abdomen ultrasound examination</td>
<td>Normal</td>
<td>Gallbladder wall thick</td>
<td>Gallbladder very big</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Conclusions: Cholestasis syndrome is not a rare entity in Kawasaki disease. Pronounced itching from the beginning of the disease could be the first symptom of cholestasis syndrome.
Outcome of virus-induced hepatocellular carcinoma: Analysis of treatment options

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Background and aims: In this retrospective-prospective study, the effect of stages of cirrhosis and tumor on the outcome of HCC (hepatocellular carcinoma) patient who underwent curative or non-curative treatment options.

Patients and methods: A total of 126 (101 male, 25 female, mean age 55.8 years, 58.7% HBV, 13.5% HBV + HDV, 25.3% HCV, 2.4% HBV + HCV) consecutive patients with a previous or recent diagnosis of virus-induced HCC were included within a 2 year period (January 2002-January 2004). Patient demographics and clinical characteristics were prospectively collected. Single tumor less than 5 cm or 3 tumors with a total tumor size less than 9 cm were accepted as early tumors while more advanced tumors (including portal vein thrombosis and distant metastasis) were accepted as late tumors. Kaplan-Meier survival analysis was performed on patient groups.

Results: 122 (97%) patients had cirrhosis (Child A/B/C: 23/42/57). 43 (34%) patients had early tumors while remaining 83 (66%) had late tumors. One year survival of all patients with different treatment strategies was 44%. Survival was better in patients with early tumors (Child A + B vs. C, mean 26 vs. 12 months, p = 0.0045) compared to patients with late tumors. Survival was also better in early stages of cirrhosis compared to advanced cirrhosis in both early (28 vs. 15 month, p = 0.036) and late tumors (15 vs. 8 months, p = 0.018). Most frequent curative treatment was percutaneous ethanol ablation (PEI) (42%), followed by resection (20%) and transplantation (13%). In patients with early tumors, resection was superior to PEI with respect to patient survival. 4 of 9 patients who underwent transplantation died during perioperative period. Interestingly, survival was better in patients with late tumors who received non-curative treatments compared to those who did not (chemoembolization and PEI) (12 vs. 5 months, p = 0.04).

Conclusion: Stage of cirrhosis has a major impact on patient survival. Resection is superior to PEI in patients with early tumors. However, this may the reflection of the fact that patients undergoing surgery are more likely have a better condition. Non-curative treatment approaches in late tumors may prolong the survive.
Long-term follow-up of genotype D-HBV-infected inactive carriers

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Background and aims: HBeAg seroconversion is partly related to infecting genotype and is usually associated with sustained remission of liver disease during the course of the HBV infection. In this study, natural course of genotype D HBV-infected Turkish inactive carriers was investigated.

Patients and methods: The clinical records of 500 HBeAg- patients with normal ALT and undetectable HBV DNA by a hybridization assay, and with no evidence of advanced liver disease on ultrasound examination were retrospectively analyzed. Among these 500 patients, patients who had at least a 2 year regular follow-up were included into the study.

Results: 81 patients (30 male, 51 female, mean age 41.8 years) were eligible. None of the 16 patients who underwent liver biopsy had fibrosis and all but one patient had inflammatory activity score less than 4. During the mean follow-up of 36.6 months (24-77 months), HBV DNA became detectable in 6 (7.4%) patients. ALT was elevated in 5 of these 6 patients. In 8 patients, there was an ALT elevation without HBV DNA increase and ALT was normalized in 6 who had a history of recent drug intake.

Conclusion: HBV reactivation was rarely observed during a mean follow-up of 3 year (up to 6.5 years). Our results suggest that more than 90% of inactive carriers remain inactive and isolated ALT elevations are transient and are more likely due to other benign causes (drug etc.) during long term follow-up in genotype D-infected Turkish population.
Spontaneous bacterial peritonitis in cirrhosis

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**Introduction:** Spontaneous bacterial peritonitis (SBP) is a life-threatening complication of advanced liver disease with ascites. The prevalence of SBP in patients (pts) with cirrhosis and ascites is 15-20%, the hospital mortality being 20-40%.

**Aim:** To estimate the incidence and the evolution of SBP under medical treatment in hospitalized cirrhotic pts with ascites.

**Material and methods:** a total of 140 consecutive pts were included in the study. SBP diagnosis was based upon elevated ascitic neutrophilic leukocytes count (> 250/mm³) in the absence of data suggesting secondary peritonitis. Cefotaxime 2 g t.i.d. IV during 7 days was the first choice therapy in severely infected cirrhotics (11 pts = first group); 11 pts received Ofloxacin 800 mg orally for 10 days in uncomplicated SBP (second group).

**Results:** The incidence of SBP in cirrhotics with ascites was 15.7%. SBP usually develops in pts with advanced liver cirrhosis (Child's class C) and particularly of alcoholic etiology. In the cefotaxime-treated group, laboratory improvement and recovery was achieved in 9 pts (81.8%), 2 pts (18.1%) died. In the second group, oral administration of ofloxacin was effective in 72.7% pts.

**Conclusions:** The incidence of SBP in cirrhotics with ascites was 15.7%. Since SBP is a severe infection (22.7% mortality for all treated pts) antimicrobial therapy should be started immediately, before identification of the causative organism. Cefotaxime was the drug of first choice, safe and effective in 81.8% of cases.
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Evolutive possibilities in hepatitis C virus infection

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Introduction: Hepatitis B and C virus infection are the most common causes of chronic liver disease and a risk factor for the hepatocellular carcinoma.

Material and methods: Our study included 44 anti-HCV positive patients (pts), hospitalised during nine years. The mean age of the pts was 46.5 ± 8.4 years (29 M, 15 F). The morphological diagnosis was made by liver biopsy, the viral markers were determinated by second-generation ELISA tests.

Results: 70.4% out of 44 anti-HCV positive pts had chronic active hepatitis lesions, 8 pts were diagnosed with cirrhosis, 3 patients were asymptomatic HCV carriers and 2 patients had sporadic acute hepatitis C. 13% of the chronic hepatitis patients developed cirrhosis in a 7 years mean period. The prevalence of alcohol consumption as a risk factor in this group was 75%. Only 29% of anti-HCV positive pts with chronic active hepatic lesions had a history of acute hepatitis C. Liver cancer due to postviral cirrhosis appeared in 2 out of 14 cases after a 2.7 years. The unfavourable prognostic factors observed in patients with hepatitis C virus infection were: male gender, advanced age, alcohol consumption, and the presence of the mixed cryoglobulinemia.

Conclusion: chronic infection with hepatitis C can range from mild non-specific changes, presumably representing a hepatitis C carrier state, to end-stage of liver disease with cirrhosis and hepatocellular carcinoma.
Porto-systemic encephalopathy as a complication of liver cirrhosis

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Introduction: Porto-systemic encephalopathy is a functional disturbance of the central nervous system which can occur in severe acute or chronic liver diseases.

Patients and Methods: The aim of this study is to estimate the incidence and the prognosis of manifest porto-systemic encephalopathy, as well as the main causes of an early hepatic encephalopathy occurrence.

The study was carried out in a group of 292 liver cirrhosis patients, during a 6 year period, in the IV Medical Clinic of the University of Medicine and Pharmacy "V. Babeș" Timișoara.

Results: There was to be noted that 29.1% of the patients presented porto-systemic encephalopathy aspects, stage II-IV. The functional classes repartition of these patients was 7.5% Child-Pugh B, and 92.95% Child-Pugh C. Latent porto-systemic encephalopathy was found in 59.9% of cases.

The causes that determined an early occurrence of porto-systemic encephalopathy were, order by their frequency, the following:

- Gastrointestinal bleeding: 40%
- Diuretic therapy: 21.17%
- Massive paracentesis: 15.29%
- Infections: 9.41%
- The use of sedatives: 8.2%
- Unknown factors (not manifested): 5.88%

Conclusions: Manifest porto-systemic encephalopathy was present in 29.1% of patients. The prognosis for porto-systemic encephalopathy was poor: 31.75% of the patients died during the first year and 81.17% during a period of six years.
The prevalence and risk factors for malnutrition in patients with cirrhosis

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Introduction: Patients with liver diseases are at increased risk for malnutrition due to unnecessary dietary restrictions, anorexia, early satiety, malabsorption, altered protein and energy metabolism. The aim of our study was to determine the prevalence and risk factors of malnutrition in patients with cirrhosis.

Methods: We evaluated the patients using subjective global assessment (SGA), a method that combines history (food intake, weight changes, functional capacity) and physical examination (loss of subcutaneous fat, muscle wasting, edema, ascites) to categorize patients as: well nourished (SGA A), moderately malnourished (SGA B) or severely malnourished (SGA C).

Results: 144 consecutive patients with liver cirrhosis (78 males, 66 females, mean age 51.84 ± 11.24 years) admitted in our unit were prospectively evaluated. The Child Pugh class was: A in 35.41%, B in 31.25% and C in 33.33% patients, and the etiology: viral in 62.5%, alcoholic in 22.22% and mixed (viral and alcoholic) in 15.27%. Overall, the prevalence of malnutrition was 52.08%: 39.58% of patients being moderately malnourished (SGA B) and 12.5% severely malnourished (SGA C). Child Pugh class significantly correlated with the presence of malnutrition (p < 0.001), while the association between the presence of malnutrition and alcohol consumption, etiology of cirrhosis, sex, age, duration of the disease was not statistically significant (p = NS).

Discussion/Conclusion: In patients with cirrhosis malnutrition is frequently encountered. Due to its known negative impact on patient’s survival, the early recognition and correction of malnutrition are essential.
The long-term efficiency of combined treatment with propranolol and endoscopic variceal ligation in the management of variceal bleeding

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Introduction: Our aim was to evaluate the efficiency of combined pharmacological (propranolol) and endoscopic (endoscopic variceal ligation, EVL) treatment in cirrhotic patients after the first bleeding episode.

Methods: All patients admitted during 24 months with variceal bleeding and treated by EVL until variceal eradication were evaluated. Only patients that received efficient propranolol dose and were followed for at least 36 months after variceal eradication were included.

Results: 128 patients (mean age 50.12 ± 11.42 years) with cirrhosis (Child class A in 42, B in 76 and C in 10 patients) were studied. To achieve variceal eradication a median of 3 (range1-9) banding sessions were required. Kaplan Meier estimates of the variceal recurrence were at one year 18.7% and at two years 37.4%. The mean time for variceal recurrence was 23.03 months (95% CI: 19.3-26.7). At the end of the follow up period, a total of 53.1% had recurrent varices. The only parameter associated with an increased risk for variceal recurrence was Child class B or C versus class A (p = 0.04). Kaplan Meier estimate of the cumulative proportion of patients that had bled by one year was 11.4%. The median time to rebleeding was 37.8 months (95% CI: 32.2-43.4). Overall, the mortality rate in the study group was 19.6%.

Discussion/Conclusion: The combined treatment with propranolol and EVL is associated with a low incidence of rebleeding in cirrhotic patients, although the variceal recurrence is not a rare event. An advanced Child class (B, C) increases the risk for variceal recurrence and in this patients a more intensive follow up should be recommended.
DNA damage caused by chronic infection and inflammation

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**Introduction:** Hepatitis B and C viruses cause chronic infection and inflammatory disease. It was investigated whether there is a difference in peripheral DNA damage in patients with chronic HCV compared with patients with chronic HBV. And show an association in the level of peripheral DNA damage with a natural history of HBV infection.

**Methods:** The DNA damage in lymphocytes research in eighteen patients with chronic hepatitis B, fifteen patients with chronic hepatitis C, 21 patients with cirrhosis secondary to hepatitis B, 9 inactive hepatitis B s antigen (HBsAg) carriers.

**Results:** The chronic hepatitis C group had similar levels of DNA damage with patients with cirrhosis due to hepatitis B and non-cirrhotic patients with chronic hepatitis B, they had higher levels of DNA damage compared with inactive HBsAg carriers. Hepatitis B cirrhotic patients and patients with chronic hepatitis B had significantly higher levels of DNA damage than inactive HBsAg carriers. Chronic hepatitis C and HBV-related cirrhosis were discriminators in determining DNA damage in lymphocytes.

**Discussion/Conclusion:** Chronic hepatitis C, based on the severity of liver disease, or cirrhosis as an advanced form of HBV infection increase DNA damage in lymphocytes.
Efficiency and prognostic markers of the ursodeoxycholic acid therapy for primary biliary cirrhosis

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Introduction: The aim of this study was to reveal the efficiency and prognostic markers of ursodeoxycholic acid (UDCA) therapy on the natural course of primary biliary cirrhosis (PBC).

Methods: We observed 22 PBC patients, (M/F 5/17, aged 25-42 years). Histologically the patients were divided behind stages: I (5 pts), II (7 pts), III (6 pts) and IV (4 pts). The serum levels of antimitochondrial (AMA), antinuclear (ANA) antibodies, tumor necrosis factor alpha (TNFα), interferon-gamma (IFN-γ) were determined by immunoassay method. Medical treatment includes UDCA therapy in a dose of 13-15 mg/kg/day during 2 years.

Results: All patients had cholestatic clinical, biochemical and histological features of PBC, high titers of AMA at treatment start. Biochemical and histological response to treatment was observed in eight (36.4%) patients with high serum levels of alkaline phosphatase, gamma-glutamyl transpeptidase and total cholesterol: all patients with stage I and 3 (42.9%) with stage II. Among the 14 (63.6%) non-responders were all patients with stages III and IV and 4 (57.1%) patients with stage II; for them have revealed fibrosis progression. In this patients were considerably increased of blood IgG, TNFα, IFN-γ levels and high titers of ANA and expressed inflammation with piecemeal necrosis in liver tissue at treatment start.

Discussion/Conclusion: Response to UDCA therapy after 2 year is associated with early diagnosis of PBC and biochemical tests of cholestasis; non-response – with fibrotic and cirrhotic stages, expressed inflammation with piecemeal necrosis in liver tissue and high titers of ANA, high blood IgG, TNFα, IFN-γ levels.
Ursodeoxycholic acid therapy and lymphocytes apoptosis in drug-induced hepatitis patients with intrahepatic cholestasis

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Introduction: The aim of this study was to delineate the effects of ursodeoxycholic acid (UDCA) therapy on lymphocytes apoptosis in DIH patients with intrahepatic cholestasis.

Methods: 32 DIH patients with intrahepatic cholestasis (M/W 21/11, aged 44-59 years) treated with 13-15 mg/kg/day of UDCA for three months. The control included 10 healthy volunteers. Fas/APO-1(CD95), CD3, CD4, CD8, CD56, CD19 and CD150 expression on peripheral blood lymphocytes were measured by immunofluorescent method.

Results: Before UDCA therapy the quantity of lymphocytes in apoptosis was increased in 25 (78.1%) DIH patients over 3.5 times (p < 0.05) vs. control to (6.89 ± 0.53)%. Fas/APO-1(CD95) expression on lymphocytes correlated with serum levels of alkaline phosphatase (AP), gamma-glutamyl transpeptidase (GGTP) and total cholesterol (r = +0.52; +0.62 and +0.56; p < 0.05). One month after the start of UDCA therapy the quantity of lymphocytes in apoptosis was increased in 15 (46.9%) DIH patients over 1.9 times (p < 0.05) vs. control to (3.74 ± 0.30)%. Three month after the start of UDCA therapy the quantity of lymphocytes in apoptosis was increased in 5 (15.6%) DIH patients over 1.5 times (p < 0.05) vs. control to (2.85 ± 0.25). The correlation between increased Fas/APO-1 (CD95) expression on lymphocytes and decreased quantity of CD3+, CD4+ and CD56+ lymphocytes in blood became smaller (r = -0.31, -0.57 and -0.44; p < 0.05 – before UDCA therapy; r = -0.15, -0.26; and -0.18; p > 0.05 – after UDCA therapy).

Discussion/Conclusion: UDCA therapy of DIH patients with intrahepatic cholestasis promotes decrease of peripheral lymphocytes apoptosis.
Expression level of VEGF and its association with tumor-infiltrating dendritic cells in primary hepatocellular carcinoma

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Introduction: There is considerable experimental evidence that the growth of the solid tumors beyond 2- to 3-mm³ depends on tumor angiogenesis. VEGF family members are recognized as key factors required for angiogenesis of tumors. The aim of this study was to assess the expression levels of VEGF in hepatocellular carcinoma (HCC) and in the adjacent “normal” liver tissue and to compare them with the density of some tumor-infiltrating dendritic cells’ (DC) subpopulations.

Methods: The expression of VEGF and the density of S-100⁺, HLA-DR⁺, CD83⁺ DCs were studied immunohistochemically in a small subset of 10 HCC biopsies obtained from 6 male and 4 female patients aged between 25 and 82 years.

Results: The expression level of VEGF in tumor cells was significantly stronger than in the adjacent “normal” liver (p < 0.004). Interestingly, an opposite tendency was found for the density of S-100⁺ and HLA-DR⁺ DC in tumors with different levels of VEGF expression: the mean numbers of S-100⁺ and HLA-DR⁺ DCs per mm² in tumors with lower VEGF expression (12.49 ± 17.5 and 12.49 ± 10.5) were higher, although not significantly, than those in tumors with very strong expression of VEGF (7.03 ± 9.7 and 8.54 ± 5.5, respectively, p < 0.05). No such difference was seen in the density of the mature DC (CD83⁺) between the tumors with lower (3.5 ± 3.7) and higher (3.9 ± 4.8) VEGF expression.

Discussion/Conclusion: In conclusion, we suggest that the increased expression of VEGF might be implicated in the HCC developing and progression not only by stimulating the tumor-associated angiogenesis, but also by suppression of tumor-infiltrating dendritic cells.
Cholestasis syndrome of CVH patients

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Introduction: Chronic virus hepatitises (CVH) are the main reason of development of hepatocirrhosis and hepatocellular carcinoma. It is well known that the presence of cholestatic syndrome is a factor that worsens the prognosis for a disease and complicates the conduction of antiviral therapy.

Methods: We have examined 183 CVH patients and have proved the presence of cholestatic syndrome of 36.6% of patients. The study of motor-kinetic function of bile-excreting tracts has detected that 89.6% of patients have functional disorders of biliary tract and 69.9% of patients have functional disorders of biliary tract with prevalence of Oddi's sphincter dysfunction. It is possible that functional disorders of Oddi's sphincter assist in aggravation or appearance of intrahepatic cholestasis of CVH due to the development of bile hypertension that is confirmed by the increase of level of AP and γ-GT in blood serum.

The ursodeoxycholic acid (UDCA; Ursofalk®) in dose 10 mg per 1 kg of weight has been used in the therapy of 18 CVH patients during 2 months. Among these patients the chronic hepatitis B has been verified in the group of 7 patients and hepatitis C has been verified in the group of 11 patients. Activity level ALT of blood serum has exceeded normal values of 10 patients (0.95 ± 0.52 mkm/l). The number of bilirubin has exceeded normal values of 12 patients (41.36 ± 23.45 mkm/l), increase of level of AP has been detected of 18 patients (425.61 ± 199.5 u/l), increase of level of γ-GT has been detected of 15 patients (6.71 ± 4.41 mm/l).

Before and after ursodeoxycholic acid therapy the intensity of biliary pains has been estimated according to three-marks system where 3 marks – pain is everyday and intensive, 2 marks – pain is not everyday and it is low intensive, 1 mark – pain is rarely appearing, 0 marks – lack of pain syndromes. Besides, all patients’ liver function tests have been done and indices qualities of life have been estimated before and after therapy.

Results: After cure of UDCA the level of biliary pains of CVH patients has decreased from 1.45 ± 0.18 to 0.36 ± 0.8 marks (average value of changes is 1.0 ± 0.19, paired Student’s test p = 0.0001).

Ursodeoxycholic acid therapy has not influenced the indices of activity of the process (p > 0.05). However, despite the fact that significant differences in bilirubin concentration in blood serum have not been detected during UDCA therapy, the level of activity of AP after therapy has decreased to 134.13 ± 30.81 u/l (average value of changes is 51.54 ± 15.54, paired Student’s test p = 0.036), γ-GT has decreased to 3.21 ± 6.78 mm/l (average value of changes is 1.98 ± 25.54, paired Student’s test p = 0.024).

After ursodeoxycholic acid therapy the patients have shown the improvement of the indices of quality of life on all 8 scales SF – 36, which reflect not only physical but also psychological health with significant increase of indices in “pain” scale from 51.0 ± 26.7 marks to 88.2 ± 11.2 marks (average value of changes is 37.1 ± 24.9, paired Student’s test p < 0.0001). Similar changes also concern the scales that reflect psychological (social) health. Significant increase of indices of quality of life...
has been marked in the scales of vitality from 44.6 ± 23.7 to 57.6 ± 11.2 marks (average value of changes is 13.0 ± 18.2, paired Student’s test p = 0.03), in the “social functioning” scale – from 56.0 ± 22.3 to 75.9 ± 14.6 marks (average value of changes is 19.9 ± 21.0, paired Student’s test p = 0.007). We have not been observing any undesirable actions of UDCA.

**Conclusion**: Thus, according to the findings UDCA is a safe and effective preparation for the treatment of cholestatic syndrome of CVH patients. UDCA therapy leads to decrease of pain syndrome, decrease of intensity of cholestatic syndrome and assists in increase of the indices of patients’ quality of life.
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What is an optimum dosage regimen of the substrate for the $^{13}$C-methacetin breath test?

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**Introduction:** $^{13}$C-methacetin breath test proved to be a valuable diagnostic tool in hepatology. We decided to compare two currently used dosage regimens of $^{13}$C-methacetin: fixed vs body mass-adjusted dose.

**Methods:** Two groups of healthy volunteers of different body mass were created: (i) $< 55$ kg (8 women) and (ii) $> 95$ kg (8 men). Every subject underwent two breath tests on separate days, taking 200 ml unsweetened black tea containing $^{13}$C-methacetin (Euriso-Top S.A., Saint-Aubin, France) either at a fixed 75 mg dose, or at a 1 mg/kg body mass-adjusted dose. Samples of expiratory air for $^{13}$CO$_2$ measurement were collected 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 40, 50, 60, 75, 90, 105, 120, 150 and 180 min after intake of the substrate. The order of the dosage regimens was randomized.

**Results:** Compared to the standard 75 mg dose, the body mass-adjusted dose was lower by 24.06 mg (range 20.30-31.00) in “light” women, and higher by 27.98 mg (range 20.60-37.00) in “heavy” men. The time to peak $^{12}$C recovery in breath amounted to 14.6 ± 1.0 min after the reduced dose and to 21.5 ± 3.2 min following the augmented $^{13}$C-methacetin dose ($p = 0.078$). Compared to the fixed $^{13}$C-methacetin dose, the maximum momentary $^{13}$C recovery after the body mass-adjusted dose increased in men (38.5 ± 2.9 vs. 32.3 ± 2.5 %dose/h) and also (paradoxically) in women (41.9 ± 2.9 vs. 36.6 ± 3.6 %dose/h). For the 3-h cumulative $^{13}$C recovery the difference between “light” and “heavy” subjects was greater with the fixed 75 mg dose (43.57 ± 3.09 vs. 39.51 ± 1.97 %dose) than with the body mass-adjusted dose (41.85 ± 3.08 vs. 43.27 ± 1.36 %dose).

**Conclusion:** The dosage regimen of the substrate may affect some results of the $^{13}$C-methacetin breath test, particularly the time to peak and the maximum momentary $^{13}$C recovery. The body mass-adjusted dosage is helpful for “normalization” of results from subjects of greatly differing body frame.
Overexpression of estrogen receptor α inhibits the negative feedback regulation of hepatic cholesterol biosynthesis and increases newly synthesized cholesterol, leading to biliary cholesterol hypersecretion

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Introduction: Biliary cholesterol hypersecretion is the primary cause of cholesterol gallstones in humans. Accumulated evidence showed that estrogen (E₂) stimulates HMG-CoA reductase, even under conditions of high cholesterol feeding, suggesting an increased delivery of cholesterol to bile from de novo synthesis. Our aims were to explore whether estrogen receptor α (ERα) stimulated by E₂ interferes with the negative feedback regulation of cholesterol biosynthesis by stimulating SREBP-2 that activates the cholesterol synthetic pathway, and to quantitate the contribution of newly synthesized cholesterol to biliary cholesterol.

Methods: At weaning, female AKR mice were ovariectomized. At 8 weeks old, the mice were treated with E₂ at 0 or 6 µg/day for 14 days with or without a combined treatment of the antiestrogenic ICI 182,780 at 125 µg/day. At 14 days of feeding chow or 1% cholesterol, expression levels of genes for SREBP-2, HMG-CoA synthase, HMG-CoA reductase, farnesyl diphosphate synthase, and squalene synthase were quantified by real-time PCR. To monitor changes in cholesterol biosynthesis and the newly synthesized cholesterol secreted into bile, the mice were treated with 2.5 mCi of [³H]water, and the incorporation into digitonin-precipitable sterols was measured in liver extracts 1 hour later and in hepatic biles every hour for 8 hours, respectively.

Results: Compared to the E₂-deficient mice, E₂-treated mice display increased expression levels of mRNAs for SREBP-2 and four major genes of cholesterol biosynthesis pathway, whatever chow or 1% cholesterol is fed. E₂ induced a massive increase in hepatic sterol synthesis (8-fold), which is associated with a significant increase in biliary secretion rates of total (28 ± 5 µmol/h/kg) and newly synthesized cholesterol (36 ± 9%). However, the E₂ effects on increasing cholesterol biosynthesis are abolished by ICI 182,780. The marked reduction in cholesterol synthesis correlates with the significant decrease in the amount of mRNAs for SREBP-2 and multiple genes of cholesterol biosynthesis and in biliary secretion rates of total (9 ± 3 µmol/h/kg) and newly synthesized cholesterol (14 ± 5%) in the mice treated E₂ plus ICI 182,780.

Discussion/Conclusion: ERα stimulates SREBP-2 that activates SREBP-2-responsive genes in the cholesterol biosynthetic pathway. During E₂ treatment, mice continue to synthesize cholesterol in spite of its excess availability from the lithogenic diet, which reflects a loss in controlling the negative feedback regulation of cholesterol biosynthesis so that mice fail to down-regulate hepatic cholesterol biosynthesis and more newly-synthesized cholesterol could be secreted into bile leading to biliary cholesterol hypersecretion.
TGF-β induces profibrogenic factor CTGF expression in hepatocytes

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Introduction: Connective Tissue Growth Factor (CTGF) plays an important role in TGF-β induced liver fibrogenesis. Hepatic stellate cells and bile duct epithelial cells were recognized as its cellular source in the liver. Here, we demonstrate CTGF expression in hepatocytes and identify a molecular mechanism responsible.

Methods: Primary cultured hepatocytes were stimulated with TGF-β. Real-Time PCR and Western blot analyses were used to detect CTGF mRNA and protein expression in hepatocytes. Immunohistochemistry was used to measure CTGF expression in liver tissue from patients with chronic hepatitis B infection or Schistosoma japonicum infection, conditional tetracycline-regulated TGF-β expressing transgenic mice, Smad7 expressing transgenic mice and Smad7 knock out mice. CCl₄ treatment was used to produce a fibrosis animal model in wild type, Smad7 expressing and Smad7 knock out mice.

Results: Immunostaining displayed strong CTGF expression in hepatocytes, bile duct epithelial and hepatic stellate cells in liver tissue from patients with chronic hepatitis B infection or Schistosoma japonicum infection. TGF-β induced CTGF mRNA and protein expression in primary cultured hepatocytes. CTGF expression is correlated with serum TGF-β levels in TGF-β transgenic mice. Adenovirus infected Smad7 partly inhibits TGF-β-dependent CTGF expression in hepatocytes. CCl₄ treatment induced significant CTGF expression in wild type mice. Furthermore, CTGF expression is downregulated by hepatocyte specific Smad7 expression in transgenic mice and increased in Smad7 knock out mice.

Discussion/Conclusion: TGF-β induces profibrogenic mediator, CTGF, expression in hepatocytes in vitro and in vivo. Smad7, an antagonist of TGF-β/Smad signaling pathway, inhibits TGF-β-dependent CTGF expression in hepatocytes.
Reye-like syndrome in one-year old girl – Case report

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One-year old girl, without gestational, labour and familial load, with normal psychomotor development thus far, was admitted to Gastroenterology Ward of Department of Pediatrics Silesian Medical University in Katowice from Intensive Care Unit with suspicion of the metabolic defect.
From history: after dietary indiscretion (rich in fat meal) and long time without feeding, weakness, increasing hypersomnia and the next coma were occured.
In the laboratory tests we found: very increased levels of aminotransferase (AIAT-1560.0 U/l, AspAT-6400.0 U/l), lactate dehydrogenase (6430.0 U/l) and creatine kinase (505000.0 U/l), hyperlactacidaemia and small hyperammonemia with normal glycaemia (on glucose intravenous infusion).
In ultrasonography of abdomen in abnormalities we found only enlarged liver.
Examinations in the direction to metabolic defects: tandem MS, aminogram in the blood serum, orotic acid in the urine were normal. Profil of organic acids in the urine using GC-MS method – only low acidosis.
We found in the child decreased level of free and total carnitine in the blood serum (free carnitine – 23.6 µmol/l, total – 38.8 µmol/l) and increased excretion of total carnitine with the urine (free – 137.0 µmol/g creatinine, total – 983.0 µmol/g creatinine).
Beta oxidation of fatty acids- oxydation of palmitate in the full blood – 0.53 nmol/h/10⁹ leucocytes – 78% of control. In the result of the treatment : continued intravenous infusion of glucose, rich in carbohydrate, fat free and low-protein diet, we observed rapid normalization of clinical state and biochemical parameters. The patient intakes supplementation of carnitine. Now the girl is in good condition, without any abnormalities in physical and neurological examination, laboratoratory tests are normal too. Medical history and results of diagnostic tests indicate to Reye-like syndrome. The patient requires the continuation of tests to establishing of diagnosis of metabolic disturbances.
Quality of life of the girl with cystic fibrosis in 5 years after liver transplantation

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Introduction: Quality of life in the aspect of health state is considered as the clinical condition (kind of disease, course of treatment, prognosis), psychical condition (personality, self-perception, dealing with disease, life satisfaction) and the ability to live in a group and social support from the family.

The aim of the study is the evaluation of quality of life in the aspect of health of the girl with CF in 5 years after liver transplantation.

Methods: We presented a case of 16-year-old girl (at present) with diagnosed in 3/12 CF manifesting itself as the syndrome of salt loss, in whom in 9/12 we observed liver steatosis with hypocarnitinemia (free carnitine = 9.9 µmol/l, total carnitine = 18.7 µmol/l). This girl underwent numerous hospitalizations which were results of developing cirrhosis and portal hypertension. At the age of 11 years she underwent a successful liver transplantation. A subjective evaluation of quality of life was based on the questionnaire of interview included in the study “Quality of life with IBD” in own modification.

Results: The present clinical state of the patient is good in spite of the features of an initial stage of bronchiectasis - the other systems, including the function of transplanted liver, are normal.
The subjective assessment of life quality is high (14 points in the questionnaire). The girl is satisfied with her life despite several restrictions resulting from the disease; she feels fully accepted by her family and peers and perceives the treatment as efficient.

Discussion/Conclusion: The presented case of 16-year-old girl evaluating her quality of life as good, despite progressing disease, may be an example for “groups of support” for people with chronic diseases.
CTLA-4 gene polymorphism and the outcome of autoimmune hepatitis treatment in pediatric population

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Introduction: A – G transition in exon 1 (+49) of CTLA-4 gene may play a role in T-cells activation and may influence the treatment outcome in children with autoimmune hepatitis (AIH).

The aim of the study was to evaluate the outcome of AIH therapy in children with different CTLA-4 genotypes.

Methods: We analyzed 54 case records of children (M-13, F-41) with AIH diagnosed at the age of 2-16 (mean ± SD 10.7 ± 3.3) years and observed for 2-11 (5.4 ± 2.5) years after diagnosis. All children were treated with prednisone and azathioprine for the first two years after the diagnosis had been established. The subsequent therapy was modified according to the efficacy and tolerability. The treatment outcome was based on the presence of clinical, laboratory, ultrasound and histopathological features of liver disease. The following categories of outcome were defined: full remission, partial remission or continuous disease. CTLA-4 polymorphism was analyzed by restriction analysis.

Results: CTLA-4 genotype AA was found in 19 (35%), AG in 26 (48%) and GG in 9 (17%) children. No differences in the liver function tests among different CTLA-4 genotypes were observed at presentation of the disease and at two years of treatment. At the end of observation period children with genotype AG presented with the higher than children with genotypes AA or GG ALT activity (respectively 113 ± 156 vs. 64 ± 54 and 51 ± 23, p = 0.04) and higher histopathological grading score (3.0 ± 0.8 vs. 0.3 ± 0.6 and 0.7 ± 0.6, p = 0.02). Full AIH remission was observed in 12 (22%) and continuous disease was observed in 11 (20%) of children. Lack of therapeutic effect was observed more often in children with genotype AG than in those with genotypes AA or GG (respectively 30.8%, 5.3% and 22.2%) but these differences were not statistically significant. 3 children required conversion to cyclosporine and 2 children had liver transplantation. All these children had genotype AG.

Discussion/Conclusion: Poor therapeutic effect was observed more often in children with CTLA-4 genotype AG.
Biochemical characteristic of liver damage in patients with chronic HCV infection and liver cirrhosis

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Liver macrophages were shown to secrete a complex of cytokines and lysosomal enzymes which can change resistance of liver cells to infections and other pathogenic factors. Among serum markers of liver fibrosis matrix metalloproteases-1,7,9 and their endogenous tissue inhibitors- TIMP-1 and TIMP-2 were introduced recently (Leroy et al.).

The aim: to investigate the correlation of lysosomal enzymes activity in serum of patients with HCV infection and liver cirrhosis from inflammatory process and stage of disease. Among lysosomal enzymes β-galactosidase (originated mainly from hepatocytes), chitotriosidase (marker of macrophages) and matrix metalloproteases-2, 7 (serum marker of collagen formation in liver) were chosen.

60 patients (37 men and 23 women) with chronic HCV infection, aged 41.4 ± 13.5 years, 9 patients of liver cirrhosis as well as 15 practically healthy blood-donors of the appropriative age were under investigation. In all cases the diagnosis of HCV infection was confirmed by ELISA and PCR analysis and data of liver biopsy. Fluorescent methods were used for determination of activity of β-galactosidase (Barrett, 1981), chitotriosidase (Guo et al., 1995) and matrix metalloproteases-2, 7 (Nagase et al., 1994). Statistical analysis was performed with help of programs SPSS 9.0; 10.0.

Comparatively to healthy blood-donors very low β-galactosidase activity in serum of patients with HCV infection was revealed (not dependent from serum ALT activity). In the same group of patients serum chitotriosidase activity was increased as a result of liver macrophage stimulation. In patients with liver cirrhosis increased matrix metalloproteases-2, 7 was registered; data were statistically significant as compare to chronic HCV infection without symptoms of cirrhosis development (p = 0.01; CI 386.1-62.4).

We can conclude that assay of matrix metalloproteases-2, 7 is useful for early diagnostic of collagen formation in liver and cirrhosis revealing in patients with HCV infection.
Serum ALT is not a reliable screening tool for NAFLD in the general population

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Introduction: ALT is considered the most specific enzyme for hepatocellular injury and therefore serves as a marker of liver status in population studies. One of the factors affecting serum ALT levels is the significant fraction of subjects with increased ALT as the only manifestation of undiagnosed NAFLD. The aim of the study was to evaluate ALT validity as compared to ultrasound (US) in the detection of primary NAFLD and to redefine the optimal upper limit normal for that purpose.

Methods: A cross-sectional study, involving 352 subjects, a sub sample of the first Israeli national health and nutrition examination survey. Exclusion criteria: any known aetiology for liver disease or elevated ALT suspected to be secondary: excessive alcohol consumption, HBsAg and anti-HCV antibodies, hepatotoxic drugs, inflammatory bowel disease, myopathic diseases, surgical interventions able to induce fatty liver and celiac disease. Each participant underwent an abdominal US and biochemical (serum transaminases, lipid profile, fasting serum glucose and insulin) and anthropometric evaluation.

Results: After exclusion, 326 subjects (53.4% male, mean age 50.5 ± 10.3 SD, mean BMI 27 ± 4.4 SD) were included in the analysis. The prevalence of primary NAFLD diagnosed by US was 30% (95% CI 25-35%). The prevalence of elevated serum ALT (above 39 U/l), after applying the exclusion criteria was only 3.7% (12/ 326). ALT sensitivity for the diagnosis of primary NAFLD, as compared to diagnosis by US in the current upper limit (above 39 U/l), was 8.2% (9/98) with specificity of 98%. According to the Receiver-operating characteristic (ROC) curve (Fig.1) the best discriminating point on the curve is 19.5 U/l as the upper limit normal of ALT, with sensitivity and specificity of 72% and 60% respectively.

Conclusions: The use of ALT in epidemiological studies as a marker for NAFLD should be discouraged since it leads to major underestimation.
Study on mode for pattern classification in posthepatitic cirrhosis

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Introduction: To investigate mathematical mode for pattern classification of Traditional Chinese Medicine in posthepatitic cirrhosis

Methods: Formula for pattern differentiation was established, through subtracting featured symptoms and symptoms by analyzing the data obtained from 900 patients with posthepatitic cirrhosis with factors analyzing, two-step clustering, C Mean data clustering and fuzzy comprehensive evaluation.

Results: The basic pathomechanism of posthepatitic cirrhosis is Qi deficiency blood stasis, and 5 patterns being of clinical implication are liver and kidney yin deficiency, damp-heat smoldering, stasis and heat smoldering, liver depression and spleen deficiency, and spleen and kidney qi deficiency.

Discussion/Conclusion: Symptoms and signs of the posthepatitic cirrhosis are classified into two categories, that are common characterized information of the disease and featured information for pattern classification; Formula for pattern differentiation provides quantization standard for judging of pattern of posthepatitic cirrhosis.
Tissue localisation of hepatic bilirubin and phospholipid export pumps, MRP2 and MDR3, in liver biopsies from patients with drug-induced liver injury (DILI)

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Introduction: DILI is an important cause of cholestasis and hepatocellular injury. Whether cholestasis and jaundice in DILI is associated with alterations of MRP2 and MDR3 expression is unknown.

Methods: Immunohistochemistry for MRP2 and MDR3 was performed on liver biopsies obtained from 16 patients with DILI. The diagnosis was based on CIOMS criteria and the exclusion of any disease-related causes of liver injury. DILI was classified according to ALT/AP ratio.

Results: According to ALT/AP ratio, four patients had a cholestatic, three a mixed and nine a hepatocellular type of DILI, however, cholestasis and jaundice were present in all patients. Four patients had DILI due to beta-lactam or macrolide antibiotic treatment. In all of these patients, MRP2 and MDR3 staining was markedly reduced in pericentral zones irrespective of the type of liver injury. Of note, MRP2 and MDR3 staining was not altered in cholestatic DILI caused by other drugs. Hepatocellular DILI commonly displayed marked architectural alterations (confluent necroses, inflammatory infiltrate) while MRP2 and MDR3 staining was preserved in 8 of 9 patients. Only in one patient, no MRP2 and MDR3 signal was detected.

Discussion/Conclusion: Reduced MRP2 and MDR3 expression in pericentral areas may contribute to cholestasis in patients with DILI due to beta-lactam and macrolide antibiotics but not in other forms of DILI. Restoring reduced MRP2 and MDR3 expression in antibiotic-induced cholestasis may represent an attractive target to overcome acute cholestatic injury and subsequent progressive bile duct destruction observed in these patients.
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