POSTER ABSTRACTS

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Steatosis in chronic viral B and C hepatitis - a prospective study

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Aim of study: To assess the etiology and the significance of the steatosic lesions that occur in liver biopsies performed in 120 patients with chronic viral B or C hepatitis.

Material and method: Liver biopsy was performed in 80 patients with chronic viral C hepatitis and 40 patients with chronic viral B hepatitis. The severity of the fatty changes (focal, mild, severe), the type of steatosis (macrovesicular or microvesicular) and the distribution of steatosis (focal, perivenular, diffuse) were observed. The patients were previously evaluated for other causes of steatosis such as alcoholic liver disease, obesity, diabetes, dislipidemia, drugs.

Results: Patients with chronic viral C hepatitis showed focal steatosis in 35%, mild steatosis in 32,5% and severe steatosis in 5%. The aspect of steatosis was macrovesicular and randomly distributed, except one single case with mild microvesicular steatosis. Patients with chronic viral B hepatitis had focal steatosis in 17,5% and mild steatosis in 7,5% with the same macrovesicular and diffuse aspect.

Conclusions: Fatty changes are common features in chronic viral C hepatitis (72,5%), especially focal and mild steatosis, but severe steatosis may occur. Fatty changes are relatively rare in chronic viral B hepatitis (25%); severe steatosis was not noticed.

Methods of Treatment in Alcoholic Hepatitis

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Background. Alcoholic hepatitis is frequent in Romania, and the corticosteroids were usually used in the treatment.
The aim of the study is to compare the treatment with Prednisone and Hepatofalk Planta in patients with alcoholic hepatitis.
Methods. Two groups of patients entered this study. Group A included 30 patients with alcoholic hepatitis (23 males, mean age *51,3)* and Group B 30 patients with the alcoholic hepatitis also (21 males, mean age 49,7 years). In all the patients the HBV and HCV were negative, and the liver biopsies showed alcoholic liver hepatitis. The liver enzymes and GGTP were elevated, in a similar way in the two groups. In group A was administrated a low dose of Prednisone 10mg/day for two months, and group B received Hepatofalk Planta 3x 250mg/day for the same period.
Results. In Group A, the liver enzymeS and the GGTP were normal after two months of treatment at 24 (80%) of the patients. 3 (10%) patients could not stand the therapy with prednisone because of pyrosis complains and 2 (6.66%) patients escaped the treatment because of the alcoholism. At 1(3.33%) patients the analyses did not improved.
In Group B the liver enzymes and GGTP were normal at 26 (86.66%) of the patients, 4 (13.33%) of the patients escaped the treatment because alcoholism.
Conclusions. There was no statistical difference between the two groups in the normalization of analyses (P > 0,05). But in the group treated with Prednisone the side effects were higher at 3 patients U0%), who had to escape the treatment and in I patients there was no improvement of the liver disease (3.33%). In the group treated with Hepatofalk all the patients stand the treatment and improved the functions of the liver, in spite of the fact that 4 (13.33%) patients escaped the treatment because the alcoholism. We concluded that Hepatofalk Planta is better tolerated by the patients with alcoholic liver disease than Prednisone and ensures a normalization of hepatic enzymes.

Type 2 Diabetes and Obesity in Patients Diagnosed with Cryptogenic Cirrhosis.

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Introduction: Cryptogenic cirrhosis accounts for *5-30%* of patients with cirrhosis. Nonalcoholic steatohepatitis (NASH) may play a role in the development of cryptogenic cirrhosis. We investigated the occurrence of diabetes mellitus and obesity, accepted risk factors for NASH, in patients with cryptogenic cirrhosis and compared them with controls.

Methods: The cases notes of patients with a diagnosis of cryptogenic cirrhotics were retrieved. Diagnosis was confirmed by absence of history of alcohol excess, negative tests for hepatitis B and C, lack of autoantibodies, normal caeruloplasmin and alpha-Iantitrypsin levels and exclusion of haemochromatosis. Controls were cirrhotic patients matched for sex and disease severity as assessed by Child's classification. The presence of type 2 diabetes and the body mass index were noted. Chi-square analysis was used to compare cases and controls.

Results: Thirty three patients fulfilled the criteria for the diagnosis of cryptogenic cirrhosis. Mean age at diagnosis was 52.1 (SEM 2.5), 16 patients were female and 17 male. At time of diagnosis 9 patients were Child's A, 16 B and 5 C. The aetiology of controls were primary biliary cirrhosis 12, primary sclerosing cholangitis 8, alcoholic liver disease 7 and chronic viral disease 6.

	Type 2 diabetes	**BMI <25**	**BMI >25**	**DM or BMI>25**
Cryptogenic	19/33 (58%)	15(45%)	18(55%)	24(73%)
Controls	2/33 (6%)	23(70%)	10(30%)	10(30%)
	p< 0.0001		p<0.05	p<0.0l

Conclusion: Patients with cryptogenic cirrhosis have an increased incidence of type 2 diabetes and obesity compared with controls. This may reflect a role for NASH in the development of cirrhosis in cases previously termed cryptogenic.

STEATOHEPATITIS IN COELIAC DISEASE CHILDREN

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Aim of this study was to evaluate the possibility of steatohepatitis in coeliac disease children. 37 patients (age: 10 month - 8 years) have been diagnosed and followed over a median of 3 years (1-6 years). All patients had no cirrosis and no viral hepatitis. In untreated CD all children had weight loss, malabsorption, positive serum antigliadin antibody and villious atrophy on intestinal hystology. After diagnosis verification the gluten free diet (GFD) was administrated. There was a good effect of treatment with weight increasing and intestinal restitution. We evaluated liver biopsy in coeliac disease children in acute stage and after 1 years of gluten4ree diet. The steatohepatitis was diagnosed in all childen with acute coeliac disease. Also semm lipids disorders was observed: decreasing of total lipids with increasing of phospholipids. Delayed peaking time in the liver and delayed clearance from the liver parenchyma in hepatobiliary scintigraphy was marked. After one years of GFD the steatohepatitis was diagnosed in 12 childrens only. There was no hepatocites dysfunctions on scintigraphy, normal semm total lipids level but increased serum phospholipids. Later sewm phospholipids had positive trend but increased levels was observed even after six years of follow-up. In conclusion, the steatohepatitis with hepatocytes dysfunction and serum lipids disorders in coeliac disease children is transient and due to malabsorption with nutrient insufficiency. About increased serum phospolipids we suppose, GFD don't stop cell lesion and the continued membrane damage after GFD administration rests.

POLYAMINE OXIDASE AND ARGINASE ACTIVITIES IN ALCOHOLIC LIVER DISEASE

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Alcohol is the most common cause of fatty liver. This disease manifests with disturbance of hepatic biosynthetic functions related with proteins, carbohydrates and lipids. A cause that there is a general tendency to hypoproteinemia, especially hypoalbuminemia; biosynthesis of triglicerydes and cholesterol decreases; metabolism of charbochid rate is compromised. The release of hepatic enzymes into the blood increased.
There are reports suggesting that chronic and acute alcohol ingestion affect polyamine metabolism in hepatic tissue. Also, literature data represent that albumin biosynthesis is stimulated by those amino acids which increase urea synthesis, especially by omithine, an amino acid produces from arginine by the action of arginase; at the same time ornithine is the precursor of polyamines spermine and spermidine. Spennine and spemidine and their natural N^1 - acetyl derivatives are degraded by the action of polyamine oxidase (PAO,EC 1.5.3.3).
In this study we investigated polyamine metabolism in patients with alcoholic liver disease through arginase and polyamine oxidase activities investigations. Patients were divided into groups: first -with short-term ingestion and second group-with long-term alcohol ingestion. Enzyme activities we have measured in serum using the spectrophotometric method: arginase activity on the base of measurement formed ornithine and polyamine oxidase by measuring the amount of formed aminoaldehydes. The degree of impairment of hepatic tissue we have evaluated by the measurement of general blood compound and enzymes activities that are important for the liver metabolic functions. Our results show that in the both group of patient's serum arginase and polyamine oxidase activities were higher then in the healthy person. The enzyme activities depend on the duration of alcohol consumption. In the second group of patients polyamine oxidase activity increases in contrary to arginase activity which decreases. These findings are in accordance with the degree of hypoalbuminemia and hipotrigliceridemia. In the lather stage of the alcoholic liver disease biosynthesis of protein and lipids is impaired. The lower arginase activity could be related with low serum albumin amount. Increased PAO activity may be a consequence of lipid disbalance and higher presence of acetyl derivatives of spermine and spermidine in alcoholic hepatic Ussue.
In conclusion, the befter understanding of polyamines metabolism in alcoholic liver disease my contribute to elucidation the mechanisms by which ethanol causes chronic liver disease.
Hypoalbuminemia is a patognomonic laboratory sign for the progression of hepatic liver disease. Albumin synthesis is stimulated by amino acid ornithine, a precursor of the synthesis of putrescine, spermidine and spermine.

THE COEXISTENCE OF STEATOHEPATITIS WITH OTHER FORMS OF CHRONIC LIVER DISEASE IN LIVER BIOPSIES

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INTRODUCTION: The histologic features of steatohepatitis (SH) include, in varying degrees, mixed steatosis, lobular acute and chronic inflammation, liver cell ballooning, and zone 3 perisinusoidal fibrosis. Portal chronic inflammation is usually absent or mild. Other findings may include lipogranulomas, Mallory's hyaline, and glycogenated nuclei. These findings are common to SH of all etiologies: alcoholic hepatitis, obesity, drugs, or idiopathic nonalcoholic steatohepatitis ~ASH), and are distinct from the characteristic portal-based chronic inflammation and spotty necrosis of many other forms of necro-inflaenmatory chronic liver disease (CLD), such as hepatitis C, hepatitis B, autoimmune liver disease, and the diagnostic changes of metabolic liver disease, such as hemochromatosis and ot-1-antitr'ypsin disease. MM: The aim of this study was to identify cases from our institution with biopsy findings of both SH and another CLD. **METHODS:** A computer search of biopsies Q)xs) evaluated at SLUHSC from 6/97 to 3/00 was performed. Of the 3102 total bxs, non-allografi bxs with the diagnosis of SH with at least stage 1 fibrosis (zone 3 perisinusoidal fibrosis) and another CLD were tabulated. RESULTS:

SH + CLD (n)	**MIF**	**Age yrs. Range (mean)**	
HCV (96)*	61/29	M: 32-73 (46)	F: 37-67 (49)
HCV + hx ofETOH (11)	10/1	M: 37-62 (46)	F: 43
PBC (5)**	1/3	M: 55	F: 40-64 (53)
a-I-AT deficiency (5)**	2/2	M: 20-45	F: 35-71
HFE mutation (3)***	2/1	M: 36-56	F: 47
HBV (3)	3/0	M: 44-56 (49)	N/A
Autoimmune (2)	1/1	M: 47	F: 59
Drug-induced (1)	0/1	N/A	F: 44

*96 bxs, 90 pts; **5 bxs, 4 pts; *** 2 C282Y/wt; 1 C282Y/H63D

SUMMARY AND CONCLUSIONS: 1) Multiple disease processes may affect the liver simultaneously. With appropriate clinical information and histologic criteria, distinguishing various forms of coexisting liver diseases is possible. 2) The microscopic features of SH, regardless of etiology, can usually be seen distinctly even when in combination with other CLD. These features include the combination of steatosis, zone 3 perisinusoidal fibrosis, lobular inflammation, and zone 3 liver cell ballooning. 3) As noted in other series, not all bxs with SH have Mallory's hyaline. In this study, Mallory's hyaline was seen in only 21/126 bxs of SH and another CLD. 4) Not all biopsies with HCV show steatosis or SH. The proportion of bxs with concomitant SH and HCV most likely reflect the frequency of these two common diseases. 5) The injury from concomitant SH may be synergistic with that of the coexistent CLD and contribute to disease progression.

TUMOR NECROSING FACTOR- ALFA (TNF-alfa) LEVEL IN ALCOHOL DEPENDENT MALES, IT'S RELATION TO LIVER INJURY AND GASTRIC pH.

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TNFα is the cytokine, which may have an importance in pathogenesis of multiple-organ disturbances, lever injury including in alcoholics. Cytokine synthesis is stimulated by endotoxins produced in the bowel. The aim of this study was to determine the TNFα level in alcohol dependent males and relationships between the cytokine level and the level of liver function test and gastric pH. METHODS: In 29 alcohol dependent males, who drank alcohol not later than 3 weeks before the study start, and in 8 males, who denied alcohol consumption for last 3 weeks the level of TNFα (using ELISA, method by ENDOGEN), biochemical determinations and 24-hours gastric pH-metry (in 26 pts) were made. RESULTS: Alcohol dependent males had higher mean TNFα level than control group (l,1±2,4 vs. 0,2±0,3pg/ml, ns). 41% of alcohol dependent patients had TNFα level above 0. After 4 weeks of abstinence period mean TNFα plasma concentration decreased not significantly (l,1±2,5 vs. 0,6±1 ,Opg/ml, p=0,26). Patients with TNFα in plasma presence had higher blood platelets count and total cholesterol, triglicerydes and TSH concentration. The TNFα level in first examination correlated (rank Spearman's correlation) with % of total monitoring time with gastric pH range 0-1 (R= -0,56), 1-2 (R= -0,64), 3-4 (R=0,66), and 7-8 (R~0,5l). No relationships between TNFα level and liver function tests levels, alcohol dependence severity score (Short Alcohol Dependence Data, Michigan Alcoholism Screening Test), quantity of alcohol drank during 90 days before the study start and age of patients were found. CONCLUSION: Alcoholics more often bad high TNFα plasma, what suggests involving of TNFα in pathogenesis of chronic alcohol abuse complications. Low gastric pH, as unspecific barrier against digestive tract bacterial colonisation may be an important factor cytokine production decreasing.

RELATIONSHIPS BETWEEN GASTRIC PH AND THE LIVER FUNCTION TESTS LEVELS IN ALCOHOL DEPENDENT MALES DURING ABSTINENCE PERIOD.

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Gastric acidity is an unspecific barrier, which protect the digestive tract against the bacterial colonisation, which can be a source of bacterial endotoxins, well documented factor of liver injury. In our work we tested the hypothesis, that alcohol induced changes in gastric pH may have an influence on the liver function tests level in alcohol dependent males after alcohol abuse period. **METHODS**: 24-hours gastric pH-metry and laboratory determinations were made in 26 alcohol dependent males, who drank alcohol not later than 3 weeks before examination

performance. **RESULTS**: Short Alcohol Dependence Data score correlated with % of total monitoring time (%tmt) with pH range 2-3 (R= -0,66); number of drinking days during 90 days before gastric pH-metry performance correlated with % of monitoring night time (%mnt) with gastric pH range 0-1 (R= -0,48) and 1-2 (R= *-0,53);* and total number of standard drinks drank during 90 days before start of the study correlated with %tmt with gastric pH range 0-1 (R= -0,58). We have also found correlation between gammaglutamyltranspeptidase (GTP) activity and %mnt with gastric pH range 0-1 (R= -0,42), bilirubin concentration correlated with %tmt with gastric pH range 2-3 (R=0,4), 5-6 (R= -0,58), 6-7 ~= -0,52) and 7-8 (R= *-0,51),* al-globulin plasma concentration correlated with

%mnt with pH range 6-7 (R= -0,43), and a2-globulin level correlated with %tmt with pH range 2-3 (R--0,5), INR correlated with %mnt with pH range 0-1 (R=0,46), and y-globulin level correlated with %tmt with pH range 0-1 (R-- -0,43). **CONCLUSION**: Liver injury and acute phase reaction may be related to alcohol consumption induced changes in gastric pH.

CHARACTERIZATION OF T-LYMPHOCYTES IN IIIF SUBSETS IN THE LIVER TISSUE IN ALCOHOLIC LIVER DISEASE

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The aim of the study was to identify T lymphocyte in the liver tissue in different forms of alcoholic liver disease (ALD) and to determine their relation with the histological signs of hepatocellular damage~ The study was prospective and includes 69 patients with different forms of ALD divided into 4 groups: gr. I - fatty liver, gr. II (AH) - alcoholic hepatitis, gr.ffi (AC + AH) - alcoholic cirrhosis with hepatitis and gr.IV (AC) - inactive cirrhosis. 10 pts. with non-alcoholic liver damage were as controls. The including criteria was an absence of hepatitis B and C virus infection.

Lymphocytes in liver biopsy specimens were stained by an indirect immunoperoxidase method using Vector mouse antihuman lymphocytes monoclonal antibodies (CD3: pan T cells; CD4: helper/inducer T cells; CD8: cytotoxicisuppressor T cells). The presence of histological features related to ALD, as well as T lymphocyte subsets were assessed semiquantitatively, using numerical score. A non-parametric Spearman test of correlation was applied to determine the relation between histological and immunohistochernical findings. Patients with ALD have shown slightly decreasing of CD3+ and CD8+ markers of peripheral blood lymphocytes, followed by decreasing of CDI+/CD8+ ratio, in comparison with control subjects, but without statistical significance. CD3+ cells in liver tissue were present in 84 %, mainly in pts with AH and AC + AH *(95.65* % and 90.90 % respectively). CD4+ cells were present in 29.82 %, equally in I, II and III group of pts with ALD. CD8+ cells have been detected in II and III group. Consequently, C1)4+/CD8+ ratio was decreased in pts with AH and AC + AH, in comparison with pts with steatosis. CD3 and CD cells were identificated mostly in periportal area, whereas CD8 cells had predominantly intralobular localization. CD8+ cells have been associated significantly with the presence and the degree of both lobular and portal necrosis ($p < 0.001$).

According our findings of T cells infiltrating the liver tissue, we can conclude that suppressor-cytotoxic T lymphocytes are involved in the pathogenesis of necrosis, as the essential sign of injury in ALD.

The clinical significance of the ASAT/ALAT ratio in differentiating nonalcoholic from alcoholic steatohepatitis

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Nonalcoholic steatohepatitis NASH) is believed to be one of the most common explanations for abnormal liver aminotransferases. Clinically NASH is a diagnosis of exclusion that should be suspected in patients who deny significant alcohol consumption and have negative viral markers. The identification of fatty liver on ultrasonography supports the diagnosis of steatosis, but it has to establish by liver biopsy.

The aim of the study was to determine whether the values of aminotransferases and the ASAT/ALAT ratio was useful to distinguish nonalcoholic from alcoholic steatohepatitis.

Methods. Forty patients with NASH were compared with 45 patients with alcoholic steatohepatitis. The diagnosis of fatty liver was suspected on ultrasonography and confirmed by liver biopsy. The diagnosis of NASH was based on exclusion of significant alcohol consumption. The Student t test and Chi square test were used for statistical analysis.

Results. The mean age of the two groups of patients was similar (43,5 yrs vs 44.5 yrs). The males were predominant in both groups. The aminotransferases levels were increased in 58% of patients with alcoholic steatohepatitis and in 37.5% of patients with NASH. The increase of ASAT was more frequently found in patients with alcoholic fatty liver (38% vs 7.5%). The mean ASAT/ALAT ratio was 1.9 (0.8 to 6.7) in patients with alcoholic steatohepatitis versus 1 (0.3 to 3) in patients with nonalcoholic steatohepatitis. Analysis of histological findings revealed that fatty liver alone is more frequently found in NASH (55%) while in alcoholic etiology the predominant lesions were steatonecrosis (40%) and steatofibrosis (33%). In alcoholic patients with necroinflamatory changes the ASATIALAT ratio was significantly higher than in patients with fatty liver alone (2.9 vs 1.4). The presence of fibrosis is also associated with higher ASATIALAT ratio (2.3). The necroinflamatory changes and steatofibrosis were rare lesions in patients with NASH (20 respective *25%).* The ASATIALAT ratio in these patients was not greater 1.1.

Conclusions. The patients with NASH have frequently fatty liver alone on histology. In these patients the ASATIALAT ratio was not higher than 1. In alcoholic steatobepatitis the predominent lesions were necroinflamatory changes and steatofibrosis. In these patients the ASAT/ALAT ratio was significantly higher. The ASAT/ALAT ratio appears to be useful in differentiating alcoholic from nonalcoholic steatohepatitis. The higher ratio could be an indicator for the necroinflamatory changes on histology.

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Nonalcoholic steatohepatitis - the underestimated diagnosis

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In the time period January 1998 to April 2000 174 liver biopsies were performed. In 35 patients with the liver impairment of unknown origin the nonalcoholic steatohepatitis (NASH) was taken into consideration in a differential diagnosis. Further examination in more detail 16 of 35 patients excluded - in 6 alcohol abuses was disclosed, in 1 gold treatment for rheumatoid arthritis, in I infectious mononucleosis, in I HBVDNA positivity, in 1 Wilson's disease was diagnosed. In further 3 a drug liver impairment was suspected. One patient was excluded for AMA positivity and 2 for missing data of glycaemia or lipid metabolism
parameters. Thus our group of 19 patients consists of 9 women and 10 men. The obesity, NIDDM, hyperlipidaemia as risk factors were present in our patients all or in various combinations. The morphologic examinations showed typical histologic findings - macrovesicular steatosis grade l-IV, PMN infiltration, the presence of Mallory hyalin, lipogranulomas,... and different stages of fibrosis, or cirrhosis. The ratio AST : ALT was 0.57-1.35 in women and 0.49-1.6 in men. In patients, where the ratio was greater than one the cirrhosis was present. Thus in our patients the NASH was present in 10.9% of them and NASH cirrhosis in 1.7%. NASH cirrhosis account for 15.7% of NASH patients. We conclude, that the greater attention should be paid to NASH than so fare particularly when the occurrence of obesity, NIDDM and hyperlipidaemia is increasing in our population.

T LYMPHOCYTES ACTIVATION IN ALCOHOLIC LIVER DISEASES

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In our study we investigated the degree of T lymphocytes activation in peripheral blood of liver alcoholic diseases patients. The tests were performed on 12 liver alcoholic steatosis (LAS) patients, 21 chronic alcoholic hepatitis (CAR) and 8 alcoholic liver cirrhosis (ALC) patients, without viral hepatitis markers in the serum, and 6 healthy persons as controls. The lymphocytes marker CD_3, the CD_4+and CD_8+, and the expression of T lymphocyte activation markers, CD_{25} (IL-2 receptor) and HLA-DR molecule were determinate by flow-cytometry.

The results indicated an insignificant decrease of CD_3 cells in all types of liver alcoholic diseases than in controls (71.7±2.08%) and also an insignificant increase of CD_4+ and CD_5+ cells than in controls CD_4+ *(45±2.24%)* and CD_8+ (22.96±1.71%) cells. The values of HLA-DR markers indicated an insignificant increase in LAS patients than in controls p = 0.366) and significant increases in CAR (10.06±3.55%) and ALC patients (15.09±3.35%) than in controls *(6.725±0.26%).* The values of CD_{25} marker showed an insignificant increase in LAS patients than in controls *p* = 0.248) and a significant increase in CAR *(25.4±1.18%)* and ALC patients (24.88±1.35%) than in controls (18.42±1.20%Y. The elevated expression of T lymphocyte activation markers, on CAR and ALC patients is correlated with the long exposure to antigenic products resulted from the alcohol metabolism and with the intensity of liver injuries.

THE EXPRESSION OF ADHESION MOLECULES ON T LYMPHOCYTES AND CYTOLITIC SERUM MARKERS IN ALCOHOLIC LIVER DISEASES

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In our study, we pursue the migration capacity of T cells in the tissues and the degree of hepatocytolysis, determining the expression of adhesion molecules -

- integrins: CD_{11}~CD_{18}, $CD_{1\ 1}$b/CD_1g and *CD_{11}~ICD_{18}* by flow-cytometry and the cytotoxic serum markers, AST and ALT by rephractometry. The tests were performed on 12 liver alcoholic steatosis (LAS) patients, 21 chronic alcoholic hepatitis (CAH) and 8 alcoholic liver cirrhosis (ALC) patients, without viral hepatitis markers in the serurm, and 6 healthy persons, as controls.

The results indicated an insignificant increase of expression of $CD_{1\ 1}$a/CD_{18} on LAS (34.29±1.98%) and LAC (35.96±3.14%) patients than in controls (30.52±0.66%), and a significant increase in CAH (42.34±3.79%) patients. There are not significant increases between the expression of $CD_{1\ 1}$/CD_8 and $CD_{1\ 1}$~/CD_{18} on T cells of ALD patients and the controls. The values of AST were greater in LAS (34±4.16 U.I/ml), CAH *(61.57±24* U.I./ml) and ALC *(43.75±26.25)* patients than in controls (22±7) as well as the values of the ALT on LAS (40±3.60), CAH (60.28±24.44) and ALC (23±7.84) patients and the controls (16±9). The lower expression of adhesion molecules on LAS and CAH patients assumes a decreased inflammatory and cytotoxic reactions than in CAH patients, that is correlated with the levels of cytotoxic serum markers, ALT and AST.

THE STAGING OF THE ALCOHOLIC LIVER

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Aim of the stady. The aim of this study was to establish the criteria for staging alcoholic liver disease.

Material and methods. This clinical trial represents a prospective anatomo-clinical study conducted on a number of 375 cases with significant alcohol consumption, followed-up over a period of 10 years.

Results. We noticed the predominance of male gender (70.13% vs. 29.86%, p<0.003). Mean age was 40±3 years in steatosis 47±4.5 years in subacute steatohepatitis, 50±6 years in steatofibrosis and 55±8 years in liver cirrhosis. 40% of cases developed liver cirrhosis, while the other 60% remained in the non-cirrhogenic stages. The mean duration of alcohol impregnation increases directly with the severity of liver affection (5 years for steatosis, 12 years for subacute steatohepatitis, 15 years for steatofibrosis and 20 years for liver cirrhosis). Hepatomegaly and dyspeptic syndrome were present in all cases. Alcoholic polynevritis, alcoholic encephalopathy and red palms are usually correlated with the degree of alcoholic impregnation. Jaundice appears constantly in steatohepatitis (100%), in *28.57%* of cases in steatosis and in 33.33% of cases in steatofibrosis. The hepatocytolitic and cholestatic syndromes were more pronounced in patients with subacute steatohepatitis. The presence of splenomegaly, decreased levels of leukocytes and of thrombocytes, the presence of signs of portal hypertension and liver biopsy differentiate liver cirrhosis from non-cirrhogenic alcoholic liver.

Conclusions. 1. The differentiation between liver cirrhosis and the alcoholic liver is made on the basis of the above-mentioned clinico-biological criteria, with portal hypertension and hepatocellular failure associated with cholestasis. 2. Our observations are similar to those found in the literature regarding the fact that yGT is correlated more with the degree of liver affection rather than with the quantity of alcohol consumption. 3. The differentiation between the 3 stages of non-cirrhogenic alcoholic liver is not possible only on the basis of clinical and laboratory data in the absence of histological criteria. 4. The cirrhogenic rate in the group that we studied was of 40%.

ALCOHOL STEATOSIS - DETERMINANT OF FIBROSIS PROGRESSION IN HEPATITIS C

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Aim of the study. The most striking feature of hepatitis C is the natural history toward fibrosis. The rate of fibrosis progression as the impact of host and environmental factors remains uncertain. We designed a study including untreated PCR-positive patients and we determined the role of host and environmental factors on liver fibrosis before any medical intervention.

Matenal and methods. 48 patients fulfilled the selection criteria. Liver fibrosis, inflammation and necrosis were graded according to the Ishak score. The degree of fatty infiltration was graded on a 3-grade scale (mild<30% of hepatocytes affected, moderate 30-70%, severe>70%). Usual liver tests, glicemia, BMI were recorded at the time of the first biopsy. Drinkers were defined as having a daily intake higher than 30 grams for men and 20 grams for women. Clinical and biochemical parameters were examined in univariate and multivariate regression analysis to identify independent predictors of increased liver fibrosis.

Results. Of 48 patients, 12 *(25%)* had no abnormal fibrosis, 18 (37%) had mild fibrosis, 5 (10%) had moderate fibrosis and 13 (27%) had bridging fibrosis. Mild steatosis was present in 13 (27%) patients, moderate in 28 (58%) patients and severe in 7 (15%) patients. 7 (15%) patients had elevated hepatic iron and transferrin saturation. Severity of steatosis was statistically associated with BMI ~<0.000l), age of

contamination (p<0.005) and alcohol intake. Identified risk factors of fibrosis progression were: male gender ~<0.01), BMI (p<0.05), combination of steatosis and alcohol intake ~<0.00l), hepatic iron (p<0.02) and necro-inflammatory score > 6 (p<0.02).

Conclusions. The alcohol steatosis is the major determinant of fibrosis progression in hepatitis C.

THE SPECTRUM OF NONALCOHOLIC STEATOHEPATITIS

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Aim of the study. The spectrum of nonalcoholic fatty liver disease ranges from fatty liver alone to nonalcoholic steatohepatitis. Although fatty liver alone is considered to be non-progressive, patients with nonalcoholic steatohepatitis NASH) can develop progressive liver disease and cirrhosis.

Material and methods. We reviewed *50* liver biopsies with fatty liver alone with or without lobular inflammation (group A) and 26 with steatohepatitis (group B). Exclusionary criteria included: (1) daily alcohol intake > 20 gr; (2) jejunoileal bypass; (3) total parenteral nutrition; (4) other known liver diseases. Obesity (BMI>30) was present in 21% and 57% of cases, respectively, diabetes mellitus (DM) in 13% versus 38%, type IV hyperlipidemia in 7% versus 11%, therapeutic agents in 4% versus 27%. The follow-up time was 64f3 1 months.

Results. The following laboratory results had abnormal mean values: ALAT (58 UIL vs. 87 UIL), GGT (105 vs. 390 UIL), cholesterol (212 mg/~ vs. 325 mg/dL) and triglycerides *(259* vs. 1156 mg/dL). Statistical analysis revealed significant correlation between macrovesicular steatosis, pericellular fibrosis and BMI value ~<0.04), hepatomegaly ~<0.0l 1), splenomegaly (p<0.00l). There was a difference regarding cirrhosis between the 2 groups: 2.4% in group A vs. 21.7% in group B (p<0.000l). There was no difference in overall survival: 3-year survival *85.6%* vs. 81.9% ~=0. 18). Patients with fatty liver alone had fewer liver-related deaths than those with NASH (4.3% vs. 25.5%, p=0.04).

Conclusions. I. Obesity and diabetes mellitus are the most frequent causes of NASH. 2. Steatonecrosis with Mallory hyaline and fibrosis is an aggressive disease with progressive potential. 3. Clinical parameters associated with significant fibrosis included obesity, hepatomegaly and splenomegaly. 4. Why some patients with NASH progress to fibrosis and cirrhosis while others have a benign course without clinical or histological sequelac is unclear.

THE EFFECTS OF ACUTE AND CHRONIC ETHANOL INTOXICATION ON LIVER ULTRASTRUCTURE AND BIOCHEMISTRY IN RATS

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This study compares the effects of acute (AEtOH) and chronic (CEtOH) ethanol intoxications on certain specific pathways of mammalian liver (gluconeogenesis and ketogenesis), and on the ultrastrocture of the hepatic tissue.

In the chronic experiment, rats were given EtOH in a daily dose of 3 g/kg b. wt., for 7 months. In the acute experiment, EtOH was infused in the perfusion medium, as to give a constant concentration of 0.5 mM for 30 mm. Livers were perfilsed with either gluconeogenic substrates (lactate + pyruvate), or with a ketogenic substrate (octanoate). Glucose, respectively acetoacetate and ~-hydroxi-butirate were determined from the liver outflow every 3 mm. After 1 hour of perfusion, a lobe of the liver was processed for electron microscopy examination.

Acute EtOH administration resulted in a slight stimulation of ketogenesis and a 10% inkibition of gluconeogenesis. In CEtOH animals, hepatic glucose synthesis only reached half of its intensity found in control rats; the stimulation of ketogenesis was more evident and the ratio AcAc/~-OH-B decreased, as compared to a C group.

Important ultrastmctural alterations were noticed at mitochondrial level. In acute-treated livers, mitochondria were condensed, while chronic EtOH administration produced swelling and vacuolisation of the mitochondrial matrix. SER proliferation in the peripheral area of the lobule and the accumulation of lipid droplets in the centrolobular area were moderate in the AEtOH group and enhanced in the CEtOR one.

Homozygosity for the presence of an alanine at position -9 in the presequence of manganese superoxide dismutase is associated with increased susceptibility to develop alcoholic liver disease

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Alcohol abuse increases the hepatic formation of reactive oxygen species (ROS) which damage mitochondrial DNA, proteins and lipids. For a similar ethanol consumption, some subjects develop alcoholic liver disease (ALD) while others do not. Manganese superoxide dismutase (MnSOD) is synthesized with a presequence and imported into the mitochondrial matrix, where it catalyses the dismutation of the superoxyde anion. An Ala/Val polymorphism at position -9 of the presequence of MnSOD has been described, which may modulate the import of MnSOD into mitochondria and susceptibility to mitochondrial ROS. We looked for this polymorphism in liver DNA of ALD patients (n=71) and blood cell DNA from blood donors (n--79). We found a significant increase in the total percentage of the Ala allele in the alcoholic patients (63%) compared to controls (45,6 %). There was a marked difference in the repartition of the three genotypes (Ala/Ala, Ala/val, Val[Val) in ALD patients and controls (p=O,0043). The percentage of homozygous Ala/Ala patients in the whole ALD group was twice that in controls (44% vs 19%, respectively). Furthermore, this percentage increased with the severity of the liver lesions. The percentage of Ala/Ala homozygotes was 17% (i.e., normal) in alcoholic patients with only macrovacuolar steatosis, 43% in those with microvesicular steatosis, 58% in patients with alcoholic hepatitis, and 69% in cirrhotic patients. Alcohol consumption was not different in Ala/Ala homozygotes, AIa/Val heterozygotes and Val/Val homozygotes. In conclusion, Ala/Ala homozygosity at position -9 of the MnSOD presequence is a major genetic factor/marker for susceptibility to develop ALD in ethanol abusers.

HISTOPATHOLOGICAL ASPECTS OF STEATOHEPATITIS

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The simple steatosis (S) can progress to steato hepatitis and then to chronic hepatitis (CH).
Methods: We studied the presence of lipid inclusions in liver specimens obtained by liver biopsies of 104 patients (pts) with CH HBV (54 pts) or HCV (60 pts). The specimens were embedded in paraffin and stained with hematoxylin - eosin, van Gieson and Perls then was examined in optical microscopy. The histopathological evaluation was made by lshak score. We excluded the main causes of hepatic steatosis: alcohol, drugs, obesity and diabetes mellitus.
Results: S was found in 40 pts, (38.4% of cases) - 33pts (30%) with HCV CH and 8pts (20%) with HBV CH -. In pts with HCV CH, steatosis was found in 53.3% of cases, sometimes associated with biliary duct lesions (12.5% of cases) or lymphoid follicles (22.5% of cases). As compare CH steatosis group vs. CH non - steatosis group, we found: piecemeal necrosis 47.5% vs. g2.8% cases, intralobular inflammation with mononuclear cells 77.5% vs. 90.8% cases, Kupifer cells hyperplasia 15% vs. 18.4% cases. The evaluation of S was made in three stages: st I - 72.5% of cases, st II - 20% of cases and st III - 7.5% of cases. The relationship between S and Ishak score was:

Steatosis index	Medium index activity	Medium fibrosis
StI	6.3	2.1
StIl	5.57	1.7
St III	8.5	4.5

The hepatocytes regeneration was found in st I of steatosis only. **Conclusions**: steatosis, present in 53.3% cases of HCV CH and only in 12.5% cases in HBV CH is less frequent associated with piecemeal necrosis and intralobular inflammation with mononuclear cells than CH without S. The hepatocytes regeneration is found only in st I of steatosis but, in st Ill of steatosis we found the highest activity and fibrosis index, this last stage being probable followed by CH.

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INSULIN RESISTANCE AND TNFz POLYMORPHISM IN ITALIAN PATIENTS WITH LIVER STEATOSIS AND/OR NON ALCOHOLIC STEATOHEPATITIS (NASH)

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It has been suggested that two recently described
TNFα promoter polymorphisms (at position 308 and 238) are associated with insulin resistance which is retained to underlay liver steatosis and NASH. The aim of this study was to define the prevalence of insulin resistance and TNFα polymorphism in Italian patients with liver steatosis. We studied 67 patients with liver steatosis (59 men, age 50.4±9.62; 8 women, age 52±9.1) diagnosed by ultrasonography. Liver biopsy, performed in 35 patients with abnormal ALT values and *I* or increased serum ferritin indicated the presence of NASH in 28/35 of them (80%). In all patients lipidemia, OGTT, insulin, uric acid and BMI were measured. TNFα polymorphisms were determined by PCR followed RFLP analysis. The prevalence of TNFα polymorphism was compared to that found in 94 normal subjects. Hyperlipidemia was present in 69%, altered values of OGTT in *19%,* hyperuricemia in *19%* and an increased BMI (>25) in 72% of the patients. Insulin resistance was present in 64/67 patients (95,5%),The prevalence of the 308 TNFα polymorphism did not differ between patients and controls (28.3% *vs* 23.4%). In contrast, the prevalence of 238 TNFα polymorpism was significantly higher in patients than in controls (40.3% *vs* 17.7%; P--0.0012). No correlation was observed between the presence of the 238 TNFα allele, insulin levels, fasting glucose, hyperlipidemia, BMI and ALT values. In conclusion 1) insulin resistance is present in almost all patients with liver steatosis 2) TNFα 238 polymorphism is strongly associated with insulin resistance and/or liver steatosis and may allow to recognize subjects with predisposition to metabolic abnormalities.

HFE MUTATIONS IN PATIENTS WITH HYPERFERRITINEMIA AND NON ACOHOLIC STEATOHEPATITIS (NASH)

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Increased values of ferritin, often in the absence of augmented transferrin saturation, have been recently reported in patients NASH. To clarify the possible relationship between iron overload, mutations in HFE, the gene responsible for hereditary hemochromatosis (HHC), and NASH, we analyzed liver iron concentration (LIC) and the mutations of HFE gene (C282Y and H63D) in 30 patients with hyperferritinemia and histology proven NASH (28 men, age 49±9; 2 women, age 61 and 66). HFE mutations were determined by PCR followed by RFLP analysis. The results were compared to those found in 128 normal subjects. Mean values of transferrin saturation and ferritin were respectively 34±8% and 693±198 nglmL. LIC was > normal values in all subjects (range 142-735 µg 1100 mg). The C282Y mutation prevalence was significantly higher in patients with NASH than in controls (10/30, 33% *vs* 2/128,1%; P<0.0001); H83D mutation was more prevalent in patients than in controls but the difference was not significant (12 /30, 40% *vs* 31/128, 24%). One or both of the mutations were found in 18/30 patients (60%) and in 33/1 28 controls (26%) (P=0.000B). Transferrin saturation and ferritin levels were similar in patients with or without HFE mutations, but LIC was significantly higher in those with the C282Y mutation (362±1 82vs 243±92; P=0.0302). In conclusion the simultaneous presence of iron overload, due to heterozygosity for HHC, and alteration of gluco-lipidic metabolism may facilitate the development of NASH.