Disease Progression and Disease Prevention in Hepatology and Gastroenterology

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Abstracts of Invited Lectures
Poster Abstracts

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DISEASE PROGRESSION
AND DISEASE PREVENTION
IN HEPATOLOGY AND
GASTROENTEROLOGY

Berlin (Germany)
October 3 - 4, 2005

Scientific Organization:
P.R. Galle, Mainz (Germany)
G. Gerken, Essen (Germany)
W.E. Schmidt, Bochum (Germany)
B. Wiedenmann, Berlin (Germany)
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121. LAPTMT4B, a hepatocellular carcinoma-associated novel proto-oncogene
Cholelithiasis and biliary cancer
Molecular mechanisms controlling bile metabolism in health and disease

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Human bile is an aqueous solution of four distinct lipids. In decreasing quantitative order these are bile salts, a group of structurally similar detergent-like and signaling molecules, a phospholipid mixture mostly phosphatidylcholines, unesterified cholesterol with traces of phytosterols, chonchoestersols and cholesterol precursors and bilirubin conjugates principally di- and monoglucuronates. All lipid components of bile are heteroaggregated via hydrophobic interactions into simple plus mixed micelles and unilamellar vesicles. The detergency of bile salts is responsible for solubilizing and dispersing phospholipids and sterols in bile and solubilizing fatty acid/monoacylglycerol products of triglyceride hydrolysis, lipovitamins and sterols in the upper small intestine. Bile salts also act as high affinity binders for bilirubin conjugates, thereby affixing the lipopigments to the exterior of biliary aggregates and abating their osmotic activity in bile.

Based principally on rodent studies, molecular mechanisms for the major pathways of hepatic bile salt, phosphatidylcholine, cholesterol and bilirubin uptake, metabolism and secretion are fully worked out. On the basolateral membrane, chylomicron remnants (primarily ApoB/E receptor) and HDL (SRBI receptor) are the primary sources of cholesterol for bile, whereas LDL (ApoB/E receptor) provides cholesterol for de novo bile salt synthesis. Most biliary phospholipids are de novo synthesized by the diacylglycerol pathway with a smaller quantity synthesized via trimethylation of phosphatidylethanolamine on the canalicular membrane. Bile salts returning to the liver are taken up principally by an Na⁺ coupled SLC (solute carrier) 10A1 (NTCP); at least four other SLCs exchanger (OATPs) have been identified including one for unconjugated bilirubin and perhaps two for bile salts. SLC22A1 is an organic cation especially choline transporter contributing to phosphatidylcholine synthesis via the diacylglyceride pathway.

The exit transporters on the canalicular membrane are rate limiting in bile formation. All transporters are of the ABC (ATP Binding Cassette) family and two molecules of ATP are hydrolyzed during their functioning in primary active transport. Individual transporters exist for monovalent bile salts (ABCB11, BSEP), phosphatidylcholine (ABCB4), divalent bile salts plus bilirubin conjugates (ABCC2), and sterols (ABCG5/G8). At least one other transporter is responsible for cholesterol secretion in the mouse, but it has not been identified. In the distal ileum, bile salts are recovered from the small intestine by an efficient membrane and transcellular transport system that maintains the enterohepatic circulation of bile salts. The three components of ileocytes include an electrogenic Na⁺ coupled transporter, SLC10A2 (ASBT) on the apical membrane, an IBABP-FABP6 binder for bile salts within the cell, and OSTα/β on the basolateral membrane which exchanges bile salt molecules with bicarbonate ions. Bile salts are delivered to the liver in the portal vein bound to albumen and HDL. In the liver, bile salts activate FXRα, a nuclear transcription factor which in association with its obligate heterodimer partner RXR, exhibits a variety of pleiotropic effects. FXRα activation is responsible for increased conjugation and secretion of bile salts from hepatocytes and decreased basolateral uptake via SLC10A1 and especially

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biosynthesis of bile salts via downregulation of CYP7A1 (rate controlling in bile salt synthesis) and CYP8B1 (leading to cholic acid). In the small intestine, FXR activation leads to increased IBABP-FABPB expression and decreased ASBT (SLC10A2) expression, facilitating loss of bile salts from the enterohepatic circulation. Similarly, activation of CAR (by bilirubin), LXRα (by oxysterols) and PXR (by xenobiotics) all upregulate phase II metabolism of their ligands as well as their canalicular secretion into bile.

In the western world the most common disease involving bile is cholesterol gallstone formation. Most afflicted individuals exhibit hypersecretion of hepatic cholesterol and less commonly hyposecretion of bile salts plus phosphatidylcholines. Irrespective of the hepatic secretory cause, the end result is supersaturation of gallbladder bile with cholesterol. Pathogenesis of choledolithiasis has genetic, environmental, dietary, and microbial components. Studies in the inbred mouse have revealed 23 Lith loci, but to date, none of the Lith genes have been unequivocally identified. Candidate genes include all of the canalicular lipid transporters as well as FXRα and megalin (a member of the LDL receptor family).

Rarely, human LITH genes can cause monogenic, i.e., Mendelian, cholesterol gallstone disease. Syndromes defined to date include defects in ABCB4 which cause "low phospholipid-associated choledolithiasis" or LPAC, and defective CYP7A1, which causes cholesterol gallstones and hypercholesterolemia resistant to HMG-CoA reductase inhibitors. Two other monogenic causes of cholesterol gallstones involve genes that are highly expressed in the gallbladder. Megalin, a candidate gene for Lith1 in the mouse, is LDL related protein 2 (LDLRP2) that mediates endocytic uptake of a variety substrates including ApoA1 bound to phospholipid and cholesterol. It may be expressed also on large cholangiocytes and be responsible for decreasing supersaturation of hepatic as well as gallbladder bile. The second defect involves the CCK1 receptor responsible for motility of the gallbladder and also small intestine. A number of case reports have shown that CCK1 receptor dysfunction is associated with cholesterol choledolithiasis from gallbladder paralysis and hypomotility of the small intestine. The former facilitates mucin accumulation and nucleation whereas the latter engenders increased cholesterol absorption and augmented deoxycholate formation and absorption from the intestine. However, in the vast majority of cholesterol gallstone subjects the disease is polygenic, but none of the responsible genes has been identified to date.

The most exciting recent discovery is that for cholesterol gallstones prevalence rates to occur with high frequency in the mouse model, there needs to be distal small intestinal and cecal infection with one or more choledolithogenic enterohepatic Helicobacter spp. These non-H. pylori helicobacters are apparently crucial for nucleating supersaturated bile in mice, most likely via polymeric IgA production since this immunoglobulin is an extremely potent inducer of cholesterol crystal phase separation from liquid crystals in supersaturated bile. Since humans are known to be "asymptomatically" infected with a wide variety of these organisms, it will be salutary to determine their choleliothogenic potential using inbred mouse models.
Etiology and pathogenesis of primary sclerosing cholangitis

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Background: Despite recent advances, the etiology and pathogenesis of primary sclerosing cholangitis (PSC) remain enigmatic. Chronic intra- and extra-hepatic peri-biliary inflammation and fibrosis leading to cholestasis and cirrhosis are its pathological hallmarks. The median survival time after diagnosis is seven to nine years. Recurrent bouts of bacterial cholangitis are common due to stricture formation and the risk of cholangiocarcinomas may be as high as 1% per year.

Etiology: An array of etiologies, incorporating different combinations of environmental, infectious, immunologic, and genetic risk factors, has been proposed for the development of PSC. However, no factor has clearly been identified as an etiologic agent in PSC. Given the increased prevalence of PSC-associated autoantibodies among unaffected family members, the development of PSC may actually be a multi-"hit" process. Perhaps, for this reason, no animal model recapitulates all of the features of PSC. The increased prevalence of PSC among family members of affected individuals and its association with certain HLA haplotypes indicate that in-depth analysis to determine if genetic factors affect susceptibility to PSC and its progression is warranted. With the exception of a family history of PSC or a personal history of ulcerative colitis, the individual odds ratios associated with other risk factors indicate a weak relationship with development of PSC.

Pathogenesis: Immune dysfunction is apparent in PSC and likely contributes to its pathogenesis, but the precise nature of the dysfunction is uncertain. The presence of increased serum titers of autoantibodies (e.g. p-ANCA) in PSC has long suggested an autoimmune pathological mechanism. Whether the cytotoxic, activated T cells surrounding the bile ducts are responding to a self-peptide is unknown. In PSC, we have observed increased cholangiocyte phagocytosis of apoptotic cells, which may be sources of a variety of immunogenic self-peptides. However, immunosuppressive agents are not effective in its treatment, except in those with overlapping autoimmune hepatitis. Progress in elucidating the pathogenesis of ulcerative colitis, which often accompanies PSC, suggests aberrant mucosal immunity plays a significant role in its pathogenesis. A similar abnormality in those with PSC, would explain its association with ulcerative colitis and a variety of infectious agents. Failure to down-regulate initially appropriate mucosal immune responses could lead to chronic inflammation and fibrosis.

Conclusions: Large, multi-center epidemiological and genome surveys are needed to make sense of the puzzling mix of genetic, infectious, environmental, and immunologic factors associated with the etiology and pathogenesis of PSC.
Etiology and pathogenesis of biliary cancer

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Cholangiocarcinoma (CCA) is the primary cancer of extra- and intra-hepatic bile ducts. Although it is a rare neoplasm, its incidence has increased. CCA derives from malignant transformation of the cholangiocyte - the cell that line the bile ducts. Extra-hepatic CCA arises in left and right hepatic ducts, common hepatic and common bile ducts. These tumors present usually with clinical and biochemical features of biliary obstruction. In contrast, intra-hepatic CCA develops insidiously within the liver parenchyma and frequently presents with characteristic solid mass lesions at an advanced stage. It is probable that the etiopathogenetic mechanism(s) of extra- vs. intra-hepatic CCA are discrete.

To date, the etiology and pathogenesis of CCA are limited. A number of risk factors have been identified although in most cases the cause remains elusive. Common features among several of the known risk factors include a milieu of chronic biliary inflammation and cholestasis. This biliary environment results in production of cytokines and reactive oxygen species resulting in cholangiocyte malignant transformation as indicated by sub-cellular and cellular changes of bile duct cells. Postulated molecular mechanisms leading to bile duct carcinogenesis include cholangiocyte self-sufficiency/proliferation, evasion of apoptosis and escape from senescence. Expression of proteins that induce CCA invasiveness and metastasis, preservation of telomere shortening and promotion of angiogenesis are additional pathways involved in CCA pathogenesis.

In the past decade, we have begun to better understand the biology of CCA. Nevertheless, more studies are in high demand to further the current knowledge before early detection and effective therapies for CCA become a reality.
Detection, surveillance and timing of intervention of primary sclerosing cholangitis

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During UDCA treatment, dominant stenoses of the major bile ducts may develop which cannot effectively be treated with UDCA and need endoscopic treatment. In a prospective trial during treatment with UDCA for up to 18 years, 50% of the patients had or developed a dominant stenosis of major bile ducts. Endoscopic dilatation of these stenoses is highly effective.

Repeat cholangiography in patients with increasing cholestasis (AP, GGT, serum bilirubin) seems essential to detect such stenoses of major bile ducts early. Endoscopic cholangiographies should be performed under antibiotic prophylaxis. ERCP is more sensitive and specific than MRCP. It is evident that mechanical obstruction of major bile ducts cannot be treated by UDCA effectively and that endoscopic measures are essential if conservative treatment shall be effective. In two prospective studies the actuarial Kaplan Meier estimate of survival after dilatation of major duct stenoses was significantly improved compared to the predicted survival.

When dominant stenoses of the larger bile ducts are detected by ERCP early endoscopic intervention with dilatation of the duct is mandatory. In most cases one single dilatation is not sufficient and repeated dilatations are necessary till the duct remains open. Intermittent stenting also has been used but the stents tend to occlude early due to the inflammatory material that is shedded from the bile ducts. Since occlusion of the stent leads to bacterial infection of the proximal biliary tree stents in general should be removed or replaced early, i.e. within 1-2 weeks. In our hands dilatation is by far the more effective form of endoscopic treatment.

We conclude that PSC may be treated conservatively by UDCA with good treatment results and prolongation of survival free of liver transplantation only when patients who develop major duct stenoses are early recognized and are additionally treated by endoscopic means. In endstage disease liver transplantation is indicated.
Surgical treatment of pancreatic cancer

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Pancreatic cancer is the fifth leading cause of cancer mortality with a rising incidence in most european countries. Due to both, the aggressive biology of the disease and the late diagnosis in many cases, Pancreatic duct carcinoma is still a disease with a poor prognosis. Today, surgical resection of localized tumor remains the only potentially curative option available for these patients. Advances in surgical technique and perioperative care has improved significantly in the last twenty years causing an extension of indications for surgical intervention. Resection in elderly patients or removal of advanced tumors including resection of the portal vein are feasible with low perioperative mortality rates nowadays. Although the spectrum of indication increased, operative mortality rates for pancreaticoduodenectomy should not exceed 5% at centers with high case-load today.

New diagnostic modalities like MRI, endoscopic ultrasound or MRI cholangiography for locoregional staging and preoperative imaging of the ampullo-pancreatobiliary region have helped to improve the preoperative assessment of resectability. However, despite new diagnostic techniques the surgical exploration still plays the key role for finally assessment of resectability. In this context the role of diagnostic laparoscopy in patients with pancreatic malignancies is controversial. For detection of liver or peritoneal metastasis laparoscopy before laparotomy may be reasonable. For evaluation of local resectability laparoscopy alone cannot generally be recommended today and explorative laparotomy is required.

Contraindications for pancreatic resection are liver metastasis, peritoneal metastasis and tumor infiltration of visceral arteries. The surgical management of pancreatic cancer consists of two phases: First, assessment of tumor resectability and second, if resectability is given, the pancreaticoduodenectomy with consecutive reconstruction. Standard surgical strategies are the classic pancreaticoduodenectomy including a distal gastrectomy and the pylorus-preserving pancreaticoduodenectomy (PPPD) preserving antral and pyloric function respectively. The PPPD as the less invasive procedure can be helpful in reducing the incidence of postgastrectomy symptoms. Both surgical procedures are equally effective for the treatment of pancreatic carcinoma.

Delicate lymphadenectomy during pancreaticoduodenectomy is important for radical oncological enforcement. An extended lymphadenectomy showed no benefit in several trials.

Although the encouraging advances in surgical treatment actuarial 5-year survival rates after pancreatic resection are only at 20%. For that adjuvant therapy concepts are needed for an appropriate postoperative treatment after pancreatic resection. Preliminary results of a randomised trial of adjuvant chemotherapy with gemcitabine vs. observation in patients with resected pancreatic cancer are promising and further clinical trials should be initialized for improving survival after successful pancreatic resection.
Gallstones represent a serious burden for our healthcare systems: over 10% of Europeans and Americans carry gallbladder stones, and the prevalence of gallstone disease seems to be rising as a result of longer life expectancy. Many gallstones are silent, but symptoms and severe complications ensue in around 25% of cases, necessitating surgical removal of the gallbladder, usually by laparoscopic cholecystectomy. Each year, an estimated 700,000 cholecystectomies are performed in the US, and 150,000 in Germany. Thus, cholelithiasis is the second most expensive digestive disease, exceeded only by gastroesophageal reflux disease. Mortality rates following cholecystectomy range from 0.1 to 0.8%, and in the US about 3,000 deaths (0.12% of all deaths) per year are attributed to complications of cholelithiasis and gallbladder disease. Non-surgical approaches, including gallstone dissolution by ursodeoxycholic acid and extracorporeal shock wave lithotripsy, have more and more lost their impact on therapy and are performed only for non-complicated symptomatic cholecystolithiasis in a very small number of selected patients.

Cholelithiasis belongs to the complex metabolic diseases affecting humans and its pathogenic mechanisms are not well known. Data from recent identical-twin, family and linkage studies provide conclusive evidence for a strong genetic component to gallstone disease. Furthermore, epidemiological studies in at-risk populations indicate that gallstone formation is caused by multiple environmental influences and common genetic factors and their interactions. Recently monogenic subtypes of cholelithiasis, such as ATP-binding cassette transporter deficiencies, have been identified. Human association studies have illustrated that common gene variants contribute to gallstone formation. The rapid identification of new lithogenic genes in knockout, transgenic and inbred mice provides the basis for studies of the corresponding genes in patients with gallstones. The transfer of findings from mouse genetics to the bedside might lead to new strategies for individual risk assessment and reveal molecular targets for the development of new strategies for prevention and medical treatment.
Future perspectives in the treatment of biliary tumour disease: A multidisciplinary approach

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Overall survival for patients with gallbladder or bile duct cancer is poor, because most tumours are diagnosed in an advanced stage. The minority is curatively resectable and long-term survival after curative resection ranges between 20% and 40%, and below 20% in pN1-positive tumours. Nevertheless, resection will remain the key to improve cure, provided the tumours will be diagnosed earlier and/or down-staged/sized with neoadjuvant therapy. This requires dedicated teams of specialists in gastroenterology-oncology, radiology, hepatobiliary surgery and radio-oncology. Imaging techniques like MRI, multi-array CT scan and PET-CT scan made much progress in recent years. Early use for rising cholestatic serum enzymes - long before the onset of jaundice - might increase the rate of curatively resectable bile duct tumours. Development of PET-CT-targeted biopsies will clarify a fluorodeoxyglucose enriched PET-focus by histology in high risk diseases such as PSC or ulcerative colitis. In addition, preoperative staging and oncosurgical planning will become standard to achieve en-bloc resection of hilar bile duct or gallbladder cancers.

Neoadjuvant protocols have been used in pilot studies for downstaging of hilar bile duct tumours classified non-resectable. The MAYO group treated unresectable stage I/II perihilar cholangiocarcinoma with neoadjuvant external beam irradiation, 192-iridium-brachytherapy and 5-fluorouracil and/or oral capecitabine prior to liver transplantation (OLT). OLT was achieved in 28/56 patients and long-term survival after OLT in 22 of them. Our own group used local photodynamic therapy of the tumour stenoses and adjacent bile ducts for down-sizing of unresectable hilar cholangiocarcinoma in 9 patients. Resection was achieved in 7 of them (all R0 resected without undue complications, including one with OLT), and long-term survival in 5 of them. Liver transplantation for unresectable hilar cholangiocarcinoma has recently resulted in 30% survival after 5 years. These approaches, including living donor-related liver transplantation, have to be evaluated for locally confined, unresectable bile duct tumours in prospective multicenter trials.

Radiochemotherapy and systemic chemotherapy are not yet evidence-based for biliary tract cancers. Phase II trials indicate poor responsiveness to external beam radiotherapy, but moderate responsiveness to 5FU- or gemcitabine-based radiochemotherapy and to chemotherapy in particular with 5FU or capecitabine, gemcitabine and cisplatin or oxaliplatin. Improved palliative biliary drainage including local tumour ablation by photodynamic therapy overcomes mechanical cholestasis and cholangitis and makes patients nowadays fit for antitumour therapies including radiochemotherapy and polychemotherapy. Growing knowledge of the relevant oncogenic pathways will lead to targeted therapies for biliary tumours. Thus biliary cancers will become better treatable within the next ten years.
Pancreatic carcinoma
Genetics of chronic pancreatitis and pancreatic carcinoma

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Pancreatic cancer is a devastating disorder of which most affected patients die. It has the worst survival rate of any major cancer. In the US and according to the latest estimates 30,700 patients will be diagnosed with and 30,000 Americans will die from pancreatic cancer in 2004. Similarly, estimates world-wide for the year 2000 by the World Health Organization showing an incidence of 201,506 cases and 200,865 deaths demonstrates the near-universal lethality of this disease. Survival rates are somewhat stage-dependent with a 5-year survival rate of only 17% for local disease. However, only a minority of patients presents with local disease. This is a result of our inability to diagnose pancreatic cancer early by symptoms alone and our current lack of a blood test or imaging test that can accurately detect a cancer prior to symptom onset in the general population. Although the low incidence of pancreatic cancer in the general population does not make it practical to screen for pancreatic cancer at this time, high-risk groups can be identified which may benefit from surveillance by detecting a tumour at an earlier, hopefully more curable stage. The most promising of those are subjects/patients from families in which a definitive gene has been identified, that conveys an increased risk of developing pancreatic cancer and can be tested for. In addition to hereditary pancreatitis, which increases the pancreatic cancer risk 40-70 fold, the following cancer syndromes fall into that category.

Hereditary Breast and Ovarian Cancer Syndrome (HBOC) Definition:

Individuals diagnosed with HBOC have a hereditary predisposition to early onset breast and ovarian cancer. Other cancers associated with this syndrome are prostate, colon, and pancreas. Women diagnosed with HBOC have an approximate 80% lifetime risk of developing breast cancer and an approximate 40% lifetime risk of developing ovarian cancer.

Relative Risk of Pancreatic Cancer: 10 fold

Gene and Chromosomal Location:
BRCA1 on 17q21
BRCA2 on 13q12-q13

Individuals should be referred for genetic counseling to identify a potential genetic predisposition to pancreatic cancer if:

1. Two or more family members (1st-degree relatives of each other) with pancreatic cancer with or without breast or ovarian cancer.
2. One pancreatic cancer case with at least two relatives with early age onset (< 50 years) onset of breast cancer or ovarian cancer at any age.
3. One pancreatic cancer case with at least 3 cases of breast cancer or one case of ovarian cancer and one or more cases of breast cancer.
4. Ashkenazi Jewish ancestry with one pancreatic cancer case and a single case (or more) of breast or ovarian cancer at any age in a first or second-degree relative (testing for the three Ashkenazi Jewish founder mutations may be sufficient).

**Peutz-Jeghers Syndrome (PJS) Definition:**

Individuals affected with Peutz-Jeghers syndrome have multiple gastrointestinal hamartomatous polyps and mucocutaneous pigmentation. Individuals with PJS are at increased risk for developing pancreas, colon, breast, endometrial, ovarian, lung, or testes.

**Relative Risk of Pancreatic Cancer:**
Up to 100 fold

**Gene and Chromosomal Location:**
STK11/LKB1 on 19p13.3

Individuals should be referred for genetic counseling to identify a potential genetic predisposition to pancreatic cancer if:

The individual can be clinically diagnosed with PJS based on the following:

1. An individual has two or more histologically verified Peutz-Jeghers polyps, small bowel polyposis, and mucocutaneous hyperpigmentation, or
2. An individual has small bowel polyposis, mucocutaneous hyperpigmentation, and a family history of PJS.

**Hereditary Non-Polyposis Colorectal Cancer Syndrome (HNPCC) Definition:**

HNPCC is a condition that is characterized by an increased risk of colon cancer and other cancers that include cancers of endometrium, ovary, stomach, small intestines, hepatobiliary tract, upper urinary tract, brain, and skin. Individuals diagnosed with HNPCC have an approximate 80% chance of developing colon cancer in their lifetime. The average age of onset for colorectal cancer in these individuals is 44 years. Women diagnosed with HNPCC have a 20-60% chance of developing endometrial cancer in their lifetime with an average of diagnosis of 46 years.

**Affected Gene:** MLH1 and MSH2

**Relative Risk of Pancreatic Cancer:** 1.5 fold

Individuals should be referred for genetic counseling to identify a potential genetic predisposition to pancreatic cancer if:

A clinical diagnosis of HNPCC can be based on one of the following 2 criteria.
A. The Amsterdam criteria states that an individual can be diagnosed with HNPCC if:
   a. At least three individuals in the family diagnosed with colorectal cancer.
   b. The affected individuals represent two generations in the family.
   c. One of the affected individuals must be a first degree relative (parent, sibling, or child) of the other two.
   d. At least one individual must be diagnosed with a colorectal cancer before the age of 50.

B. The Amsterdam II criteria states that an individual can be diagnosed with HNPCC if:
   a. At least three individuals in the family diagnosed with an HNPCC-related cancer.
   b. The affected individuals represent two generations in the family.
   c. One of the affected individuals must be a first degree relative (parent, sibling, or child) of the other two.
   d. At least one individual must be diagnosed with an HNPCC-related cancer before the age of 50.

Familial Atypical Multiple Mole Melanoma (FAMMM) Definition:

Individuals with FAMMM have a familial predisposition to developing atypical moles that can develop into melanoma. Melanoma can also develop de novo in these individuals. The average age of initial melanoma diagnosis is 34. Those diagnosed with FAMMM may have an increased risk of developing pancreatic cancer and astrocytomas.

Relative Risk of Pancreatic Cancer:
13-22 fold

Gene and Chromosomal Location:
P16INK4 on 9p21

Individuals should be referred for genetic counseling to identify a potential genetic predisposition to pancreatic cancer if:

Background: Whelan et al. described the occurrence of a p16 mutation in a pancreatic cancer-prone family that showed an excess of malignant melanomas. Genetic testing of asymptomatic members of these families has identified several healthy individuals who carry this mutation and thus are at high risk for the development of pancreatic cancer. Goldstein et al. found a 13-fold prospective risk of pancreatic cancer in FAMMM kindreds with the p16 mutation. Vasen et al. estimated the cumulative risk of developing pancreatic cancer of 17% by the age of 75 in putative p16 mutation carriers from 19 FAMMM families.
However, the Melanoma Genetics Consortium has recommended that clinical testing be confined to confirming research results and not routinely offered. With that in mind individual diagnosed with FAMMM have the option of enrolling in research protocols if:

1. An individual has a personal history of melanoma and 1st degree relative with melanoma.
2. An individual has three or more primary melanomas.
3. An individual has 2 or more first degree relatives with melanoma.

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Surveillance and diagnostics of chronic pancreatitis

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In 80% of all cases chronic pancreatitis is preceded by long-term alcohol abuse. Idiopathic chronic pancreatitis may be associated with mutations of SPINK (serine protease inhibitor type Kasal) or CFTR (cystic fibrosis transmembrane conductance regulator). Tropical pancreatitis is associated with SPINK mutations in about 50%. Our knowledge on genetic causes or associations with chronic pancreatitis has not led to new therapeutic concepts. However, decades of existing chronic inflammation of the pancreas may impose a considerable risk to develop pancreatic cancer. The prognosis of patients with alcohol induced chronic pancreatitis is not good. After 10 years of the disease up to 50% of all patients may have died. However, death is often not due to complications of chronic pancreatitis such as severe relapses or complications of diabetes but due to the general "life style", id est consequences of alcohol and smoking (lung cancer, heart infarction, infectious complications such as pneumonia, accidents). Thus, the risk to develop pancreatic cancer is especially high in patients at the age above 40 to 50 with hereditary pancreatitis with early onset of chronic pancreatitis.

Unfortunately we can not offer convincing surveillance programs to detect cancer at an early stage. Clinical trials are planned to measure tumour suppressor genes (such as p 16, p 53) and oncogenes (such as ki ras) in pancreatic/duodenal secretions collected by either ERCP or a duodenal tube. We do not know whether annual CT and/or MR scans and/or endosonography may lead to an early diagnosis. Abstinence from alcohol and smoking are the only preventive measures which may decrease the cancer risk.

For diagnosis of chronic pancreatitis the role of function tests is decreasing. Measurements of elastase in stool or indirect function tests such as fluorescein dilaurate test have a disappointing sensitivity. The sensitivity of cerulein-secretin test is rather good. However, the test is cumbersome, expensive and not standardized. Furthermore, results of function tests are not very important in decision making. Indication for prescription of porcine pancreatic extracts can be based on clinical symptoms, such as steatorrhea, weight loss, diarrhea.

Various imaging procedures are mandatory not only in diagnosing the disease but in decision making regarding further therapeutic options. There is no evidence based algorithm which procedure should be chosen under various clinical conditions. Sonography is mostly the first option guiding the indication for further imaging techniques such as MRCP, ERCP, endosonography, CT. MRCP as a rather non-invasive procedure is helpful in detecting bile and pancreatic duct stenosis. ERCP is needed for interventional endoscopic techniques such as stenting of bile and pancreatic duct, extracorporeal shock wave lithotripsy. Endosonography allows to measure the distance between a pseudocyst and the gastric and duodenal wall and to exclude vessels in the pseudocyst wall which could cause major bleeding in cases of endoscopic drainages. CT scans may display an inflammatory mass of the head of
the pancreas encouraging duodenum preserving pancreatic head resections. Unfortunately, all techniques including PET scans are not really helpful in diagnosing early pancreatic cancer or in decision making whether a pancreatic mass in long standing chronic pancreatitis is malignant or not. It has to be clarified whether endosonography guided punctures followed by histological evaluation will solve this dilemma.

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Medical management of pancreatic carcinoma

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Pancreatic carcinoma is one of the best studied solid tumors, hence this has not been able to translate into therapeutic strategies providing us with response rates known for other carcinomas such as breast or colorectal. The vast majority of patients with pancreatic adenocarcinoma (PDAC) are diagnosed in an inoperable state. Therefore, palliative treatment is the only option. During the last years, gemcitabine has become the quasi standard in chemotherapy, however, with response rates about 15% the median survival with monotherapy oscillates around 6 months for the advanced (metastatic) stages. The modern cytotoxic drugs as irinotecan or oxaliplatin have also not provided a break-through, both alone or in combination with gemcitabine. Fixed-dose regimen may improve the response rates and survival.

The hope of both medical oncologists and patients relies on novel substances termed "targeted therapy". This refers to four levels at and within a tumor cell: ligands, receptors, receptor tyrosine kinases, downstream signal transduction pathways. Based on the intimate understanding of tumor biology, several systems have been tackled such as the ligands (EGF, VEGF), receptors (EGF-R, VEGF-R), mutant ras (FTI), receptor tyrosine kinaseses (gefitinib, imatinib), folate enzymes (permetrexed) and cyclooxygenases (celecoxib). Beyond the tumor cell, targets such as the stroma (MMP-inhibitors) and the endothelial cell (VEGF-inhibitors) have been addressed. Non of those has yet demonstrated a break-through response rate. Some results, e.g. for the antiangiogenesis with VEGF-antibody bevacizumab) are pending.

Besides chemotherapy, supportive care is pivotal in these patients, however, often neglected. This refers to an aggressive nutritional therapy including supplements for cachectic patients such as ω3-fatty acids and pancreatic enzyme supplementation, adequate pain therapy as well as psycho-oncologic aid.

In summary, the understanding of tumor biology leading to targeted therapy - in combination with conventional chemotherapy offers a tremendous chance. For the time being, the promise of this rather rational approach has not been fulfilled.
Pancreatic cancer: Surgery, palliative resection and value of lymph node resection

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Pancreatic cancer remains with an overall long-term survival rate of less than 1% one of the most difficult cancers to treat. It is the 4th to 5th leading cause of cancer related mortality in the Western world and is responsible for around 28,000 deaths per year in the USA and 40,000 per year in Europe. In only 10-15% of patients potentially curative surgery is possible, and even in these patients, the median survival is only 10-18 months with 5-year survival rates of 17-24%. Nonetheless, surgery remains the only treatment option with the chance of cure. Pancreatic surgery has significantly changed during the past years. Pancreatic resection remains an intervention of particular significance, often technically challenging and with logistic demands for preoperative diagnostics and perioperative management. Recently, the value of centralization of pancreatic surgery in "high volume institutions" has been demonstrated. The current mortality rates following pancreatic resections are below 5% in specialized surgical centres. Pancreaticoduodenectomy is the surgical option for tumors of the pancreatic head, which account for the majority of pancreatic cancers. Tumors in the body or tail of the pancreas are treated by pancreatic left resection or occasionally by total pancreatectomy. Organ preserving resection procedures such as pancreatic segmental resection are performed more frequently for selected cases. In this context, pylorus-preserving pancreaticoduodenectomy has been proven to be equal to the classical pancreaticoduodenectomy in terms of tumor recurrence or long-term survival, and should therefore be considered the standard procedure for tumors of the pancreatic head. Various forms of radical lymphadenectomies for pancreatic cancer do not improve the long-term outcome according to randomized controlled trials.

Since October 2001, we have performed more than 1300 pancreatic operations including more than 900 major pancreatic resections at the Department of General Surgery (University of Heidelberg, Germany). 70% of the patients underwent pancreatic resection because of pancreatic malignancy, 23% of the patients were operated for chronic pancreatitis, and the rest for other indications.

A classical Whipple operation was performed in 94 patients; a pylorus-preserving Whipple operation was performed in 410 patients. 119 patients underwent a duodenum-preserving pancreatic head resection. A left resection was performed in 158 patients, a segmental resection in 44 patients, and a total pancreaticoduodenectomy in 64 patients. The 30-day hospital mortality was 1.6%. The pancreatic fistula rate was 3.7%. The median postoperative hospital stay was 11 days.
In conclusion pancreatic resections can be performed with considerable safety and very low pancreatic complication rates. However, even since pancreatic surgery remains the only treatment with curative potential, the long-term survival for pancreatic cancer is not more than 20 - 30% after radical resection. In the future, these results can hopefully be significantly improved by novel multimodal treatment concepts for pancreatic malignancies.

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Development compounds with different modes of action for the treatment of pancreatic and hepatocellular carcinomas: ZK 304709 and L19-IL2


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Two novel development compounds (ZK 304709 and L19-IL2) will be presented which have shown strong anti-tumor efficacy in a variety of standard human xenotransplantation models as well as orthotopic models for pancreatic and hepatocellular carcinomas.

ZK 304709 is the first oral Multi-target Tumor Growth Inhibitor™ (MTGI) acting via inhibition of cell cycle progression and tumor-induced angiogenesis. ZK 304709 is a pyrimidine-based nanomolar inhibitor of CDKs1, 2 and 4, VEGF-RTKs1-3 and PDGF-RTKβ, undergoing phase I clinical trials. Inhibition of VEGF-induced vascular permeability, as measured by Evans blue dye extravasation (Miles assay) in nude mice, indicates that ZK 304709 blocks the VEGF-RTK system in vivo. Oral treatment with ZK 304709 of mice bearing xenografted estrogen-independent human MaTu tumors (volume approx. 350 mm³) resulted in inhibition of phosphorylation of the intratumoral retinoblastoma protein, induction of massive apoptosis, and tumour regression. Additionally, ZK 304709 was found to be highly efficacious in orthotopic as well as subcutaneous human pancreas cancer transplantation models. These data clearly show that the compound is able to quantitatively inhibit the anticipated pathways in vivo resulting in strong antitumor effects in all investigated animal models.

L19-IL2 is a recombinant fusion protein consisting of the human L19 antibody moiety selective for ED-B fibronectin and human IL2. The compound is able to target ED-B fibronectin-expressing human tumors and to activate IL2-associated effector functions.

Hepatocellular and pancreatic cancers show perivascular and stromal ED-B fibronectin expression within the tumor tissue whereas corresponding normal tissues and liver biopsies from patients with pancreatitis or liver cirrhosis do not. Three clinically relevant orthotopic animal models for hepatocellular carcinoma and renal cell carcinoma have been used to establish the therapeutic potential of L19-IL2. We could demonstrate that ED-B-fibronectin expression in these animal models is comparable to the human situation, L19-IL2 has a superior efficacy compared to non-targeted IL2, and that already small amounts of ED-B expression within tumor tissue are sufficient for therapeutic efficacy of L19-IL2.
Tumor stasis was achieved at clinically relevant doses of L19-IL2, whereas animals treated with non-targeted IL2 showed only minor or no signs of therapeutic efficacy.

**Conclusion:**
These preclinical results support the ongoing clinical investigations of ZK 304709 and initiation of clinical studies with L19-IL2 in solid cancer indications with high medical need including pancreatic and hepatocellular cancer.
How does pancreatic cancer develop?

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Pancreatic cancer is one of the most common causes of cancer related death. Despite the advances of the molecular pathogenesis, pancreatic cancer remains a major unsolved health problem. Still, the 5-year survival rate is below 5% and of the 10% of patients with respectable disease only around one fifth survive 5 years. Current therapeutic approaches have no impact in respect to overall survival.

Cancer develops through stepwise accumulation of genetic alterations. Although similar genetic changes can be detected in specimens of different tumors, the type of mutations, the sequence and the frequency of these alterations depends on the tumor type. Colon carcinogenesis has for years served as a model disease for the multi-step model of cancer. Lately a multi-step model for pancreatic carcinogenesis has been evolved and put forward by Ralph Hruben and coworkers. Pancreatic intraepithelial neoplasia (PanIN 1 to 3) has been identified as precursor lesions. It is believed that genetic and epigenetic mechanisms work together to overcome intrinsic tumor suppression during tumorigenesis. The structural alterations involve mutations of the proto-oncogene KRAS and the tumor suppressors CDKN2A, TP53, BRCA2 and SMAD4/DPC4 at different stages. The epigenetic changes are less well characterized and include promotor methylation of genes controlling cell cycle progression. Pancreatic cancer has some feature which distinguish it from other cancers. Pancreatic cancer is very aggressive and highly infiltrative. Even small tumors metastasize to lymph nodes and perineurally. The molecular mechanisms responsible for this aggressiveness are so far unknown. Pancreatic tumor cells display a ductal differentiation although the cellular origin of pancreatic cancer is unknown. Recent studies using genetically defined mouse models resembling the human disease suggest that stem cells rather than terminally differentiated duct cell are the origin of tumor development. Treatment of pancreatic cancer remains difficult. Recent clinical trials using "modern drugs" including antibodies and small molecules which target signaling molecules shown to be important for pancreatic cancer biology are disappointing. Therefore the development of new strategies to prevent pancreatic cancer will be important in the future.
Anti-apoptotic interventions as a novel treatment option in liver diseases

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Cell death by apoptosis occurs in diverse human liver diseases. For example, liver damage by apoptosis has been well-documented in alcoholic hepatitis, non-alcoholic fatty liver disease, viral hepatitis, and ischemia-reperfusion injury. Apoptosis in liver diseases is often initiated by death receptor signaling cascades, especially the Fas and tumor necrosis factor receptor-1 death receptors. These cascades involve activation of zymogen proteases referred to as caspases. Full activation of caspases in death receptor signaling requires mitochondrial dysfunction. Once activated, these caspases cleave a variety of intracellular target molecules culminating in cell death by apoptosis. Recently, the use of caspase inhibitors for the treatment of human liver diseases has become a reality. A pharmaceutical caspase inhibitor has been developed and is currently in clinical trials for the treatment of viral hepatitis. Preclinical studies have demonstrated that this caspase inhibitor will not only attenuate liver injury by Fas but also ischemia-reperfusion injury and cholestatic liver injury following bile duct ligation. More importantly, caspase inhibitors have also been found to abrogate hepatic fibrogenesis in this model. These preclinical clinical studies with a caspase inhibitor are exciting and have the prospects for truly employing hepatoprotective therapy.

References:


Session Liver I
Diagnosis and surveillance in liver disease
Primary prevention of HCC is feasible through vaccination against HBV infection. Universal vaccination of infants in Taiwan reduced the prevalence of HBV carriers in childhood (from 15% to 1%) and simultaneously of HCC (60% reduction compared with non-immunized children). Secondary prevention can be achieved in non-cirrhotic individuals considered "sustained responders" to new anti-viral strategies. On the contrary, with established cirrhosis the preventive effect of these agents is unproven. Although some randomized controlled studies suggested that retinoids, interferon and internal radiation -131 may prevent HCC recurrence after curative therapies (tertiary prevention), these results need further validation in the West. Surveillance programs enable early detection of HCC in targeted populations (mainly cirrhotic patients), although there is no unequivocal proof of a clear decrease in cancer-related deaths. The diagnosis of HCC is established either on the basis of pathological criteria or non-invasive criteria. The later criteria were established in the year 2000, in cirrhotic patients presenting 2 coincidental imaging techniques showing a nodule > 2 cm in diameter with arterial hypervascularization. These criteria are being revised by a recent consensus of the EASL-AASLD-JSH.
Imaging modalities

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Diagnostic confirmation and assessment of disease extent are crucial for proper clinical management of patients with hepatocellular carcinoma (HCC). For years, the diagnosis of HCC was based on percutaneous biopsy, and intrahepatic tumor staging required invasive procedures, such as angiography and Lipiodol computed tomography (CT). Currently, owing to the advances in imaging techniques, a reliable diagnostic assessment can be based in most instances on noninvasive examinations in combination with clinical and laboratory findings. Nevertheless, imaging cirrhotic patients with suspected HCC is a challenging issue. HCC shows a variety of imaging features, that reflect the variable gross and microscopic characteristics of this malignancy. In addition, pathologic changes inherent in cirrhosis - such as large regenerative nodules and dysplastic nodules (DNs) - may be indistinguishable from a small HCC. One of the key pathologic factors for differential diagnosis that is reflected in imaging appearances is the vascular supply to the lesion. Through the progression from regenerative nodule, to low-grade DN, to high-grade DN, to frank HCC, one sees loss of visualization of portal tracts and development of new arterial vessels, termed nontriadal arteries, which become the dominant blood supply in overt HCC lesions. It is this neovascularity that allows HCC to be diagnosed and is the key for imaging cirrhotic patients. While ultrasound (US) is widely accepted for HCC surveillance, spiral CT or dynamic magnetic resonance (MR) imaging are required for diagnostic confirmation and intrahepatic tumor staging. However, despite recent technological advances, CT and MR imaging remain relatively insensitive for the detection of tiny satellite lesions and tumor vascular invasion into peripheral portal vein branches.
Role of pathology in neoplastic liver disease

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Pathology plays a decisive role in the assessment of nodular hepatic lesions. It includes biopsy evaluation of focal lesions of uncertain significance and the evaluation of resection or explantation specimen. Indication for biopsy diagnosis is set by the clinician and has to weigh the diagnostic benefit against potential biopsy related complications.

Biopsy diagnosis of focal liver lesions is demanding and covers a broad range of benign and malignant diseases. Hepatic metastases may occur in almost any type of malignancy but is most frequently seen in gastrointestinal tumors, mammary carcinoma and lung cancer. Nevertheless, it has to be kept in mind that the majority of solitary focal liver lesions even in the context of a known primary liver tumor do not represent metastases. Biopsy diagnosis is especially difficult in cases of highly differentiated hepatocellular lesions; they include liver cell adenoma, focal nodular hyperplasia, regenerative nodules, premalignant dysplastic nodules, and highly differentiated hepatocellular carcinoma. Differences can be subtle and require detailed analysis of cytological and mainly histological details. Thus aspiration cytology is of little help in these cases and histology should be obtained. Histologic algorithms for the diagnosis of highly differentiated hepatocellular lesions have been developed and together with the so-called matrix diagnosis have greatly improved the differential diagnosis.

Resection of focal liver lesions is mostly performed in cases of primary or secondary malignant liver tumors and infrequently for benign lesions such as liver cell adenoma or large haemangiomas. In cases of primary malignant liver tumors pathomorphology has to clarify relevant questions: final TNM-classification including vascular invasion, grading, resection margins and state of the nontumorous liver. Resection of secondary liver tumors is especially performed in synchron or metachronous metastases of colorectal cancer. Here the questions to the pathologist are the confirmation of the metastasis and the relation to the resection margin.

Differential diagnosis of liver tumors has gained enormously from the increased availability of immunohistological markers that help to differentiate primary liver tumors from metastases. There is currently no need for molecular pathological diagnosis but most likely in the future target structures will be assessed comparably to mammary carcinoma and adenocarcinoma of the lung in order to identify patients suited for specific therapeutic approaches. First molecular approaches such as broad spectrum screening by cDNA- or oligonucleotide-arrays or evaluation of known targets have been performed and have gained several promising candidates such as IGF-II, EpCAM, COX-2 and others, that await further clinical evaluation.
| Session Liver II | Metabolic liver disease |
Fat, diabetes and liver injury

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Nonalcoholic steatohepatitis (NASH) is a condition characterized by excessive deposition of fat in the liver (steatosis), inflammation and hepatocellular necrosis. While steatosis alone is generally a benign and stable condition, NASH can have a dire prognosis in a minority of patients, mainly because of fibrosis occurrence and progression to cirrhosis. Life-threatening complications such as liver failure and hepatocellular carcinoma have been described in NASH-induced cirrhosis. Insulin resistance is almost universally found in patients with NASH and the main risk factors for this condition are overweight and diabetes. Improvement in insulin sensitivity, whether achieved by diet, exercise and/or pharmacological interventions, results in a dramatic reduction of liver fat and inflammation and fibrosis as well. Therefore NASH should be viewed as the hepatic phenotypic manifestation of insulin resistance and a bona fide component of the metabolic syndrome. Liver injury should be assessed in diabetic and/or obese patients and the mechanisms by which insulin resistance promotes liver damage needs to be elucidated. Diet and lifestyle modifications are key to the treatment of this disease. Substantial weight loss significantly improves histological lesions in NASH but often requires a surgical intervention. The encouraging results of the use of insulin sensitizers in particular PPARγ agonists in human or experimental models of NASH, justifies future large-scale, randomized controlled trials. The efficacy of anti-oxidants and/or hepatoprotective agents has yet to be proven.
Nonalcoholic fatty liver disease (NAFLD) is the hepatic component of the metabolic syndrome, a systemic state of insulin resistance caused by abnormal production of fat- and liver-derived factors that modulate energy substrate flux to coordinate tissue anabolism and catabolism. These factors are produced by multiple types of cells in both tissues. Many, if not all, of the intermediary metabolism regulators are also potent regulators of immune system functions. Conversely, acknowledged immune regulators, such as cytokines, modulate the production and biological activity of the metabolic factors. Steatosis, steatohepatitis and insulin resistance develop as a result of excessive proinflammatory factors. Because the metabolic syndrome is a chronic inflammatory state, early stage NAFLD is very common in individuals with this condition. However, although chronic exposure to inflammatory mediators generally promotes the generation of various profibrogenic factors, progression from steatohepatitis to cirrhosis is actually relatively uncommon. This paradox may reflect the requirement for additional factors, such as certain Th-2 cytokines, that are selectively induced in subpopulations of individuals with the metabolic syndrome who have particular hepatic innate immune system defects. Defective hepatic innate immunity may also contribute to the evolution of hepatocellular carcinoma in some patients with chronic NAFLD. More research is needed to delineate mechanisms that govern liver remodeling during conditions of metabolic stress in order to clarify the pathogenesis of NAFLD and prevent its progression.
Steatosis and hepatitis C - does it matter?

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Pathogenesis of steatohepatitis: the "two-hit" theory

Since 1998 the pathogenesis of steatohepatitis associated with excessive alcohol intake and obesity/insulin resistance has been explained by the so-called "two-hit" theory, with steatosis - the first "hit" - sensitising the liver to the injurious effects of the second "hits" required for the progression from to necroinflammation, apoptosis and fibrosis. The strongest candidates for these second hits are oxidative stress (and subsequent lipid peroxidation) and cytokines. Evidence has emerged from both animal models of steatosis and patients with fatty liver that, when compared to lean livers, steatotic livers develop more lipid peroxidation in response to oxidative stress and more necroinflammation when exposed to endotoxin. In heavy drinkers the most obvious source of oxidative stress is alcohol metabolism. The principal source of intrahepatic cytokines (principally TNFα) are Kupffer cells stimulated to express cytokines in response to portal endotoxaemia, itself arising from an alcohol-induced increase in gut permeability. Evidence for increased oxidative stress and lipid peroxidation in non-alcoholic fatty liver disease (NAFLD) has been provided by both human and animal studies. The principal source of reactive oxygen species (ROS) is considered to be increased free fatty acid (FFA) oxidation by mitochondria, peroxisomes and microsomes, coupled with the inhibitory effects of TNFα on mitochondrial electron transport. The source of intrahepatic cytokines in NAFLD may be hepatocytes responding to increased levels of FFA, Kupffer cells responding to portal endotoxaemia (arising as a result of increased gut permeability) or peripheral adipose tissue now known to synthesise and secrete a variety of hormones and cytokines (known as adipocytokines) in obesity.

The two-hit theory 2005

Since the publication of the two-hit theory in 1998 there have been at least two important modifications. First, it is clear that steatosis per se may contribute to the second hit with the (assumed) increased concentration of intrahepatic FFA increasing the synthesis and secretion of TNFα by hepatocytes. Furthermore, the increased TNFα, FFA or their metabolites interfere with insulin signalling and contribute to steatosis-associated insulin resistance which will further increase FFA oxidation by mitochondria and microsomes and lead to oxidative stress. Secondly, it has become clear that the correlation between steatosis severity and necroinflammation/fibrosis may not be due to fat being involved directly in the pathogenesis of advanced disease but may be explained by other "factors" leading to both steatosis and liver injury and fibrosis. Of these "factors", obesity and the associated peripheral insulin resistance can both lead to an increased supply of free fatty acids and insulin to the liver which will cause steatosis. The increased ratio of TNFα to adiponectin observed in obesity will, via their effects of hepatic lipid metabolism, also contribute to the development of steatosis. The increased FFA and TNFα/adiponectin ratio will directly contribute to the development of hepatic necroinflammation, while insulin, TNFα and
other adipocyte-derived factors, including angiotensinogen, norepinephrine and leptin, (along with low levels of adiponectin) are all capable of stimulating fibrogenesis. Clearly this alternative explanation for the correlation between steatosis and advanced disease has implications for therapy, since if fat is directly involved in the pathogenesis of advanced fatty liver disease then therapy should be directed at the steatosis per se, whereas, if (as seems more likely), the fat simply reflects the presence of other injurious factors associated with obesity and/or peripheral insulin resistance then therapy should be directed at these factors.

**Steatosis and the progression of liver disease in chronic HCV infection**

Can this emerging information on the pathogenesis of steatohepatitis and fibrosis in ALD and NAFLD be used to explain the association between steatosis severity and disease progression in HCV? Initial reports of a correlation between the severity of steatosis in patients with chronic HCV infection and either the coexistence of fibrosis or the risk of subsequent progression, coupled with observations that NAFLD type lesions were not uncommon in patients with HCV\(^9\), including the pattern of fibrosis, suggested that NAFLD-like mechanisms may play a role in the development of fibrosis in HCV-associated fatty liver. Further evidence supporting the role for steatosis in the progression of liver injury in chronic HCV was provided by a recent study demonstrating that in patients with chronic HCV, increasing steatosis was associated with increased hepatocyte apoptosis. The classical "two-hit" theory certainly provides a plausible explanation for a direct causal relationship between steatosis and disease progression in HCV. Lipid peroxidation has been observed in liver biopsy specimens from patients with chronic HCV and its magnitude and location correlate with the stage and site of fibrosis. With respect to cytokine mediated "second hits", serum TNF\(\alpha\) levels are frequently raised in patients with chronic HCV. Clearly the anti-viral hepatic inflammatory response will provide an excellent source of ROS and free radicals and more recently it has been shown, both *in vitro* and *in vivo* that the HCV core protein may induce oxidative stress directly through an effect on mitochondrial electron transport. However, as in NAFLD, the association between steatosis and disease progression could also be indirect with some factor or combination of factors leading to both steatosis and to steatohepatitis and fibrosis. As in NAFLD, this clearly has important implications for therapy since if steatosis is directly involved in disease progression then it is an obvious target for therapy, whereas if steatosis is simply a reflection of the magnitude of some other pathogenic factor then treatment should be directed at that factor along with the HCV itself.

Studies that have emerged over the last 12 to 18 months largely argue against a direct role for steatosis in disease progression in chronic HCV implying instead that the presence of steatosis is a marker of the severity of alternative mechanisms that are directly involved in the development of necroinflammation, apoptosis and fibrosis. First, although genotype 3 has been consistently shown to be associated with the greatest risk of steatosis, and appears to have direct steatogenic effects, there is no convincing evidence that this genotype is associated with more progressive HCV disease. Second, in non-genotype 3, the severity of steatosis correlates with the "usual" fatty liver risk factors of obesity and insulin resistance. When studies have
included some measure of insulin resistance in their multivariate analysis, it is this rather than steatosis per se that predicts fibrosis. Third, studies that have examined viral genotype-specific associations between fat and fibrosis severity have come up with contradictory results with at least one study showing a genotype 1 specific association and two showing a genotype 3 specific effect. This clearly argues against some general profibrogenic or proinflammatory effect of steatosis. Taken together, these studies suggest that the link between steatosis severity and disease progression in hepatitis C is indirect. In non-genotype 3 patients increased body mass index leads to the development of peripheral insulin resistance via FFA and TNFα dependent mechanisms with HCV infection per se possible contributing to the increase in TNFα. The obesity and insulin resistance, via the mechanisms outlined above, lead to both steatosis and hepatocyte injury, inflammation and fibrosis. In genotype 3 patients, any relationship between steatosis and advanced disease seems unlikely to reflect a general pro-inflammatory/fibrotic effect of steatosis and more likely to reflect the viral load or the general pathogenicity of the virus which leads directly to both steatosis and inflammation/fibrosis. The therapeutic implication of these conclusions are that in patients with the genotype 3, therapy should be directed at the virus alone whereas in patients infected with non-genotype 3, therapy should be directed both at the virus and at the associated obesity and insulin resistance. Recent data also suggest that the apparent resistance to anti-viral therapy associated with steatosis in genotype 1 infected patients may also be indirect with the steatosis simply reflecting insulin resistance and hyperinsulinaemia with the high levels of insulin inhibiting the effects of interferon rather than any direct effect of steatosis.
Hereditary hemochromatosis: The genes and the disease

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Hereditary hemochromatosis (HH) or hemochromatosis (HC) is a common autosomal recessive iron loading disorder characterized by excessive deposition of iron in parenchymal organs that can cause tissue damage, and disease. The most common form of HC is characterized by gradual iron accumulation potentially leading to organ disease, particularly liver cirrhosis, during the 4th-5th decades of life. This adult-onset form is usually caused by pathogenic mutations in the \( HFE \) gene (1), or, in rarer cases, in the gene encoding the serum transferrin receptor 2, \( Tfr2 \) (2). In the phenotypic form known as juvenile hemochromatosis iron loading occurs at a greatly accelerated rate: Individuals are more likely to present with cardiomyopathy and/or endocrine disease. This disorder was originally linked to chromosome 1q, where the hemojuvelin gene, \( HJV \), has recently been identified (3). The function of hemojuvelin, \( HFE \) and \( Tfr2 \) is currently unknown, but patients with pathogenic mutations in these genes seem to produce inappropriately low levels of hepcidin, a peptide that limits iron transfer from enterocytes and macrophages into the circulation. Indeed, rare cases of juvenile hemochromatosis have also been linked to mutation of the hepcidin gene \( (HAMP) \) itself (4). Recently, reports have been also presented of uncharacteristically severe "juvenile-like" disease in patients who have "adult" gene mutations in combination with "juvenile" gene variants (5) or have combined mutations of "adult" genes in the absence of juvenile gene mutations (6). This indicates that a variety of genotypes can produce a hereditary hemochromatosis phenotype and highlights the shortcomings of current attempts to classify hereditary hemochromatosis into sub-types based on underlying single-gene defects, e.g., the OMIM database, or to consider the adult form distinct from the juvenile form.

In conclusions, hereditary hemochromatosis should be defined as a unique clinical/pathophysiological entity that can be provoked by pathogenic mutations of one of the four known hemochromatosis genes, \( HFE, Tfr2, HJV \) and \( HAMP \) (7). It is important to recall, however, that numerous host and environmental factors can modify the natural history of the disease. Furthermore, mutations can occur in two or more of these genes, and depending on the roles of the affected genes and the homo- vs. heterozygous nature of the mutations, the resulting phenotype may vary. In vitro and in vivo studies will be needed to dissect the biochemical consequences of each hereditary hemochromatosis allele and increase our understanding of the precise contribution of each gene to the hereditary hemochromatosis phenotype.

References:

1. Feder JN, et al.
   A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis.
The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22.  

Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis.  
Nat Genet 2004; 36: 77-82.

Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis.  

Digenic inheritance of mutations in HAMP and HFE results in different types of haemochromatosis.  

Juvenile hemochromatosis associated with pathogenic mutations of adult hemochromatosis genes.  

7. Pietrangelo A.  
Hereditary hemochromatosis - a new look at an old disease.  
Wilson disease: The impact of molecular advances

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Wilson disease (WND) is a recessive disorder of copper transport with highly variable clinical manifestations. Excess copper storage leads to liver disease, that can express as fulminant hepatic failure, or slowly progressive disease, with onset from 3 to 55 years of age. Neurological features also vary widely, with onset from 8 to 55 years. Patients can have predominantly neurologic or hepatic symptoms, or both.

Identification of the causative gene (ATP7B, encoding a copper transporting hepatic P-type ATPase) in our laboratory (1993) has led to increased understanding of the copper transport pathway, and offers potential for molecular diagnosis. Clinical and biochemical features often do not provide a secure diagnosis for this treatable disease. Mutation analysis using leukocyte DNA is now feasible, with the development of rapid methods for mutation analysis. More than 260 mutations have been reported (http://www.uofa-medical-genetics.org/wilson/index.php). We have applied mutation analysis to more than 300 clinically affected individuals as an aid to diagnosis. Mutation analysis is particularly important for confirmation of clinical suspicion in the many cases that do not have all of the copper abnormalities typical for WND patients (low serum ceruloplasmin, high urinary copper, high hepatic copper, Kayser-Fleischer rings). Mutation analysis can be performed by sequencing of selected exons, by sequential sequencing of up to all of the 21 exons, or by other mutation detection methods. We identified at least one ATP7B mutation in 98% of patients diagnosed through typical clinical and biochemical features, and have demonstrated the effectiveness of mutation analysis for the many patients with uncertain biochemical assessment. However caution is required for interpretation of mutation results, as not all missense mutations (160+) have been shown with certainty to be disease-causing; more characterization is needed. DNA mutation analysis is feasible for accurate diagnosis and timely treatment. New types of treatment are currently being evaluated.

Because of the uncertainty of biochemical signs in asymptomatic patients, and overlap with heterozygous carriers, DNA analysis using markers flanking the ATP7B gene is essential for reliable diagnosis of sibs of patients and does NOT require knowledge of the specific mutation present. There are currently no other disease genes known to mimic the clinical signs and symptoms of WND. The MURR1 (COMMD1) gene, defective in canine copper toxicosis, has not been found to be associated with disease in patients with various signs of copper storage.
Session Liver III
Viral hepatitis
Immune pathogenesis of hepatitis B and C

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More than 500 million people are persistently infected with the hepatitis B virus (HBV) and hepatitis C virus (HCV) and are at risk of developing chronic liver disease, cirrhosis and hepatocellular carcinoma. The host immune response has a unique role in viral hepatitis because it contributes not only to viral control, clinical recovery and protective immunity but also to chronic hepatitis and liver cirrhosis. Although the determinants of HBV and HCV clearance and persistence have only been poorly defined, it is widely accepted that cellular immune responses play an especially important role in viral clearance and disease pathogenesis of both viral infections. Indeed, spontaneous elimination of HBV and HCV during acute infection is associated with multispecific and strong virus-specific CD4+ and CD8+ T cell responses that accumulate in the infected liver. The central role for T cell responses in HBV and HCV clearance has recently been directly demonstrated in the chimpanzee model by the finding that in vivo depletion of CD4+ or CD8+ T cell prevents HBV and HCV clearance and clinical recovery. The mechanisms that lead to the failure of the virus-specific T cell response and thus to viral persistence are only poorly defined. Various mechanisms of virus-specific T cell failure, such as primary T cell failure, T cell exhaustion and emergence of viral escape mutations, have been suggested. A detailed and better understanding of the immunological mechanisms of the virus-host interactions will be central for the development of a vaccine against HCV infection and immunotherapies that eliminate persistent HBV and/or HCV infection.
Therapeutic vaccination strategies in chronic hepatitis B

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Since more than two decades prophylactic vaccine against hepatitis B (HBV) has been introduced which very successfully prevents infection with HBV. Vaccines that are used in humans are subunit vaccines consisting of 22 nm empty subviral particles composed of the envelope proteins of HBV. Many efforts are made to improve the current vaccine against hepatitis B with respect to the enlarge humoral and cellular immune response in order to reduce number of re-vaccinations for low and non-responders and expand time intervals for revaccination.

The need for improved types of prophylactic vaccines against HBV is not only the priority in the developed countries but also efforts are made to improve the health care in other less developed countries. Despite the existence of a safe and efficient vaccine to prevent infection with HBV in humans DNA based vaccines have been evaluated and might be used for preventive or therapeutic vaccination in the future.

The development of therapeutic vaccines against chronic HBV infection is of general interest, both in the basic science and clinical use. Up to date, several attempts to stimulate the specific immune response to HBV to reach the control over HBV in chronic carriers by vaccination were undertaken but showed only limited success. Through the progress on the characterization of the woodchuck immune system and the development of specific immunological assays, the woodchuck model became an informative animal model for vaccine development. Woodchucks with chronic WHV infection provide excellent opportunities to test the effectivity of new options for therapeutic vaccinations. Among the new types of vaccines, genetic vaccines based on purified plasmid DNA provide features in contrast to classical protein vaccines and seems to be the most promising candidates for future development. The woodchuck is an excellent model to assess prototyp prophylactic or therapeutic vaccines as efficacy can be tested by challenge experiments or viral elimination in chronic carriers respectively.

No antiviral effects were achieved in chronically WHV-infected woodchucks during the early immunization trials with WHcAg only or in combination with famciclovir. In subsequent experiments, a T helper cell determinants were added to the vaccine preparation, in an attempt to circumvent the T-cell unresponsiveness to WHV proteins. However, only a small number of animals developed high titres of anti-WHsAg and suffered from severe liver damage. Thus, foreign T helper cell determinant peptides might provide help to overcome the deficiency of specific T-cell responses to viral proteins in chronic carriers.

In another immunization experiment, chronically WHV-infected woodchucks were treated with plasma-derived WHV surface antigens (WHsAg) adsorbed to aluminum salt with monophosphoryl lipid A. Anti-WHsAg antibodies were detected in all immunized woodchucks and persisted for a time period of up to 2 years after immunizations.
However, neither WHV DNA nor WHsAg titers in immunized woodchucks changed significantly. These results indicate that immunizations with WHsAg could partially induce specific B-cell responses to WHV proteins in chronically WHV-infected woodchucks. However, additional components for the stimulation of T-cell responses are necessary to achieve therapeutic effects against chronic hepatitis B.

The high replication level of hepadnaviruses in chronic carriers may maintain the immunotolerance to viral proteins. The T-cell response to HBV was successfully restored in patients treated with lamivudine or interferon-α. Thus, a reduction of the viral load by antiviral treatments may enhance the effect of therapeutic vaccines. A combination of an antiviral treatment and immunization with WHsAg led to a dramatic decrease of viremia and induced a WHV-specific lymphoproliferation in chronic carriers. The immunizations with WHsAg consistently induced a low level of anti-WHsAg in chronic carrier woodchuck. This finding hints to the usefulness of a combination of antiviral treatment and immunizations.

Very recently, a novel prototype therapeutic vaccine was developed by the group of Wen et al. This therapeutic vaccination using an antigen-antibody complex has been successfully tested in transgene mice and is now under clinic phase I evaluation. In the woodchuck model, a combination of antiviral treatment with lamivudine and therapeutic vaccination with DNA vaccines or antigen-antibody complexes was carried out to evaluate their efficacy. Interestingly, woodchucks immunized with WHsAg-anti-WHs complexes and pWHsIm developed anti-WHs antibodies and show a further decrease of serum WHV DNA and WHsAg concentrations. The anti-WHs antibody persisted in two woodchucks for a period of 8 weeks. These results indicated that immunization with antigen-antibody complexes in combination with nucleosid analogs seems to be an effective treatment against chronic HBV infection.
Viral hepatitis: The preventive potential of antiviral therapy

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There are about 380 million respectively 170 million people who are infected either with the hepatitis B virus (HBV) or the hepatitis C virus (HCV). Both virus infections have to be regarded as the major cause for the development of liver cirrhosis and hepatocellular carcinoma (HCC). As compared to uninfected persons chronically infected patients have a high risk to develop HCC and liver cirrhosis increases this risk considerable. Thus, in 2-6% of the latter patients the presence of HCC could be detected at an annual basis.

Preventive strategies in HBV infection.

Since the first introduction of an extremely safe and highly effective hepatitis B vaccine twenty years ago there is now convincing evidence that a public health vaccine program as conducted for instance in Taiwan significantly can reduce the rate of HBV infection in children.

In chronic HBV infection, goal of antiviral therapy is the long-term suppression of hepatitis B viremia which could be taken as end point of beneficial outcome. Seroconversion from HBeAg to anti-HBe in HBV wildtype infection often goes along with this type of response and is a good surrogate marker for treatment efficacy. Pegylated interferon alfa-2a (PEG-IFNα) as well as the nucleoside/nucleotide analogues lamivudine and adefovir have been approved for the treatment of chronic hepatitis B. Although these drugs have been shown to suppress HBV DNA their mode of action differs to some extent as far as the efficacy of HBV DNA suppression and HBeAg seroconversion is concerned. Quiet importantly; the antiviral effect is associated with a lower risk of HCC and hepatic decompensation as could be demonstrated in long-term lamivudine treated patients. However complete control of the infection defined by the loss of HBsAg and the appearance of anti-HBs occurs only rarely and in less than 5% of the infected patients.

Preventive strategies in HCV infection

In patients with acute HCV infection, monotherapy with (PEG)-interferon generally leads to a rapid and sustained virologic response hereby preventing in most instances (> 80%) a chronic course of the disease. However prevention of HCV infection by vaccines is still not possible.

Patients with chronic HCV infection can nowadays be successfully treated by the combination of PEG-IFNα and ribavirin and cure rates are now more than 50%. Sustained virologic response may not only stop disease progression but can also lead to fibrosis regression.
The well established antiviral therapy is also effective in patients already suffering from cirrhosis and can delay the progression to Child B stage and the development of HCC as documented by a metaanalysis conducted in 4614 patients assembled from 18 controlled clinical trials.

Besides the antiviral effect of the (PEG)-IFNα therapy there is great interest in its antiproliferative potency. From preliminary studies on a limited number of patients there is evidence that long-term IFNα therapy could positively affect fibrosis progression and also HCC development and mortality in patients with advanced fibrosis even if there is no complete virologic response. The concept of long-term PEG-IFNα treatment in virologic nonresponders to prevent fibrosis progression and clinical complication (HCC) is now investigated in several large scale prospective trials.
Session Liver IV
Hepatocellular carcinoma
Genomics of hepatocellular carcinoma

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Much is known about both the sequential cellular changes that precede the formation of hepatocellular carcinoma (HCC) and the etiological agents (i.e. HBV, HCV infection, and alcohol) responsible for the majority of HCC. Nevertheless, the molecular pathogenesis of HCC is not well understood. Also, a staging system that reliably separates patients with early HCC as well as intermediate to advanced HCC into homogeneous groups with respect to prognosis does not exist. This is important because the natural course of early HCC is unknown and the progression of intermediate and advanced HCC are quite heterogeneous. Thus, improving the classification of HCC patients would at minimum improve the application of currently available treatment modalities and at best provide new treatment strategies.

We have investigated the possibility that variations in gene expression of HCC at diagnosis would permit the identification of distinct subclasses of HCC patients with different prognoses. We applied three independent but complementary approaches for data analysis to uncover subclasses of HCC and the underlying biological differences between the subclasses. Unsupervised classification methods based solely on gene expression patterns revealed two subclasses of HCC strongly associated with the length of patients' survival. Also, when the classifiers used in a training set to optimized classification of the tumors were applied to the validation set, all five models successfully separated poorer survival patients (cluster A) from longer survival patients (cluster B). Furthermore, application of a univariate Cox regression model was used to identify individual genes whose expression is highly correlated with the length of survival. Application of survival associated genes for subclass prediction was highly accurate as illustrated by the fact that averaged gene expression indices from the selected 406 "survival genes" were sufficient to segregate the two subclasses even without the use of sophisticated prediction models. Information obtained from knowledge-based annotation of the 406 survival genes provided insight into the underlying biological differences between the two subclasses of HCC. Out of several biological groups of the survival genes, the cell proliferation group was the best predictor of an unfavorable outcome of the disease. Expression of typical cell proliferation markers like PNCA, and cell cycle regulators such as CDK4, CCNB1, CCNA2, and CKS2 was greater in subclass A than subclass B. Not surprisingly, many genes that are expressed more in subclass A are anti-apoptotic. Interestingly, higher expression of genes involved in ubiquitination and sumoylation was observed in subclass A. The ubiquitin system is often deregulated in cancers. In HCC, the degree of ubiquitination is highly correlated with cell proliferation and survival of patients and has also been proposed as a possible predictive marker for recurrence of human HCC. Also, enhanced activation of ubiquitin-dependent protein degradation may account for deregulation of cell cycle control and faster cell proliferation in the poor survival group (subclass A). This result is highly concordant with a recent study with mouse models of liver cancer demonstrating a much higher ubiquitination index in the models that mimic poor prognosis human subclass A.
HCC - A cancer of developed countries? Risk factors and cofactors

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Hepatocellular carcinoma (HCC) is one of the main complications of liver cirrhosis and a serious health problem worldwide. HCC is the fifth most common solid tumor in the world and accounts for approximately 500,000 deaths each year. The incidence of HCC in North America and Western Europe is low (approximately 3-9/100,000), while in some regions of Africa and Asia HCC is the major cause of death among malignant diseases with an incidence up to 100/100,000. These epidemiological data reflect the influence and the distribution of risk factors for HCC in high and low incidence areas. A causal relationship between liver cirrhosis and the development of HCC is obvious. Worldwide over 80% of HCC occur in liver cirrhosis. Although cirrhosis per se appears to be the prime risk factor for HCC, hepatocarcinogenesis strongly depends also on the cause of the underlying cirrhosis and on secondary risk factors. Based on the cause of liver cirrhosis one can distinguish patients with high risk (viral hepatitis, hemochromatosis, tyrosinemia), moderate risk (alcohol, α₁-antitrypsin deficiency, autoimmune hepatitis) and low risk for HCC (M. Wilson, PBC, PSC). Secondary risk factors for HCC among patients with chronic liver diseases are male sex, age and cigarette smoking.

Only few risk factors are known which may induce hepatocarcinogenesis in patients without liver cirrhosis. The best studied and most potent carcinogens inducing HCC are aflatoxin B1, a natural product of the Aspergillus fungus, and thorotrast. Exposure to aflatoxin B1 has been linked to a specific mutation on the codon 249 of the p53 tumor suppressor gene, which occurs frequently in HCCs of patients from Africa and China. Although estrogens are capable of causing HCC in laboratory animals as promoting factors, the epidemiologic association between steroids and human hepatocarcinogenesis is still controversial. Case control studies showed in non-endemic hepatitis regions a higher risk for HCC among women who were users of contraceptives, while among endemic hepatitis populations no association between contraceptives and HCC were observed.

Hepatitis B and C virus infection are closely associated with the development of hepatocellular carcinomas. The geographic variation in the prevalence of hepatocellular carcinoma actually provides some of the most convincing evidence linking the disease and chronic hepatitis C and B infection. There is a striking correspondence between areas where hepatocellular carcinoma is common and where hepatitis B or C virus is hyperendemic. A second line of evidence linking hepatocellular carcinoma and viral hepatitis infection is the high rate of serological markers for hepatitis C and B in patients with this tumor. In China and Korea 85% to 95% of patients with hepatocellular carcinoma are HBsAg positive, whereas in Japan, Spain and Italy 94.4%, 75% and 65% of the patients with this tumor were positive for anti-HCV.
A prospective study of more than 22,000 male government workers in Taiwan has shown the incidence of HCC to be more than 100 times higher in HBsAg positive than in HBsAg negative persons. Although both HBV and HCV appear to be capable of inducing HCC in noncirrhotic livers, epidemiologic data suggest that HBV associated HCC does not require cirrhosis as much as the HCV associated counterpart does. HBV associated HCC tends to evolve more frequently in younger individuals than HCV related HCC and vice versa HCV associated HCC emerges more often in advanced cirrhotic liver in older patients. In agreement with the hypothesis that HCV-associated hepatocarinogenesis needs the environment of cirrhotic liver, it has been shown that the risk of HCV-associated HCC is increasing with alcohol consumption in a dose-dependent manner.

In selected areas of some developing countries, the incidence of primary liver cancer has decreased, possibly as a result of the introduction of hepatitis B virus vaccine. In contrast to developing countries, studies from cancer registries have shown a strongly rising incidence of hepatocellular carcinoma in several developed countries, including United States, France, Australia, Scotland and Italy. This increase appears to be mainly due to an increase in the incidence of HCC related to hepatitis C, while the numbers of HBV- and alcohol-associated HCC remain nearly constant.

HCV was introduced in the US population (and presumably also in Western Europe) around 100 years ago and widely disseminated in the 1960s. In contrast, HCV was introduced into Japan >100 years ago and widely disseminated in the 1930s. In Japan the numbers of deaths from HCC showed a sharp increase in 1975. As the development of hepatocellular carcinoma in patients with chronic hepatitis C infection needs approximately 20-30 years, it has been predicted that the burden of hepatocellular carcinoma in developed western countries will further increase in the next two to three decades, possibly to equal that currently experienced in Japan (HCC incidence in Japan: 30/100,000).

It has been revealed that a significant proportion of the increasing incidence of HCC in developed countries cannot be attributed to hepatitis C virus. In general approximately 15-40% of HCC cases remain idiopathic, suggesting that other risk factors, in addition to HCV, are responsible for the recognized strong increase of HCC-incidence. Diabetes mellitus and obesity have been suggested as potential causes of the rising cases of HCC in developed countries. Diabetes mellitus and obesity are risk factors for non-alcoholic steatohepatitis (NASH), which can lead to liver cirrhosis and subsequently HCC. Compared with other kinds of cancers, obesity has the strongest impact on the carcinogenesis of primary liver cancer. Recently, a case control study demonstrates that diabetes mellitus is associated with a 2-3-fold increase in the risk of HCC, regardless of the presence of other major HCC risk factors. Given the current epidemic of obesity and diabetes, these risk factors will be increasingly important contributors to the HCC burden in developed countries.
New therapeutic approaches - anti-angiogenesis, immunotherapy

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Introduction
HCC is a growing clinical problem making it up to the fifth most common cause of cancer. Usually arising from a cirrhotic liver - with an additional disease-specific risk of malignant transformation - cells of HCC are particularly resistant towards chemotherapy. Although the enigma of this particular resistance is only partially understood, a large body of data suggests that the process of oncofetal dedifferentiation enables hepatocytes to evade immune surveillance by refining their intracellular set of proteins, which orchestrate the subtle balance of apoptotic death and survival. Evading the immune surveillance by developing resistance to apoptosis is a classic principle, which is of particular importance in a number of malignancies including HCC, where increased levels of anti-apoptotic proteins such as c-FLIP and survivin or decreased levels of pro-apoptotic proteins such as FADD have been described. Another hallmark of HCC is its high degree of neovascularization. Having the above in mind, three new therapeutic approaches to fight HCC are currently pursued: restoring an effective immune response towards tumor cells, restoring sensitivity of tumor cells towards apoptosis and the deadly attack of killer cells and interfering with the tumor driven neovascularization by anti-angiogenic means.

Immunotherapy of HCC
Although obviously attractive and promising, so far there are no firm clinical data demonstrating a benefit of immunotherapy for patients with HCC. It is not clear why immune response towards HCC tumor cells is primarily down-regulated. Analysis of tumor infiltrating lymphocytes insinuated that a subgroup of suppressive CD4(+) CD25(+)Foxp3(+) T(reg) cells prevent a more vigorous immune response. However, variety of specific and non-specific immunostimulatory strategies against HCC have been applied in preclinical experimental models with some promising results. The molecular characterization of HCC associated tumor antigens such as alpha-fetoprotein (AFP) and the increased understanding of the immunological pathways involved in liver and tumor immunology paved the way to design promising gene-based cancer vaccines. The most promising of these techniques is based on the use of dendritic cells, which are able to process and present antigens to activate naive T cells and, when loaded with tumor antigens, can stimulate a specific and durable anti-tumor response. The first phase I and II immunotherapeutic clinical trials, based on dendritic cell immunotherapy and peptide vaccines, are ongoing in HCC-patients.

Sensitizing towards apoptosis
Several reports ascribe the group of histone deacetylase inhibitors such as sodium butyrate (SB) or valproic acid (VA) the capacity to re-differentiate and re-sensitize malignant cells towards apoptotic stimuli. A number of studies demonstrated that SB and VA are able to induce apoptosis by itself or increase sensitivity of malignant cell lines from different tissue origin towards receptor induced apoptosis. A combinatorial
treatment approach with HDACi together with apoptosis inducing agents, such as death receptor ligands like TRAIL, or classical chemotherapeutics merits further evaluation in pre-clinical and clinical studies.

**Antiangiogenic approaches**

It was Judah Folkman back in 1971, who firstly described the idea of blocking vessel formation as a therapeutical principle to fight tumor growth. It took 3 decades until the monoclonal antibody bevacizumab an anti-angiogenic biological entered successfully the clinical stage as part of treatment patients with colon carcinoma. Bevacizumab targets soluble VEGF and there is evidence from pre-clinical studies that also in HCC animal models, directly interfering with the VEGF system reduces tumor growth. However, interfering with angiogenesis probably will not be enough - in a phase II trial, treatment with thalidomide, which down-regulates VEGF production, resulted in stable disease in one third of the patients only. Sorafenib, a small molecule which inhibits VEGF and raf kinase signaling, showed initial anti-tumor activity and is now tested in a randomized phase III trial in patients with advanced HCC.

**Conclusion**

Treatment of patients with HCC remains a clinical challenge. New approaches, which target the Achilles' heel of HCC namely its resistance towards killer cells and apoptosis as well as its hypervascularity, are about to enter the clinical stage. Due to the usually underlying liver disease, HCC patients will remain a difficult-to-treat and heterogeneous population - we will have to work on our partners in the pharmaceutical industry to support more strongly the transfer of promising preclinical results into randomized trials with our patients.
Living donor liver transplantation: Extended indications?

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Living donor liver transplantation (LDLT) represents nowadays an established procedure for pediatric and adult recipients. The indications are the same of deceased donor liver transplantation (DDLT) and the results similar and sometimes better (children) than DDLT. In consideration of actual donor scarcity the indications are results-oriented in term of guarantee of best survival (overall and disease free). At this regard some indications are considered not standard or better defined as extended indications: 1) HCC beyond Milan criteria, 2) Decompensated end-stage liver disease (UNOS 2A), 3) HCV cirrhosis. For these reasons these patients are going to be excluded from the waiting list of DDLT and the only alternative which can be offered to them is LDLT.

1) HCC beyond Milan Criteria

Until the start of the 90s the results of DDLT were very poor since the main indication to LT was advanced HCC. As result HCC became a contraindication to DDLT till the introduction of Milan Criteria (MC) by Mazzaferro in 1996: no extrahepatic metastasis, no macroscopic vascular invasion, single tumor nodule ≤ 5 cm or ≤ 3 tumors ≤ 3 cm. Applying the MC a 4-year-survival of 83% and a disease free survival at 4 years of 75% was reached. Similar results were observed in LDLT in different centers. Unfortunately, the actual pre-operative tumor screening and tumor staging is not always reliable: the consequence is that sometimes patients are over staged before LT with exclusion of high number of patients who could benefit from LT. Additionally, probabilities to drop out because tumor progression during the waiting time range between 40-50% at 2 years after diagnosis. To escape the dilemma of limited organ availability LDLT is a good alternative offering a short waiting time with consequent less drop-out and reduced mortality in waiting list. Additionally, more than 50% of patients in published series of LDLT for HCC were beyond MC. For these reasons Yao et al. proposed to expand the MC in case of LDLT: single nodule ≤ 6.5 cm, ≤ 3 nodules ≤ 4.5 cm. The authors reported a 1- and 5-year-survival of 90% and 75% respectively.

Actually, it seems to be that a number of tumors represent a less important factor than diameter, presence of vascular infiltration and histological type associated with different grade of malignancy. At this regard Lee et al. suggested to extend MC in selected cases with higher number of tumor nodules as long as the HCC were small without macrovascular invasion. Recently, also the size of the tumor has been put under discussion. Gondolesi et al. reported recently good results also in case of LDLT for large HCC. Overall and in patients with HCC > 5 cm (n = 12), there were no
statistically significant differences in survival or in freedom from recurrence between recipients of living donor and cadaveric grafts. LDLT allows timely transplantation in patients with early or with large HCC. In conclusion, although complicated factors such as donor voluntarism and selection criteria limit the role of LDLT for HCC, LDLT allows more patients to undergo early transplantation and to reach results in a better outcome also in cases beyond MC.

2) Extended end-stage liver disease
LDLT for patients with decompensated end-stage liver disease (UNOS 2A, MELD > 30) is controversial. Nevertheless, these patients are most in need of a timely liver transplant. In our series patient and graft survival rates were only 43%. Regardless the high mortality rate no donor had regrets about the procedure, and all donors stated that they would donate again if presented with the same decision. LDLT represents a timely and effective alternative to DDLT in case of decompensated end-stage liver disease. Nonetheless, the ethical concerns regarding risk a benefits for both donor and recipient should be discussed.

3) HCV cirrhosis
Approximately 170 million people worldwide have been infected with HCV. By the year 2020 current estimates suggest that nearly 14 million people will have cirrhosis due to chronic HCV. HCV-related disease accounts for more than half of the indications for LT in most transplant programs. As waiting list continues to expand, the time to transplantation is becoming increasingly prolonged. At the moment the current number of deaths on the waiting list is higher for patients with diagnosis of chronic HCV infection than for other diagnosis.
Liver cirrhosis secondary to HCV actually represents 30-50% of the indication to LT in European and American countries.
Relapse of HCV occurs virologically in 100% of recipient. Histological recurrence is approximately 50% of recipients with ensuing graft failure in 10% of patients by the 5th postoperative year. Additionally, 8% to 31% of patients with post transplant HCV-recurrence develop cirrhosis within 5 to 7 years resulting in reduced long-term survival rates.
In contrast to whole DDLT, survival outcomes and effects of recurrence following LDLT for HCV are not yet defined. Preliminary reports showed earlier and severe recurrence within the first year after transplantation with higher incidence of cholestatic hepatitis. In this case the advantages of early transplantation may be offset by the risk of graft failure imposed by early recurrent disease. Nevertheless, an emerging strategy for preventing recurrent HCV infection is pre-transplant treatment to achieve viral eradication (especially in patients with HCC and compensated cirrhosis with a good viral profile: non-1-genotype, or genotype 1 with low viral load) followed by timed LDLT. If such strategies become successful, LDLT may exhibit an advantage over DDLT.

The extension of the indication to LDLT should be drawn carefully and individually based on both patient's and donor's safety. Nevertheless, LDLT opens new perspectives for patients with advanced HCC, decompensated end-stage liver disease and HCV cirrhosis.
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POSTER ABSTRACTS

Poster Numbers 1 - 121
Autoimmune pancreatitis: An underdiagnosed condition in Caucasians

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Introduction: Autoimmune pancreatitis is uncommon in the western world, particularly in Europe. Recognizing autoimmune pancreatitis as new clinical entity in Europe will change the management for many patients who have been labeled as acute or chronic pancreatitis.

Methods: We report the first case of autoimmune pancreatitis in the United Kingdom and highlight the classical features of the condition including its management, which differs considerably from either acute or chronic pancreatitis. We studied our case of autoimmune pancreatitis against the autoimmune pancreatitis criteria.

Characteristic findings of autoimmune pancreatitis
- Increased IgG
- Autoantibodies presence
- Diffuse enlargement of the pancreas
- Diffuse irregular narrowing of pancreatic duct
- Fibrotic pancreas with lymphocyte infiltration
- No or mild symptoms without attacks of acute pancreatitis
- Rare calcification or pancreatic cysts
- Occasional association with other autoimmune diseases
- Effective steroid therapy
- Diabetes often improves with steroids

Results: Our case manifested all of the characteristic findings of autoimmune pancreatitis including the rarer features of symptomatic pancreatitis, pancreatic fibrosis and pancreatic cyst formation. He also manifested other autoimmune associations including interstitial nephritis and Sjögren's syndrome.

Discussion/Conclusion: Autoimmune pancreatitis was first recognized as a pathological entity by Sarle and colleagues in 1961. By the end of the 20th century, there were several hundred cases documented in the literature the vast majority of which were Japanese patients. This disease appears very rarely outside of Japan, Autoimmune pancreatitis is now established as a definite clinical entity and as the features of the condition become more familiar to physicians, the prevalence of the condition in the Western World will undoubtedly increase. Our recognition of autoimmune pancreatitis in the Europe will have a significant impact on our approach and management for patients with pancreatitis.
**New chemotherapeutic regimen for hepatocellular carcinoma**

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**Introduction:** A liver metastatic model was established by the orthotopic transplantation of human gastric carcinoma into nude mice, and a human specific beta-globin-related sequence in the DNA extracted from the nude mice's liver was amplified by polymerase chain reaction (PCR) and detected by Southern blot method to detect metastasis.

**Methods:** Liver metastasis was macroscopically observed from 3 to 5 weeks after transplantation, but the PCR amplified fragments in the liver already detected in the 2nd week. Uracil-Tegafur (UFT) 20 mg/kg were administered per os for 2 weeks from 1st, 2nd and 4th week on this model. The group UFT was given from 1st week, completely inhibit liver metastasis and the PCR amplified fragments in the liver were not detected. These results suggest, UFT could inhibit metastasis of liver, also useful for earlier evaluation.

**Results:** Clinically: advanced hepatocellular carcinoma (HCC) has poor prognosis. HCC's with tumor thrombus in portal vein were treated by arterial infusion of chemotherapeutic agents and oral administration of Tegafur/5-fluorouracil (5-FU) in 24 cases. The regular course consisted of administration once a week of cisplatin (0.4 mg/kg) and doxorubicin (0.2 mg/kg) and daily administration of Tegafur/5-FU (6/13 mg/kg). Most patients received 25-30 courses of chemotherapy. The serum levels of alpha-fetoprotein and des-gamma-carboxy-prothrombin (DCP) were reduced to normal range in 4 cases and much reduced in 8 of the remaining after treatment. Some cases showed much necrosis of HCC and 4 cases showed complete disappearance of HCC and tumor thrombus. There has been no recurrence of HCC for 6 months, adverse reactions were tolerable.

**Discussion/Conclusion:** This new regimen may be useful in treating patients with HCC in advanced phases including tumor thrombus in portal vein.
Non-invasive parameters for prediction of oesophageal varices

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Introduction: Portal hypertension commonly accompanies the presence of liver cirrhosis and the development of oesophageal varices is one of the major complications of portal hypertension. Cirrhotic patients should be screened for the presence of oesophageal varices when portal hypertension is diagnosed. In order to reduce the increasing burden that endoscopy units have to bear, some studies have attempted to identify parameters for non-invasive prediction of oesophageal varices presence.

The aim of our study was to evaluate the value of biochemical and ultrasonography parameters for prediction of oesophageal varices presence.

Methods: This report included 58 cirrhotic patients underwent a complete biochemical work-up, upper digestive endoscopy and ultrasonography examination. Albumin/right liver lobe diameter and platelet count/spleen diameter ratios were calculated and their correlation to the presence and degree of oesophageal varices, and Child-Pugh score of liver cirrhosis explored.

Results: The mean age of patient included in the study was 53.07 ± 13.09 yrs, 40 males and 18 females. In Child-Pugh class A were 53.4% patients, class B 39.7% whereas 6.9% were in class C. In 24.1% patients no oesophageal varices (OE) were identified by upper digestive endoscopy, 19% had OE grade I, 34.5% grade II, 20.7% OE grade III, and 1.7% OE grade IV. The mean value of right liver lobe diameter/albumin ratio was 5.43 ± 1.79 (minimum 2.76; maximum 11.44), while the mean platelet count/spleen diameter ratio was 1064.55 ± 775.14 (minimum 118.42; maximum 3320.99), respectively. Statistically significant correlation (p < 0.01) was proofed by Pearson test between OE grade and calculated indexes. The values of Pearson's test correlation were 0.461 and -0.636, respectively. Using Spearman test we also got a statistically significant correlation (p < 0.01) with Child-Pugh score, ρ values were 0.498 and -0.424, respectively.

Discussion/Conclusion: The right liver lobe diameter/albumin ratio and platelet count/spleen diameter ratio are non-invasive parameters which provide accurate information pertinent to determination of oesophageal varices presence and their grading in patients with liver cirrhosis.
Delta viral hepatitis: A serious health problem in Bulgaria

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Delta hepatitis has a worldwide distribution. There are three zones of HDV (hepatitis D virus) endemicity - low, intermediate and high. Viral hepatitis D (VHD) is a serious health problem in Bulgaria that deserves special attention.


Material and methods: Viral hepatitis was diagnosed by conventional laboratory and instrumental analysis. HDV infection was proved by routine immune methods for diagnosis. All patients have positive HBsAg and HDV AB. In some patients liver biopsy and specific immunological analysis were performed.

Results: All 100 patients were classified into eight age groups irrespective of their sex. These included 33 women and 67 men aged from 5 months to 73 years, hospitalized in the Clinic of Infective Diseases at the "St. George" University Multipurpose Hospital for Active Treatment - Plovdiv, in the period 1986-2003. The patients were classified irrespective of the type of HDV infection - coinfection and superinfection. We graded them according to the severity of the course of the disease into 3 groups: I group - mild cases, II group - moderate and III group - severe disease. Acute hepatic failure was observed in 9 patient and 6 patient had fatal outcome of the disease. In 62 patients the HDV infection became chronic. Some of the results are shown in table 1.

Conclusions: Patients with hepatitis D seem to suffer from a more severe and progressive disease than do those patients with other forms of viral hepatitis. VHD is an example of an infection from the past that has its imprint on the present and its management and eradication remain to be achieved in the future. Further immunization against HBV of risk groups will resolve the problem of HDV infection.
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Legend: m-male, w-women.
Hepatic effect of insulin sensitizing agents, with both ezetimibe, and valsartan combination therapy in rats with experimental NAFLD

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Background: Non-alcoholic liver disease (NAFLD) is a component of the metabolic syndrome, with a clinical spectrum ranging from fatty liver to steatohepatitis, cirrhosis, and hepatocellular carcinoma. The primary event of NAFLD is the accumulation of TG in hepatocytes due to insulin resistance and therapeutic modalities for this condition are needed.

Aim: To assess whether a combination of insulin sensitizing agents (ISAs) with both ezetimibe, and valsartan is superior to ISAs alone for treatment of NAFLD.

Methods: Adult male Sprague-Dawley rats (n = 6/group) were treated for 9 wks with methionin choline deficient diet (MCDD, NASH Controls) only, or MCDD diet with either metformin (200 mg/kg rat), rosiglitazone (3 mg/kg) or metformin plus rosiglitazone (200 mg/kg and 3 mg/kg, respectively), ezetimibe (2 mg/kg), valsartan (20 mg/kg), and combination of all drugs for a total of 15 weeks. Six additional rats served as healthy controls. Rats were then sacrificed and liver histology, hepatic and plasma lipid content, parameters of oxidative stress, and TNF-alpha were measured.

Results: NASH Control rats with MCDD diet only had severe hepatic fatty infiltration (> 90% fat), with increase in hepatic TG (+1263%) and hepatic cholesterol (+245%), increase in hepatic MDA levels (+225%), but a decrease in hepatic alpha-tocopherol (-74%), and no changes in plasma TNF-alpha as compared to healthy control rats. Combination therapy of all study drugs produced a significant decrease in liver steatosis (-56%), hepatic TG (-68%), hepatic cholesterol (-30%), hepatic MDA (-67%), but increased hepatic alpha-tocopherol (+128%), and no change in TNF-alpha levels. Combination therapy of ISAs produced the same decrease in liver steatosis (-33%), and hepatic lipids content (TG -64%, TC -27%) but to a lesser extent and was better than ISA alone. Plasma TNF-alpha levels increased significantly only with MCDD + rosiglitazone therapy (+130%, p < 0.05) as compared to NASH controls.

Conclusion: The combination therapy has a greater effect on the extent of fatty infiltration and on hepatic lipid composition than ISAs alone. The clinical implication of this finding is that polypill policy hold promise for the treatment of NAFLD in the future.
NTCP-mediated bile acid transport is regulated by cholesterol content in lipid rafts

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Introduction: Bile acid (BA) transport and secretion is a key metabolic function of the liver. Sinusoidal uptake of BA is mainly mediated by the Na⁺/taurocholate cotransporting polypeptide (NTCP). NTCP seems to localize into lipid rafts in vivo. However, it is not well known if NTCP localization in specific membrane microdomains regulates its function. This study assesses the effects of changes in plasma membrane lipid content on NTCP function.

Methods: NTCP was stably transfected in HEK-293 cells. Immunofluorescence, membrane fractionation techniques of detergent soluble and insoluble (rafts) fractions and Western blots were used to study NTCP localization. Taurocholate (TC) uptake assays were used to assess NTCP function. Modulation of cholesterol content in membrane lipid rafts was achieved using beta-cyclodextrin (BCD) treatment.

Results: NTCP expression conferred Na⁺-dependent-BA transport capability to HEK-293 cells. NTCP localized in the plasma membrane in IF studies. Under basal conditions NTCP localized in both, rafts and non-rafts microdomains. Cholesterol depletion of rafts (50%) determined a 2.5-fold increase in TC transport, which correlate with a shift of NTCP localization into non-rafts microdomains. On the other hand, cholesterol loading of HEK-293 cells with cholesterol-BCD complexes was associated to a significant decrease in TC transport (-20%), which correlate with a shift of NTCP localization into rafts microdomains.

Discussion/Conclusion: NTCP localization in specific plasma membrane microdomains seems to be regulated by membrane cholesterol content. Our results suggest that NTCP localization in specific microdomains at the plasma membrane might be a novel mechanism of regulation of BA transport in the liver.
Features of cirrhosis and liver cancer in a decorin knock out and TGF-β1 transgenic mouse model

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Introduction: The accumulation of connective tissue is characteristic for chronic liver diseases. Cirrhosis increases the risk of hepatocellular carcinoma. TGF-β1 is a major stimulator of fibrogenesis, therefore its neutralization is a principal therapeutic aim. Decorin regulates connective tissue proliferation by binding to collagen and inhibiting TGF-β1. Furthermore, it also acts as a tumor suppressor. The role of TGF-β1 in carcinogenesis is contradictory.

Methods: To better understand the role of decorin and TGF-β1 in cirrhosis and hepatocarcinogenesis, an animal model was created. Decorin KO (dec−/−) mice were mated with TGF-β1 transgenic mice overexpressing the growth factor in their liver (TGF-β1⁺). Cirrhosis and cancer have been induced by thioacetamide (TA) and diethyl-nitrosamine (DEN), respectively. Results were assessed by immunohistochemistry, morphometry, and real time RT-PCR.

Results: The severity of cirrhosis increased in the order Wt < dec−/− < TGF-β1⁺ < dec−/−/TGF-β1⁺. Seven months after TA administration, the number of tumors in the cirrhotic dec−/− animals was 3 x higher than in the dec−/−/TGF-β1⁺ mice. In contrast, the carcinogenic effect of DEN proved to be more potent in the dec−/−/TGF-β1⁺ group where tumors developed earlier and in a greater number than in the dec−/− animals.

Discussion/Conclusion: This disparity might be explained by the different biological effect of TA and DEN. The genotoxic DEN acts as an initiator, while TA behaves as a promoter in the process of hepatocarcinogenesis. TGF-β1 inhibits initiation, but it facilitates the events of promotion. Hence, our mouse strains respond differently to the treatments, depending on their levels of decorin and TGF-β1 expression.
The use of Ursofalk® in patients with reflux disease

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Introduction: In spite of considerable results that have been achieved in the treatment of reflux disease, the study of the impact of Ursofalk® on pathogenesis of the disease has been rather actual.

The goal: To define the worth and the effectivity of Ursofalk® in complex therapy of patients with reflux disease.

Methods: Fifty-five patients with reflux disease have been under observation. The diagnosis was made relying on the clinical data and Video gastroduodenoscopy. The patients were divided into two groups. The first group - 30 patients that together with the ordinary medicines received Ursofalk® 500 mg per day. The second group - 25 patients received regular treatment. The therapy lasted 6 months.

Results: The first group - the syndrome of bowel dyspepsia, pain in the epigastria, heartburn, vomiting with bile and nausea has disappeared at 27 patients (90%). The decrease of hyperemia and edema of stomach and oesophagus mucosa and absence of bile in stomach at gastroduodenoscopy - at 25 patients (83.3%). The changes at the second group are analogical but they are less expressed in terms of quantity. The syndrome of bowel dyspepsia, pain in the epigastria, heartburn, vomiting with bile and nausea has disappeared 19 patients (76%). The decrease of hyperemia and edema of stomach and oesophagus mucosa and absence of bile in stomach - at 17 patients (68%).

Discussion/Conclusion: The use of Ursofalk® in the complex therapy of patients with reflux disease leads to achievement of positive results during shorter periods of time and for a greater number of patients than the use of therapeutically schemes of treatment. This is due to the expressed action of Ursofalk® on the bile acids in stomach and oesophagus which transform in hydrophilic form, that less irritate stomach and oesophagus mucosa.
Pancreatic cancer in patients with chronic pancreatitis: Incidence and risk factors

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Introduction: Patients with chronic pancreatitis (CP) have an increased risk for pancreatic cancer (PCc), a malignancy with late diagnosis and fatal outcome.

Purpose: To assess frequency and risk factors for PCc in our patients with non-hereditary CP.

Methods: We performed a retrospective record analysis and a subsequent prospective follow-up of 136 patients operated in our clinic between 1993 and 2002 for non-hereditary CP. At laparotomy, 3 males (38, 51 and 56 years old) were found to have ductal adenocarcinoma developed on CP (final tissue diagnosis). Standardized incidence ratio (SIR) was calculated for 2121 patient years of follow-up. To find risk factors for PCc, we applied a Pearson correlation test and a nonlinear estimation for the following factors: age, sex, occupation, alcohol intake (dose, years of drinking), cigarette smoking (number of cigarettes, years of smoking), coffee consumption, fat diet, CP duration, number of CP complications, presence of diabetes and calcifications. For significant associations, relative risk (RR) was calculated.

Results: SIR of PCc was 12.76. We found that male sex, cigarette smoking, fat diet, CP duration, alcoholic etiology and number of complications are strong risk factors for PC (p < 0.005). Factors association leads to higher RR.

Discussion/Conclusion: CP is an independent risk factor for development of PCc. We describe a new risk factor for PCc in patients with CP: number of previous CP complications requiring operation, which possibly accelerates dysplasia. Presence of risk factors in CP patients suggests the need for closer follow-up and an aggressive surgical approach if malignancy is suspected.
High expression of agrin in hepatocellular and cholangiocellular carcinoma


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Introduction: The synthesis of proteoglycans, as one of the major component of ECM, was shown to be changed during neoplastic transformation both in tumors and surrounding tissues. Agrin, being a heparan sulfate proteoglycan constituent of the basement membrane, was implicated in the regulation of cell growth, differentiation, adhesion and motility.

Our goal, therefore, was to investigate mRNA and protein expression of agrin in hepatocellular carcinoma (HCC) and cholangiocellular carcinoma (CCC) to analyse its involvement in tumorigenesis.

Methods: Twenty-one cases of CCC and HCC, respectively, and 7 normal liver samples were studied. mRNA expression was evaluated by real-time PCR using relative quantification and beta-actin as reference gene. Protein expression was detected by immunohistochemistry in tissue sections and by Western blot analysis in tissue homogenates.

Results: 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. HCC presented intense expression along the neovascular basement membrane. In CCC well differentiated areas showed strong agrin expression in tumor specific basement membrane, while in less differentiated areas and at infiltration sites the staining was fragmented, decreased or even absence. Western blot analysis and real-time PCR confirmed the increased expression of agrin in HCCs and CCCs compared with normal and surrounding nontumorous liver.

Discussion/Conclusion: Agrin may play an important role in neoangiogenesis in human HCCs, as part of the newly formed vasculature. In CCCs, however, agrin might be involved in tumor progression.

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Molecular characteristics of pancreatic ductal adenocarcinoma in young patients

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) rarely affects patients before the age of 40 years. The aim of the present study was to determine if the clinical, pathomorphological and genetic features of PDAC occurring in young patients (≤ 40 years) differ from those in elderly patients.

Methods: PDAC obtained from 7 patients (mean age 38 years) were analysed clinically, histomorphologically, and immunohistochemically. Mutational analyses were performed using SSCP and direct sequencing.

Results: All seven patients were females. Four patients were smokers and one patient suffered from non-hereditary chronic pancreatitis. There was no association to cancer predisposing genetic syndromes. Pathomorphologically, three tumors displayed moderate differentiation and four tumors showed poor differentiation, the latter group including one adenosquamous carcinoma. All tumors presented overexpression of TGFβ1 and loss or significant reduction of Smad4. Accumulation of p53 and overexpression of EGFR were seen in five and four cases, respectively. There was no expression of p16, estrogen hormone receptors or progesterone receptors. Mismatch repair gene products (MLH1, MSH2, MSH6) were expressed in all tumors. Mutational analyses revealed K-ras mutations in only three of the seven tumors.

Discussion/Conclusion: PDAC of young patients shows a large clinical, pathomorphological and genetic overlap with PDAC of elderly patients. However, the low rate of K-ras mutations suggests the existence of yet undefined initiating events of pancreatic carcinogenesis in at least a subgroup of young patients, possibly making them eligible for alternative therapeutic approaches.
Chronic liver diseases associated with Sjögren syndrome

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

**Aim:** To describe the Sjögren syndrome and risk factors in association with chronic liver diseases.

**Patients and methods:** The study was made on 45 patients divided in: a group of 15 patients with chronic liver disease and Sjögren syndrome, other group of 15 patients with chronic liver disease without Sjögren syndrome and a group of 15 with Sjögren syndrome without chronic liver disease. The methods for diagnosis were: clinical, biological, immunological, bioptic, ultrasound, TC, x-ray and endoscopic investigations. It was determined in the same time sicca tests and antibodies against Helicobacter pylori (H.P.)

**Results:** The chronic liver diseases were represented by: viral hepatitis C (11 patients) and B (11), autoimmune hepatitis (3), viral cirrhosis C (6) and B (6), alcoholic cirrhosis (5), primary biliary cirrhosis (3 patients). Sjögren syndrome was associated in chronic liver diseases 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 suggest 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 liver disease (12 patients). The TNF-α, IL-1, IL-6 were increased in Sjögren syndrome and chronic liver diseases. The Sjögren syndrome was described in 7 patients with viral hepatitis C and 2 with B, 1 with autoimmune hepatitis and primary biliary cirrhosis, 2 patients with viral cirrhosis C and B.

**Conclusions:**
1. Sjögren syndrome associated with active chronic liver disease 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 liver diseases, the increase of immunoglobulins G, M and against H.P., CD4 cells and cytokines (TNF-α, IL-1, IL-6).

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Estimates of digestive affectations in patients with systemic lupus erythematosus

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The presence of digestive manifestations in the evolution of systemic lupus erythematosus (SLE) is important in the assessment of disease implications, complications, treatment and associated diseases.

Patients and methods: The study was performed in a group of 28 patients diagnosed with SLE, aged 16-50, which were supervised on a 3-5 years period. The digestive manifestations were observed in the same time with specific features of SLE. The investigations were clinical, imagistical (endoscopy, ultrasound, X-ray, CT) and pathological.

Results: The digestive manifestations observed in the evolution of patients with SLE were: stomatitis with erythematous lesions, oral ulcerations and xerostomia - in 13 patients (46%), dysphagia and odynophagia - in 3 patients (10%), lupus peritonits - in 1 patients, infectious hepatitis (B and C) - in 2 patients, lupus hepatitis - in 1 patients, pancreatitis - in 1 patients and splenomegaly - in 4 patients (14%).

Conclusions: The digestive affection in SLE patients is marked by symptoms and signs at clinical exam and investigations in the same time with diagnosis parameters of lupus.
The digestive manifestations in evolution of SLE are produced by disease (gastrointestinal vasculitis), complications, treatment or associated diseases. The disorders in active SLE can be at any site of gastrointestinal tract or of annexed organs.
Determination of hepatitis Delta virus RNA by polymerase chain reaction among chronically infected hepatitis B patients from Northern Poland

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²Department of Infectious Disease, Medical University of Gdansk

Introduction: HDV/HBV coinfection and superinfection may be associated with a higher rate of fulminant hepatitis as a consequence of more progressive infection. The objective of this study was to estimate the presence of hepatitis delta virus RNA in chronically infected HBV patients. To our knowledge, these are the first such studies of HDV infection in Poland performed by PCR based method.

Methods: A total group of 40 patients randomly selected from the group of 120 chronically infected hepatitis B patients (32 men, 8 women, age: 11-77 years) were enrolled into this study. At entry, age, sex, mode of transmission, duration of infection, serum biochemical markers and HBV DNA were evaluated. Virus RNA from patients' serum was isolated using QIAamp Ultrasens Virus Kit and than reverse transcribed by Omniscript Reverse Transcription Kit. cDNA was amplified by "home-made" nested PCR method.

Results: Forty HBV positive patients' serum samples were examined. Of them 3 samples (7.5%) were HDV-RNA-positive. All three patients were men, one was HCV co-infected. One was treated with interferon, none of them with nucleoside analogs. In all 3 HDV-RNA+ patients serum HBsAg was present and HBeAg absent. Those HDV carriers had high levels of serum ALT (72-281 IU/l). Their HBV viremia level was relatively low (940-28,000 cp/ml).

Discussion/Conclusion: HDV RNA detected in this study by RT-PCR is relatively high in comparison to results obtained in other laboratories using serological methods of HDV detection.

Acknowledgements: This study was supported by the VIRGIL European Network of Excellence (LSHM-CT-2004-503359).
Changes in redox-homeostasis during Raphacol (black radish) treatment in IBD

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Introduction: Chemoprevention is one of the strategies of cancer prevention. Research justified, that natural antioxidants could act on signal transduction and phase II enzyme activities indirectly. These molecules can also influence the apoptotic genes. Raphacol contains bioactive components, isothiocyanates, flavonoids, and vitamins of black radish, therefore we wish to know, how these molecules can act on the redox-homeostasis in IBD patients.

Methods: Valuable data came from 32 patients (13 male, 19 female) suffered from moderate active IBD, and the half of patients (6 male, 10 female) was treated with 0.2 g/day Raphacol granule for 12 months. Together with routine laboratory measurements, redox parameters such as reducing power, H-donating ability, free SH-groups and IL-6-, IL-1-, TNF-alpha, red blood cell chemiluminescence and HbA1c level were determined.

Results: Raphacol did not influence the activity of IBD. Beside beneficial subjective judgement, bile acid level was elevated continuously and the values of redox parameters were decreased in the plasma during the term. The granule diminished the red blood cell chemiluminescence significantly and the HbA1c level moderately until the beginning of the ninth month. After this, the trend has changed until the end.

Conclusion: Data showed, that antioxidant and isothiocyanate-rich Raphacol could influence the redox-homeostasis of IBD patients even at low dose.

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Different expressions of claudins in endocrine and exocrine tumors of the pancreas

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Introduction: A new family of tight junction (TJ) proteins called claudins are responsible for cell adhesion, polarity and paracellular permeability. Although changes in permeability of TJs were noted in several cancers, the role of claudins in carcinogenesis is controversial. Claudin 4 overexpression was reported in primary and metastatic pancreatic adenocarcinomas. These findings support use of claudin 4 as target for novel therapeutics of pancreatic cancers.

Aims: To analyse different claudin expressions in human pancreatic endocrine tumors and ductal carcinomas using a panel of polyclonal (claudins 1, 3, 7) and monoclonal (claudins 2, 4) antibodies.

Methods: Twenty formalin fixed, paraffin embedded pancreatic endocrine tumors and ten pancreatic ductal carcinomas were studied, and immunohistochemical evaluation performed to analyse claudin 1, 2, 3, 4 and 7 expressions. Semiquantitative evaluation was used for the area of extension (0-5) and intensity (0-3) of reaction.

Results: Claudins 1, 2 proved negative in all endocrine tumors. The majority (7/10) of ductal carcinomas was positive for claudin 4, while endocrine tumors were negative. In contrast, claudin 7 showed high expression in all endocrine tumors, however, ductal carcinomas did not express the protein. Claudin 3 was detected in 80% (16/20) of endocrine tumors, while ductal carcinomas were negative. Claudin 7 expression was significantly higher in endocrine tumor cells, compared with claudin 3 (p < 0.0032).

Conclusion: Our findings support that claudins 3 and 7 are specific marker for endocrine pancreatic tumors in contrast to ductal carcinomas. Further studies are necessary to assess the biological role of these proteins in pancreatic carcinogenesis.

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Hepatoprotective dose-dependent effects of ursodeoxycholic acid in rats fed methionine-choline deficient diet

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Ursodeoxycholic acid (UDCA) is widely used for treatment of cholestatic liver diseases. Many authors believe that administration of UDCA as a preparation to treat non-alcoholic steatohepatitis (NASH) may have a beneficial effect. However, this problem has been extensively studied and the data from clinical trials are controversial. The aim of this study was to evaluate the effects of UDCA on rats with NASN induced by feeding a methionine-choline deficient diet (MCDD).

Male Wistar rats (180-200 g) were fed MCDD during 10 weeks. Rats from 4 MCDD-fed groups were daily administered with UDCA (10, 20, 40 and 80 mg/kg b.w./day, i.g.) during the last two weeks on the experiment. For base line values before starting UDCA, one of the MCDD-fed groups was decapitated after 6 weeks of the feeding. The severity of liver damage was evaluated morphologically, including electron microscopy, and by biochemical methods.

The rats were fed a MCDD diet for 6 weeks to induce NASH, demonstrating that about 25% (score stage 1) of the liver parenchyma had steatosis and were ballooning. At that stage, the liver did not show any inflammation and had a normal structure. The animals fed a MCDD diet for 10 weeks developed severe steatohepatitis of score stage 3 (51-75% of the parenchyma developed steatosis and were ballooning), and inflammatory scoring stage 1 with less than 5% of inflammatory foci. Electron microscopy showed severe fatty degeneration at the lobular central and intermedial zone and, to a lesser degree, at the periportal zone with a lot of fine-structural changes in hepatocytes, which led to a strong increase the liver function parameters ALAT and ASAT in serum and to that in alkaline phosphatase. The liver steatosis was biochemically characterized not only by elevation of triglyceride content, but also by an increase in cholesterol and phospholipid concentrations, while the serum triglyceride and cholesterol contents decreased. The changes in the liver were also associated with a strong rise of the serum concentration of inflammatory cytokine TNF-α and a significant increase in the serum leptin concentration and a marked reduction of the serum insulin and adiponectin concentrations, while the blood glucose content was increased. There also seemed to be an increase in the total colonic anaerobic flora which paralleled to the pronounced increase in the gut mucus and blood ethanol content.
For most of the morphological, histological and biochemical parameters UDCA dose-dependently caused a reversal or even a normalization, comparable to the data obtained for a control group without a MCDD diet. A normalization of the parameters was mostly seen in the UDCA high-dose groups (40 and/or 80 mg/kg b.w./day), but sometimes even in the 10 and 20 mg dose group, while the histology was mainly reversed to normal in the 80 mg/kg b.w./day group.

We can conclude that even under therapeutic conditions in the presence of the MCDD-diet UDCA dose-dependently caused a reversal of the changed liver parameters after 4 weeks of the therapy. Most of the biochemical parameters, especially biochemical ones, were already normalized at the UDCA dose of 40 mg/kg b.w./day.
Ski/SnoN as negative regulators of distinct TGF-β signalling pathways in liver cells

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The discovery that cytokines control liver fibrogenesis, has opened an important area of research and provides potential for therapeutic intervention. TGF-β1 is a critical factor promoting stellate cell transformation into myofibroblasts, with subsequent shift of balance in the extracellular matrix degradation toward synthesis. Since hepatocytes are the predominant cell type of the liver and are clearly responsive to TGF-β, they may also actively contribute to fibrogenesis. Because TGF-β activity is tightly regulated by multiple levels of control, liver-protective and anti-fibrotic strategies are often aiming at neutralization of TGF-β effects.

Aims: To study the TGF-β1 pathway in isolated hepatocytes and cell lines derived from these, with special impact on the regulation of cell proliferation and apoptosis. Further, to investigate the Ski and SnoN interference in hepatocyte specific TGF-β signaling.

Methods: Primary cultured hepatocytes were carefully characterized using RT-PCR to show expression of typical cell markers. RNA and protein expression were quantified using RT-PCR, Northern blot and Western blot analysis. Implication of Ski/SnoN as negative regulators for TGF-β signalling was investigated by proliferation and apoptosis assays. Finally, TGF-β target gene regulation was investigated by transient transfections and reporter gene experiments.

Results: Mouse hepatocytes at early times of culture (2 days) express little or no TGF-β, Smad7, Ski and SnoN. By reporter gene assays and Western blot analyses, we found that addition of TGF-β to primary cultured mouse hepatocytes and hepatoma1-6 cells activates both classical Smad cascades, Smad1/5 and Smad2/3. SnoN expression is rapidly but transiently induced by TGF-β treatment in hepatoma1-6 cells and hepatocytes.

Overexpression of Ski/SnoN does not interfere with TGF-β mediated phosphorylation of Smad2 and Smad3. Overexpression of Ski and/or SnoN in hepatoma1-6 cells prevents activation of an artificial Smad3/4 reporter construct, (CAGA)₅-MLP-Luc, plasminogen activator inhibitor (PAI) and collagen promoters. Furthermore, high levels of Ski/SnoN interdict TGF-β antiproliferative effects, but not its induction of apoptosis. Smad7 expression is rapidly induced by TGF-β and can inhibit TGF-β induced apoptosis in this cell type. In this case, parallel overexpression of Ski and SnoN restores the TGF-β apoptotic effect.

Conclusions: These results suggest that Ski and SnoN specifically interfere, directly or indirectly with apoptotic, proliferative and profibrogenic actions of TGF-β in hepatocytes and hepatoma1-6 cells. Our data define new details of TGF-β-induced cellular responses in hepatocytes, which may help to define more specific TGF-β neutralizing strategies to prevent or effectively treat liver damage in the future.
Clinical and diagnostic value of serum-ascites albumin gradient in diseases with ascites

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Introduction: The serum-ascites albumin gradient (SAAG) has been proved in multiple studies to categorize ascites better than the total protein concentration, which classifies ascites into exudate (≥ 25 g/l) and transudate (< 25 g/l). If the SAAG is greater than 1.1 g/dl, the patient has portal hypertension with approximately 97% accuracy. SAAG is the difference between the serum and ascites albumin concentration. This difference correlates directly with portal pressure, which is based on oncotic-hydrostatic balance.

Methods: SAAG was measured in 96 patients with ascites. All patients were divided in two groups. One group included 76 patients who had ascites and portal hypertension (55 patients with decompensated liver cirrhosis, 9 with heart failure, 4 with massive liver metastases, 6 with mix ascites and 2 with peritoneal carcinomatosis). The other group included 20 patients who did not have portal hypertension (13 with peritoneal carcinomatosis, 3 with peritoneal mesothelioma, 3 with pancreatic ascites and 1 with TBC peritonitis). SAAG was determined by a person who was unaware of diagnosis (blind study).

Results: SAAG values were greater than 1.1 g/dl in all patients who had ascites and portal hypertension (average value: 2.3 g/dl ± 0.5 g/dl). SAAG values were less than 1.1 g/dl in the patients without portal hypertension (average value: 0.9 g/dl ± 0.5 g/dl).

Discussion/Conclusion: SAAG values above 1.1 g/dl point to portal hypertension as the cause of ascites. SAAG values below 1.1 g/dl point to the other causes of ascites. SAAG has significant clinical and diagnostic value in diseases with ascites.
Introduction: Anti-cholesterol antibodies (ACHA) are natural antibodies against the 3beta-OH group of free, LDL/VLDL cholesterol, but not of the cell membranes. ACHA is subject of research in atherosclerosis. Hypercholesterolemia is common in PBC, although its significance is not clarified. We aimed to investigate ACHA and its correlation with clinical parameters in PBC compared to healthy controls, non-alcoholic steatohepatitis (NASH) and Wilson disease (WD).

Methods: 108 patients with PBC, 31 with NASH and 52 with WD were investigated. Age-matched healthy persons served as controls. ACHA was measured in sera by ELISA. Correlation between ACHA and lipid parameters, liver function tests, immunoglobulin levels was analyzed.

Results: Higher ACHA level was found in PBC patients (41.9 ± 25.1 AU/ml) compared to both healthy controls (28.8 ± 11.3; p < 0.0001) and NASH (34.2 ± 15.5; p < 0.05). The ACHA values showed an increasing tendency with severity of PBC. Comparing stages III+IV with stages I+II the difference was significant. The serum cholesterol was slightly elevated in PBC.

No correlation was found between serum ACHA concentration and either serum cholesterol, immunoglobulin levels or any liver function test.

In NASH there was no difference in the level of ACHA compared to the controls. In WD ACHA level was lower compared to age-matched controls (17.4 ± 8.9; p = 0.000005). The serum ACHA levels in the three control groups were similar.

Discussion/Conclusion: Our novel finding on stage-dependent elevation of serum ACHA might stimulate further research to clarify its clinical significance. The complexity of ACHA phenomenon is illustrated by the normal ACHA level in NASH and the very low ACHA level in WD.
The impact of steatosis in fibrosis progression of hepatitis C

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Introduction: Steatosis is common in patients infected with hepatitis C (HCV) and occurs in 30-70% of liver biopsies. The pathophysiology of steatosis in HCV and its role in disease progression is controversial.

Aims and methods: To assess whether steatosis in chronic hepatitis C patients is an innocent bystander or contributes to liver disease progression. Our histopathology database was searched and 382 HCV patients who had a single liver biopsy retrieved. Patients with concomittent disease or inadequate biopsies were excluded. Biopsies were staged using modified Ishak fibrosis score and Brunt classification for steatosis by blinded histopathologists. Age, gender, ethnic minority, body mass index (BMI), alcohol intake, betal nut chewing and genotype were recorded.

Results: Steatosis was found in 58% of liver biopsies. In multivariate analysis steatosis was more common and more severe in Asians than in Caucasians (p < 0.001). Steatosis was most severe in patients infected with genotype 3 (p < 0.001). There were no significant relationships between steatosis and sex, age or BMI. In multivariate analysis factors associated with higher fibrosis scores included age, Asian ethnicity, high alcohol consumption, male sex. There was no independent relationship between steatosis and fibrosis score.

Conclusion: Older age, high alcohol intake, male sex and Asian ethnicity are important factors associated with hepatitis C fibrosis progression. Factors such as steatosis, betal nut chewing, and genotype do not play a role in fibrosis progression in hepatitis C.
Correlations between biochemical, histological and virological parameters in chronic viral hepatitis C

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Background: A retrospective study was carried out in order to assess some correlations between biochemical, histological and virusological parameters in chronic viral hepatitis C.

Patients and methods: A number of 219 patients with chronic hepatitis C, without previous treatment, were studied. The criteria of eligibility for therapy were represented by the presence of anti-HCV antibodies, positive viremia (Cobas Amplicor HCV Monitor Test), histological changes of chronic hepatitis (Knodell's score of necro-inflammatory activity ≥ 6 and fibrosis < 3) and increase serum of transaminase. The statistic was performed with chi² Test.

Results: Demographic characteristics (age and sex): 40.11% male patients and 59.88% female patients have a mean age by 45.96 ± 10.79. ALT more than 2.5 upper normal limit was find in 55% of the patients. From the histological point of view mild hepatitis was find in 65.6% of the patient and middle hepatitis in 30.2% of the cases. Regarding fibrosis score 3 for fibrosis was find in 53% patients and score 1 for fibrosis in 41% of the cases. 62% of the patient had the viral ARN-HCV below 800,000 UI/ml.

We find the follow statistic correlations:
- between patients with ALT > 2.5 ULN, mild chronic hepatitis (49.6%) and middle chronic hepatitis (67.3%);
- between patients with fibrosis1 and mild hepatitis (54%) analysed with patients with middle hepatitis and fibrosis 1 (15%) 
- between patients with fibrosis 3 and mild hepatitis (38.9%) analysed with patients with middle hepatitis and fibrosis 3 (84.6%)
- between patients with fibrosis1 and mild hepatitis (85.9%) analysed with patients with mild hepatitis and fibrosis 3 (48.4%)
- between patients with fibrosis1 and middle hepatitis (11.3%) analysed with patients with middle hepatitis and fibrosis 3 (48.4%)

Conclusions: These findings shows that there are some correlations between these parameters but they are very heterogeneous.

Key words: virus C infection - chronic hepatitis
Unusual association between liver adenocarcinoma and non-Hodgkin malignant lymphoma

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Introduction: The existence of hyperecogenous images localized intrahepatically detected by ultrasonography in a patient with non-Hodgkin malignant lymphoma suggests in the first place the existence of lymphomatosis determinations, but guided hepatic biopsy is also necessary for an accurate diagnosis.

Methods: We present a patient with unusual association between liver adenocarcinoma and non-Hodgkin malignant lymphoma

Results: P. A., a male patient aged 66, came to hospital with weight loss, asthenia, night sweat and fever. The patient had generalized peripheral, retroperitoneal and mediastinal adenopathies. On abdominal ultrasonography we detected diffuse splenomegalia and global hepatomegalia. In segment VI the patient presented a clearly shaped, 6.2 cm diameter, predominantly hyperecogenous diffuse nodule formation. We diagnosed the patient with diffuse non-Hodgkin's malignant lymphoma with small B cells, stage IVB, with peripheral discharge, based on the ganglion and osteo-medullary biopsy, we decided to perform diagnostic and therapeutic splenectomy. Histopathological examination confirmed the existence of multi-nodulary lymphomatosis determinations, with a confluent tendency. The alpha-fetoprotein was normal. Under COP polychemotherapy, the adenomegalias were diminished, but the hepatic node formation increased progressively, therefore the patient underwent hepatic punction-biopsy ultrasonographically guided, whereby a hepatic adenocarcinoma was detected. The patient is not a hepatic virus carrier and does not have metabolic syndrome. He has continued chemotherapy parallel with the treatment with tamoxifen. At present he has survived 20 months since the diagnosis was put.

Discussion/Conclusion: The lack of the risk factors for hepatocarcinoma and of the growth of the alpha-fetoprotein cannot refute a possible hepatocarcinoma when the patients with malignant lymphomas present hepatic tumor formations.
Liver vascular index in NASH, chronic hepatitis C and liver cirrhosis

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Introduction: The liver vascular index (LVI) is a sensitive and specific Doppler US parameter in the diagnosis of cirrhosis and portal hypertension.

Methods: We assessed LVI in 25 NASH (group I) and 40 chronic hepatitis C (CHC group II) with different stages of liver fibrosis. The results were compared with those of 40 patients with liver cirrhosis (LC group III) and 50 healthy volunteers (group IV). Portal and hepatic arterial Doppler US was performed in 3 consecutive days. LVI was calculated as the ratio of portal venous velocity to hepatic arterial pulsatility index.

Results: The LVI was up to 12 cm/s (the best cut-off value) in all healthy persons and CHC. In NASH group there was a large deviation and overlap in different stage of liver disease (from controls to liver cirrhosis) -50%. The LVI was significantly lower (p < 0.001) in patients with LC than healthy controls and other liver diseases. It is under 13 cm/s in all cases. The intraindividual deviation between 3 investigations was observed in all investigated group from 2 to 6 points. No significant changes of Doppler US parameters between patients with mild (stage I or II) and severe fibrosis (stage III or IV).

Conclusion: Our results confirm that LVI is a good parameter to distinguish the liver cirrhosis from noncirrhotic stage of liver disease. It is not valid surrogate marker of liver fibrosis, especially in patients with chronic hepatitis C. Future studies are need to assess the potential diagnostic value and clinical impact of LVI in NASH.
Effect of molsidomine and sin-1 on trypsinogen activation in taurocholate pancreatitis in rats

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Introduction: Liver function in acute pancreatitis (AP) is impaired. Effect of nitric oxide donor sin-1, hepatic metabolite of molsidomine (M) on trypsinogen activation in severe AP was not investigated.

Methods: Taurocholate AP was induced in 76 male Wistar rats; 12 were sham operated (SO). AP rats were treated with M (0.1, 0.5, 1.0 mg/kg i.p.) or sin-1 (0.02, 0.1, 0.2 mg/kg i.p.) at 0, 6, 12, and 18 h of AP. After 24 h, free (F) and total potential trypsin (T) in 12000xg supernatant of pancreatic homogenate was assayed. %F/T indicated trypsinogen activation.

Results: F trypsin (mcg/mg protein) increased in untreated AP (2.49 ± 0.32 vs. 0.60 ± 0.10 in SO, p < 0.001). M attenuated this increase: 0.97 ± 0.16, 0.58 ± 0.09, 0.63 ± 0.17 (p < 0.001) dose respectively, whereas sin-1 to 0.42 ± 0.10 (p < 0.001), 1.56 ± 0.25 (p < 0.05), 1.27 ± 0.31 (p < 0.02). %F/T increased to 29.2 ± 5.0 in AP vs. 5.4 ± 0.9 in SO (p < 0.001). Its values in M groups were lower: 11.0 ± 2.2, 7.6 ± 2.6 and 6.5 ± 2.7% (p < 0.01). In sin-1 groups these values were also diminished: 5.4 ± 2.2 (p < 0.001), 15.8 ± 3.3 (p < 0.05), 11.3 ± 2.9 (p < 0.01).

Discussion/Conclusion: Both molsidomine and its hepatic metabolite sin-1 (5 x lower doses) attenuated trypsinogen activation in severe taurocholate AP in rats. Effect of higher dose molsidomine was comparable to the effect of low dose sin-1. Sydnonimine NO donors could act beneficially in severe AP by attenuation of premature trypsinogen activation in pancreas.
IFN-γ abrogates profibrogenic TGF-β signaling in liver by targeting expression of inhibitory and receptor Smads

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In chronic liver disease, approaches opposing profibrogenic activities of TGF-β may be amenable. According to experimental models IFN-γ counteracts several TGF-β effects. Here, the impact of IFN-γ on hepatic fibrosis development was investigated in patients with chronic hepatitis B viral (HBV) infection and hepatic stellate cell (HSC) activation mechanisms were elucidated.

In a randomized open-labeled multicenter trial 83 patients with chronic HBV infection were enrolled. 54 patients received a nine-month IFN-γ treatment, whereas 29 patients served as controls. Histology and serum indices displayed a beneficial outcome in the IFN-γ treatment group with decreased fibrosis scores according to the modified Chevallier criterion and reduced number of α smooth muscle actin-positive HSC.

In vitro studies with HSC demonstrated that TGF-β-dependent activation of (CAGA)$_9$-MLP- Luc, a highly sensitive TGF-β reporter construct, was significantly decreased by IFN-γ, indicating a TGF-β antagonizing function. IFN-γ induced the activity of the Smad7 promoter and Smad7 protein expression via STAT-1 signaling. Immunostainings of liver biopsies indicated that disease-dependent TGF-β signaling was abrogated by IFN-γ-treatment as nuclear phospho-Smad2 and Smad3 staining was predominant in damaged tissue and absent after IFN-γ treatment, whereas strong cytoplasmatic Smad7 staining was only observed after IFN-γ application.

In summary, IFN-γ displays beneficial anti-fibrotic effects in patients with chronic HBV infection via STAT-1 phosphorylation, upregulation of Smad7 expression and impaired TGF-β signaling.
Concentration of antioxidative vitamins and selected microelements in the serum of patients with chronic hepatitis C

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Introduction: The aim of the study was to determine the concentration of selected pro and antioxidation factors in the serum of patients with chronic hepatitis C.

Methods: The group that underwent the investigation consisted of 19 patients (6 males and 13 females) aged from 20 to 73 years with diagnosed chronic hepatitis C. The diagnosis was made due to physical, serological (positive: a-HCV, HCV-RNA, genotype 1) and histopathological (liver biopsy G1S2) examinations. The group control consisted of 20 healthy volunteers. Patients and volunteers negated tobacco smoking, alcohol consumption and the use of vitamins or microelements. In the serum of the both groups there was determined the concentration of following vitamins: A, C, E and β-carotene (spectrometer Lambda 14P-Perkin Elmer Co.) and microelements: zinc, selen, copper, iron (atomic emission spectrometer PU-Philips Co.).

Results:
Vitamins' concentration:

<table>
<thead>
<tr>
<th></th>
<th>A (µmol/l)</th>
<th>β-carotene (µmol/l)</th>
<th>E (µmol/l)</th>
<th>C (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>1.26 ± 0.26</td>
<td>1.50 ± 0.26</td>
<td>29.67 ± 3.2</td>
<td>67.29 ± 6.96</td>
</tr>
<tr>
<td>Group control</td>
<td>1.7 ± 0.49</td>
<td>2.87 ± 0.75</td>
<td>32.95 ± 4.96</td>
<td>69.77 ± 12.8</td>
</tr>
<tr>
<td>p</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>p &gt; 0.05</td>
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Microelements' concentration:

<table>
<thead>
<tr>
<th></th>
<th>Fe (µg/dl)</th>
<th>Cu (µg/dl)</th>
<th>Zn (µg/dl)</th>
<th>Se (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>181.68 ± 21.22</td>
<td>107.05 ± 14.74</td>
<td>62.05 ± 19.27</td>
<td>6.07 ± 1.63</td>
</tr>
<tr>
<td>Group control</td>
<td>137.75 ± 15.30</td>
<td>87.45 ± 14.21</td>
<td>114.4 ± 17.67</td>
<td>9.88 ± 1.61</td>
</tr>
<tr>
<td>p</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
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Discussion/Conclusion: The results of our studies show that HCV infection disturbs the functioning of the antioxidative barrier of the host. They also suggest the need of vitamins' A, E and β-carotene supplementation and reduction of amount of iron and copper in the diet.
Peripheral blood dendritic cell subsets and serum interleukin-12 levels in patients with chronic hepatitis C virus infection: Relation to disease activity

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Introduction: Hepatitis C virus (HCV)-specific T-cell immune responses appear to influence the outcome of HCV infection and require activation by antigen presenting cells like dendritic cells (DC). Therefore, the present work was designed to study the DC subsets (myeloid and lymphoid) in peripheral blood and to assess the serum levels of interleukin-12 (IL-12) in patients with chronic hepatitis C in relation to disease activity.

Methods: 28 patients with chronic hepatitis C (15 with elevated serum levels of alanine aminotransferase (ALT) and 13 with persistently normal ALT levels) and 12 healthy subjects were included in the study. All patients had seropositivity for anti-HCV antibodies and HCV RNA. The percentages of DC subsets in peripheral blood were detected using 3-color flow cytometric assay. Dendritic cells were identified as lineage marker negative (lin-)/HLA-DR positive cells and the differentiation of myeloid DC subset from lymphoid DC subset was based on the expression of CD11c or CD123 on the cell surface respectively. Quantitative determination of IL-12p70 heterodimer in serum was performed using a solid phase sandwich enzyme-linked immunosorbent assay kit.

Results: The percentages of peripheral blood DC subsets (CD11c⁺ and CD123⁺), the CD11c⁺ DC/CD123⁺ DC ratio and the serum IL-12 levels were significantly lower in chronic hepatitis C patients than in healthy subjects and in patients with elevated ALT than in those with normal ALT (p < 0.05). The reduction in circulating DC subsets and serum IL-12 levels showed negative correlations with serum levels of ALT and the histopathological grading and staging scores (p < 0.05) but not with serum HCV RNA levels (p > 0.05). The serum IL-12 levels were positively correlated with the percentages of peripheral blood CD11c⁺ DC subset (p < 0.05).

Discussion/Conclusion: Patients with chronic HCV infection had significant deficiencies in circulating DC subsets, particularly the myeloid subset and in IL-12 production, which were well correlated with disease activity and hepatocellular injury. These findings suggest that DC and IL-12 may play a key role in the progression of liver disease in chronic HCV infection and may provide a potential new goal for HCV immunotherapy.
An assessment of cardiovascular morbidity and mortality following orthotopic liver transplantation

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Introduction: Cardiovascular (CV) disease is a major cause of morbidity and mortality in the first year post OLT and, in the limited studies performed to date, it accounts for between 30 and 70% of major clinical events.

The American College of Cardiology (ACC) has issued guidelines aimed at identifying patients at risk of cardiac disease. The aim of this study was to a) document the prevalence of CV risk factors pre-transplantation in OLT recipients and b) the incidence of CV events following OLT. We also evaluated the use of ACC clinical predictors as a guide to identifying patients in a high-risk group.

Method: Single centre retrospective observational study. We studied 100 consecutive patients who underwent OLT from July 2000-December 2002. Only patients with chronic liver disease who were undergoing elective OLT were included. Cardiac risk factors were identified at transplant assessment and patients were followed for 6/12 post OLT. The incidence of CV events or cardiovascular death was recorded. Predictors of CV risk as defined by ACC guidelines; two or more of the following (obesity, hypertension, smoking, elevated total cholesterol, family history of premature CV disease, age > 50 years) or one of the following (previous MI or CVA, abnormal echocardiogram, evidence of an arrhythmia, LBBB, ST or T wave changes on ECG). A cardiac event was defined as CV death, non-fatal myocardial infarction, hospitalisation for myocardial ischaemia or cardiac failure, stroke or transient ischaemic attack, or coronary revascularisation.

Results: 93 patients (56 males and 37 females) were studied in total. Seven patients were excluded (3 transplanted for acute liver failure and 4 retransplants). Indications for transplant were ALD 22 patients (23.7%), PBC 20 patients (21.5%), HCC 12 patients (12.9%), PSC 11 patients (11.8%), cryptogenic cirrhosis 11 patients (11.8%), hepatitis C 7 patients (7.5%), CAH 3 patients (3.2%) and other causes such as haemachromatosis, Wilson’s disease and Caroli’s syndrome accounted for the remaining 7 patients (7.5%). The mean age at transplant was 54.8 years. 21.5% of patients were smokers, 20.4% had a diagnosis of DM and 10.8% of patients had documented hypertension. Mean BMI was 26.6 with 28% of patients classified as obese with a BMI > 30. During the 6/12 follow up period 7 patients died (7.5%), with 2 deaths attributable to CV events. Non fatal CV events occurred in 10 patients (10.8%) (3 had MI, 1 CCF, 4 documented arrhythmias, 1 new onset angina and 1 CVA). Preoperatively 38.7% of patients were deemed to be at high risk of CV events with only 50% of total CV events occurring in this group.

Conclusion: 12.9% of our patients had a CV event within six months of OLT. The American College of Cardiology clinical predictors of CV risk did not identity the group of patients who are at increased risk of CV events post OLT, with half the patients being in the low risk group.
TGFβ1 and decorin induced extracellular matrix protein production of hepatic stellate cells and myofibroblasts

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Background and aims: Previous studies suggested that decorin might act as an inhibitory agent of fibrogenesis in experimental glomerulonephritis (1). We hypothesised a similar decorin effect in the liver on hepatic stellate cells (HSC) and myofibroblasts (MF), possibly via the inhibition of TGFβ1 effect using the MAPK signal-transduction pathway. Therefore we measured the expression, production of a few important extracellular matrix proteins after decorin, TGFβ1 stimulation and costimulation on HSCs and MFs.

Materials and methods: Primary cultures of rat HSC and MF from passages 3, 8, 12 (P1-14) were treated and cotreated with decorin and TGFβ1 (Sigma) on day 7. The protein synthesis was assessed by immunocytochemistry and Western blot analysis of cells and culture medium of HSC and MF using antibodies against collagen type I, III, IV (Col I, III, IV), laminin (Lamc1), fibronectin (Fn1). Relative messenger RNA level of the same genes, and also the TGFβ-inducible early growth response (Tieg), syndecan 2 (Sdc2), syndecan 1 (Sdc1), was assessed by Real-time PCR using the ABI 7000 sequence detector system with either SYBR green® dye or TaqMan® probes (number of different real-time PCR runs: 1-5/gene). Primer pairs were designed for each gene using the Primer Express (version 2.0) software. Primer and probe sequences are available upon request. The mRNA expression was also assessed by Northern blot hybridization.

Results: The different decorin concentrations used resulted slight increase in mRNA levels of Col III and Lamc1 and did not alter the other mRNAs studied. Dose dependent negative feedback of decorin mRNA was observed. Higher mRNA expression of Col IV, Col I, Sdc1, Sdc 2 and Tieg was found in TGFβ1 treated hepatic stellate cells. In contrast, co-treatment of HSCs with decorin and TGFβ1 resulted in nonsignificant changes at mRNA level compared to untreated Ito cells nearly in all genes examined. We could not detect any remarkable change in myofibroblasts in mRNA expression of Col IV, Col II, Sdc 2, Tieg, and Lamc1 genes neither after TGFβ1 nor after decorin treatment and co-treatment (Figure 2 and 3). Protein expression on Western blots and on immunocytochemistry were in concordance with the mRNA results. The most remarkable effect after TGFβ1 treatment was observed on collagen type I protein.
Conclusions:

1. Hepatic stellate cells and myofibroblasts demonstrated different response to TGFβ1 exposure. In contrast to HSCs in which TGFβ1 upregulates different matrix protein mRNA levels and also increases the protein synthesis no significant changes could be detected in myofibroblasts.

2. Decorin itself has a limited effect on the matrix production of HSCs and MFs. However, when we applied it in combination with TGFβ1 it was capable to inhibit the action of the growth factor in HSCs.
Function of TGF-beta induced autocrine PDGF signaling in hepatocellular EMT

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Introduction: Upon cooperation of active Erk/MAPK and transforming growth factor (TGF)-beta signaling, differentiated MIM hepatocytes undergo an epithelial to mesenchymal transition (EMT), which is accompanied by acquisition of tumorigenic potential as well as metastatic properties. Thereby, TGF-beta induces an autocrine platelet derived growth factor (PDGF) secretion through upregulation of the PDGF-A ligand and both PDGF receptor subunits.

Methods: In order to elucidate the role of PDGF in hepatocellular EMT, we interfered with PDGF signaling either by employing hepatocytes expressing the dominant negative PDGF-alpha receptor and on the other hand by using the pharmacological inhibitor STI571.

Results: We found that in EMT converted hepatocytes, PDGF signals via PI3K - Akt and via STAT3, and that inhibition of both downstream pathways was associated with the strongly reduced capability of fibroblastoid cells to grow on plastic due to altered synthesis of ECM components and to changes in integrin expression patterns. Moreover, suppression of PDGF induced STAT3 signaling alone was sufficient to strongly decrease TGF-beta induced migration through transwell filters. Tumor formation after subcutaneous injection of cells into SCID mice showed that interference with PDGF signaling predominantly diminished tumor growth of TGF-beta mediated EMT hepatocytes, whereas tumor formation of epithelial cells remained unchanged. The contribution of PDGF to TGF-beta induced EMT was furthermore confirmed in vitro by pharmacological inhibition of the PDGF receptor with the tyrosin kinase inhibitor STI571.

Discussion/Conclusion: In conclusion, these results provide evidence for an essential role of PDGF in TGF-beta mediated tumor progression and suggest PDGF as a target for therapeutic intervention in liver cancer.
Heme oxygenase-1 over-expression increases liver injury after bile duct ligation in rats

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Introduction: Chronic cholestasis leads to liver injury and will finally progress to portal fibrosis, cirrhosis and end-stage liver disease necessitating liver transplantation. Heme oxygenases are the rate-limiting enzymes that catalyze the conversion of heme into biliverdin, CO and iron. Recently, there were several reports suggesting protective effects of heme oxygenase-1 (HO-1) against oxidant-induced injury and the induction of HO-1 as an adaptive response against oxidative damage. Accordingly, this study investigated if cobalt protoporphyrin (CoPP), a HO-1 inducer, would reduce hepatic injury and fibrosis caused by bile duct ligation (BDL).

Methods: Either CoPP or saline were injected intraperitoneally in male SD-rats. Three days later, BDL or sham-operations were performed. Rats were sacrificed 3 weeks after BDL and livers were harvested for histology. Fibrosis was evaluated by sirius red staining and image analysis. Alpha-smooth muscle actin was detected by immunohistochemical staining, and cytokine and collagen-I mRNA were detected using RNase protection assays.

Results: As expected, serum alanine transaminases increased about 8-fold above normal levels one day after BDL. Surprisingly, enzyme release was not reduced in rats receiving CoPP before BDL. Liver fibrosis was evaluated 3 weeks after BDL and the sirius red-positive area was found to be increased to about 8.7%. However, in livers from bile duct ligated rats pretreated with CoPP sirius red-positive areas were significantly increased to about 11.7%. Col-Iα mRNA increased about 20-fold by BDL, consistent with increases in collagen synthesis. Again, this effect was significantly increased by HO-1 over-expression. Transforming growth factor-β and tumor necrosis factor-α were increased significantly after BDL but no significant difference was observed between the CoPP or saline pretreated rats.

Discussion/Conclusion: Taken together, it is concluded that hepatic injury and fibrosis due to BDL is not reduced by the HO-1 inducer CoPP. In contrast, our data indicate that HO-1 over-expression increases liver injury in rats under conditions of experimental chronic cholestasis.
Reduced hepatic expression of PPAR-gamma coactivator 1 (PGC-1) in human cholelithiasis

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Introduction: the prevalence of cholesterol cholelithiasis in the Western world is high. Even if laparoscopic cholecystectomy represents a satisfactory treatment in most instances, some drawbacks need to be considered such as stone recurrence, pancreatitis due to biliary sludge and postoperative complications. Further insight in the pathophysiology and molecular regulation of biliary lipid secretion is therefore highly desirable.

Aim of the present study was to analyze the hepatic expression of a number of nuclear receptors involved in bile acid metabolism in human cholelithiasis.

Methods: surgical liver biopsies were obtained in 11 patients with untreated cholesterol cholelithiasis and 9 gallstone-free subjects; mRNA levels of CYP7A1 and related nuclear receptors and coactivators were assayed by real-time quantitative RT-PCR.

Results: No differences between the two groups were detected in gene expression of CYP7A1 and other nuclear receptors, with the exception of PGC-1 which was significantly (p < 0.01 on a log scale) less expressed in gallstone subjects. Expression of PGC-1 was linearly correlated with FXR both in the whole population (r = 0.55 on a log scale, p < 0.05) and in gallstone patients (r = 0.87, p < 0.01); in gallstone patients PGC-1 expression was also correlated with HNF-4 (r = 0.78, p < 0.01).

Discussion/Conclusion: PGC-1 appears to play a role in the prevention of cholesterol gallstone disease in humans; this might take place via interaction with the bile acid receptor FXR, whose protective role in cholelithiasis has been suggested by recent evidence in animal models. Involvement of target genes coding for biliary lipid transporters is likely. PGC-1 and related genes might therefore represent molecular targets for the prevention and/or treatment of gallstone disease.

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Autoimmune processes and alpha-interferon therapy of patients with chronic HCV-infection

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**Aim:** The study of autoimmune processes dynamics in the patients with chronic HCV infection in the background of alpha-interferon therapy.

**Material and methods:** 113 patients aged 18-49 years (46 men and 67 women) with chronic HCV infection were treated with alpha-interferon (alpha-IFN) preparations. The diagnosis of the ANA, AMA, ASMA, LKM, anti-GPSA autoantibodies was carried out by immunofluorescence method, antibodies to thyroglobulin by the radioimmune method, the level of cryoglobulinemia was determined by spectrophotometric method.

**Results:** From classical autoimmune diseases 13 (11.5%) patients developed autoimmune thyroiditis, 2 patients having it in combination with diabetes mellitus. Total frequency of the autoantibodies recording in patients before therapy made up 39.7% (26/68) in titers range 1:10-1:60 (ANA - 5.9%, AMA - 8.8%, anti-GPSA - 26.8%, LKM - 0%). During antiviral therapy the frequency of ANA recording increased in the group of "respondents" - in 21.9% patients in 6 months (p < 0.05) and in 23.3% in 12 months (p < 0.05) which subsequently - 6 months after therapy termination - didn't differ truly from initial data. Initially increased level of cryoglobulinemia in the patients with chronic HCV infection both in the group of "respondents" and "non-respondents" in relation to healthy persons (0.056 ± 0.007 opt. units; p < 0.001 and 0.033 ± 0.04 opt. units, p < 0.05 correspondingly) by 12 months of antiviral therapy decreased in compassion with initial data and didn't differ from the values of healthy persons.

**Conclusions:** Autoimmune reactions of temporary character were observed during alpha-interferon therapy. After completion of antiviral therapy many indices achieved the level of healthy persons.
Aagenaes syndrome - A case in unusual geographic area

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Introduction: The combination of neonatal recurrent intrahepatic cholestasis and lymphoedema represents a specific syndrome. The development of small lymphoid vessels is probably deficient around the small biliary tracts and in general. It is a rare entity outside Norway. We report a case with Aagenaes syndrome in a Bulgarian patient with no Norwegian ancestry.

Methods: The patient is a full-term male born with non-consanguineous parents. On the third day after birth hepatomegaly and severe jaundice presented. The liver biopsy showed giant cells and intrahepatic cholestasis. The icteric episode lasted several months and resolved. Psychomotor development was normal. At 8 years the child showed edema of the legs, at 12 years the right hand became edematous and at 13 years a part of the face and the eyelids in particular developed edema. Serum proteins, electrolytes, adrenal, thyroid, cardiac and renal functions were normal. The diagnosis was established at 29 years when jaundice reappeared with pruritus and lasted 4 months. The biopsy established cholestasis and moderate portal fibrosis. At 31 years severe cholestasis recurred for 3 months and responded to ursodeoxycholic acid. Since the appearance at age 8, the lymphoedema presents so far, becoming more prominent after physical strain. At 34 years the patient is anicteric with a moderate physical disability.
Therapy in non-alcoholic steatohepatitis (NASH). A challenge for the future

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Introduction: The aim of the study consisted in evaluating the efficiency of ursodeoxycholic acid, pentoxifillin, losartan, and atorvastatin in treatment of NASH.

Methods: Forty-eight patients with histologically confirmed NASH were enrolled between 2001 and 2005 (17 with type II diabetes, 12 hypertensive and 19 obese/dyslipidemic, non-hypertensive, and non-diabetic). The batch was divided in four groups: A (10 dyslipidemic patients, receiving atorvastatin 10 mg/day), P (13 non-hypertensive/non-dyslipidemic patients receiving pentoxifillin 400 mg bid.), L (12 hypertensive patients, treated with losartan, 50 mg/day) and U (12 non-diabetic/non-hypertensive patients receiving ursodeoxycholic acid 15 mg/kg/day). Mean duration of treatment was 37.8 ± 5.4 weeks, and mean age 55 ± 7.54 years old. Body mass index was determined at inclusion (mean BMI 31.45 ± 5.54) as well as alanine aminotransferase (ALT = 65.75 ± 19.55 UI/l), gamma-glutamyl transpeptidase (GGT = 46.12 ± 12.34 UI/l), total cholesterol (TC = 297 ± 31.34 mg/dl), triglycerides (TG = 156.2 ± 58.12 mg/dl) and alkaline phosphatase (AP = 259.76 ± 32.12 UI/l). Hepatic biopsy was performed at inclusion and at the end of treatment, evaluating necroinflammatory score, steatosis, and fibrosis using Brunt's criteria.

Results: In group A significant reduction of ALT, GGT, TC and AP was noticed. Histology showed diminution of steatosis but no fibrosis and necroinflammatory improvement. In group P we observed a reduction in mean ALT and GGT values and in necroinflammatory score, as well as in group L. Group U presented significant reduction in mean ALT and GGT values, without improvement in steatosis, necroinflammation or fibrosis.

Discussion/Conclusion: Atorvastatin and losartan showed efficacy in treatment of dyslipidemia- and hypertension-associated NASH, improving both biochemical parameters and steatosis/necroinflammation. Pentoxifillin showed similar efficacy in non-hypertensive and non-dyslipidemic patients with NASH, while ursodeoxycholic acid did not improved histologic aspects although determined significant amelioration of biochemical parameters.
Child-Turcotte scoring system and hepatorenal syndrome in cirrhosis. No positive correlation at all

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Introduction: The aim of the study was to identify any correlation between hepatorenal syndrome (HRS) and Child's score, as well as certain functional renal parameters as urinary density, urinary Na, serical/urinary urea nitrogen ratio, seric/urinary creatinine ratio, fractional excretion of Na (FENa) which might predict imminence of HRS.

Methods: From a cohort of 104 cirrhotic patients we selected 28 in which HRS was diagnosed by classic criteria: severe hepatic insufficiency with severe portal hypertension, absence of renal disease or fluid depletion, seric creatinine > 1.5 mg/dl, proteinuria < 500 mg/dl, lack of response at diuretic cessation, oliguria, serical Na under 40 mEq/l and urinary Na under 10 mEq/l. Thirteen patients were staged Child B and 15 class C. The following aspects were studied: 1) correlation between Child's score and HRS; 2) urinary abnormalities which might be candidate as alarm criteria for imminence of HRS.

Results: No significant correlation (p = 0.125) was identified between Child's score and HRS. The risk for developing HRS was similar in class B (12.5 ± 3.43%) and C (14.42 ± 4.32%). Among urinary indices with valuable predictability for development of HRS, significant correlation was identified between diminishing of urinary Na excretion below 20 mEq/l, augmentation of seric/urinary creatinine ratio to more than 40, and diminishment of FENa < 1 (p < 0.05). No significant correlation was identified between HRS and urinary density or seric/urinary urea nitrogen ratio.

Discussion/Conclusion: HRS did not correlate with Child's score in B and C stages. Urinary indices with predictive value for imminence of HRS are urinary Na excretion, seric/urinary creatinine ratio, and FENa.
A case of hepatocellular carcinoma associated with paraneoplastic erythema nodosum and polyarthritis

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Introduction: Hepatocellular carcinoma (HCC) may manifest various paraneoplastic syndromes. Such syndromes have been reported to be present in 10-20% of the patients with HCC. Sometimes combination of paraneoplastic syndromes in one HCC patient has been observed. We report a rare case of HCC associated with erythema nodosum and polyarthritis.

Methods: Case report.

Results: A 23-year-old woman was hospitalized for a liver tumour detected at abdominal ultrasonography. She had a 2-year history of erythema nodosum and polyarthritis, which poorly responded to corticosteroids and NSAIDs and were still present at admission. Fine needle aspiration biopsy revealed moderately differentiated HCC. No other reasons for erythema nodosum were revealed. Atypical resection of liver segments III, IV, V and part of segment II was performed and the diagnosis of HCC was confirmed by histology. Erythema nodosum and the polyarthritis disappeared completely 10 days after surgery without any treatment. Four years after the operation the patient is free of any symptoms.

Discussion/Conclusion: The clinical course of the disease strongly suggests that erythema nodosum and polyarthritis in this patient appeared as paraneoplastic syndromes of HCC. The case reminds the necessity of taking into account HCC as a possible cause for inexplicable and poorly controlled erythema nodosum and polyarthritis.
Does the type of diabetes mellitus influence the prevalence of NASH?

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Introduction: Non-alcoholic steatohepatitis (NASH) is becoming one of the most important aspects of metabolic liver disease. One area in which NASH has an important role is diabetes mellitus (DM).
The aim of our prospective study was to investigate if the type of DM, I - insulin-dependent or II - non-insulin-dependent is influencing the prevalence of NASH.

Methods: We studied a batch of 803 consecutive patients with DM, with a mean age of 52.31 years. From this, 272 had DM type I, mean age 46.38 years, 11.11% F - 88.89% M, with the mean BMI of 27.3. The other 531 subjects had DM type II, mean age 57.39 years, 57.99% F - 42.01% M, BMI - 31.62. We also calculated the mean value of cholesterolemia, triglyceridemia, HDL, STGO, STGP. Steatosis was determined by ultrasonography in all patients.

Results: NASH was present in 18 (6.6%) of the DM type I and 269 (50.66%) DM type II subjects (p < 0.0001 - ES). For the other compared variables there were not significant differences.

Discussion/Conclusion: NASH is present in a much higher prevalence in DM type II compared to DM type I subjects - 50.66% vs. 6.6% (p < 0.0001 - ES). Subjects with DM type I were younger, males were twice more frequent in DM type I as in DM type II. We can conclude that non-insulin-dependent diabetes mellitus has a much higher role in appearance of NASH as insulin-dependent diabetes.
Antigen-presenting cells in small bowel mucosa in patients with elevated level of anti-gliadin antibodies

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Introduction: The aim of our study was to determine the difference in quantity of antigen-presenting cells (APC) in small bowel mucosa between anti-gliadin antibodies (AGA)-positive and AGA-negative patients.

Methods: Macrophages and dendritic cells were determined using immunohistochemical staining with antibodies to CD68 and protein S-100 respectively. AGA (IgA and IgG) level was determined by ELISA in serum. 10 AGA-positive and 13 AGA-negative patients were studied.

Results: All the AGA-negative patients had normal small bowel mucosa. Distribution of the AGA-positive patients according to Marsh staging of celiac disease was the following: Marsh 0-5 cases, Marsh I - 2, Marsh IIIA - 1, Marsh IIIC - 2. Number of macrophages was significantly higher in AGA-negative group than in AGA-positive group (95% CI: 22.04-27.35 vs. 15.51-24.11 cells/hpf; p = 0.047) but number of dendritic cells didn't differ significantly (95% CI: 13.00-18.38 vs. 12.47-36.05 cells/hpf; p = 0.317). There was no correlation between Marsh grade and APC number in AGA-positive group. We found a significant reverse correlation between number of macrophages and dendritic cells (r= -0.558, p = 0.016; Spearman).

Discussion/Conclusion: High AGA level is associated with decreased quantity of macrophages in small bowel mucosa. We suggest that the disbalance between macrophages and dendritic cells could be responsible for AGA rising and play role, at least partially, in the pathogenesis of celiac disease.
Students, alcohol and the risk of liver disease

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Introduction: Alcohol consumption is a reality among teenagers and students.

Aim: To estimate the alcohol consumption, among Timisoara's students.

Methods: A questionnaire about alcohol consumption, diet, lifestyle was distributed to 105 students.

Results: The results revealed some concerning aspects. The students were informed about the complications of alcohol abuse. Although most of the students are occasional drinkers, for some of them, the motivation of alcohol consumption is the need of alcohol. Youths drink alcohol to get free from their shyness, to escape from their own inhibitions and consider it as a way to be accepted in a group. The main occasions of alcohol consumption were: social and official events (67%), followed by going out with friends (61%). In the top of students' preferences, beer is the first choice (59%), and they usually drink two bottles.

Discussion/Conclusion: Alarming is the fact that some students admit that they drove after they drank alcohol. While car crashes risks are double after one glass of wine, we can easily imagine, the danger they are assuming.
Informing teenagers and students about the liver consequences of alcohol abuse and educate them for a moderate consume of alcohol and for a healthy diet are very important, because they have to know when to say NO to alcohol.
Spatial complexity analysis of the hepatitis B virus infection on liver tissue sections

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Introduction: Although more than 400 million people worldwide are chronically infected by the hepatitis B virus, and it is responsible for more than 300,000 cases of liver cancer every year, the complex dynamics underlying the infection in humans is still today widely debated.

The aims of the present study are: a) to introduce a new index of the spatial complexity of the hepatitis B virus infection; and b) to investigate its behaviour during computer-simulated changes in infected cell density and distribution.

Methods: Prototypical liver biopsy sections were immunohistochemically treated to discriminate infected from uninfected hepatocytes using specific antibodies raised against the core antigen (HBcAg) polypeptide. The sections were subsequently digitised and the surface fractal dimension (Dₛ) automatically estimated. A computer-aided model was developed to simulate the geometrical complexity of a two-dimensional section of the liver tissue that automatically generates an unlimited number of images with a variable density of infected hepatocytes randomly distributed on a planar surface and separated from each other by uninfected cells.

Results: We found that Dₛ measure the geometric complexity arising from the intricate relationships of infected/uninfected cells. Moreover, Dₛ not only depends on the number of infected cells and their different degrees of contiguity and continuity (the two characteristics determining the so-called connectivity of the cellular component: i.e. from unconnected infected cells to the continuous cell network), but also on their density and spatial distribution.

Discussion/Conclusion: Our results show that Dₛ may be a useful index of assessing the spatial complexity of the hepatitis B virus infection. Furthermore, it could be of help in human viral pathology not only because of its ability to quantify drug-correlated density changes, but also because it can stage virus-related alterations and predict disease evolution.
Survival time after onset of ascites in liver cirrhosis - Retrospective analysis of outpatients

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Introduction: Ascites, esophageal varices, hepatic encephalopathy (HE), and hepatocellular carcinoma (HCC) are among the complications of liver cirrhosis, and each of them causes deterioration in the outcome of the disease. Ascites results not only from increased sinusoidal pressure but also from sodium retention that in turn results from vasodilatation and activation of neurohumoral systems. Ascites affects not only hepatic but also renal functions. Ascites is becoming a condition that can be treated with diuretics, albumin preparations, ascites reperfusion, and portacaval shunting, but the prognosis of patients with ascites is still poor.

Patients, methods and aim of the study: In the present study the survival time after onset of ascites was investigated by means of a retrospective analysis in 39 (35 men, 4 women) from a total of 292 patients with decompensated liver cirrhosis treated from 1992-2004 in a practice specializing in gastroenterology and hepatology.

Results: The cumulative survival rate after the onset of ascites was 59% after 1 year, 35.9% after 2 years, and 12.8% after 5 years respectively. The cause of death was liver failure in 13 (33.3%), HCC in 8 (20.5%), rupture of gastric or esophageal varices in 4 (10.3%), renal failure, liver coma, extrahepatic tumor in each case 3 (7.7%), hepatorenal syndrome and heart failure both in 2 (5.1%), and cerebral hemorrhage 1 (2.5%). Episodes of HE between state I-III occurred in 37 of the 39 patients.

Conclusions: Results confirm that ascites, which often complicates liver cirrhosis, is a factor that deteriorates the outcome of the disease. Renal function is usually reduced in liver cirrhosis, particularly in patients with ascites. In addition, ascites is a risk factor of variceal bleeding, and for the development of HE. Therapy of ascites is aimed at creating a negative sodium and water balance and, if this strategy proves inadequate, at decreasing portal pressure by portosystemic shunting. It requires a high level of compliance in drinking and taking of medication and good cooperation between patient and doctor to ensure better long-term success in ascites management.
Endocrine cells in the large bile duct in obstructive jaundice

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Introduction: Endocrine cells (ECs) have been found to be widely distributed in the epithelial layer in organs that originated from primitive gut. As to the biliary tract in humans, there have been several reports describing ECs only in the gallbladder. We examined the presence of ECs in the lower part of the human large bile duct in obstructive jaundice.

Methods: Surgical biopsy specimens were obtained from 43 patients and 7 controls (from autopsies). 24 patients had acute obstruction and chronic exacerbated cholangitis (CAC) and 19 had chronic sclerotic cholangitis (CSC). ECs were investigated immunohistochemically with anti-gastrin, anti-serotonin, anti-somatostatin, anti-secretin, anti-chromogranin A (CHA) and antisynaptophysin antibodies.

Results: The numbers of CHA-positive ECs were as followed: 0.856 ± 0.666 cells/mm² in CAC; 1.03 ± 0.863 cells/mm² in CSC; and 0.33 ± 0.157 cells/mm² in controls. In controls we observed gastrin-, serotonin-, somatostatin-, secretin-, CHA- and synaptophysin-positive ECs in the intramural glands. In patients we observed increased number of the same cells. Ultrastructural immunohistochemistry revealed the characteristic granules for gastrin-, somatostatin- and secretin-positive cells. Serotonin-positive cells had two types of granules ovoid or discoid and pleomorphic. M-cells were also observed electronmicroscopically. They had the smallest granules.

Discussion/Conclusion: It can be concluded that the lower part of the human large bile duct contains a great diversity of EC types, which are usually met in the stomach and the intestine. ECs may be involved in the motility, secretion and bile drainage of the choledochus.
Antioxidant status of non-alcoholic fatty liver patients with non-organ specific autoantibody positivity

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**Introduction:** Autoantibodies are natural or pathological immunoglobulins that react against normal host protein. Non-organ specific autoantibodies (NOSA) can be found in the general population as well as in a variety of non-autoimmune chronic liver disease. Importance of NOSA positivity in non-alcoholic liver disease (NAFLD) is uncertain. Increased prevalence of NOSA in NAFLD may be the result of primary immune-mediated mechanism or secondary hepatocellular injury as a consequence of free radical reaction and cytokine production. Our aim was to investigate the NOSA prevalence and the redox status as well as cytokine level in NAFLD patients.

**Methods:** Plasma free SH-group concentration, total antioxidant status were measured by colorimetric methods. Chemiluminescence intensity was determined by a chemiluminimetric method. IL-6 concentration was measured by ELISA in various group of NAFLD patients.

**Results:** NOSA prevalence was found to be 55%. NOSA-positive patients showed a decrease of plasma free SH-group concentration, total antioxidant status and strengthening of free radical reaction. Elevated IL-6 concentrations were measured in both patient groups, but IL-6 concentration was higher in ANA-negative group with better antioxidant status.

**Discussion/Conclusion:** The pathogenesis of NAFLD undoubtedly have immune-mediated component. We suppose that the appearance of autoantibodies in NAFLD is triggered by free radical reaction, but further investigations are needed to understand the significance, role and the exact mechanisms of NOSA production in NAFLD. We suppose that similarly to alcoholic liver disease the aldehyde products of lipid peroxidation can react with proteins and form stable protein adducts, which are very immunogenic and capable of inducing immune response, resulting in generation of antibodies.
Clinical features associated with spontaneous viral clearance in mother-to-infant hepatitis C

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Introduction: The aim of the study - to investigate clinical features associated with outcome of mother-to-infant hepatitis C (spontaneous viral clearance or chronic hepatitis development).

Methods: 32 children (14 male, 18 female) with mother-to-infant hepatitis C not treated with interferon were observed in Minsk Children's Infectious Hospital, Belarus. Mother-to-infant hepatitis C diagnosis was confirmed in case of serum HCV-RNA detection by PCR in 3-9 months old infant 2 times with 3 months interval (n = 29) or anti-HCV detection in > 18 month old infant (n = 3). Spontaneous viral clearance was confirmed if previously HCV-RNA positive infant was found to be HCV-RNA negative 2 times. US-scanning and routine biochemical tests data were analyzed.

Results: Spontaneous viral clearance had 37.5% (12 patients) at mean age 12.8 ± 3.0 months. We’ve shown statistically significant differences in some clinical features in group with viral clearance compared to group with chronic mother-to-infant hepatitis. There was high max serum ALT level (mean = 154.3 ME/ml, 95% CI = 97.6-210.9 vs. mean = 80.4 ME/ml, 95% CI = 46.7-114.1, p = 0.0262) and presence of unconjugated bilirubin level > 80 mkmol/ml in 1-month infant (28.6% vs. 1.98%, p = 0.0477). Spontaneous viral clearance correlated with max serum ALT level (gamma = 0.38, p = 0.0128), bilirubin level in 1-month infant (gamma = 0.34, p = 0.0221) and presence of unconjugated bilirubin level > 80 mkmol/ml in 1-month infant (gamma = 0.77, p = 0.0039).

Discussion/Conclusion: High max serum ALT level and high bilirubin level in 1-month infant associated with spontaneous viral clearance in mother-to-infant hepatitis C.
Expression of claudins in human hepatoblastoma

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Introduction: Claudins are recently described transmembrane proteins of the tight junctions and might play an essential role in carcinogenesis. We aimed to characterize the expression of different type of claudins as 1, 2, 3, 4 and 7 in human hepatoblastoma with respect to expressional differences between the fetal and embryonal epithelial components.

Methods: Fourteen surgically resected (formalin fixed, paraffin embedded) specimens were analysed by immunohistochemistry. Monoclonal mouse antibodies were used to detect claudin-2, -4, PCNA, Ki-67 and HSA, rabbit polyclonal antibodies for claudin-1, -3 and -7 expression. Claudin-1, -3, -4, -7 mRNA expressions were measured by real-time (RT)-PCR.

Results: Immunohistochemistry showed strong staining of claudin-1 and -2 in the fetal components, while embryonal cells showed scattered positivity only. Claudin-3 and -7 expression was mostly negative. Claudin-4 protein was negative in the tumor cells. By real-time PCR claudin-1 and -2 were found significantly upregulated in the fetal cell types dissected from hepatoblastomas compared to the embryonal cell types (23-fold; p = 0.001, 8.5-fold; p = 0.001 respectively). Claudin-3, -4, -7 did not show significant difference between the fetal and embryonal components by RT-PCR. PCNA and Ki-67 were significantly increased in the embryonal cells, while HSA expression was stronger in fetal cells.

Discussion/Conclusion: Our results suggest that claudin-1 and -2 are characteristic markers of differentiation in hepatoblastomas and associated with lower proliferation rate. The latter findings indicate an association between the activation of the wnt pathway in the more differentiated phenotype.

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Effect of losartan on early liver fibrosis development in a rat model of non-alcoholic steatohepatitis

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Introduction: Non-alcoholic steatohepatitis (NASH) is a metabolic disorder of the liver which may evolve to fibrosis or cirrhosis. Recent studies have shown reduction of experimental liver fibrosis with the use of angiotensin converting enzyme inhibitors or angiotensin receptor antagonists.

Aim: To study whether losartan can influence the early phase of fibrogenesis in an animal model of NASH

Methods: To induce NASH, a choline-deficient diet (CDD) was given to Sprague-Dawley rats for 12 weeks. Rats were allocated to three groups: control group receiving choline-supplemented diet (CSD), CDD group fed with choline-deficient diet (CDD) and NASH-L fed a CDD and receiving losartan (10 mg/kg/day) in drinking water. Biochemical [serum levels of alanine aminotransferase and aspartate aminotransferase] and histological evaluation of fatty liver was performed by conventional techniques. Hydroxyproline content in liver tissue was assayed by spectrophotometry. In addition, mRNA levels of Pro-collagen I and transforming growth factor \(\beta\) were assessed by semi-quantitative RT-PCR and stellate cell activation by actin immunofluorescence stain.

Results: After 12 weeks CDD induced a marked elevation of serum aminotransferases, a severe fatty liver infiltration with mild histological inflammation and fibrosis. These findings correlated with a significant increase in mRNA levels of both Procollagen I and TGF\(\beta\)-1 and significant increased liver hydroxyproline content. No differences were seen between rats receiving CDD alone and rats receiving CDD diet and losartan with regard to the biochemical, morphological or molecular alterations induced by the CDD.

Discussion/Conclusion: Losartan does not seem to influence liver injury and fibrogenic events in the CDD model of NASH.
Characteristics of HBV genotypes in the Republic of Karelia

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Introduction: Genotype A HBV is the most prevalent one in Northern Europe, and chronic hepatitis caused by genotype A is characterized by a mild course according to literary data. HBV genotypes prevalence in the North-West of Russia has not been studied sufficiently. The objective of our study was to investigate the prevalence of HBV genotypes in the North-West of Russia and to determine the effect of HBV genotype on the course of the disease.

Methods: 60 chronic hepatitis patients were examined. Serological tests were carried out and HBV DNA in the serum and liver tissue was analyzed by PCR. Genotyping was performed using PCR-RFLP of the preS/S- and precore/core-regions.

Results: Genotype D HBV was revealed in the majority of cases (68.3%), genotype A - in 18.3% of cases, and in 13.3% of patients the genotype could not be determined because of the low viral load. Chronic hepatitis caused by genotype A was characterized by a more severe course and progressed into liver cirrhosis in 18.2% of patients in the second decade of the disease. In contrast, chronic hepatitis caused by D-genotype had a milder course and progressed into liver cirrhosis only in 9.8% of patients in the third decade of the disease. The necrotic-inflammatory syndrome was more pronounced in genotype A, than in genotype D.

Conclusion: Our results show that genotype D HBV, which is found in 68.3% of hepatitis B patients, is the most prevalent in the republic of Karelia. Genotype A HBV causes a more aggressive disease course than genotype D.
Assessment of histological features of the changes in lipid metabolism in chronic hepatitis C

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Introduction: Liver steatosis is common in patients with chronic hepatitis C (CH-C), occurring in ca. 50% of biopsy specimens. Several data suggest interactions between HCV and lipid metabolism. Nevertheless steatosis is a frequent histological lesion in CH-C, this phenomenon was not analyse as an integral part of the grade in the modified Knodell scoring system.

Aim of this study was to assess relationhip between degree of steatosis and serum lipid levels in CH-C patients in correlation with the histological picture.

Patients and methods: 137 CH-C patients were studied and compared to patients with steatosis of other reasons and negative for HCV. Paraffin-embedded biopsy materials were analysed using Knodell scoring system. Steatosis was graded semiquantitatively as: absent (grade 0), mild (involving less than 10% of hepatocytes, grade 1), moderate (involving 10-30%, grade 2), severe (involving more than 30%, grade 3). Alanine aminotransferase (ALT) activity, total cholesterol and triglyceride concentrations, presence of HCV RNA were measured in sera of patients. Statistical analysis was performed by one way ANOVA.

Results: Steatosis was detected in 85 CH-C specimens (62%). ALT levels were significantly higher in patients with grade 3 steatosis in the CH-C group, compared to grade 0 steatosis with CH-C (p < 0.01). Total cholesterol and triglyceride concentrations were significantly higher (p < 0.0001) in patients with steatosis only (without HCV) than in patients with CH-C with or without steatosis. Inserted the degree of steatosis (grade: 0-3) as a fourth character of the grade of the inflammatory process, there was significant correlation between the ALT level and grade of the inflammatory activity in CH-C patients not only in the group without steatosis, but in CH-C patients with steatosis too.

Conclusion: Steatosis in CH-C patients is associated with a significant decrease of cholesterol and triglyceride levels compared to patients with steatosis only. The degree of steatosis is an integral part of the morphological lesions in the liver biopsy induced by viral infection. These changes are confirmed in the ALT levels. Our results suggest modification of the Knodell scoring system used previously.
Increased cholesterol and sitostanol absorption and reduced biliary cholesterol secretion in ATP-binding cassette transporter Abcg8 (-/-) mice

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Introduction: Sitosterolemia is caused by mutations in either ABCG5 or ABCG8 alone, but concurrent mutations of the heterodimer ABCG5/G8 pair have not been observed. Whether the phenotypes induced by each individual gene differ is unknown. In this work we explored the consequence of targeted deletion of the Abcg8 gene.

Methods: Abcg8 (-/-) mice in a C57BL/6J background were generated by targeted mutation of the Abcg8 gene. Using chow-fed male mice (n = 5 each) with intact biliary lipid secretion, intestinal absorptions of cholesterol and sitostanol were determined by direct measurements of lymphatic transport and by a sterol balance method. Uptake of trace [14C]cholesterol and [3H]sitostanol by the enterocyte were determined at 45 min after the duodenal infusion. Expression of the cholesterol influx transporter Npc1l1 in the intestine was assayed by real-time PCR.

Results: Lymph flow (260-290 µl/h) was constant and similar in both mouse strains over the 12 h period. Absorption and lymphatic transport of radiolabeled cholesterol and sitostanol in (-/-) mice (56.2 ± 8.7% and 24.6 ± 2.9%) was increased by 40% and 500%, respectively, compared with wild-type mice (39.8 ± 3.7% and 4.0 ± 0.4%). Both (-/-) and wild-type mice ate similar amounts of food containing 0.81 mg/day of cholesterol. However, the sterol balance study revealed that daily biliary cholesterol outputs and fecal total neutral steroid excretion in (-/-) mice (1.30 ± 0.08 mg/day and 1.60 ± 0.08 mg/day) were significantly (p < 0.0001) decreased compared with wild-type mice (2.14 ± 0.10 mg/day and 2.58 ± 0.10 mg/day). An input-output analysis determined that absorbed cholesterol mass in (-/-) mice (0.52 ± 0.09 mg/day) was significantly higher than in wild-type mice (0.37 ± 0.05 mg/day). In agreement with lymphatic transport data, calculated percent cholesterol absorption in (-/-) mice was 63 ± 10%, significantly greater than in wild-type mice (45 ± 4%). However, at 45 min after the duodenal infusion, the intestinal uptake rates of trace [14C]cholesterol and [3H]sitostanol were similar between chow-fed (-/-) and wild-type mice, as were relative mRNA levels for Npc1l1 in the duodenum, jejunum, and ileum.

Discussion/Conclusion: Disruption of Abcg8 alone significantly increases intestinal mass cholesterol and sitostanol absorption and reduces but does not eliminate hepatic secretion of cholesterol. Knockout of Abcg8 does not influence intestinal expression of Npc1l1 nor acute uptake of cholesterol and sitostanol by the enterocyte. Hepatic secretion of biliary cholesterol is reduced by 40% in (-/-) mice, suggesting that an Abcg5/g8-independent pathway plays an important role in biliary cholesterol secretion.
Gene expression analysis identifies novel genes participating in early murine liver development and liver regeneration

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Since the current knowledge of parallels between liver morphogenesis and tissue repair is still incomplete, we studied gene expression at different stages of embryonic liver development using chip technology. This approach resulted in the detection of 44 transcripts that were strongly up-regulated in the liver at embryonic day 9.5. To analyse a subset of the newly identified transcripts in liver regeneration, gene expression was analysed after partial hepatectomy by quantitative RT-PCR.

Introduction: Liver morphogenesis is characterised by the co-ordinated expression of many genes and molecular cascades involved in adult tissue regeneration may recapitulate molecular events of organogenesis.

Methods: RNA was prepared of embryonic liver tissues at d9.5, d11.5 and d13.5 p.c. and used for chip hybridization (Affymetrix). To study a subset of the identified genes in liver regeneration, BALB/C mice were subjected to partial hepatectomy. Therefore, RNA was prepared from the liver lobes (n = 3/stage) at 0, 2, 4, 6, 12, 24, 48 h post-hepatectomy. Expression of selected genes normalized to GAPDH was measured by "real-time"-PCR.

Results: First results show that two of the genes that were strongly expressed in the fetal liver GAP43 and PITX2 are exclusively expressed 24 hours posthepatectomy, while the genes Twist1 and Zic1 each showed a specific expression profile in the regenerating liver with peak expressions at 4 and 6 hours, respectively.

Discussion/Conclusion: Pathway analysis of the newly identified genes indicate the involvement of GAP43, Zic1 and PITX2 in liver development/differentiation, e.g., PITX2 is regulated by FGF8 that is described to contribute to the outgrowth of the hepatic endoderm.
In summary the present data reveal that the analysis of the genetic program of the developing liver not only lightens the embryonic morphogenesis of the liver itself, but also may raise new insights into diseases.
Non-alcoholic fatty liver disease (NAFLD): Specific G-protein β3 subunit polymorphism (C825T) associates with higher degrees of steatosis and ALT levels

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Introduction: Obesity is the main risk factor for developing NAFLD. The spectrum of NAFLD ranges from simple uncomplicated steatosis to steatohepatitis, which correlates with high aminotransferase levels. Genetic factors might explain why only some patients progress to fibrosis. As recent studies revealed that a G-protein β3 subunit gene polymorphism (C825T) associates with obesity, we earlier postulated that altered G-protein receptor coupling may provide a clue for explaining this bifurcation.

Methods: Histopathology and aminotransferase levels were assessed in 50 patients with NAFLD. In genomic DNA isolated from blood leukocytes, C825T polymorphisms identified by PCR and restriction analysis were correlated with aminotransferase levels, age, BMI, and lipids.

Results: In all subjects with NAFLD, genotype distribution was 24 CC, 19 TC, and 7 TT. In patients carrying the TC genotype, the degree of steatosis was 36.2% more pronounced than in CC carriers and 74.1% higher than in TT carriers. Importantly, elevated ALT values indicating liver damage significantly correlated with the TC genotype (100%), but not genotypes CC (68%) or TT (56%). We found no correlation with age, BMI, or lipids.

Discussion/Conclusion: Our preliminary data indicate that a heterozygous GNB3 825T carrier status associates with elevated steatosis and ALT. We suggest that genotyping the GNB3 locus may provide a future tool for identifying individuals at risk for obesity and the progressive form of NAFLD, thus enabling preventive changes in lifestyle.
Radical versus conservative treatment of hepatic hydatidosis - Twenty-five years experience

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The author report the experience leaded above one hundred patients who underwent surgical procedures for hepatic hydatidosis.

Introduction: Hydatid disease is quite rare in European country outside the endemic area around the Mediterranean Sea. The liver is the most frequent seal of hydatidosis and occurs about 70% of cases. Surgery remains the principal treatment.

Methods: Between 1980 and 2005 one hundred patients underwent surgery for hydatid liver disease between 3 and 78 years (median age 49). 65 (65%) underwent radical treatment-resection and cystopericystectomy; 35 patients underwent non-radical treatment-partial peripheral excision, cystectomy, dissection of the cyst with aspiration of the contents.
49 patients had unique hydatid cyst: 26 total peripheral excision, 7 hepatic resection, 5 partial peripheral excision and 11 cystectomy. 16 patients had multiple hydatid cysts: 3 left hepatectomy, 9 peripheral excision plus cystectomy, 2 left hepatectomy plus cystectomy, 2 left hepatectomy and peripheral excision.

Results: First group showed a mobility of 11% (3 pleural effusions, 3 pneumonias and 1 hyperpyrexia). Second group showed a mobility of 30% (p = 0.02): 6 infections of residual cavity, 3 biliary fistula and 1 subphrenic abscessus and 1 pleural effusion. The recurrences were observed in second group in 6 cases and no observed in the first group (p = 0.05).

Discussion/Conclusion: The radical treatment is the technique of choice if compared with the non-radical treatments in terms of mobility and eventual recurrences. In childhood method of choice is conservative surgical procedures.
Helicobacter pylori infection and microscopic colitis

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Introduction: We noted that H. pylori is frequently found in gastric biopsy specimens taken from patients with microscopic colitis. In addition, metronidazole, a drug used for treatment of H. pylori infection may also treat microscopic colitis. Therefore, we decided to study the prevalence of H. pylori in patients with microscopic colitis, wishing to find out is there an association between these two disorders.

Methods: Gastroscopies and gastric biopsies with H. pylori staining were performed in 29 patients with histologically proven microscopic colitis. A group of 40 patients with irritable bowel syndrome who underwent the same procedure served as a control group.

Results: H. pylori was present in 27 of 29 patients with microscopic colitis, but only in 17 from 40 patients with irritable bowel syndrome. This difference was statistically significant (p < 0.05).

Discussion/Conclusion: The results of our study confirm the association between H. pylori infection and microscopic colitis. This association does not have to be aetiological, but it is also possible that microscopic colitis could be the result of H. pylori infection (for instance, that it is related to H. pylori antigens present in the stool). In the case if those two disorders are related, treatment of Helicobacter pylori infection could also be an effective therapy for microscopic colitis and currently, for different indication, employed eradication therapies for Helicobacter pylori might result in decreased incidence and prevention of appearance of microscopic colitis in the future.
Autonomic neuropathy and QT-interval prolongation in alcoholic liver diseases - Possible markers of survival?

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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 (> 440ms) 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.

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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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Level of some proinflammatory cytokines and apoptotic activity of peripheral lymphocytes as a prognostic markers of unfavorable clinical course of chronic pancreatitis in elderly patients

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**Introduction:** Availability of control of the programmed cell death of pancreas cells in order to find new ways of effective prophylaxis and treatment of chronic pancreatitis (CP) could be considered.

**Methods:** The purpose of our research was determining of the level of tumor necrotic factor α (TNF-α), interleukin 6 (IL-6) and CD95 on peripheral lymphocytes of blood in CP patients for enhancing diagnostic and treatment. 84 elderly patients with CP and 25 practically healthy persons were studied.

**Results:** Reliable (p < 0.05) rise of IL-6 and TNF-α level was found in all CP patients. In factor analysis TNF-α > 80 picogram/ml and IL-6 > 400 picogram/ml are prognostically important and describe unfavorable course of CP. Hyperproduction of TNF-α stimulates apoptosis in TNF-dependent way. TNF-dependent apoptosis develops slower then CD95-dependent one (reliable (p < 0.05) rise of surface expression in all studied groups). This causes correction of the ratio of functional subclasses of T-cells in immune response. The maximum level of CD95 expression is connected with easy course of disease. With disease progress this level reduces. Necrosis of acinar cells causes hard course.

**Discussion/Conclusion:** Considering raise of apoptotic activity of peripheral lymphocytes accompanies the raise of apoptotic activity of pancreas, apoptosis of acinar cells reduces damage of tissue while exacerbation of CP and causes atrophy of acinar tissue, fibrosis and sclerosis of pancreas in the following course of disease.
Natural history of chronic hepatitis C in children

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Introduction: The aim - to investigate natural course of chronic hepatitis C (CHC) in children.

Methods: The course was analysed in 14 children with CHC, comparative analysis of morphological data among children (95) and adult (94) patients with CHC was performed. The diagnosis of HC was established according clinical, biochemical methods, US-scanning, identification of serologic markers of VHC, RNA HCV identification with PCR. Genotype 1b was revealed in 80% of children, 3a - 10%, 1B+3a - 4%, not identified - 6%. Morphologic data were analysed due score system for grading and staging by Knodell.

Results: HC infection progression was observed in 42.9% of children according Doppler measurements and in 14.3% - according liver biopsy. Spontaneous clearance of serum HCV was not observed. The duration of the disease in children was shorter than in adults, biochemical activity was less prominent. Morphologic grading revealed that moderate (12.2% in children, 28.3% in adults) and severe (0 and 6.5% simultaneously) activity were observed more often in adults. No significant difference was revealed in the stage of disease among children and adult pts according morphologic investigation: fibrosis was absent in 42.4% of children pts and 56.5% adults, fibrosis 1 - 42.4 and 32.6%, fibrosis 2 - 0 and 6.5%, fibrosis 3 - 2.2% and 13.6%, cirrhosis - 1.5 and 2.2% respectively (p < 0.05).

Discussion/Conclusion: The results of the investigation demonstrate the probability of HC-infection progression in children.
Port-site metastasis after laparoscopic cholecystectomy for benign gallbladder disease with an occult cholangiocellular carcinoma. A case report

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Introduction: Port-site metastasis after laparoscopy, well-known entity, after benign diseases is such a rare condition. We report a case of port-site metastasis after laparoscopic cholecystectomy for benign gallbladder disease with an occult cholangiocellular carcinoma.

Case Report: LS, is a 73-years-old, male patient. Laparoscopic cholecystectomy was performed in December 2002 for chronic cholecystitis due to cholelithiasis. The pathological examination of the surgical specimen was benign. 18 months later from the surgery there had been a granulation on the trocar site at the umbilicus. It was excised and was reported as well differentiated adenocarcinoma. On October 2004, there had been another nodular lesion on the abdominal wall, which was reported, malignant also. On December 2004 the patient has presented to us with jaundice, abdominal pain and high body temperature. The liver enzymes were elevated. The markers for acute hepatitis were negative. Intrahepatic biliary tract dilatation was seen at abdominal US. ERCP was done. The main choleduct was measured 9 mm, and 1.5 cm distally from the hilus there was a stricture for 2 cm distances. Intrahepatic biliary tract was dilated. After endoscopic sphincterotomy, a plastic biliary stent was implanted. The diagnosis after ERCP was cholangiocellular carcinoma. For six months from the diagnosis, stenting was done for three times due to cholangitic attacks. At the thoracic CT scan there was a metastatic lesion on April 2005.

Discussion/Conclusion: Port-site metastasis is a well-known entity after laparoscopy for malignant diseases. There were three cases of metastasis after benign diseases. In our case it was thought that there was an occult cholangiocellular carcinoma while the first operation done. Before the symptoms of cholangiocellular carcinoma, the port-site metastases were seen. In our opinion all pathologies on the port-site should be investigated. It may be the first sign of an intraabdominal occult malignancy.
Non-invasive assessment of liver fibrosis - Make it simply

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Introduction: In chronic viral hepatitis liver biopsy provides important information that guides treatment decision, but is invasive, associated with possible complication and in some patients is contraindicated.

Aim: 1. To determine the diagnostic utility of biochemical markers for assessment of liver fibrosis in patients with chronic viral hepatitis. 2. To test the predictive value of an introduced by us alpha2-globulin score, representing the difference between the levels of alpha2-macroglobulin and haptoglobin. 3. To compare alpha2-globulin score with Fibrotest - an accurate and already in practical use, noninvasive index of viral fibrosis, which includes alpha2-macroglobulin, haptoglobin and in addition apolipoprotein A1, gamma-GT, and total bilirubin, but is calculated in rather complicated formula.

Methods: Routine liver function tests and serum levels of alpha2-macroglobulin, haptoglobin, apolipoprotein A1, ceruloplasmin and IgG (measured by immunoturbidimetry) were compared with liver histology (METAVIR) in 103 patients with chronic viral hepatitis - 75 HCV and 28 HBV. Sixty-six patients had F2-F4 fibrosis and 22 of them had F4 fibrosis. The primary outcomes were significant fibrosis (F2-F4) and cirrhosis. Statistical analyze was done by receiver operating characteristic curves.

Results: The AUROC values of the parameters predicting significant fibrosis (p < 0.05) are present in ascending way as follows: alpha2-macroglobulin (0.667 ± 0.08), AST (0.716 ± 0.07), haptoglobin (0.740 ± 0.07), Fibrotest (0.740 ± 0.07) and alpha2-globulin score (0.772 ± 0.06). Cirrhosis was predicted by AST (0.709 ± 0.08), GGT (0.755 ± 0.07), IgG (0.781 ± 0.6), haptoglobin (0.832 ± 0.05) and alpha2-macroglobulin (0.895 ± 0.05). Alpha2-globulin score and Fibrotest more accurately predicted fibrosis (AUROC 0.941 ± 0.04 and 0.935 ± 0.03, respectively). Apolipoprotein A1, ceruloplasmin and ALT did not shown significant predictive values (p > 0.05).

Discussion/Conclusion: The proposed by us simple, cheap and easily calculated alpha2-globulin score is promising noninvasive test, which shows similar to Fibrotest predictive value for assessment of significant and advanced fibrosis.
The influence of active prophylaxis on epidemiology of hepatitis B

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Introduction: In 1993 in accordance with the intensive program of prevention and combating hepatitis B in Poland, the following groups received vaccinations: patients scheduled to undergo an operation, chronically ill patients and persons who have been in close contact with those infected with HBV. Carrying out all the guidelines of the program resulted in the decrease in contracting hepatitis B from 34.6/100 thousand in 1993 to 4.43/100 thousand in 2003. The purpose of the article was to analyze the epidemiological aspects and the way in which active prophylaxis was run in the patients hospitalized due to hepatitis B.

Methods: In the years 1998-2004 142 patients were hospitalized in Infectious Diseases Clinic in Bydgoszcz. On the basis of medical history and the results of biochemical and serological examinations the patients were diagnosed with hepatitis B. In the group of 142 patients there were 59 women and 83 men in the age bracket of 16-84 years old (average age 47 years old). In each case attempts were made to establish the way of contracting hepatitis B. Moreover, earlier vaccinations against hepatitis B were taken into consideration in anamnesis.

Results: The results have been presented in the table below.

<table>
<thead>
<tr>
<th>Infection route / year</th>
<th>1998</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical procedures</td>
<td>13</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Minor medical procedures</td>
<td>13</td>
<td>6</td>
<td>12</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Dental treatment</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Intravenous drug abuse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tattooing, piercing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Sexual contacts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HBV carrier in close family</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>13</td>
<td>5</td>
<td>7</td>
<td>6</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>43</td>
<td>19</td>
<td>28</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>• women</td>
<td>21</td>
<td>8</td>
<td>12</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>• men</td>
<td>22</td>
<td>11</td>
<td>16</td>
<td>9</td>
<td>7</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

Number of persons vaccinated against HBV before the disease (age)

<table>
<thead>
<tr>
<th></th>
<th>1998</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete vaccination</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Number of persons vaccinated with one dose of the vaccine before scheduled surgery (age)</td>
<td>2</td>
<td>1</td>
<td></td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* - a patient undergoing chemotherapy due to acute lymphocytic leukaemia
Discussion/Conclusion:

1. Nosocomial infections, including minor medical procedures, still remain the predominant way of contracting hepatitis B in Poland.

2. It is essential that a high sanitary standard should be pursued in all health care institutions and the protection of patients should not be limited to vaccinations.
Progressive familiar intrahepatic cholestasis (Morbus Byler): Different natural course and treatment in siblings of one family

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Introduction: Byler’s syndrome is a progressive familiar intrahepatic cholestasis, often manifested as neonatal cholestasis in the majority of cases.

Methods: In this work, we describe a case of a family with three children suffering from Byler’s disease. Genetic examination of these siblings (in 1984) has confirmed the diagnosis of Morbus Byler. There have been several cases of infant mortality among the fathers ancestors.

Results: The first child from the described family (P.P.) has suffered from a serious progressive icterus as well as liver cirrhosis and died in the age of five. The second child (I.P.) had an icterus on the third day after birth, with high levels of aminotransferases, alkaline phosphatase and with slightly increased levels of aminotransferases. Later on, the levels of bilirubin and alkaline phosphatase were varying, together with pruritus which remained the most serious subjective problem. Repeatedly performed liver biopsy (in 1979, 1984, 1997) has confirmed the presence of intrahepatic cholestasis with changes of hepatocytes. Laparoscopic examination in 1984 has revealed an increased brown-green liver. Long-term observation of the patient has revealed varying in the disease’s course. The levels of alkaline phosphatase were varying from double of the normal value to more times the normal value. Similar course of the disease is present also at the third child from this family (K.P.). The levels of bile acids in this case were extremely high.

Discussion/Conclusion: Genetic examination of these siblings (in 1984) has confirmed the diagnosis of Morbus Byler. I.P. has had a milder course of the disease when compared to P.P. and K.P. Since treated with ursodeoxycholic acid, I.P. has had an improvement of biochemical parameters, most of all there was a decrease in the levels of alkaline phosphatase. K.P. is now after liver transplantation in good shape.
Liver transplantation in 23-year-old patient with Crigler-Najjar syndrome

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Introduction: Crigler-Najjar syndrome (CN) belongs to familiar unconjugated hyperbilirubinemas caused by deficiency of liver enzyme UDP glucuronyltransferase (B-UGT).

Methods: 23-year-old patient with CN syndrome was until his 17 years treated with phenobarbital and phototherapy. The treatment with phenobarbital was temporarily interrupted in 1991 when it was substituted with zoxorin, but it later continued with phenobarbital.

Results: Since 1993, the bilirubin levels have been constantly increasing and reached the value of more than 500 μmol/l. In 1991 and in 1995, epi paroxysms were discovered repeatedly and the EEG finding was getting worse. The bilirubin levels raised up to 680 μmol/l, therefore treatment with orally administrated calcium hydrogenphosphate dihydrate and calcium carbonate was started. The usual therapeutic management (phototherapy and phenobarbital) was continued.

Discussion/Conclusion: The principle of the effect of calcium phosphate therapy is assumed to be in intestinal binding of unconjugated bilirubin to precipitate intestinal calcium phosphate. During this treatment, the bilirubin levels decreased by ca. 100-150 μmol/l. Patient tolerated the treatment well and it was continued until the liver transplantation in August 1996 in Hamburg. The patient is in good condition with good liver function. It can be assumed, that calcium phosphate enhances the efficacy of phototherapy by intestinal binding of unconjugated bilirubin which was secreted as bilirubin monoisomers into bile but reverted to unconjugated bilirubin in the intestine. In patient with CN syndrome, this treatment resulted in positive change in bilirubin level and can be used as an adjuvant therapy. Calcium phosphate supplementation may even provide a new therapy for CN patients. Patient tolerated the treatment well and it was continued until the liver transplantation in August 1996 in Hamburg. He is in good condition with good liver function.
The survival rate among the Slovak patients after liver transplantation


Background and study aims: Some data of Slovak patients with transplanted liver in various liver transplantation centers are presented in this work. The authors have introduced a survey of elective liver transplantation indications of these patients for the period under review, i.e. between IV/1991 and IV/2005; the most frequent complications, as well as actually administered immunosuppressive therapy. In the article the authors evaluate the most frequent chronic liver diseases in adults and children, which were indicated for liver transplantation in the Slovak Republic during the period under review.

Patients and methods: Patients after liver transplantation underwent objective examination with assessing the general status, complementary laboratory examinations (haematological, biochemical, immunological, virological, microbiological examinations), X-ray, morphologic liver examination, imaging examinations - in particular ultrasonographic examination including Duplex ultrasonography and further other examinations (gastrofibroscopy, ERCP, CT, MR, angiographic examination etc.), if necessary.

Results: 62 elective liver transplantations were accomplished in 59 Slovak patients at various transplantation centers (Prague, Bratislava, Vienna, Essen, Hamburg, Brno, Lyon, Tübingen, Leiden) during the time period between IV/1991 and IV/2005. In the years under review, various numbers of liver transplantations were performed: in 1991 (1), in 1995 (3), in 1996 (3), in 1997 (5), in 1998 (4), in 1999 (7) in 2000 (6), in 2001 (8), in 2002 (6), in 2003 (8), in 2004 (6) and by April 2005 (2). Totally - in the period under review (between IV/1991 and IV/2005) - the most transplantations resulting from chronic liver disease were accomplished in Prague in IKEM (22), Bratislava (11), Vienna (7), Essen (6), Hamburg (5), Brno (5), Tübingen (1), Leiden (1) and in Lyon (1) - in this case it was a combined liver and kidney transplantation.
In 53 cases, there was the transplantation of cadaverous organ, in 6 cases segmental liver transplantation from living donor. Out of the above mentioned 6 cases in 4 patients, transplantation of 2 liver segments was made to a child recipients, 2 times it was transplantation of 4 liver segments to adult recipients. Re-transplantation was performed 3 times, there of 2 times in children and 1 time in an adult.

**Conclusion:** From 59 elective transplanted Slovak patients for chronic liver disease in more transplantation centers between April 1991 and April 2005, totally 46 patients have survived by now, 13 patients died, there of 9 adult recipients and 4 child recipients. It means 78% surviving in the period under question. The most common indication for liver transplantation in adult patients was Chronic sclerosing cholangitis (27.1%), in child patients Biliary atresia (18.7%).
ILEI, a novel key component and regulator of hepatocellular carcinoma

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*IMP - Research Institute of Molecular Pathology, Dr. Bohrgasse 7, A-1030 Vienna, Austria

Introduction: Interleukin-like EMT Inducer (ILEI) has been identified as a novel key regulator of carcinoma progression. ILEI shows structural relationship but no homology to cytokines, and represents a potent inducer of tumor formation and epithelial to mesenchymal transition (EMT), the latter process closely mimicking late stage tumor progression and metastasis. Intriguingly, secreted ILEI on its own causes differentiated epithelial cells to become tumorigenic and metastatic.

Methods: We recently established a cellular tumor model linked to preclinical animal studies which gives us the unique opportunity to analyze the molecular mechanisms involved in hepatocellular carcinoma (HCC) progression. In this model, the functional synergy of Ras activation and transforming growth factor (TGF)-β signaling is operative to induce and maintain an increase in the malignancy of hepatocytes. This progress in neoplastic transformation is accompanied by the reprogramming of regulatory networks involved in epithelial tissue homeostasis and the progressive loss of cell type specificity. Importantly, these molecular alterations upon hepatocellular EMT include the secretion of TGF-β, the loss of the tumor suppressor E-cadherin, and the accumulation of nuclear β-catenin which represent hallmarks of human HCCs.

Results: The current investigation presents first evidence that ILEI is highly upregulated in a translationally controlled fashion during EMT of hepatocytes. In vivo conducted experiments performing hepatocellular EMT after orthotopic transplantation demonstrate specific expression of ILEI in cytoplasmic areas of cancerous and locally infiltrative hepatocytes. ILEI expression is most abundantly observed in spreading hepatocytes which localize in distant organs such as the lung.

Discussion/Conclusion: In further studies we will employ comprehensive gain- and loss-of-function studies to examine the impact of ILEI in the experimental tumor model of HCC progression. Investigations will focus on the molecular mechanisms underlying the putative potential of ILEI for neoplastic transformation and alterations in the plasticity of epithelial hepatocytes. These results might support the development of novel therapeutic strategies for HCC treatment.
Prevalence and association with predisposing conditions of NAFLD and NASH in general population from the west part of Romania

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Introduction: Because the etiology, pathogenesis, natural history and therapeutic approach of NAFLD/NASH are not fully understood, the study is proposing to detect the prevalence of NAFLD and NASH - as a component part - in the population from the west part of Romania, and also the association with some predisposing conditions.

Methods: Our study included a group of 1548 persons, considered representative for general population, in which we investigated the presence of NAFLD and NASH. The diagnosis was made on the presence of steatosis at abdominal ultrasonography, in the absence of alcohol consumption or other causes of increased aminotransferases (eg viral hepatitis). Patients with NAFLD/NASH were evaluated regarding age, sex and the presence of major risk factors, such as type 2 diabetes, obesity and hyperlipidemia.

Results: 369/1548 (23.83%) persons presented steatosis at ultrasonography. From these, 72/369 were considered as alcoholic steatohepatitis (ASH) and 297/369 (80.48%) NAFLD - 144 (48.48%) men and 153 (51.52%) women. 63/297 (21.21%) patients presented NASH, 36 (57.14%) men and 27 (42.86%) women, mean age 50.57 years.

Prevalence in general population and association with predisposing conditions:

<table>
<thead>
<tr>
<th></th>
<th>NAFLD (n = 297)</th>
<th>NASH (n = 63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence</td>
<td>297/1548 (19.18%)</td>
<td>63/1548 (4.06%)</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>69/297 (23.23%)</td>
<td>18/63 (28.57%)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>24/297 (8.08%)</td>
<td>6/63 (9.52%)</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>69/297 (23.23%)</td>
<td>15/63 (23.8%)</td>
</tr>
<tr>
<td>Obesity</td>
<td>108/297 (36.36%)</td>
<td>186/297 (62.62%)</td>
</tr>
<tr>
<td></td>
<td>(27/63 (42.85%)</td>
<td>(39/63 (56.89%)</td>
</tr>
<tr>
<td>Overweighted</td>
<td>78/297 (26.26%)</td>
<td>12/63 (14.04%)</td>
</tr>
</tbody>
</table>

9/63(14.28%) patients presented splenomegaly at ultrasonography.

Discussion/Conclusion: Our region encounters an increased rate of NAFLD (19.18%) and NASH (4.06%), in association with metabolic disorders such as type 2 diabetes and hypertriglyceridemia in over 20% of cases. Over one third of the patients presenting hepatic steatosis were obese. The disorder occurs with an approximately equal frequency in men and women, most often in middle-aged persons.
Clinical and pathological significance of hepatic steatosis in chronic hepatitis C virus infection

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²Department of Pathology, University of Medicine and Pharmacy, Timisoara, Romania
³Department of Biostatistics, University of Medicine and Pharmacy, Timisoara, Romania

Introduction: Hepatic steatosis represents a histological characteristic of chronic HCV infection. However, its pathophysiology and implications in disease progression have to be established.

Methods: To evaluate the prevalence of hepatic steatosis in HCV patients and to find correlations with various histopathological and clinical features. We studied 137 patients with chronic hepatitis C, 52 (38%) men, 85 (62%) women, mean age 48.93 ± 10.66 years (range 19-67). Histological, clinical and biochemical data were compared between patients with/without hepatic steatosis; the statistical analysis included unpaired "t" test, ANOVA and Chi square test.

Results: 106 patients (77.4%) had steatosis: grade 1(< 30% hepatocytes involved) in 84 (61.3%), grade 2 (30-70% hepatocytes) in 22 (16.1%); grade 3 (> 70%) was absent. The mean BMI of patients with no steatosis was 23.57 ± 3.4, for grade 1 steatosis 26.17 ± 3.78, for grade 2 steatosis 27.53 ± 3.09. There was a very significant relationship between steatosis grade 1 and 2 and BMI (p = 0.0022). There was no significant association between the level of triglycerides (p = 0.6585), cholesterol (p = 0.5091), gamma-glutamyl transpeptidase (p = 0.2617), alkaline phosphatase (p = 0.6489), diabetes mellitus (p > 0.05), age (p = 0.1788), gender (p = 0.692502), presence of lymphoid aggregation/follicle (p = 0.1979) or bile duct damage (p = 0.3775) and steatosis. Patients with steatosis had a significantly higher mean fibrotic score than those without steatosis (1.41 ± 0.99 vs. 0.90 ± 0.90, p = 0.0112). Necroinflammation was more severe in these patients (8.95 ± 2.55 vs. 6.96 ± 3.35, p = 0.0005 - extremely significant). A slight correlation was found between the level of total bilirubin (p=0.0992 - marginally significance), aspartate aminotransferase (p = 0.0668) and steatosis.

Discussion/Conclusion: Hepatic steatosis was found in 77.4% patients with HCV. Patients with steatosis were more frequently obese and had more severe hepatic necroinflammation and fibrosis.
Serum apolipoprotein A-I level predicts advanced liver fibrosis in children with chronic hepatitis B

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¹IIIrd Department of Pediatrics, ²Department of Children Allergology, ³Department of Clinical Pathomorphology, Medical University of Bialystok, Poland

Introduction: Liver biopsy is thought mandatory for staging fibrosis in patients with chronic HBV infection, however there are no serum parameters that reliably predict the stage of liver fibrosis in children.

The aim of the study was evaluation of diagnostic accuracy of potent serum biochemical fibrosis markers: apolipoprotein A-I (ApoA-I), haptoglobin (HPT) and alpha-2-macroglobulin (A2M) in children with chronic hepatitis B evaluated by ROC analysis.

Methods: We determined serum level of ApoA-I, HPT and A2M with an automatic nephelometer (BNII, Dade Behring) in 63 children (age range 4-17, mean 10 years) with biopsy-verified chronic HBeAg-positive hepatitis B. Fibrosis stage and inflammation grade were assessed in a blinded fashion according to Batts and Ludwig. We defined advanced fibrosis as a score > 2. ROC analysis was used to calculate the power of the assays to detect advanced fibrosis (AccuROC, Canada).

Results: Serum concentration of the examined markers were not significantly different in patients with chronic hepatitis B compared to controls (n = 16). However, ApoA-I level of 1.19 ng/l had a sensitivity of 85.7% and a specificity of 60.7%; AUC (95% CI) = 0.7117 (0.5049-0.9186), p = 0.035 to predict advanced fibrosis. Correct classification proportion of staging fibrosis was 63.5%. All other markers and their combination did not allow a useful prediction. None of these markers was a good predictor of histologic inflammation.

Conclusion: ApoA-I may be a suitable serum marker to predict advanced liver fibrosis in children with chronic hepatitis B. Application of this marker may decrease the need for liver biopsy in children with chronic HBV infection.
High expression of claudin-7 during liver regeneration


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Introduction: Hepatic injury combined with impaired regeneration of the involved hepatocytes (HCs) triggers the activation of hepatic progenitor cells (HPCs), which play an important role in differentiation and regeneration of the liver. Claudins, the main proteins of tight junction, are involved in changes of cell adhesion during liver regeneration. Out of the 24 types of recently known claudins, type 7 has been associated with bile duct cells (BDCs). For this reason, the aim of our study was to analyse the expression of claudin-7 in HPCs and to compare the found expression with mature HCs and BDCs.

Methods: Rats were treated with 2-acetylaminofluorene (AAF) followed by 70% partial hepatectomy (PH). Claudin-7 expression was detected in paraffin sections by immunohistochemistry and further investigated on snap frozen specimens by Western immunoblot analysis. Double immunofluorescence-staining of claudin-7 and of oval cell markers (CK7, OV-6) was performed on frozen sections and detected by confocal microscopy.

Results: Weak immunoreactivity of claudin-7 was found in normal BDCs whereas HCs were negative. Large number of oval cells with strong claudin-7 immunostaining was observed in regenerating liver. Double immunofluorescence revealed that the cells expressing claudin-7 were indeed progenitor cells, since they also expressed CK7 and OV-6. Western blot confirmed the increased expression of claudin-7 in the AAF/PH treated rat liver.

Discussion/Conclusion: Our data demonstrate a strong expression of claudin-7 in HPCs during hepatic regeneration. Based on our results claudin-7 protein could well become a new marker for the identification of hepatic progenitor cells.

Supported by grant: NKFP-1/0023/2002.
Polyenylphosphatidylcholine effects in in vitro ethanol-induced programmed cell death of blood lymphocytes in patients with alcoholic liver cirrhosis

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Introduction: Soybean polyenylphosphatidylcholine (PPC) has been shown to prevent further development of both early (i.e. fatty liver) and late (i.e. liver fibrosis and cirrhosis) alcohol induced changes in the liver in alcohol-fed animals as well as in humans with alcoholic liver disease. Moreover PPC has been proved to attenuate ethanol-induced apoptosis of hepatocytes in rats.

The aim of this study was to examine if, in vitro, PPC may have an impact on apoptosis (spontaneous or ethanol-induced) and necrosis of peripheral blood mononuclear cells (PBMCs) in patients with alcohol-induced cirrhosis.

Methods: PBMCs were isolated from the blood of 21 patients with cirrhosis and of 6 healthy controls, then, incubated or not (spontaneous apoptosis) in vitro with 80 mM ethanol. The influence of PPC application (100 μg/ml of PPC) on PBMCs apoptosis was examined.

Results: In comparison to healthy controls, PBMCs in cirrhotic patients exhibited accelerated spontaneous (without treatment) apoptosis of CD3+ lymphocytes, CD4+ cells including. Ethanol treatment induced apoptosis of immune cells, especially CD4+ cells in cirrhotic patients and apoptosis of CD8+ cells of healthy controls. PPC decreased ethanol-induced apoptosis, but it stimulated necrosis of PBMCs after 24 hrs of in vitro incubation. PPC antiapoptotic activity was not lymphocyte subpopulation specific.

Discussion/Conclusion: The results of our study suggest that alcohol consumption during PPC therapy may induce mainly necrotic immune cell death, especially in patients with alcohol-induced cirrhosis.
Microhemorrheological disturbances in patients with chronic liver diseases

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Introduction: Microhemorrheological disturbances play an important role in development of tissue hypoxia and fibrosis. Current investigations revealed that the most significant microhemorrheological disturbances are associated with the most severe and complicated course of kidney, cardiovascular and autoimmune disorders. The aim - to study the hemorrhological parameters in patients with chronic hepatitis (CH) and liver cirrhosis (LC).

Methods: 100 patients (pts) were included into this study - 64 CH (95% - hepatitis of low activity) and 36 LC (Child - Pugh class A - 69%, class B - 31%). Both groups were comparably equal at sex, age and basic laboratory characteristics (blood count, serum transaminases, total protein). Viral etiology was confirmed in 92% pts CH and 67% pts LC. To characterize the hemorrhology we determined the following parameters: blood and plasma viscosity, index density of erythrocytes and the rate of erythrocyte aggregation. The control group consisted of 20 healthy persons.

Results: Blood and plasma viscosity in pts CH and LC had no significant differences from those in control group. Index density and the rate of erythrocyte aggregation were increased in 48% and 36% pts LC. These parameters were significantly higher than those in pts CH and health control. Statistic analysis revealed significant correlations between hemorrhological parameters and following laboratory characteristics: anemia, bilirubin, alkaline phosphatase, serum iron and hyperimmunoglobulinemia.

Discussion/Conclusion: The increased values of index density and rate of erythrocyte aggregation indicate the microhemorrheological disturbances, which are associated with block of microcirculation and tissue hypoxia in experiment. The correlation between the microhemorrheological disturbances and cholestasis allows to suggest that anticholestatic therapy may lead to the improvement of erythrocytes rheology and tissue oxygenation. This mechanism could be a part of the antifibrotic effect of the ursodeoxycholic acid.
Effects of proven and potential novel antifibrotic agents, singly and in combination on experimental rat liver fibrosis reversal

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We studied the effects of proven and of potential novel antifibrotic agents and their combinations in experimental model of rat liver fibrosis reversal induced by thioacetamide (TAA). The agents singly investigated were losartan (LOS), sylimarin (SYL), interferon α2 (IFN), mycophenolate mofetil (MMF) and pioglitazone (PGZ). The last four compounds were combined with LOS. According to the literature data, in vitro studies demonstrated potent antifibrotic effects of all the investigated compounds. Since many of the single agents are orally available, in part with a well known clinical safety profile, a rapid transfer of the best combinations to clinical studies in patients with liver fibrosis is feasible.

Advanced liver fibrosis was induced by TAA treatment (200 mg/kg, i.p.) during three months, 2-times per week. Resolution of fibrosis was monitored after 2 months of TAA withdrawal. During the last two months of the experiment, the TAA-treated groups were daily administered i.g. with LOS (5 mg/kg), SYL (50 mg/kg), PGZ (3 mg/kg), MMF (5 mg/kg) and 3-times per week - with IFN (150000 IU/rat). The combinations of LOS with other agents were applied in similar dosage. The severity of liver fibrosis was assessed by morphometric evaluation of liver slides stained with Azan-Mallory, hydroxyproline (Hyp) determination and mRNA the steady state levels of collagen I, TGF-β1, MMP-3, -13, -14, TIMP-1 and PAI-1 in the liver were quantified in total liver RNA by QRT-PCR using the TaqMan technique.

The TAA treatment during 3 months induced micronodular liver fibrosis with expressed deposition of collagen fibers. The treatment with LOS, IFN, MMF, LOS+IFN, LOS+MMF significantly decreased the square of liver connective tissue stained by Azan-Mallory compared to the resolution untreated group. Only LOS decreased the total Hyp content, whereas MMF lowered relative Hyp in the liver. However, their combination did not change these parameters. IFN, LOS, MMF as well as LOS+IFN and LOS+MMF increased mRNA expression of MMP-14. The expression of MMP-13 was enhanced in rats treated with IFN, LOS+IFN and LOS+MMF, whereas SYL and LOS+PGZ decreased this parameter. The profibrogenic gene expression of TGF-β1 in animals treated with MMF, SYL and LOS+SYL and the PAI-1 expression in rats treated with LOS+PGZ were significantly down-regulated. No effects on collagen I and TIMP-1 expression were found in all the experimental groups.

The preliminary testing suggests that two combinations, LOS+MMF and LOS+IFN, are most effective in this experimental situation. These combinations, which are effective in TAA-induced rat fibrosis model in vivo, can be easily tested in a clinical setting, since single agents have a satisfactory clinical safety profile.
High level of vitamin C in rat liver after intoxication of CCl$_4$ and galactosamine

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**Introduction:** Numerous recent studies aimed at explaining the pathomechanism of many diseases of oxidative stress which is caused by excessive production of reactive oxygen forms. It has been found out that the organism is equipped with the system, called the antioxidative barrier, protecting it against free radical forms. One of its elements is vitamin C which shows strong reductive properties. In the reactions with ascorbate oxidants it forms the non-reactive ascorbic radical as a result of monoelectron reduction and is involved in the regeneration of another important antioxidant (vitamin E) reducing the tocopheryl radical. Therefore it seems interesting to determine the vitamin C level in various tissues of animals exposed to CCl$_4$ or galactosamine - factors resulting in the disorders of homeostasis.

The **aim** of our study was to determine the level of vitamin C in the liver of the rats exposed to CCl$_4$ and galactosamine.

**Methods:** The examinations were conducted on Wistar rats weighing 250-350 g. Group I (control) was administered 2 mg of olive oil, group II - CCl$_4$ in the single intraperitoneal dose of 0.5 ml/kg b.w. dissolved in 2 ml of olive oil, group III - CCl$_4$, 0.5 mg/kg b.w. in 3 successive days intraperitoneally, group IV - galactosamine in the single intraperitoneal dose of 800 mg/kg. The animals were decapitated 24 hours after the administration. The level of vitamin C in the tissues was determined using the Ale-Ky method.

**Results:** The highest levels of vitamin C were observed in group III (3 days intoxication of CCl$_4$).

**Discussion/Conclusion:** The differences in vitamin C levels found in the experimental groups may be explained by the fact that vitamin C acting synergistically with vitamin E may be involved in the first line of antioxidative defense.
The usefulness of fine needle aspiration in diagnosis of pancreatic tumors

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The aim of this study was evaluation of usefulness of fine needle aspiration (FNA) in diagnosis of pancreatic tumors.

The studies were performed on a group of 132 patients (55 women and 77 men) with clinically diagnosed pancreatic tumor 103 cases, and 23 cases of pancreatitis, as well as 6 patients with pancreatic cyst. Patients' age ranged from 20 to 87 years (mean 56.03 year). In all cases FNA was done under ultrasound or computerized tomography control. In 36 cases a biopsy was done during surgery. In 26 cases cytological diagnosis was verified by postoperative histological studies. FNA samples were routinely stained using H&E method. In cases with diagnosed or suspected malignancy immunohistochemical studies of CA19.9, p53 oncoprotein using ABC/HRP method were performed.

On basis of cytological examination of FNA samples the following diagnoses were made: normal cytological picture in 25 cases; chronic pancreatitis in 32 cases; pancreatic cyst in 7 cases; malignant tumor in 39 cases (they included adenocarcinoma in 30 patients and non-differentiated carcinoma in 8 cases, and anaplastic carcinoma in 1 patient) and pus in 1 case. In addition in 14 cases suspicious neoplastic cells were found, and in next 14 cases FNA material was non-diagnostic.

CA19.9 expression was found in 27/39 (69%) cases with malignant tumor and in 7/14 (50%) patients in cases with suspected cells. Overexpression of p53 was found in 18/39 (46%) cases with malignant tumor and in 5/14 (36%) patients with suspected cells. Immunohistochemical studies of CA19.9 and p53 allowed for diagnosis of malignant tumor in next 8 cases, in which cytological examination revealed suspected cells. In a group of 26 FNA (21 neoplastic lesion, 5 chronic pancreatitis) histological study verified one case of false positive diagnosis (fibrous chronic pancreatitis) and two false negative cases (adenocarcinoma G1).

Controlled FNA is good diagnostic method of tumorous pancreatic lesions. In difficult cases immunohistochemical studies help in diagnosis of neoplastic lesions.
Epidemiological analysis of HBV, HCV infections in patients with dermatological diseases

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Introduction: Detection of HBV, HCV infections in a group of patients with dermatological diseases, assessing the prevalence of HBsAg and HCV Ab and epidemiological analysis of the risk factors.

Methods: We investigated a group of 322 patients with dermatological diseases, and another group of 302 patients without dermatological diseases concerning the seroprevalence of HBsAg and HCV Ab. We used the enzyme immunoassay (ELISA) for detection of HBsAg and HCV Ab and the Epiinfo program for the analysis of epidemiological factors (sexual, parenteral, surgical, transfusional). As a epidemiological methods we used descriptive analysis and case-control study.

Results: The seroprevalence of HBsAg was 11.8% in the case lot and 5.5% in the control lot (p < 0.001, OR: 2.13). The mean of age was 45 and the difference between male and female was insignificant. The seroprevalence of HCV Ab was 12.4% in the case lot and 1.6% in the control lot (p < 0.0001, OR: 7.62). The analysis of epidemiological factors in HBV, HCV infections, showed an association of 2 factors in up to 60% of cases. Sexual risk was present in 13.2% of HBV-positive cases and 11% of HCV Ab-positive cases. Parenteral risk was present in 21% of HBV-positive subjects and 31% in HCV Ab-positive subjects. Surgical risk was positive in 18% of HBV cases and 40% of HCV Ab-positive cases. Concerning the transfusion, just 17.5% of HCV Ab-positive subjects had a history of transfusion.

Discussion/Conclusion: Our study shows an increased prevalence of HBV and HCV infection in patients with dermatological disorders compared with the general population (p < 0.0001). This situation asks for a continuous monitoring of these infections, the improvement of sexual educations methods and further studies on this subject.
Pegylated interferon alpha plus taurine treatment in experimental liver fibrosis

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Introduction: Hepatic stellate cells (HSC) are the main source of excessive collagen in the fibrotic liver. Oxidative stress (OS) has an important role in fibrogenesis. HSC apoptosis may be involved in spontaneous recovery (SR) of liver fibrosis. We studied individual and combined effects of peginterferon alpha2b (PegIFNα2b) and antioxidant taurine over SR in experimental liver fibrosis.

Methods: Liver fibrosis was produced with CCl₄ in 60 Sprague-Dawley rats. Then the rats were divided into four groups. Group I (n = 15) was left for SR, Group II was treated with PegIFNα2b (1.5 microg/kg/week), Group III with taurine (1200 mg/kg/day), and Group IV with their combination. All rats were killed after four weeks. Histopathological fibrosis scores, numbers of alphaSMA(+) HSCs, apoptotic HSCs and hepatocytes, and OS parameters were determined.

Results: Fibrosis scores and alphaSMA(+) HSC counts reduced significantly in Groups II, III and IV compared to Group I. AlphaSMA(+) HSC counts were similar in Groups II and III, and were significantly lower in Group IV than Group III. Whereas PegIFNα2b had no effect, taurine alone and in combination induced hepatocyte apoptosis significantly. HSC apoptosis increased significantly in Groups II, III and IV compared to Group I, and in Group IV compared to Groups II and III. Apoptotic HSC counts in Groups II and III were similar. Tissue SOD, MDA, and GSHPx levels improved significantly only in Group III.

Discussion/Conclusion: PegIFNα2b and taurine both improved experimental liver fibrosis, whereas combination provided no further benefit. Induction of HSC apoptosis and thereby accelerating SR may be potential approaches in treatment of liver fibrosis.
Evaluation of Ursofalk®'s effect on hepatic haemodynamic parameters in treatment of patients with compensated liver cirrhosis

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The aim of our study was to evaluate the effect of the Ursofalk® administration on the hepatic haemodynamic parameters in patients with compensated liver cirrhosis.

Patients and methods: We studied 41 patients with liver cirrhosis of viral C etiology in stage A (according to Child-Pugh score). All the patients were evaluated by duplex Doppler ultrasonography of portal system and hepatic artery before and after 1 month of treatment. 29 patients received Ursofalk® (750 mg daily) and 12 patients did not receive Ursofalk® (control group).

Results: Ursofalk® administration significantly decrease the diameter of the trunk of the portal vein with 9.9 ± 0.5% (p < 0.05), the diameter of the splein vein with 11.2 ± 0.7% (p < 0.05). The portal blood flow velocity in the trunk of the portal vein significantly increase with 10.3 ± 1.2% (p < 0.05). Was not discovered significantly change of the portal blood flow in the portal and splein veins (p > 0.05). Hepatic artery resistance index significantly decrease with 16.2 ± 3.1% (p < 0.05). In control group we did not observe any significantly change (p > 0.05).

Conclusion: Our study indicates that the administration of Ursofalk® in patients with compensated liver cirrhosis (class A by Child-Pugh) improves the hepatic haemodynamic, probably, because they contribute to decrease the intrahepatic resistance in cirrhotic patients.
Insulin and glucagon resistance in patients with alcoholic and non-alcoholic steatosis, steatohepatitis and steatocirrhosis

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Introduction: Insulin and glucagon resistance were discussed in both alcoholic and NAFLD, but the differences between them is not clear yet. The aim of this study was to characterize and compare the insulin and glucagon resistance in patients with alcoholic and NAFLD.

Methods: Serum glucose, IRI, C-p and IRG were measured in 146 patients with alcoholic and non-alcoholic steatosis (20 and 20), steatohepatitis (15 and 13), and cirrhosis (40 and 38) without diabetes mellitus (DM). OGTT, number and affinity of mononuclear cells insulin receptors (n = 50), liver biopsy glycogen content (n = 60) were also investigated. Control group included 20 healthy persons.

Results: IRI and IRI/glucose ratio (fasting and during OGTT) were significantly increased in all groups (p < 0.01-0.001) compared to the healthy controls. Insulin resistance, decreased number of insulin receptors and increased serum levels of C-p were significantly more expressed in non-alcoholic steatosis and steatohepatitis (p < 0.05-0.001). C-p/IRI equimolar ratio was decreased in liver cirrhosis (p < 0.001) compared to other groups despite of etiology. On the opposite, glucagon resistance was presented only in patients with liver cirrhosis (p < 0.001), especially with alcoholic etiology (p < 0.01). There was significant correlation between the alterations in carbohydrate metabolism and the severity of liver damage (p < 0.001). In advance disease the hyperglucagonaemia become predominant (catabolic phase).

Conclusion: Insulin resistance is present in both alcoholic and non-alcoholic FLD. The most significant changes occur in non-alcoholic cases. Glucagon resistance increased with advance of the liver disease, especially in cases with alcoholic etiology.
Is the corticotherapy of malignant hemopathies a risk factor for the fatty liver disease?

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Introduction: At many patients with malignant hemopathies the polychemotherapeutic regimes include the corticotherapy - a risk factor for metabolic syndrome entities, including steroidic diabetes and liver steatosis. We propose to examine the ultrasonographic aspects suggestive for fatty liver disease at a group of patients with malignant hemopathies treated with polychemotherapeutic regimes that included corticosteroids versus a witness group.

Methods: 32 patients with malignant hemopathies admitted in the last year to compartment of Hematology from the County Clinical Hospital Sibiu and treated with corticotherapy based regimes (group A) were examined versus 167 patients with other diseases admitted in the last 3 months to Medical Clinic II from the same hospital (group B) from point of view of next parameters: age, body mass index, weekly average of stools, liver echogenity, posterior attenuation, triglyceridemia, number of plaquettes, transaminases and gamma-glutamyl transpeptidase. The ethanol drinkers were not included in the study. The results were statistically analyzed with Student t test for unpaired observations.

Results: The age was significantly higher and the weekly average of stools was significantly lower at group A versus group B (p < 0.05, respectively p < 0.001). Although the body mass index was not significantly different between the 2 groups, the liver echogenity and ultrasound posterior attenuation were significantly more important at the group A (p < 0.05, respectively p < 0.0001). The average of alanine aminotransferase and aspartate aminotransferase were near between the groups. There were no statistic significant differences between the groups concerning the other investigated parameters, including triglyceridemia.

Discussion/Conclusion: The more important ultrasound posterior attenuation at the patients with corticosteroid-treated malignant hemopathies suggests the presence of liver fibrosis, element component of non-alcoholic fatty liver disease. The possible role of constipation in its pathology deserves to be thoroughly.
Comparative characteristics of hepatites B and C patients with lymphoma and solid malignant tumours

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Introduction: The aim of the present study was the comparison of serological markers of hepatitis B (HBV) C (HCV) in 300 patients with lymphoma (LP) and solid malignant tumors (SMT).

Methods: After the comparison of these results we have determined the differences in patients with LP and SMT. In particular, in LP patients HBsAg, anti-HBc and anti-HCV were detected statistically more often than in SMT patients. Replication of HBV and HCV was also more often occurred in patients with LP. As well as other researchers we have noticed that in patients both with LP and SMT in the course of the oncological disease the frequency of HBsAg detection increases, but not of IgM-anti-HBc.

Results: Similar pattern was found in the relation with frequency of anti-HCV detection, which increased due to the clinical stage of the disease. They have been expressed more in LP patients. These facts indirectly specified that with the promotion of LP and SMT clinical stages the increase in the frequency of chronic replicative cases of infections and decrease in the frequency of acute forms was marked. At the same time, there was no statistical significance in the ratio of manifested and subclinical forms in LP and SMT patients.

Discussion/Conclusion: Thus, according to the serological results we can confirm that HBV and HCV prevalence in LP patients is essentially higher than in SMT patients. This is the evidence that the LP patients are in "high risk" group in terms of hepatitis B and C.
Transdifferentiation of hepatocytes to metastatic myofibroblasts during late stage liver tumorigenesis

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Introduction: Changes in epithelial cell plasticity associated with a dramatic increase in malignancy are frequently observed in various types of carcinomas. Here we present evidence for the first time that transforming growth factor (TGF)-beta1 induces transdifferentiation of malignant hepatocytes to myofibroblastoid cells in vivo.

Methods: We established a murine tumor model based on cooperation of Ras with TGF-beta1, the latter representing a hallmark in hepatocellular carcinoma (HCC). Orthotopic transplantation of immortalized and genetically modified hepatocytes allows us to faithfully reflect the progression of HCC, and to monitor signaling pathways responsible for tumor invasion and metastasis.

Results: Upon crosstalk between TGF-beta and Ras, expression profiling revealed upregulation of proteins associated with fibrosis and metastasis. Experimentally generated tumors strongly increase in size which was accompanied by reduced survival of mice. This increase in malignancy could be correlated with downregulation of E-cadherin and cytokeratin as well as with nuclear accumulation of PCNA, Smad-2 and beta-catenin. Most importantly, exogenous hepatocytes exhibit an intra and peritumoral localization of alpha-SMA and showed fibrotic marker expression such as fibulin 2, GFAP and desmin. This transdifferentiation could be completely blocked by overexpression of Smad-7 which abrogates TGF-beta signaling. This indicates that a subpopulation of hepatocytes transdifferentiates into myofibroblastoid derivatives during the acquisition of invasive and metastatic properties in a TGF-beta dependent manner.

Discussion/Conclusion: Taken together these data provide evidence for a direct link between fibrosis and liver tumor progression which might be taken into consideration for the pathological assessment of and therapeutic intervention in liver carcinoma progression.
Liver hemodynamics before and after radiofrequency ablation (RFA) assessed by Doppler-ultrasound

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Introduction: RFA-induced hyperthermia is considered to be relatively safe for hepatic vasculature. However, the precise liver haemodynamic actions of the generated heat are not well established.

The Aim of the study is to evaluate the haemodynamic changes related to RFA.

Methods: Liver haemodynamics was evaluated by Color and Pulse Doppler-US examinations of the portal vein (PV), hepatic artery (HA) and hepatic veins (HV) prior to, 1 hour, 1 and 7 days post-RFA both in the peritumoral zone and at the main branches in 48 patients with 63 malignant nodules treated by RFA.

Results: No significant spectral changes, neither thrombosis were observed in HV. HA revealed significantly reduced Resistive Index 1 h post-RFA (0.684 vs. 0.651, p < 0.05). Portal blood flow was the most affected with increased mean velocity (18.3 cm/s vs. 26.8 cm/s). In all cases the haemodynamic alterations of HA and PV returned to pretreatment values one day post-RFA. In the peritumoral zone in 7 pts with cirrhosis/HCC portal vessels with reversed flow were observed, in 2 more the portal spectrum was arterialized, probably due to cirrhotic-related collateral shunting. Segmental portal thrombosis, adjacent to the tumor zone, was visualized in one case.

Conclusions: A transient increase of hepatic blood flow is observed after RFA. Peritumoral vascular changes are more distinct in patients with cirrhosis/HCC and affect mostly the portal flow. Major vascular complications are rare.
The study of risk groups for cholangiocarcinoma

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Introduction: Cholangiocarcinoma is a neoplastic disease arising from the intrahepatic and extrahepatic bile ducts. The disease is associated with known risk factors: bile duct inflammation accompanying Caroli’s disease, choledochoch cysts, Clonorchis sinensis infections, hepatolithiasis and primary sclerosing cholangitis (PSC); however, most patients have no known identifiable predisposing conditions.

Aim of study: Recognized etiological factors in the development of cholangiocarcinoma.

Material and methods: This prospective study involving a group of 96 patients with cholangiocarcinoma developed during a 10 years period January 1995-January 2005. Statistically the majority were male patients (60.41% men comparative with 26.5% women); and the mean ages is 59.05 ± 8.56 years. The clinical, biochemical (serum CA 19-9) and imagistic evaluation has been complete (PET scans, magnetic resonance or ERCP).

Results and discussions: We recognized etiological factors in the development of cholangiocarcinoma in 39 cases (40.62%). Primary sclerosing cholangitis (PSC) was recognized in 11 cases (11.45%), Caroli’s disease in 5 cases (5.20%), choledochoch cyst in 8 cases (8.33%), hepatolithiasis in 10 cases (9.6%) and liver fluke infestation in 1 case (1.04%). In 57 cases have no known indentifiable predisposing condition.

Conclusions: Primary sclerosing cholangitis (PSC) is the major risk factor associated with the development of cholangiocarcinoma. Another risk factors were hepatolithiasis and choledochoch cyst. This information suggest screening strategies for cholangiocarcinoma in PSC.
Pancreas cancer in Northern Ireland (2001): A population-based audit of 1.7 million inhabitants

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Objective: To document the presentation, clinical course and outcome of pancreas cancer in Northern Ireland in 2001.

Design: Population-based, retrospective, cross-sectional, observational study.

Methods: The Northern Ireland Cancer Registry (NICR) receives notification from all pathology laboratories, hospital patient administration systems, radiology sites and the Registrar General's Office of all patients diagnosed with pancreas cancer. The computerised and hand-written records of all patients diagnosed during 2001 were reviewed by trained researchers.

Results: In a population of 1.7 million, 169 people were diagnosed with pancreas cancer in 2001. 13 patients were excluded due to a paucity of reliable data. 156 cases were analysed in depth (84 males and 72 females). The median age at diagnosis was 70 years (males) and 77 years (females). 97% of patients were symptomatic as follows: loss of appetite (67%), nausea (56%), weight loss (65%), back pain (26%), jaundice (56%), dark urine/pale stools (60%), itching (21%), abdominal pain (54%), fatigue (33%), and diarrhoea (15%). 39 patients underwent surgery (19 biliary bypasses, 16 gastric drainage procedures, 4 completed pancreaticoduodenectomies). Median survival following surgery was 119 days (95% CI, 63-175 days) and without surgery was 68 days (95% CI, 48-88 days).

Conclusions: The incidence and clinical presentation of pancreas cancer in Northern Ireland is similar to the rest of Europe. The resection rate is low, and the prognosis is poor. The increased survival seen in patients undergoing surgery is probably due to selection bias and an earlier stage at presentation. This comprehensive dataset establishes a baseline for monitoring change.
Thrombocytosis as a prognostic factor in pancreatic cancer - Facts or fictions

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Introduction: The purpose of this study was to determine the significance of thrombocytosis as a prognostic factor in patients with pancreatic cancer (PC).

Methods: There were 69 patients - 44M/25F with pancreatic cancer (44 resectable and 25 unresectable cancers), mean age 62.5 yrs. The clinicopathologic factors studied for prognostic value were: gender, age, tumor size, pain, preoperative and postoperative levels of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9), metastases, the extent of weight lost and biochemical and morphological parameters. Platelet counts were recorded and any patients with at least one platelet count of greater than 450 x 10^9/l was classified with thrombocytosis. A platelet count of 450 x 10^9/l were classified with a normal platelet count.

Results: Thrombocytosis were found in 22/25 (88%) pts with unresectable PC, and in 26/44 pts (59%) with resectable PC after operation (p < 0.001). In all patients with thrombocytosis were found very high level of CA 19-9 (76% vs. 35%) compared with pts with normal platelet count (p < 0.001). Multiple metastases with pain and weight lost were in 42/48 pts with thrombocytosis vs. 6/25 pts with normal platelet count (p < 0.001). No significant difference was found for age, gender, CEA level and tumor size between pts with high or normal platelet counts (p < 0.06). Prognostic factors were evaluated by univariate and multivariate analysis.

Discussion/Conclusion: Our clinical data shown that thrombocytosis in preoperative and postoperative time is associated with poor survival outcome in patients with PC. The clinical value of PC further investigated. Longterm survival from PC is possible if the disease is identified in its early stages.
Viral hepatitis - Health care problem that can be solved

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Adopted in the 1995 State Program of the President of Turkmenistan S. A. Niyazov "Health" has a strategic aim in improving the general health condition and in increasing the population productive life span. One of the priority tasks for Turkmenistan health care improvement is the implementation of measures on disease prophylaxis. Viral hepatitis is a special group of diseases that requires the development of a National Program, based on the scientific advances worldwide and the recommendations of World Health Organization (WHO).

The aim of our studies was the identification of the etiological and clinical characteristics associated with hepatitis B virus (HBV) and hepatitis C virus (HCV) infection and to develop strategies for their treatment and prevention in Turkmenistan.

From 1991-2005 we analyzed 375 patients with chronic liver diseases: hepatitis B, C and D viruses were the cause for more than 73% of liver diseases. Further analyses of patients with chronic viral hepatitis revealed that more than half of the patients were between 20 and 39 years old. Blood transfusions as well as therapeutic and diagnostic medical interventions were the major routes of transmission, even though we could frequently not identify a specific risk factor (sporadic hepatitis).

The natural history of viral hepatitis is frequently characterized by an asymptomatic course with clinical manifestation at later stages with decompensated liver cirrhosis and/or HCC.

Implementation of high-sensitivity makes it possible to diagnose liver diseases early and in time for effective therapies thereby preventing its progression to cirrhosis and HCC. At the present time there are reforms going on in screening blood donors in the transfusion service.

Treatment of patients with chronic liver diseases is a difficult, expensive and one of the top priorities in practical healthcare. During the last 10 years major advances have been made in the treatment of chronic hepatitis B and C.

Considering the different modes of HBV transmission the most promising way for prophylaxis of HBV infection that is first vaccine in the world to also prevent a malignancy.

A high priority task for Turkmenistan is the prevention of viral hepatitis with a National Program for Newborns Vaccination (Order N350 dd.27.11.01 "On measures for hepatitis virus sickness rate decrease in Turkmenistan" Ministry of Health and Medical Industry of Turkmenistan). Vaccination of all newborns against hepatitis B is included in the vaccination calendar and was started in January 2002 in Turkmenistan.
Assessment of Helicobacter genus DNA in the bile from gallbladder and bile ducts in patients with cholestasis and cholecystolithiasis

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Introduction: The presence of helical bacteria Helicobacter pylori (H. pylori) in the mucous layer of gastric juice was combined with chronic gastric and duodenal ulcer diseases. The role of autoimmunity in the H. pylori infection pathogenesis became well known. There was a few data about the influence of H. pylori on the primary biliary cirrhosis (CBP), primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH).

The aim of the study was an assessment of presence 16S rDNA Helicobacter genus in the bile from gallbladder and bile ducts in patients with chronic cholestasis and cholecystolithiasis.

Methods: 63 patients were admitted to hospital because of cholestasis - Group-1 (44 patients), and cholecystolithiasis - Group-2 (19 patients). Aetiology of cholestasis was: choledocholithiasis in 14 cases, AIH in 4 cases, PSC in 4 cases, suppurative cholangitis in 4 cases, CBP in 2 cases and unknown aetiology in 16 cases. The samples of bile were collected during endoscopic retrograde pancreatobiliary cholangiography and cholecystectomy. DNA from bile was extracted by QIAamp DNA tissue kit (Qiagen) and amplified by nested PCR reaction with Helicobacter genus specific primers.

Results: The Helicobacter genus DNA was detected in Group-1 in 10/44 cases (0/4 with AIH, 1/2 with CBP, 2/4 with PSC, 3/14 with choledocholithiasis, 0/4 with suppurative cholangitis and 4/16 with unknown aetiology of cholestasis) and in Group-2 in 7/19 cases.

Discussion/Conclusion: Helicobacter genus bacterium could take part in pathogenesis of CBP and PSC, but its role in pathogenesis of gallstones is still unknown and needs more clinical trials.
Biliary atresia in Republic Sakha (Yakutia)

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Aim: To evaluate the clinical presentation, complications and outcome of infants with biliary atresia.

Methods: Retrospective chart analysis of medical records of infants with biliary atresia was made for period 1999-2004.

Results: A total of 9 patients were studied, 4 pts (44%) diagnostic over last year. There were 8 boys and 1 girl. The median age of presentation was 60 days (range 40-90 days). Infection of cytomegalovirus was found in 8 patients (89%). In 44% cases (4 pts) was intrahepatic biliary atresia, in 64% cases - extrahepatic biliary atresia. All cases is non-syndromic the biliary atresia. The same surgical team performed Kasai operation on 7 infants (hepatic portoenterostomy: 5 pts, hepatic portocholecystostomy: 2 pts). The median age at time of operation was 66 days after birth (range 47-106 days). 6 pts had no drainage and 1 patient had good drainage. Among the 6 patients with no drainage had died, 4 pts with biliary cirrhosis is on the waiting list for liver transplantation. The median age when the patient became jaundice-free was 2 months of age (range 1-3 months). Complications included cholangitis (22%), portal hypertension (44%). The age at surgery did not significantly influence the outcome of the patients.

Conclusions: Incidence of biliary atresia increased over last 1 year and composed 4:14000 live births in Yakutia. In 89% cases was founded association of biliary atresia with cytomegalovirus infection. Liver transplantation is the complementary therapy option in 60% patients.
Ursofalk® in cholestatic liver diseases in infancy

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**Aim**: To investigate the effect of Ursofalk® in different cholestatic liver diseases in infancy

**Material and methods**: From 1998 until 2005 17 consecutive infants (age: 15 days - 3 months; average 35 days) with cholestatic liver diseases were admitted and treated at the University Children’s Hospital Belgrade. The average follow up was 16 months (range: 8 months to 7 years). The diagnosis were follow: congenital biliary hypoplasia (2), α1-antitrypsin deficiency (2), inherited defect of bile acid synthesis (1), cystic fibrosis (2), neonatal hepatitis (3), biliary atresia (2), and functional cholestasis after small intestinal resection(2) and systemic infections associated with circulatory failure (2), and neonatal sclerosing cholangitis (1). All diagnosis were proven by genetic studies, cholangiographics, variety of metabolic investigations, and liver biopsies. Beside other sorts of liver and nutritional support measures, all infants were treated with Ursofalk® 15 mg/kg/day.

**Results**: Follow up studies demonstrated that Ursofalk® has had strikingly good effect of the degree of hyperbilirubinemia, GGT, and alkaline phosphatase levels and other liver function tests in the following group of infants: syndromatic biliary hypoplasia, α1-antitrypsin deficiency, cystic fibrosis, both form of functional cholestasis, neonatal hepatitis, and neonatal sclerosing cholangitis. In some of them it seems to be the life-long treatment option, eg. neonatal secerusing cholangitis, syndrotnatic biliary hypoplasia, cystic fibrosis, α1-antitrypsin deficiency associated with biliary hypoplastic changes.

**Conclusion**: Beside cholestasis caused by biliary atresia and inherited defect of bile acid synthesis, the major categories of infantile cholestatic diseases demonstrate positive clinical and laboratory response to Ursofalk®. To control the disease process, in some of them Ursofalk® seems to be life-long therapeutic option.
Vaccination against hepatitis B in heterozygous children with α₁-antitrypsin deficiency

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Introduction: To evaluate antigenic response to recombinant DNA vaccine against hepatitis B in children with chronic liver disease. Vaccination against hepatitis B in Croatia is obligatory for some risk groups and 12-year old children.

Methods: Six children (two girls and four boys) with prolonged jaundice and elevated liver enzymes during infancy due to α₁-antitrypsin deficiency (all heterozygots PiMS or PiMZ) were vaccinated against hepatitis B with recombinant DNA vaccine in 0, 1, 6 months schedule. Two months after third dose aminotransferases and titre of anti-HBs antibodies was determined as an indicator of successful vaccination.

Results: All children develop good protection level of anti-HBs antibodies (> 100 mIU/mL) with no significant increase of liver enzymes (p < 0.05)

Discussion/Conclusion: Although number of vaccinated patients is small, vaccination with recombinant DNA vaccine against hepatitis B is safe with no influence to liver enzymes level. Since no one vaccine is immunogenic in all vaccinated persons, in some risk groups control of antibodies titre is obligatory. Vaccination against hepatitis B is very important in children with possible chronic liver disease and current recombinant vaccine is safe with immunologic response.
Activated hepatic stellate cells and myofibroblasts accelerate TGF-beta dependent tumor progression of hepatocytes in a dual transplantation model

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Introduction: The progression of hepatocellular carcinomas (HCCs) derived from parenchymal hepatocytes is almost exclusively accompanied by the intra- and peritumoral accumulation of connective tissue known as fibrosis. For both hepatic tumorigenesis and fibrogenesis, transforming growth factor (TGF)-beta executes a key role and thus represent a hallmark in these pathophysiological events.

Methods: Making use of a dual transplantation model we investigated that the interaction of the fibrotic tumor microenvironment, containing either non-tumorigenic activated hepatic stellate cells (HSCs) or HSC-derived myofibroblasts (MFBs), together with neoplastic MIM-R hepatocytes.

Results: Co-transplantation of HSCs or MFBs with MIM-R hepatocytes drastically accelerates their progression in malignancy. Contrary to carcinomas derived from co-inoculated HSCs and MIM-R cells, co-transplantation of MFBs with aberrant MIM-R hepatocytes yielded strongest tumor formation along with undifferentiated cancerous tissue displaying constitutive TGF-beta signaling and nuclear localization of $\beta$-catenin in hepatocytes. Genetic interference of TGF-beta signaling through expression of inhibitory Smad7 in MIM-R hepatocytes revealed that dual transplantation of these cells with TGF-beta1 secreting MFBs fails to enforce tumor progression. Functional analysis of experimental tumors further showed that their reduced malignancy due to disrupted Smad signaling was devoid of nuclear beta-catenin accumulation.

Discussion/Conclusion: Together these data provide direct evidence that HSC, and to a larger extent, MFBs are capable to govern late stage carcinogenesis of hepatocytes in a paracrine fashion which might ultimately lead to the fatal autocrine regulation of TGF-beta signaling observed in HCCs.
Peculiarities of viral hepatites C in children

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Introduction: Recently the problem of hepatitis C (HC) in children has become very topical due to enhanced rate of hepatitis C infection and that of chronic form development. The aim of our research was to study epidemiologic and clinico-biochemical peculiarities of hepatitis C courses and outcomes in children.

Methods: We observed 65 children with VHC aged from 1 year to 14 years: acute viral hepatitis was diagnosed in 9 patients, chronic hepatitis C - in 56 patients. There were no lethal outcomes in the patients observed. Boys (52.2%) and city dwellers prevailed. The greatest number of patients was recorded in elder age groups (7 to 14 years, 67.2%).

Results: Chronic viral hepatitis (CVH) was very often combined with severe somatic diseases (chronic glomerulonephritis, chronic decompensated tonsilitis, operations). Blood was transfused to some of the children within the proposed incubation period. Chronic HCV infection was latent, without a pronounced clinical picture in half the patients and was characterized by hepatomegaly very frequently combined with splenomegaly and the presence of extrahepatic signs. In our observations, the extrahepatic signs were cholecystitis and cholecystocholangitis and a vascular system injury as teleangioectases. The peripheral blood of the patients with CHC showed anemia (46.4%), leukopenia (95.2%), lymphocytosis (85.35%), a decrease in ERS. The liver ultrasound investigation demonstrated echo signs of consolidation in Glisson's capsule strata, sclerosing cholangitis and gallbladder thickening in 38.6% of the children examined. CHC proceeded with periods of exacerbation in 68% of the patients, and 85.7% of the children with CHC were discharged in a satisfactory condition at a stage of remission. We believe the main risk factors and causes of a chronic process to be an early discharge from the hospital, the absence of a complete normalization of the clinico-biochemical parameters (14.5%) and a violation of the regimen and diet (12.1% of the children). The majority of the children with CH had an unfavourable premorbid background (frequent acute respiratory infections, asthmatic bronchitis, angines, chronic infection foci, non-differentiated lymphadenopathy, operations). The patients were treated with recombinant interferons (intron and reaferon). A remission occurred only after 6 months following the treatment and only in 27 patients (41.5%). They endured the treatment satisfactorily: side effects developed in the majority of the patients, but they were not very pronounced.

Discussion/Conclusion: Thus, HC and concomitant injuries occurring in childhood may be retained in adults, inducing serious impairments in health. This requires development of more effective methods for treatment of this category of patients to decrease the percentage of chronic hepatitis.
What is the actual prevalence of the D virus infection in chronic hepatitis and liver cirrhosis in Romania?

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Introduction: Virus D hepatitis continues to represent a public health problem in the south-eastern European countries, but the spread of the D virus infection in Romania is not completely elucidated. The paper proposed to assess the prevalence of the hepatitis D virus infection in patients with HBsAg-positive chronic hepatitis and liver cirrhosis in Romania.

Methods: A number of 219 patients with chronic hepatitis and 168 with liver cirrhosis, all testing positive for HBsAg were studied. Viral markers were determined by immunoenzymatic methods. Demographic characteristics (age and sex) as well as the distribution by Child-Pugh classes for liver cirrhosis were analyzed.

Results: In HBsAg-positive chronic hepatitis the prevalence of the D virus was 37.9% and in liver cirrhosis 51.19%. The great majority of the cases infected with hepatitis B virus were HBeAg-negative.

Discussion/Conclusion: These findings situate Romania among the countries with a high prevalence of the hepatitis D virus infection, but decreasing as compared to the data communicated by previous reports.

Keywords: virus D infection - chronic hepatitis - liver cirrhosis

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Endoscopic surveillance for hereditary non-polyposis colorectal cancer (HNPCC) families

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Introduction: The current guidelines for the endoscopic surveillance of members of HNPCC families suggest a colonoscopy every 2 years for all the first degree relatives of the index case, starting at 25 years of age.

Methods: We selected 11 families satisfying the Amsterdam criteria I and we collected data from 107 first degree relatives of 11 index cases. Of these, 15 died, 14 were younger than 25 years and 33 refused surveillance program. Eventually, 35 subjects were enrolled in the endoscopic surveillance program, together with 10 index cases.

Results: At the time of the first colonoscopy 26 out of 45 subjects had no colorectal lesion; on the other hand, 19 subjects had 26 lesions at colonoscopy: 3 hyperplastic polyps (HP), 11 low grade adenomas (LGD), 1 high grade adenomas (HGD) and 11 cancer (K). Thirty-six out of 45 enrolled subjects underwent 127 colonoscopies with a median interval of 23 months. Seventy-one endoscopies resulted negative, while 56 colonoscopies, performed in 25 patients, had a total of 97 lesions: 4 inflammatory polyps (IP), 38 HP, 43 LGD, 6 HGD and 6 K. The median interval between two positive endoscopies was 27.4 months for both IP (range 6-37) and HP (range 4-135), 33.6 months for LGD (range 4-168), 56.6 months for HGD (range 4-168) and 20.5 months for K (range 9-48).

Discussion/Conclusion: Our data suggest that our surveillance program is consistent with the international guidelines for surveillance. Moreover, our results support the hypothesis of an increased neoplastic risk associated with hyperplastic polyps.
Hepatectomy for huge HCC

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Introduction: Hepatocellular carcinoma (HCC) is the more common cancer and the second cancer death in China.

Methods: From 1995 to 2002, 179 cases of hepatectomy for huge HCC were performed in our hospital. Of them, 155 (86.6%) were males and 24 (13.4%) females; ages ranged from 15 to 77 years (mean ± SD, 56.3 ± 13.7 years); 138 (77.1%) were HBsAg-positive; 144 cases (80.4%) with liver cirrhosis; in 63 patients (35.2%) serum α-fetoprotein (AFP) was higher than 400 ng/ml; the tumor sizes ranged from 5 to 30 cm. TNM Staging revealed that 75 in stage II, 60 in stage III, 37 in stage IVa and 7 in stage IVb. The procedures were all performed under normothermic interruption of porta hepatis at single time. Interruption lasted 15 to 40 minutes. The procedures of hepatectomy included right trisectionectomy in 23 patients, left trisectionectomy in 4, extended right hepatectomy in 11, extended left hepatectomy in 3, central hepatectomy in 4, right hepatectomy in 30, left hepatectomy in 14 and others.

Results: Postoperative complication rate was 10.6% (19/179); operative mortality within 30 days was 1.1%. Postoperative overall and cancer-free survival rates at 1-, 3- and 5-years were 82.0%, 51.1% and 40.2%, and 73.1%, 46.0% and 38.1%, respectively.

Discussion/Conclusion: Hepatectomy should be regarded as an effective and safe procedure for huge HCC.
Assessment of MDR1/MRP1 transporter function in lymphocyte-subpopulations of inflammatory bowel disease

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Background: Most current drugs to treat inflammatory bowel disease (IBD) are substrates of ABC-MDR transporters. These proteins significantly alter intracellular drug availability and thus efficacy. Our aim was to determine the CD markers of choice to monitor induction of ABC-transporter proteins in IBD patients.

Patients & Methods: 50 patients were included in the study: 24% of pts were non-responsive to conventional pharmacotherapy, 22% of them were currently on steroids. 33% were in remission with steroids at the time of testing. Lymphocytes were labeled by anti-human CD3, CD4, CD8, CD19, CD45 and monocytes by anti-human CD14 antibodies. In all cases MDR1 and MRP1 activity was assessed as described in the original assay.

Results: Both MDR1 and MRP1 activity was within normal limits in CD4, CD14 and CD19 positive lymphocyte sub-population. Total (MDR1 and MRP1 related) resistance values were significantly elevated in both CD3 and CD8 positive cells compared to the rest of the lymphocyte-subsets.

Conclusion: CD3 and CD8 positive lymphocyte subsets seem to express ABC-MDR transporters. Based on our findings, MRP1 activity of CD8 positive (cytotoxic) T cells may confer resistance to conventional drugs like steroids and AZA. Prospective trials have to address the role of this phenomenon in clinical resistance.
Increased expression of geranylgeranyl pyrophosphate synthase is associated with hepatitis C virus RNA persistence in peripheral blood mononuclear cells

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Introduction: Although the liver is the main place of HCV replication, HCV can persist in peripheral blood mononuclear cells (PBMC) long after apparently complete hepatitis C resolution [Pham (2004) JVI 78]. Our previous studies have demonstrated that in some patients, who responded to IFN therapy with HCV RNA elimination from sera, HCV genome in PBMC was still detectable. From the other hand recent papers have shown that HCV replication seems to be regulated by geranylgeranylation process [Kapadia (2005) PNAS 102] which in turn requires geranylgeranyl pyrophosphate (GGPP) presence in host cell and that HCV replication could be disrupted by the inhibition of geranylgeranylation process [Ye (2003) PNAS 26].

As geranylgeranyl pyrophosphate synthase is an enzyme critical for GGPP synthesis, the aim of our study was to investigate the relationship between expression level of GGPP synthase and persistence of HCV RNA in PBMC after elimination HCV RNA from sera.

Methods: HCV RNA was determined in "one-tube" RT-PCR reaction in PBMC originated from the patients that eliminated HCV RNA from sera after IFN-alpha therapy. PBMC were isolated from venous blood by centrifugation on Histopaque-1077 gradient. GGPP synthase -specific PCR and beta-actin-specific (control) PCR were performed on 100-times diluted cDNA that was synthesized in RT reaction (Improm, Promega) from 6 microgram of RNA isolated from each PBMC sample in the presence of random primers.

Results: HCV RNA determinations in PBMC originated from 27 patients that have lost HCV RNA as a result of IFN therapy, let us select two groups of PBMC samples: first group, where HCV RNA was eliminated (15 samples) and the second, where HCV genome was still detectable (12 samples). In both group stable expression of beta-actin gene was confirmed by RT-PCR. The semiquantitative RT-PCR analysis showed that GGPP synthase expression was markedly altered depending on the selected group. cDNA originated from the PBMC samples that maintained HCV genome revealed over 10-times higher GGPP synthase gene expression then cDNA originated from PBMC samples, where HCV RNA was eliminated.

Discussion/Conclusion: Despite of the elimination HCV RNA from sera, HCV genome can persist in PBMC which is associated with significant elevation of GGPP synthase. GGPP synthesis seems to be essential for sustained HCV replication in PBMC.
Occurrence of HFE gene mutations in HCV-infected and non-infected patients with disorders of iron metabolism

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Introduction: Up to now there are no data about occurrence of HFE gene mutations in Poland and their association with iron disorders and liver diseases.

Methods: 107 patients observed because of liver pathology with increased above normal values levels of iron and ferritin, were included to the study. 56/107 patients were infected with HCV, among them 40 were treated with interferon, 27 without success. In 95/108 cases histopathological examination of the liver biopsy specimen was performed with appointment of inflammation activity, fibrosis, steatosis and iron deposits. PCR and RFLP methods were used for detection C282Y and H63D mutations.

Results: C282Y mutation was detected in 26/107 (24%) and H63D in 36/107 (34%) studied patients. Non-infected patients were C282Y carriers significantly more often than HCV-infected. Two patients were carriers of both mutations. No statistical significance between HCV infection and occurrence of H63D mutation was proved. In the group of HCV-infected patients 4/7 H63D carriers and 4/5 C282Y carriers appeared non-responders to interferon therapy. HCV-infected patients were older, they presented higher ALT values and inflammation activity in liver specimens. Non-infected patients presented more intensive liver iron deposits and more frequently liver steatosis. There were no difficulties in values of inclusion parameters in both analysed groups.

Discussion/Conclusion: Presence of iron disorders stated on increased values of iron and ferritin in serum is a sensitive mark in suspicion of HFE gene mutations. HCV infection may cause iron disorders independently on HFE gene mutations. Influence of H63D mutations on chronic hepatitis C needs further analysis.

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Helicobacter pylori eradication significantly decreases dyspeptic symptoms in chronic renal failure

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Introduction: Helicobacter pylori (H. pylori) has been implicated in the development of gastrointestinal lesions in chronic renal failure (CRF). The indication for eradication therapy and the best therapeutic approach in CRF patients are still controversial matters. The aim of this study was to assess the efficacy of two therapeutic regimens.

Methods: One hundred and six patients were included in the study: 55 patients on chronic haemodialysis (group I), and 51 patients with CRF (10 < ClCR < 60 ml/min; group II). Interviews, clinical examinations, and gastroduodenoscopies were performed, gastric biopsies were obtained for rapid urease test, histology and culture, and serum H. pylori specific IgG were measured. In 23 patients (41.8%) of the group I, and 24 (47.1%) of the group II, H. pylori was demonstrated by either histology or culture. Forty-seven CRF patients infected with H. pylori received pantoprazole 40 mg bid and amoxicillin 1000 mg bid during 7 days, and were randomly assigned to either metronidazole 500 mg bid/7 days (group A - 23 patients) or azithromycin 1000 mg od/3 days (group B - 24 patients). Four weeks after the treatment all measures were repeated.

Results: Success in H. pylori eradication was evaluated in 44 patients and achieved in 16/21 (76.2%) of patients from the treatment group A, and in 19/23 (82.6%) patients from the group B (p = 0.59). Eradication of H. pylori significantly improved both ulcer- and oesophagitis-like dyspeptic symptoms in both groups (p < 0.05 for each symptom). The average GDSS was significantly decreased in both treated groups: from 9.22 ± 3.59 to 5.14 ± 2.57 in group A, and from 9.25 ± 3.94 to 5.96 ± 2.55 in group B, respectively. There were no significant differences between two regimens in attenuation of dyspeptic symptoms. Eradication led also to the gradual improvement of gastric mucosa, independent of therapy combination, showing reduction of acute and chronic inflammation (p < 0.0001).

Discussion/Conclusion: Both triple therapies showed comparable efficacy, safety and tolerability, azithromycin regimen allowing much easier (once daily) and shorter (3 days) course. The treatment of H. pylori should be carefully chosen regarding antibiotic resistance and compliance in CRF patients.
The durability of lamivudine-induced virological response in children with chronic hepatitis B unresponsive to previous interferon-alpha therapy

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Introduction and aim of the study: Since data regarding the efficacy of lamivudine treatment in children with chronic hepatitis B who failed to respond to previous IFN-alpha therapy are lacking, the durability of lamivudine-induced virological response in children up to 6 months after treatment termination were determined.

Methods: The prospective observation was carried out on 37 children, aged 4-17 years, with biopsy-proven chronic hepatitis B (HBeAg+, HBV DNA+), who were nonresponders to previous IFN-alpha therapy (3 MU tiw for 20 weeks). Lamivudine in the dose of 3-4 mg/kg/day up to 100 mg/day was applied for 24 months.

Results: After completion of 24-month therapy 70.3% of children normalized ALT activity, 45.7% cleared HBV DNA and 27% seroconverted to anti-HBe. Three months after treatment termination the reseroconversion to HBeAg was not observed, but ALT flare (8 x ULN) with reappearance of serum HBV DNA was noticed in one child. 3 months later the reappearance of HBV DNA was observed in 9 out of 26 examined children (34.5%) but seroconversion was still permanent in this group except one child who reseroconverted to HBeAg with concomitant increased activity of ALT (7 x ULN).

Conclusions: The 2-year treatment with lamivudine in children with chronic hepatitis B who did not respond to IFN-alpha normalized ALT activity in most children and caused seroconversion to anti-HBe in 27% of them. Seroconversion rate was permanent up to 6 months after therapy completion. However, after 6 months after treatment termination the reappearance of viral replication was observed in 1/3 patients with concomitant ALT flare in selected cases.
Treatment of pediatric metabolic diseases by liver transplantation

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Metabolic diseases (MD) caused by inborn defects in liver synthesized enzymes are one of the most common indications for liver transplantation in pediatric population, just after biliary atresia. We report our experience and results of pediatric liver transplantation for metabolic diseases in a single European Center.

Materials and Methods: In Bergamo Liver Transplantation Center 305 cadaveric liver transplant were performed in 285 pediatric recipients from October 1997 to March 2005. The most frequent indication was biliary atresia (65%); in 12 patients (about 4%) indication was a MD: neonatal hemochromatosis (2), type IV glycogenosis (2), hyperoxaluria (1), Wilson disease (1), ornithine transcarbamylase deficiency (1), Crigler-Najjar syndrome (2), deficiency of H factor with hemolytic-uremic syndrome (HUS) (1), α1-antitrypsin deficiency (1), propionic acidaemia (1). In few instances LFTs were within normal values and liver morphology was nearly normal (Crigler-Najjar syndrome, hyperoxaluria, propionic acidaemia, deficiency of H factor with hemolytic-uremic syndrome) thus indicating a pure biochemical defect. The median age at liver transplantation of these latter patients was 5.4 yrs (range 0.2-13), median weight was 30 kg (range 3-55); 6 patients were UNOS status 4 at time of transplantation, 6 in UNOS status 1. Three patients received a whole organ, 9 received a split liver (segment II and III in 6 cases and segment IV-VIII in one case). In two patients a simultaneous kidney and liver transplantation was performed due to concomitant chronic liver and kidney failure. After a post-surgery short term course (8 months) of steroids plus tacrolimus, long-term immunosuppression was performed by tacrolimus in all. HUS patient developed an humoral rejection and needed to be rush retransplanted.

Results: Global actuarial liver recipients patients survivals are respectively 87% and 84%, 78% and 74% at 1, 2, 3 and 4 yrs after surgery. Patients and grafts survival of liver transplanted children for MD are 91% and 75%, respectively, at 1 and 2 yrs after OLTx. Two patients have been retransplanted, one for chronic rejection and one for hyperacute-humoral rejection. No patient developed vascular complications, while one underwent surgical revision of a bilo-digestive anastomotic stenosis. No patient had recurrent of native disease.

Conclusions: Liver transplantation may be therapy of choice for children affected by inherited hepatic enzymes deficiency, causing sometimes end stage liver disease. In few cases hepatic histologically proven damage is mild in spite of a severe systemic disease.
Our experience shows very good results in term of patient survival similar to pediatric patients liver transplanted for non metabolic diseases.

Correction of metabolic defect can be obtained.

Simultaneous kidney and liver transplantation has also cured a child with familiar HUS. This result is the first reported demonstration that HUS is caused by a defect in the hepatic based synthesis of H factor.
A 5-years single center experience in liver transplantation for paediatric end-stage cholestatic diseases

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Chronic cholestatic liver diseases are the leading causes of cirrhosis and hepatic failure in pediatric groups. Orthotopic liver transplantation (OLTx) is now a safe curative procedure in these diseases. We present a 5-years single center experience with encouraging long-term results.

Materials and Methods: Between October 1997 and August 2003, 206 pediatric patients (median age at OLTx: 1.4 yrs - range 0.1-17 yrs; median weight: 9 kg - range 2-65 kg) underwent 229 OLTx for end-stage liver disease in our center. Most of them (73%) received a split liver graft and an entero-biliary anastomosis were performed in all of them.

161 were transplanted for end-stage cholestatic liver disease (123 extrahepatic biliary atresia, 17 Alagille syndrome, 12 familial progressive intrahepatic cholestasis, 3 Crigler-Najjar syndrome) after a median time of 40 days period in waiting list. Pre-OLTx LFT's values were very high levels of total bilirubin (mean 18.5 mg/dl; range 0.8-46), cholesterol (mean 215 mg/dl; range 22-1120) and other laboratory findings of liver failure. Most of them (85%) suffered from severe malnutrition and were above 5th percentile at OLTx (mean weight 8 kg; range 4-44). Incidental HCC was discovered in explanted liver in 3/161 and chemotherapy was performed; no tumor relapse was experience in a medium time follow-up of 3 yrs. Patients were discharged from Intensive Unit in a median time of 3 days and from Pediatric Department in a median time of 3 weeks. Growth indexes come back to normal values at a mean time of 0.8 yrs from surgery. Post-OLTx immunosuppression was carried on drugs combination in first 8 months (steroid and cyclosporin or tacrolimus) and on cyclosporin or tacrolimus basis in long term follow-up. Follow-up was realized in all children as outpatients with a previously definite schedule; access to Liver Unit for sudden matters was anyway warranted.

Results: Patients' survival rate was 87% at one year from surgery, 85% at 3 years and 83% at 5 years from surgery. Main post-OLTx complications were acute cellular rejection steroid-sensitive (24%), vascular thrombosis (11%) and mechanical cholestasis due to anastomotic stricture (43 pts, 27%); this latter complication was successfully treated by PTC procedure and long-term administration of ursodeoxycholic acid at maximal dosage in 38 pts. PTLD-EBV related was observed in 4 children (2 case of polymorphic PTLD, 2 cases of large cell B lymphoma) and were successfully treated by stopping immunosuppressive drugs. Re-transplantation rate was 10% (16 pts) and mortality rate during and around surgery was 8%. 

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Conclusions: Liver OLTx is a safe curative procedure in liver end stage disease, that are mainly cholestatic. Without surgery prognosis of these pathologies is extremely severe; patients death is expected before puberty. Extensive use of split liver warrants a fast OLTx with no mortality in waiting list. Most of post-operative biliary complications can be easily managed by minimal invasive procedure. OLTx seems to be also curative for incidental HCC in few patients.
Tissue factor expression in intestine during peptidoglycan induced enterocolitis in Lewis rats

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Introduction: Activation of the extrinsic coagulation pathway through the tissue factor (TF) as well as intrinsic pathway via factor XI with concomitant generation of the kallikrein-kinins (K-K) may mediate intestinal inflammation. We have shown that the K-K system activation mediates acute (2 days) and chronic granulomatous (19 days) phase of peptidoglycan-polysaccharide induced enterocolitis in Lewis rats. Now we investigate the TF expression and localization in normal and inflamed intestine in Lewis rats.

Methods: Lewis rats were injected intramurally in the intestine either with PG-APS (disease group) or human serum albumin (control group). TF was identified in the intestinal homogenates by functional assay of procoagulant activity (PCA). The tissue sections were immunostained for TF.

Results: Intestinal inflammation evaluated by gross and histological score as well as hemorrhage signs. PCA was significantly increased in both acute (191.6 ± 23 SE pg, p < 0.001) and chronic (92.6 ± 16, p < 0.02) phase in disease groups as compared with controls (47.3 ± 6.5 and 36.56 ± 8, respectively). TF was identified by immunostaining in apical part of the crypts in normal intestinal tissue. In inflamed tissue staining for TF was more prominent in the crypts and TF was also localized in macrophages inside granulomas and in vascular endothelium.

Discussion/Conclusion: Increase of PCA in the inflamed intestine indicates TF up-regulation during inflammation. Intestinal hemorrhage observed in the acute phase in this model may be mediated by local TF-dependant extrinsic coagulation activation. Our data suggest that local TF expression in macrophages and endothelium may play a role in inflammatory bowel disease.
Evaluation of bile reflux in patients with gallstones

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Introduction: Dyspeptic symptoms in patients with gallstones and persistence of dyspeptic symptoms after cholecystectomy is common. There is evidence that some symptoms may be attributed to duodenogastric reflux.

Methods: The aim of the study was to quantify duodenogastric reflux in patients with gallstones and in patients with cholecystectomy. The method of 24-h esophageal and gastric Bili-tec monitoring was performed in 25 patients: 5 healthy, 15 with gallstones and 15 patients with cholecystectomy. Bilirubin reflux was judged when absorbance was 0.15 or more. The duration of reflux compared to the total observed period was obtained to determine percentage of time. 24-hour monitoring was completed in all of 35 subjects.

Results: Biliary colic was present in 6 patients with gallstones and in 2 patients with cholecystectomy; dyspepsia was present in 8 patients with gallstones and in 6 patients after surgery. Only 6 cases had symptoms that was correlated with the presence of biliary reflux. The time percentage of esophagus bilirubin reflux was less than 5% in all controls. In 7 patients with gallstones and also in 9 patients with cholecystectomy the time percentage of bilirubin reflux was more than 8%; in 2 cases with gallstones and also in 4 cases after surgery the duodenogastric reflux was greater than 20%.

Discussion/Conclusion: 24-hour bile monitoring provides the clinician evidence of excessive biliary reflux in patients with gallstones and in patients with cholecystectomy. According to this study, there are poor correlations between the presence of the biliary reflux and dyspeptic symptoms.
Expression of matrilin-2 in liver cirrhosis and hepatocellular carcinoma


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Introduction: The recently described matrilin protein family is a part of the extracellular matrix, however their pathophysiological role as well as distribution in liver diseases has not yet been studied. Considering that matrilins have been found to play role in cell growth and tissue remodeling, their possible involvement in carcinogenesis has been raised.

Our goal was to study the expression of matrilin-2 by real-time PCR, immunohistochemistry and Western blot analysis in hepatocellular carcinoma (HCC), surrounding liver parenchyma and normal liver.

Methods: Seven normal human livers, 10 HCC with cirrhosis and 10 without cirrhosis including respective surrounding tissues were investigated. Matrilin-2 rabbit polyclonal antibodies and laminin mouse monoclonal antibodies were used for immunohistochemistry, confocal laser scanning microscopy and Western blot detections. mRNA expression was measured by real-time PCR using relative quantification to beta-actin expression.

Results: Normal liver showed weak matrilin-2 expression around bile ducts, blood vessels and central veins, while sinusoids were negative. Cirrhotic surrounding tissue of HCC samples showed intensive matrilin-2 staining along sinusoids. Tumorous neovasculature was found to be positive by immunohistochemistry. Co-localization of matrilin-2 with laminin was proved by confocal microscopy. However, neither Western blot analysis nor PCR showed significant differences in matrilin-2 protein and mRNA quantity between normal and tumorous samples.

Discussion/Conclusion: Co-localization of matrilin-2 with laminin confirms the presence of this protein in basement membrane. Matrilin-2 appears during capillarization in liver cirrhosis and during tumorigenesis, and might be a potential target for influencing vascularization.

The project was supported by grants: Biol 14/2001, NKFP-1/0023/2002, ETT-077/2003, OTKA-T037838
The role of laparoscopic surgery in the treatment of focal liver diseases

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Introduction: The laparoscopic resection of focal liver diseases such as benign or malignant tumours is a more often used technic. The authors present the laparoscopic approach to liver resections.

Methods: 33 liver resections were performed in a five year period. The indications of the laparoscopic resections were single colorectal metastases in 3 cases, focal nodular hyperplasia in 5 cases, hemangioma in 8 cases, cyst in 6 cases, angiodysplasia in 2 cases and other benign tumour in 9 cases. In all cases US and CT was performed preoperatively to localised the tumour. This technique was used whenever the tumour was located within the lower segment or periphery of the liver. The average age of the patients was 49.9 (24-80) years. The mean size of tumour was 3.1 (1.5-9) cm. There were performed anatomical segment resection in 5 cases, pericystectomy in 2 cases, enucleation in 2 cases and non anatomical resection in 24 cases. Harmonic scalpel was used for dissection of the liver parenchyma in all cases.

Results: There was no complication related to the operation. The postoperative morbidity was 6.6%. One reoperation was performed because of the bile leakage, but there was other complications such as fever, temporary elevation of the liver necroenzymes. The average nursing time was 5.8 days.

Conclusions: Laparoscopic resection of single focal diseases of the liver may be performed safely with acceptable postoperative complications.
The significance of immunohistochemical study in diagnosis and prognostic evaluation of the papillary cystic and solid tumor of the pancreas: A case report

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Introduction: Papillary cystic and solid tumor of the pancreas is a rare neoplasm of benign or low malignant potential generally found in young women.

Methods: We report on a 32-year-old woman with a two-year history of upper abdominal pain and occasional fever. CT scan examinations revealed a heterogeneous mass of 7 cm in diameter with solid and cystic components, localized in the tail of the pancreas. The tumor was excised by distal pancreatectomy. Immunohistochemical staining was performed by LSAB method using antibodies to vimentin, S100, pan-CK, synaptophysin, neuron-specific enolase, chromogranin, actin, desmin, Ki-67, p53, estrogen receptor and progesterone receptor.

Results: Histologically, we established a solid and cystic papillary tumor of the pancreas with low mitotic activity. Immunohistochemical study revealed a diffuse expression for vimentin and a focal positivity for pan-CK. Neuroendocrine markers were negative in the tumor cells. There was no accumulation of p53 protein. We counted 5% Ki-67-positive cells. The tumor cells were negative for estrogen receptor but 50% expressed progesterone receptor. The patient is well without any signs of recurrence 5 years after the operation.

Discussion/Conclusion: Papillary cystic and solid tumor of the pancreas appears to have limited malignant potential, with good prognosis after surgical resection. The immunohistochemical staining fails to reveal a clear phenotypic relation with any of the define cell lineages of the pancreas. Positive immunoreactions for progesterone receptor suggest a possible implication in tumorigenesis and the usefulness in treatment of unresectable tumors.
Hepatocellular carcinoma caused by loss of heterozygosity in *Lkb1* gene knockout mice

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**Introduction:** Germline mutations of the *LKB1* gene are associated with Peutz-Jeghers syndrome, which is characterized by mucocutaneous pigmentation and gastrointestinal hamartoma with an increased risk of cancer development.

**Methods:** To investigate the role of LKB1 *in vivo*, we have recently constructed *Lkb1* gene knockout mice.

**Results:** Here we demonstrate that the *Lkb1* (+/-) mice develop hepatocellular carcinomas (HCCs). In *Lkb1* (+/-) mice > 50 weeks of age, > 70% of the male mice developed HCC, whereas only 20% of the females had HCCs, showing a sex difference in the susceptibility. Histological examinations revealed various types of HCCs, such as "trabecular", "clear cell", "pseudoglandular", and "sarcomatous" types, which were strikingly similar to those found in human HCCs. Western blotting and PCR analyses showed loss of *Lkb1* heterozygosity in all of the HCC tissues examined, indicating a tumor suppressor role of LKB1 in the mouse liver.

**Discussion/Conclusion:** These results suggest that lack of LKB1 is a novel mechanism for HCC development. Thus, the *Lkb1* (+/-) knockout mutant should be an important and useful model for human HCC.
Hepatocellular carcinoma in patients with occult hepatitis B - Potential impact on liver transplant

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Introduction: To study the presence of occult hepatitis B in our patients with hepatocellular carcinoma (HCC).

Methods: Between 2002 and 2004, 22 patients with HCC who consented, were recruited. HCC was defined by the EASL criteria. Standard blood investigations for hepatitis B and hepatitis C serology were performed. HBV DNA was extracted from the serum using QIAGEN test kit. HBV DNA detection was done by PCR. Surface antigen (HBsAg) and core antigen (HBcAg) primers were used. Two rounds of PCR was performed for each gene segment using two different sets of primers. Specimens were considered positive only if they were positive for both the surface and core antigens. The detection limit of our laboratory was > 500 copies/ml.

Results: Of the 22 patients with HCC, HBsAg by serology was positive in 11/22 (50%). 2/22 (9%) tested positive for HCV IgG. In 9 patients (41%), HBsAg and HCV IgG were both negative and there was no other etiology for chronic liver disease. HBV DNA by PCR was found to be positive for 2 patients who were tested HBsAg negative by serology.

Discussion/Conclusion: In our series of patients with HCC, 50% of patients had chronic hepatitis B. In patients with unknown etiology, occult hepatitis B infection was found in 22% (2/9). Occult HBV infection may be a significant cause of HCC. This may have an impact in the management of these patients in the light of liver transplant for HCC.
Taurine enhances spontaneous recovery of experimental liver fibrosis

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Introduction: Liver fibrosis is characterized by excessive production and decreased removal of matrix proteins in the hepatic sinusoids. Hepatic stellate cells (HSC) are the main producers of excessive collagen in the liver. Oxidative stress (OS) has an important role in hepatic injury and fibrogenesis. Spontaneous recovery (SR) of fibrosis after cassation of injurious agent was shown to be possible and associated with apoptosis of HSCs. We studied the effects of a potent antioxidant taurine on experimental liver fibrosis in comparison with SR.

Methods: Thirty Sprague-Dawley rats were injected with CCl⁴ for 12 weeks to induce liver fibrosis. Afterwards, CCl⁴ administration was stopped and the animals were divided into two groups. Group I (n = 15) was left for SR, and Group II (n = 15) was treated with taurine 1200 mg/kg/day. All the rats were killed after four weeks. Histopathological fibrosis scores, alphaSMA(+) HSC counts, apoptotic HSCs and hepatocytes, and oxidative stress parameters were determined.

Results: Taurine improved fibrosis in vivo. Fibrosis scores were reduced significantly in Group II when compared to Group I (p < 0.001). As the indication of activation, alphaSMA(+) cell counts were reduced in Group II when compared to Group I (p < 0.001). Taurine induced hepatocyte apoptosis significantly when compared to spontaneous recovery (p < 0.01). HSC apoptosis increased significantly in Group II compared to Group I (p < 0.001). Tissue SOD, MDA, and GSHPx levels improved significantly in Group II compared to Group I (p < 0.001, for all).

Discussion/Conclusion: Taurine improved experimental liver fibrosis better than SR mainly through induction of apoptosis resultant probably from decreased nitric oxide production.
Agrin accumulates in the liver during chronic liver injury and in hepatocellular carcinoma in human and rats

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**Introduction:** Agrin is a multifunctional heparan sulfate proteoglycan originally discovered in the neuromuscular junctions and later observed in numerous other localizations. Agrin is capable of binding various growth factors, eg. the proangiogenic bFGF, and it can directly interact with cell membrane receptors such as alphav-integrins. The presence of agrin in the liver, either healthy or diseased, has formerly not been reported.

**Methods:** Immunochemistry, immunoblotting, reverse real time PCR, cell isolation and cell culturing.

**Results:** In the healthy human liver, we detected agrin in minor amounts in the basement membranes (BMs) of blood vessels and bile ducts. In liver cirrhosis, agrin is seen overall in the BMs of proliferated bile ductules and of newly formed blood vessels. Agrin is also present in the neovascular BMs in the hepatocellular carcinoma. Hence, agrin accumulates in the liver in the above pathological conditions. An analogous distribution of agrin was observed in the liver of rats subjected to a combined fibrogenic/carcinogenic regimen, and agrin overexpression was verified by RT-PCR experiments. The putative sources of agrin in the liver are vascular smooth muscle cells, activated mesenchymal cells, biliary epithelial cells and perhaps endothelial cells. In all BMs, agrin was found in close proximity with alpha-integrin.

**Discussion/Conclusion:** Agrin in the BMs may bind growth factors, and it may activate cell surface receptors on biliary epithelial cells and endothelial cells. Therefore, we hypothesize a stimulatory role for agrin in neoangiogenic processes such as tumor vascularization, and in bile ductule proliferation.
Evaluation of the effect with propranolol, enalapril and losartan on the renin-angiotensin system in patients with liver cirrhosis

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Introduction: The renin-angiotensin system (RAS) is activated in most patients with liver cirrhosis and portal hypertension. Angiotensin II infusion increased portal pressure in cirrhotic patients. Therefore, the RAS might contribute to the degree of portal hypertension in cirrhosis.

The aim of the study was to evaluate the effect of the treatment with propranolol, enalapril and losartan on plasma renin activity (PRA), plasma aldosterone, sodium and potassium levels.

Methods: PRA, plasma aldosterone, sodium and potassium levels were determined in 88 patients with liver cirrhosis (class A and B according to Child-Pugh score) initial and after 1 month of treatment with propranolol (40-60 mg daily, n = 28), enalapril (5-10 mg daily, n = 28) and losartan (12.5-25 mg daily, n = 20). 11 patients did not receive these drugs (control group).

Results: Propranolol administration significantly decreased plasma aldosterone levels (196.4 ± 45.0 vs. 176.8 ± 39.1 pg/ml, p < 0.05) and increased PRA (0.86 ± 0.29 vs. 1.73 ± 0.90 ng/ml/h, p < 0.01). Treatment with enalapril significantly increased sodium secretion (140.0 ± 1.3 vs. 135.1 ± 0.8 mmol/l, p < 0.0001), decreased plasma aldosterone levels (111.8 ± 19.9 vs. 71.4 ± 15.8 pg/ml, p < 0.01) and increased PRA (0.67 ± 0.15 vs. 1.48 ± 0.49 ng/ml/h, p < 0.01). Losartan administration significantly increased sodium secretion (139.2 ± 1.5 vs. 134.7 ± 0.84 mmol/l, p < 0.0001), very significantly decreased plasma aldosterone levels (182.9 ± 45.7 vs. 119.0 ± 30.8 pg/ml, p < 0.0001) and increased PRA (0.69 ± 0.15 vs. 1.45 ± 0.44 ng/ml/h, p < 0.01).

Conclusion: Propranolol, enalapril and losartan have a beneficial action on the RAS in patients with liver cirrhosis and portal hypertension. However, the treatment with losartan had the advantages over propranolol and enalapril administration.
Prophylaxis of encephalopathy in patients with destructive forms of pancreatitis and cholecystitis on the background of alcoholic disease of liver

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One of the most important causes of development of fat liver is alcoholism. These patients develop malfunction of metabolism and the liver becomes more susceptible to toxic loading. There develops hepatic encephalopathy, especially in case of diffuse peritonitis, which is a criterion of decompensated hepatic insufficiency.

With the purpose of preventing development of encephalopathy in this category of patient, in case of peritonitis, we used nasal-intestinal intubation of the intestine and programmed video-laparoscopic abdominal sanitary cleansing in dynamics.

Development of fat liver had been confirmed in all the patients previously. Destructive forms of cholecystitis and peritonitis have caused the development of peritonitis. The patients were divided into two groups: the first (the main) group, 17 persons, were those for whom measures of prophylaxis of encephalopathy had been used; the second (control) group, 21 persons, were those for whom the measures hadn't been used.

Dynamics of the laboratory indices of the patients of the main group and the control group before a surgery and on the 7th day after it.

Tab. 1

<table>
<thead>
<tr>
<th></th>
<th>GGT ME/L</th>
<th>CHE U/L</th>
<th>ALP ME/L</th>
<th>5-NT E/L</th>
<th>Bil. Dir. µMol/l</th>
<th>CRB mg/dl</th>
<th>Alb g/l</th>
<th>IL-6 pg/dl</th>
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<td>148.6 ± 11.8</td>
<td>127.7 ± 8.4</td>
<td>5020.17 ± 103.6</td>
<td>161.3 ± 11.7</td>
<td>12.4 ± 0.5</td>
<td>4.0 ± 0.9</td>
<td>36.8 ± 1.9</td>
<td>490.7 ± 102.6</td>
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<td>control</td>
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<td></td>
<td>151.3 ± 12.1</td>
<td>152.5 ± 10.3</td>
<td>4985.6 ± 97.3</td>
<td>166.1 ± 9.9</td>
<td>12.1 ± 1.3</td>
<td>3.8 ± 0.4</td>
<td>359.1 ± 1.9</td>
<td>788.2 ± 141.9</td>
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</table>

*P < 0.05 as compared to the group of comparison
On admission to the hospital in 11 patients (64.7%) of the main group and 13 patients (61.9%) of the control group decompensated hepatic insufficiency was revealed. Development of destructive forms of cholecystitis and pancreatitis in combination with surgical intrusion aggravated the hepatic insufficiency. During the first two days encephalopathy on the background of decompensated hepatic insufficiency was revealed in 13 patients (76.5%) of the main group and 16 patients (76.2%) of the control group. At the same time on the 7th day it was proved clinically that the condition of 13 patients of the main group was better as compared with the patients of the control group. Encephalopathy was revealed in 6 (35.3%) patients of the main group and in 9 (42.9%) patients of the control group. Lethality was in 41.2% and 52.4% correspondingly.

Concentration of IL-6, 5-NT may serve the diagnostic criterion of prophylaxis of decompensated hepatic insufficiency with development of encephalopathy in patients with alcoholic disease of the liver in case of surgical treatment of destructive forms of cholecystitis and pancreatitis.

**Conclusion:** In patients with fat liver on the background of alcoholism in case of development of destructive forms of cholecystitis and pancreatitis in 76.5% of cases hepatic encephalopathy develops. Using nasal intestinal intubation with enterosorption and programmed video-laparoscopic abdominal cleansing in dynamics allows to decrease the development of encephalopathy up to 35.3%. IL-6 and CPB and 5-NT are the criteria of development of encephalopathy in this case in 84.6% patients.

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Metadoxine modifies both apoptotic and necrotic processes in hepatic ischaemia-reperfusion

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Introduction: Metadoxine has beneficial physiological properties and is considered effective in the treatment of chronic liver diseases. Our aim was to determine the antioxidant activity of the drug and its impact on cellular pathways in the model of ischaemia-reperfusion of the rat liver.

Methods: Free radical intensity (FRI), H-donating ability (HDA), reducing power (RP), Randox-TAS, GSHPx and SOD activities were detected. Liver metal ions were measured with ICP-AS. Histological examination was carried out to investigate necrotic changes of the liver, parallel with applying the TUNNEL-reaction. Wistar rats were divided into control (n = 16) and metadoxine-treated (n = 16; 200 mg/bw kg for ten days) groups. During narcosis hepatic ischaemia was induced for 45 min, the experiment was terminated after 24 hours.

Results: Preconditioning reduced the FRI of plasma significantly. The antioxidant plasma parameters were increased in treated rats. HDA and RP were increased in the liver due to metadoxine. The drug increased the values of TAS, SOD and GSHPx in liver. Metal- element concentrations showed close correlations with changes of redox parameters. Histological investigation showed the supposed protective effect against necrosis. The TUNNEL-reaction showed significant decrease of the apoptotic rate.

Discussion/Conclusion: Based on these data it can be concluded, that metadoxine can protect the liver from the oxidative damage caused by ischaemia-reperfusion. With our short-term model new perspectives may rise.

Supporting: ETT-002/2003, NKFP-1B/047
Ursodeoxycholic acid therapy and lymphocytes apoptosis in alcohol liver disease patients with intrahepatic cholestasis

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Introduction: The alcohol liver diseases (ALD) accompanied by immunodeficiency through activation of lymphocytes apoptosis. The aim of this study was to delineate the effects of ursodeoxycholic acid (UDCA) therapy on lymphocytes apoptosis in ALD patients with intrahepatic cholestasis.

Methods: 28 ALD patients with intrahepatic cholestasis (M/F 18/10, aged 39-55 years) treated with 10 mg/kg/day of UDCA for three months. Clinically and histologically all patients were separated: steatosis (6 pts), hepatitis (10 pts), cirrhosis (12 pts). Fas/APO-1 (CD95), CD3, CD4, CD8 and CD19 expression on peripheral blood lymphocytes were measured by immunofluorescent method.

Results: Before UDCA therapy the quantity of lymphocytes in apoptosis was increased in 18 (64.3%) ALD patients over 3.1 times (p < 0.05) vs. control. Fas/APO-1(CD95) expression on lymphocytes increased from steatosis (1.97± 0.28) to hepatitis (5.10 ± 0.43) and to cirrhosis (7.42 ± 0.52); (p < 0.05). After UDCA therapy the quantity of lymphocytes in apoptosis was increased in 9 (32.1%) ALD patients over 1.7 times (p < 0.05) vs. control. The correlation between increased Fas/APO-1(CD95) expression on lymphocytes and decreased quantity of CD3+ and CD4+ lymphocytes in blood became smaller (r = -0.37 and -0.60; p < 0.05 - before UDCA therapy; r = -0.19; p > 0.05 and -0.42; p < 0.05 - after UDCA therapy).

Discussion/Conclusion: UDCA therapy of ALD patients with intrahepatic cholestasis promotes decrease of peripheral lymphocytes apoptosis.
Expression of the xenobiotic- and reactive oxygen species-detoxifying enzymes, GST-pi, Cu/Zn-SOD and Mn-SOD, in primary hepatocellular carcinoma

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Introduction: Hepatocellular carcinoma (HCC) is the most common primary cancer of the liver. Etiologically HCC is a complex and multifactorial disease that is linked to both viral and chemical carcinogens. The pathology processes underlying the liver cancerogenesis include generation of oxidative stress and the consequent up-regulation of inflammatory cytokines or vice versa. In this respect, the aim of our current study was to assess the expression levels of enzymes involved in detoxification of xenobiotics and oxygen reactive species (ROS), particularly GST-pi, Cu/Zn-SOD and Mn-SOD, and to compare them with the expression levels in the adjacent "normal" liver tissue.

Methods: The expression of the enzymes was 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 GST-pi in tumor cells was significantly stronger than in the adjacent "normal" liver (p < 0.008, Wilcoxon signed rank test). On opposite, the expression of Cu/Zn-SOD was more prominent in "normal" liver tissue than in the HCC (p = 0.008, Wilcoxon signed rank test), whereas the immune signal for Mn-SOD was equally available and strong in both studied areas (p > 0.999). Interestingly, the biopsies with stronger expression level of Cu/Zn-SOD in "normal" liver was associated with lower serum levels of AlAT and AsAT (p = 0.030, and p = 0.190, respectively, ANOVA).

Discussion/Conclusion: In conclusion, we suggest that the changes in the expression of xenobiotic- and ROS-detoxifying enzymes might be implicated in the HCC developing via compromising the response to the injuring factors and their cancerogenetic intermediators.
Impaired gallbladder motility and accelerated cholesterol crystallization and gallstone formation in mice overexpressing human mucin gene 1

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Introduction: Mucin secretion and accumulation in the gallbladder is determined by multiple mucin genes, and gel-forming mucins play an important role in the early stages of cholesterol gallstone formation. Our aim was to study whether overexpression of the gallbladder epithelial mucin encoded by MUC1 influences susceptibility to cholesterol gallstones.

Methods: A C57BL/6J mouse strain transgenic for human MUC1 (hMUC1) was generated. Before and during feeding male transgenic and wild-type mice (n = 20 each) with a lithogenic diet containing 1% cholesterol and 0.5% cholic acid for 4 weeks, the common bile duct was cannulated for measurement of biliary lipid secretion rates. Gallstones and mucin accumulation in gallbladder biles were quantified by microscopy. Expression of the gallbladder mucin genes was determined by quantitative real-time PCR. Biliary lipid compositions were analyzed by HPLC.

Results: On chow (day 0), gallbladder volumes were significantly greater in transgenic mice (35 ± 7 μl) than in wild-type mice (25 ± 5 μl). At 4 weeks on the lithogenic diet, gallbladder sizes in transgenic mice were increased to 68 ± 17 μl, significantly larger than in wild-type mice (45 ± 12 μl). The administration of CCK-8 significantly increased gallbladder emptying in wild-type but not in transgenic mice. The human MUC1 expression was detected only in the gallbladders of transgenic mice. Compared with chow, feeding the lithogenic diet induced significant increases in expression levels of murine Muc1, Muc3, Muc4, Muc5ac and Muc5b mRNAs in transgenic mice, all being 2- to 5-fold higher than those in wild-type mice. By week 1, mucin accumulation was found in 60% of transgenic mice and 35% of wild-type mice. At week 2, 90% of transgenic mice and 40% of wild-type mice formed cholesterol monohydrate crystals in gallbladder biles. At 4 weeks on the lithogenic diet, 50% of transgenic mice and 30% of wild-type mice formed gallstones. Furthermore, gallstone sizes in transgenic mice (0.43 ± 0.26 mm) were significantly larger compared with those in wild-type mice (0.24 ± 0.14 mm). Notably, the frequency distribution of gallstone number at 4 weeks fell between 5 and 9 in transgenic mice, whereas, the corresponding values were 2 and 4 in wild-type mice. However, cholesterol saturation indices of gallbladder biles and hepatic secretion of biliary lipids were comparable in transgenic and wild-type mice.
Discussion/Conclusion: Compared with the wild-type mice, hMUC1 transgenic mice display significantly increased cumulative susceptibility to cholesterol gallstone formation by impairing gallbladder motility and increasing mucin secretion and accumulation in the gallbladder. Our findings support the notion that the gallbladder epithelial mucin MUC1 plays an important role in the formation of cholesterol gallstones.
Small bowel obstruction related to 10 phytobezoar cases

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Introduction: Mechanical bowel obstruction due to phytobezoar cases especially in previous gastric surgery is seen rarely therefore this study was to investigate aetiology, treatment options and the role of phytobezoars in all operated mechanical bowel obstruction cases.

Methods: Between 1998 January-2003 October patients who were operated for intestinal obstruction especially phytobezoar cases reviewed in SSK Göztepe Educational Hospital 1st Surgery Clinic retrospectively.

Results: 254 operated patients out of 10 (4 male, 6 female) had surgery for phytobezoars. Seven patients had previously undergone surgery for peptic ulceration (truncal vagotomy pyloroplasty or resection).

Discussion/Conclusion: Phytobezoar should be kept in mind as a cause of small bowel obstruction in patients especially previous ulcer surgery. Wherever possible milking of a phytobezoar to the caecum should be performed then whole gastrointestinal tractus begin with stomach till ileocecal valve should be explorated. Prevention of phytobezoar is dependent upon dietary counselling of patients by surgeons after gastric resection or vagotomy and drainage for peptic ulcer.
Functional and morphological injury of rat liver tissue by carbon tetrachloride. Hepatoprotective effect of melatonin


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Introduction: The potential protective effects of melatonin on carbon tetrachloride (CCl₄)-induced acute liver injury in rats were investigated in this work.

Methods: CCl₄ was intragastrically administered to male Wistar rats (4 g/kg body weight) at 20 h before the animals were decapitated. Melatonin (15 mg/kg body weight) was intraperitoneally administered 30 min before and at 2 and 4 h after CCl₄ injection.

Results: Rats injected with CCl₄ alone showed significant lipid and hydropic dystrophy of the liver, massive necrosis of hepatocytes, marked increases in free and conjugated bilirubin levels, elevation of hepatic enzymes (alanine aminotransferase and aspartate aminotransferase) in plasma, as well as NO accumulation in liver and in blood. Melatonin administered at a pharmacological dose diminished the toxic effects of CCl₄. Its administration ameliorated some of the structural damage and functional disorders in rat liver during acute intoxication with CCl₄ and clearly exerted hepatoprotective effects. Melatonin administration also reduced CCl₄-induced NO generation.

Discussion/Conclusion: These results of histological and biochemical measurements suggest that the effect of melatonin on CCl₄-induced acute liver injury may be due both to its radical scavenging properties and indirect effects as a regulator of antioxidant systems.
**LAPTM4B**, a hepatocellular carcinoma-associated novel proto-oncogene

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**Introduction**: The *LAPTM4B* (lysosome-associated protein transmembrane 4 beta) gene was cloned and identified in our lab. It was over-expressed in 87.3% hepatocellular carcinoma (HCC) and the up-regulating degree of LAPTM4B at either mRNA or protein levels were correlated with the pathological grades of HCC tissues. Here we report the biological characteristics of LAPTM4B-overexpressed HCC cells.

**Methods**: Molecular and cellular techniques, such as cDNA transfection, Co-IP etc., were applied.

**Results**: In LAPTM4B over-expressed cells the proliferation, migration and invasion were all promoted, and apoptosis was inhibited; also the immediate early proto-oncogenes (*c-myc, c-fos, c-jun*) were up-regulated. The LAPTM4B-overexpressed cells were tumorigenic when they were inoculated into nude mice. The antibody against LAPTM4B inhibited proliferation and migration of HCC cells in a dose dependent manner. It was demonstrated that LAPTM4B protein interacted with several signaling molecules in plasma membrane and cytoplasm. Thus it is proposed that LAPTM4B may be a platform for assembling of some signal molecules.

**Discussion/Conclusion**: LAPTM4B is a novel HCC-associated proto-oncogene. It is predicted that the LAPTM4B gene and protein might be important targets for gene therapy and immunotherapy of HCC.
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