Liver, pancreas, gastrointestinal diseases, microbiome

W205

IMPORTANCE OF FECAL CALPROTECTIN IN THE ASSESSMENT OF HEPATIC ENCEPHALOPATHY (HE) IN PATIENTS WITH LIVER CIRRHOSIS

J. Djordjevic[1], S. Dragasevic[2]
[1]. *Center for medical biochemy Clinical Center Serbia, Belgrade*
[2]*Clinic for gastroenterology Clinical Center Serbia Belgrade*

BACKGROUND-AIM

Calprotectin is a cytosolic protein with immunmodulatory, antimicrobial and antiproliferativeaction that is predominantly found in neutrophils, monocytes and macrophages as well as in T and B lymphocytes. The measurement of fecal calprotectin (FC) level is a sensitive and non-invasive marker that determines an active inflammation in the gastrointestinal system.The aim of this study was correlation between values of fecal calprotectin and degree of liver cirrhosis and hepatic encephalopathy

METHODS

Fecal calprotectin concentrations were determined in 40 patients with liver cirrhosis and 37 healthy patients as controls. All patients were hospitalizing on Clinic for gastroenterology. Patients revealing other causes of abnormal calprotectin results were excluded. F-calproteictin were measure with Buhlmann Quantum blue imunoenzime tests in human fecal samples on Bulhlmann Quantum Blue riader.

RESULTS

The analyzed values, mean ± SD, of fecal calprotectin in patients with liver cirrhosis was 224.7 ± 197.3.0 μg/g, and 35.0 ± 26.0 μg/g in the control group, respectively. We have confirmed significantly higher fecal calprotectin in patients with cirrhosis ($p < 0.001$). We observed statistically significant difference comparing fecal calprotectin by West-Haven criteria of hepatic encephalopathy ($p < 0.001$).

CONCLUSION

We confirmed significantly higher values of fecal calprotectin in patients with liver cirrhosis, especially in hepatic encephalopathy according to West-Haven criteria. Assessment of FC may faciliate grading of HE-severity. And we recommend the use FC as a promising, simple, non-invasive and rapid screening test to make a diagnosis of these complications.

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W206

HYALURONIC ACID CONCENTRATION IN LIVER DISEASES OF DIFFERENT ETIOLOGIES

L. Chrostek[1], A. Panasiuk[2], M. Gudowska[1], E. Gruszewska[1], B. Cylwik[3], M. Rogalska[2], R. Flisiak[2], M. Szmitkowski[1]
[1]*Department of Biochemical Diagnostics, Medical University, Bialystok*
[2]*Department of Infectious Diseases and Hepatology, Medical University, Bialystok*
[3]*Department of Pediatric Laboratory Diagnostics, Medical University, Bialystok*

BACKGROUND-AIM

Serum hyaluronic acid (HA) has been identified as a potential marker of fibrosis or cirrhosis in different studies. There are many papers concerning the diagnostic value of HA in viral liver diseases but only a few studies in patients with alcoholic etiology of disease. The aim of this study was to evaluate the effect of liver diseases of different etiologies on the serum level of hyaluronic acid.

METHODS

Serum HA concentration was measured by the immunochemical method (WAKO) in 84 cirrhotic patients (57 alcoholic and 27 non-alcoholic) and in 22 patients suffering from toxic hepatitis (19 caused by alcohol). Etiology of non-alcoholic cirrhosis was HCV -10, HBV -1, primary biliary cirrhosis – 4, autoimmune hepatitis -1 and undefined factors -11 subjects.

RESULTS

Liver diseases affect the serum HA concentrations (ANOVA rank Kruskal-Wallis test: H=26.21; P=0.000). The patients with alcoholic cirrhosis had higher serum HA concentration (mean±SD; 1081±1134 ng/mL) compared with non-alcoholic cirrhosis (428±361 ng/mL; P=0.024) and toxic hepatitis (193±188 ng/mL; P<0.001). In each diseases the serum HA concentration were higher than in the control group (34.3±10.1 ng/mL; P<0.001 for each comparisons). The severity of liver cirrhosis (alcoholic and non-alcoholic) significantly affects the HA concentration (ANOVA rank Kruskal-Wallis test: H=39.22; P<0.001). In score C HA level (2216±2399 ng/mL) was higher than that in score B (869±946 ng/mL; P=0.001) and in score A (307±316 ng/mL; P<0.001). Additionally, in score B HA level was higher than that in score A (P=0.007).
When we separately tested etiology of disease there was only significant effect of the severity of cirrhosis on the level of HA in alcoholic patients (ANOVA rank Kruskal-Wallis test: H=19.47; P<0.001). It means, in score C HA level (1747±1307 ng/mL) was higher than that in score B (744±806 ng/mL; P=0.002) and score A (367±404 ng/mL; P<0.001). The correlation analysis confirmed the existence of association between severity of alcoholic cirrhosis and HA concentration (R=0.591; P<0.001).

CONCLUSION

Serum hyaluronic acid concentration alters in liver diseases of different etiologies and correlates with the severity of liver cirrhosis depending on its etiology.

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W207

COMPARISON OF ELF WITH FIBROTEST AS THE THE NON-INVASIVE LABORATORY MARKERS OF LIVER FIBROSIS

Z. Mijušković[3], J. Pejović [3], V. Ratić[3], N. Perišić[1], Z. Kostić[2]
[1]*Clinic of Gastroenterology, Military Medical Academy, Belgrade, Serbia*
[2]*Clinic of General Surgery, Military Medical Academy, Belgrade, Serbia*
[3]*Institute of Medical Biochemistry, Military Medical Academy, Belgrade, Serbia*

BACKGROUND-AIM

The Enhanced Liver Fibrosis (ELF) test consists of an algorithm of three fibrosis markers (hyaluronic acid, amino-terminal propeptide-of-type-IIIcollagen, tissue-inhibitor of matrixmetaloproteinase-1). FibroTest (FT) is the most frequently used serum fibrosis marker and consists of an algorithm of five fibrosis markers (alfa2 macro-globulin, apolipoproteinA1, hapto-globin, GGT, bilirubin). Systematic review has shown comparable results for both individual markers. The aim of this evaluation is not only based to the correlation analysis but also to the cost-benefit analyses of both markers.

METHODS

In our study, the ELF-test was analyzed retrospectively in patients with chronic liver disease, who received a liver biopsy and the FibroTest using histology as the reference method. Histology was classified according to METAVIR (F0-F4) for patients with chronic hepatitis and primary biliary cirrhosis (PBC), respectively. Twenty-four sera of mentioned patients were analyzed for FT parameters by Nephelometry (SIEMENS DADE BNII) method and the ELF parameters were tested by (SIEMENS ADVIA Centaur XP) system. Obtained results for both tests were calculated according to adequate mathematical algorithm formulas.

RESULTS

Both tests showed well correlation. Spearman's correlation coefficient (rho) between FibroTest and ELF was 0.63 ($p < 0.001$).The diagnostic accuracy (AUROC) for the diagnosis of significant fibrosis ($F \geq 2$) for ELF and FibroTest was 0.53(95%-CI: 0.08-0.97) and 0.79(95%-CI: 0.46-0.99), respectively. Fibroscore had greater sensitivity but lower specificity and vice versa.

CONCLUSION

FT and ELF can be performed with comparable diagnostic accuracy for the non-invasive staging of liver fibrosis. More precise comparison could be done If a higher number of patients for ELF and FT testing should be included. Cost-benefit analysis showed an advantage of FT which was about ten times less expensive than ELF. Decision to one or another test also depends to the existing laboratory analytical platform.

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W208

L3 AS A MARKER FOR EARLY DIAGNOSIS OF HEPATOCELLULAR CARCINOMA

M. Salem Khalil[1]
[1]*Departements of clinical & chemical pathology and Internal Medicine* Faculty of Medicine - Cairo University*

BACKGROUND-AIM

The percentage of alpha-fetoprotein (AFP) binding to Lens culinaris agglutinin (AFP-L3%) is proposed as a diagnostic and prognostic marker for hepatocellular carcinoma (HCC). We evaluated the utility of AFP-L3% for diagnosis of HCC in an Egyptian referral population.

METHODS

This retrospective study included 80 patients: 25 with HCC on top of cirrhotic liver, 41 with cirrhotic liver and 14 healthy volunteers as a control group. AFP-L3% was measured using the Wako LiBASys clinical auto analyzer.

RESULTS

On exploring AFPL3% in patients with total AFP (10-200 ng/ml), an AFPL3% cut off of 36.5% had the same sensitivity of 91.7% as AFP at cut off 20.4ng/ml but an increased specificity of 72.5% rather than 42.5%. The high specificity of AFP-L3% at a cut-off 36.5% allowed the confident diagnosis of additional HCCs patients that were not diagnosed with total AFP < 200 ng/ml.

CONCLUSION

Conclusions AFP-L3 % increases the specificity of diagnosis of HCC especially in individuals with indeterminate elevations of total AFP (10-200 ng/ml), it could be used as a reliable early HCC biomarker. It is possible to achieve particularly accurate results for HCC screening with the use of AFP-L3 % in combination with AFP.

Liver, pancreas, gastrointestinal diseases, microbiome

W209

INFLUENCE ON POSTRANSPLANT SURVIVAL OF ALCOHOLIC CIRRHOSIS PATIENTS WITH PREVIOUS CLINICAL COMPLICATIONS.

I. Legaz[5], E. Navarro-noguera[3], J. Bolarin[4], A. Garcia-narbón[1], A. Luna[2], E. Osuna[2], M.D. Pérez-cárceles[2], M. Alvarez-lopez[4], M. Miras[3], A. Minguela[4]

[1]*Biochemistry Service, Clinic University Hospital ''Virgen de la Arrixaca'', Murcia*
[2]*Department of Legal and Forensic Medicine, University of Murcia.*
[3]*Digestive Medicine Service. Clinic University Hospital Virgen de la Arrixaca, Murcia.*
[4]*Inmunology Service, Clinic University Hospital "Virgen de la Arrixaca". Murcia.*
[5]*Instituto Murciano de Investigación Biosanitaria (IMIB), Murcia, Spain.*

BACKGROUND-AIM

Decompensate cirrhosis is associated with a poor prognosis, with a survival rate of 85% patient 1 year, 56% and 50% at 5-10 years respectively. In this final stage of cirrhosis a increased hepatic vascular resistance caused by the distortion of the liver architecture resulting in portal hypertension with the occurrence in the patient of ascites. Besides increased ammonia produced by intestinal bacteria, which produce toxicity brain, leading to neurological symptoms called hepatic encephalopathy can occur. The main objective of our study was to determine the survival of alcoholic cirrhosis patients undergoing liver transplantation with ascites and encephalopathy in the final stages cirrhosis.

METHODS

A total of 281 medical records of male patients with alcoholic cirrhosis with or without viral infections were analyzed. The ascites was diagnosed by physical examination and diagnostic abdominal imaging, establishing three degrees. Encephalopathy was diagnosed by physical examination and laboratory data, as well as different symptoms established by the scale of West-Haven. The data were analyzed using SPSS 20.0. χ2-Pearson's and Fisher's exact test were used to compare variables. P-value less than 0.05 were considered significant. The Kaplan-Meier and log-rank test were used to compare differences in survival at 1.5 and 10 years.

RESULTS

The analysis of patient survival according to presence/absence of ascites in patient prior to transplantation does not seem to influence in the short or long term survival. Below survival was analyzed according to the ascites degree, noting that patients with ascites type II had a greater survival than other patients, although these differences are not statistically significant. Similarly, analysis of patient survival according to the presence or absence of encephalopathy does not appear to influence patient survival. Interestingly, patients with encephalopathy of grade III showed better survival.

CONCLUSION

Our results show that the presence complications in alcoholic cirrhosis patients, such as ascites or encephalopathy not appear to have a significant impact on postransplant patient survival in our cohort of patients.

Liver, pancreas, gastrointestinal diseases, microbiome

W210

SERUM COPPER AND ZINC IN PATIENTS WITH CHRONIC HEPATITIS C

I. Ivanova[3], B. Atanasova[2], I. Valkov[1], A. Ilieva[3], K. Tzachev[2]
[1]*Clinic of gastroenterology, St.Ivan Rilski University Hospital, Sofia, Bulgaria*
[2]*Department of Clinical Laboratory and Clinical Immunology, Medical University-Sofia, Alexandrovska University Hospital, Sofia, Bulgaria*
[3]*Department of Clinical Laboratory, St.Ivan Rilski University Hospital, Sofia, Bulgaria*

BACKGROUND-AIM

Trace elements are of a great importance for many physiological processes in human body. Their abnormal distribution may contribute to hepatic damage and from the other hand, liver disorders may lead to their disbalance. Disturbed trace-element status in patients with chronic hepatitis C (HCV) is related to stronger oxidative stress and inflammation thus concerning the therapy. Recently in the literature, the data about disordered copper (Cu) and zinc (Zn) homeostasis in HCV are contradictory. The aim of this study was to compare Cu and Zn serum concentrations in patients with chronic hepatitis C and healthy controls.

METHODS

The study included 20 patients with HCV and 40 age and gender matched controls of healthy Bulgarian individuals. Copper/zinc ratio (Cu/Zn ratio) was also studied. Blood was drawn 7:30 – 9:30 am by standard collection procedure followed 12-hour fasting pause overnight. Serum samples were separated and immediately stored at -2/8°C until analysis. Serum copper and zinc were measured by flame atomic absorption spectrophotometer AAnalyst 400, Perkin Elmer. The results were expressed as mean±SD and statistically processed by Student's t-test as $p < 0,05$ was considered significant.

RESULTS

Statistically significant differences ($p<0,001$) between serum metal levels and Cu/Zn ratio of healthy controls and patients with HCV were found. Serum Cu and Zn and Cu/Zn ratio of the healthy individuals were 15,7±3,0 μmol/L; 13,4±1,9 μmol/L and 1,2±0,3 respectively. The same parameters for the patients with HCV were 19,9±4,1 μmol/L; 10,7±3,3 μmol/L and 2,0±0,7 respectively. Significantly higher serum copper, lower serum zinc and increased Cu/Zn ratio were observed for HCV patients in comparison to healthy group.

CONCLUSION

In summary, our data imply that serum levels of copper and zinc and copper/zinc ratio might serve as biomarkers for viral hepatic damage.

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W211

RED CELL DISTRIBUTION WIDTH (RDW) CORRELATES WITH THE SEVERITY OF ACUTE PANCREATITIS DURING THE EARLY PHASE OF DISEASE

P. Dumnicka[1], B. Kuśnierz-cabala[2], A. Gurda-duda[3], J. Kulig[3], J. Tisończyk[1], R. Drożdż[1], B. Solnica[2]
[1]*Department of Medical Diagnostics, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland*
[2]*Diagnostic Department, Chair of Clinical Biochemistry, Jagiellonian University Medical College, Kraków, Poland*
[3]*Ist Chair of General and Gastrointestinal Surgery, Jagiellonian University Medical College, Kraków, Poland*

BACKGROUND-AIM

RDW is a readily available parameter included in every complete blood count (CBC) assessed with the use of a hematological analyzer. Recent studies have shown the usefulness of RDW as a predictor of unfavorable prognosis and death in various diseases, including acute inflammatory states (e.g. acute pneumonia, myocardial infarction and acute kidney injury). However, little is known about RDW changes in the course of acute pancreatitis (AP). Our aim was to assess the usefulness of RDW in early prediction of the severity of AP.

METHODS

We recruited 40 AP patients admitted to the surgical department: 28 with mild and 12 with intermediate to severe form of the disease, 24 men and 16 women, age 46.6 +/- 13.4 years. Blood was collected at admission and then after 48 hours. The CBC was assessed with 5-diff Sysmex SE analyzer. RDW was expressed as a coefficient of variation. Tumor necrosis factor alpha (TNF-alpha) and its soluble receptor (sTNFRII), procalcitonin (PCT), tumor necrosis factor related apoptosis-inducing ligand (TRAIL), interleukins 6 and 18 (IL-6, IL-18) were measured by ELISA. Mann-Whitney test and Spearman correlation coefficient were used as appropriate and results at $p<0.05$ was considered statistically significant.

RESULTS

RDW was significantly higher in patients who died (median 14.3 and 15.0% at admission and after 48 h versus 13.6 and 13.5% in survivors, respectively). In patients with severe AP, RDW was higher at 48 h than at admission (14.3 versus 13.5%) and at 48 h it was higher than in patients with mild AP (14.3 versus 13.5%). RDW positively correlated with the length of hospital stay (R=0.47). Also, RDW correlated with TNF-alpha (R=0.61 on admission and 0.59 after 48 h), sTNFRII (R=0.51 after 48 h), PCT (R=0.39 and 0.40), TRAIL (R=0.40 at admission), IL-6 (R=0.38 at admission) and IL-18 (R=0.43 after 48 h).

CONCLUSION

RDW correlated with early mediators and markers of inflammation in AP. RDW value increased dynamically during the first 48 h after admission in patients with the severe form of AP and in those who died. Although limited by the low number of patients, our study provides the evidence of RDW usefulness in prediction of the severity of the AP. It is important in the context of high availability of RDW.

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W212

THE PROTECTIVE EFFECTS OF MELATONIN IN STEROID INDUCED OSTEONECROSIS MODEL IN RABBIT LIVER

B. Ozyurt[2], H. Ozyurt[3], A. Akbas[3], O. Atis[1], M. Uysal[2], I. Benli[3]
[1]*Ataturk University, Medical Faculty, Department of Medical Biology and Genetics, Erzurum, Turkey*
[2]*Gaziosmanpasa University, Medical Faculty, Department of Anatomy, Tokat, Turkey*
[3]*Gaziosmanpasa University, Medical Faculty, Department of Biochemistry, Tokat, Turkey*

BACKGROUND-AIM

In this study, we investigated oxidative stress in liver steroid induced osteonecrosis model rabbit model and whether melatonin can rescue steroid induced liver toxicity.

METHODS

Twenty one adult female rabbits were divided randomly three groups. Five rabbits were injected once with physiologic saline as control group. Eight rabbits were intramuscularly injected once with 20 mg/kg of metylprednisolone acetat as steroid group, and eight rabbits were intraperitoneally injected every other day 25 mg/kg of melatonin as treatmet group in addition to metylprednisolone acetate.The rabbits in three groups were killed after 14 days and their livers removed for biochemical analyzes. In order to examine antioksidant status and lipid peroxidation, and protein oxidation, we assayed CAT, SOD, GSH-Px enzyme activities and, MDA, PC, NO levels.

RESULTS

CAT, SOD, GSH-Px activities were found to be decreased significantly in steroid group ($p<0.005$) compared to control and melatonin groups, whereas MDA and PC levels were found to be increased in steroid group ($p<0.05$) compared to control and melatonin group.

CONCLUSION

The results showed that steroid caused oxidative stress in liver of rabbits and treatment with melatonin reduced this effect.

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W213

DIAGNOSTIC UTILITY OF THE SELECTED GRANULOCYTES MARKERS , NEOPTERIN AND INTESTINAL FATTY BINDING PROTEIN IN CROHN'S DISEASE AND ULCERATIVE COLITIS

D. Pawlica-gosiewska[1], D. Owczarek[2], D. Cibor[2], K. Gawlik[1], T. Mach[2], B. Solnica[1]
[1]*Department of Diagnostics, Chair of Clinical Biochemistry Jagiellonian University Medical College, Krakow*
[2]*Department of Gastroenterology, Hepatology and Infectious DiseasesThe Krakow University Hospital, Krakow*

BACKGROUND-AIM

Diagnosis of Crohn's disease (CD) and ulcerative colitis (UC) is based on histopathology, endoscopy or radiography, which makes the diagnostics time consuming, expensive, and sometimes invasive. For this reason new laboratory parameters that can be an objective tool for assessing disease activity, prediction of severity and treatment monitoring are needed. The aim of this study is to evaluate the diagnostic usefulness of selected proteins: lactoferin, calprotectin, I-FABP, leucocytes elastase and neopterin.

METHODS

The study population was a group of 33 patients with UC and 27 with CD and 20 healthy subjects in the control group. The UC patients were divided into active phase of disease (23 patients) and inactive phase of disease (10 patoients) subgroups according to the Truelove-Witts index. The CD patients were divided into active phase of disease (20 patients) and inactive phase of disease (7 patients) subgroups according to the Crohn's disease activity index. Determination of choosen parameters were determined using ELISA method. This study was funded by a National Science Center Grant (number: UMO-2011/01/N/NZ5/02757).

RESULTS

Lactoferin, Calprotectin, Leukocytes elastase were significantly higher in the group of patients with CU than controls. Comparison of the studied parameters in patients with CD and patients with UC were not statistificly significant.It has been shown that the comparison of the studied parameters useful for differentiation of patients with CD from the control group (the highest area under the ROC curve were:Lactoferin=0,866, Elastase=0,926 and Calprotectin=0,892) and in group of patients with UC from control group (the highest area under the curve were: Lactoferin=0,853, Elastase=0,897 and Calprotectin=0,833).

CONCLUSION

It was found that CRP, Lactoferin, Calprotectin and elastase levels in patients suffering from CD and UC were higher than in the control group. Thus, these parameters may be considered useful biomarkers in the diagnosis of CD and UC. It has been shown that only CRP and neopterin can be useful in the assessment of clinical activity of the respective disease, but because of the small number of patients in remission, this requires further study.

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W214

PRESEPSIN LEVELS, REACTIVE OXYGEN AND NITROGEN SPECIES IN EXPERIMENTAL SEPSIS MODELS

O. Unay Demirel[2], M. Yuksel[3], D. Ozbeyli[4], E. Kalemoglu[5], T. Orug[1]
[1]*5 Department of General Surgery, Medical Park Goztepe Hospital, Faculty of Medicine Bahcesehir University*
[2]*Department of Medical Biochemistry Goztepe Medical Park Hospital Faculty of Medicine Bahcesehir University*
[3]*Department of Medical Laboratory Techniques, Vocational School of Health Services, Marmara University*
[4]*Department of Physiology, School of Medicine, Marmara University*
[5]*Medical Student Faculty of Medicine Bahcesehir University*

BACKGROUND-AIM

Presepsin is a protein, which is a fragment of CD14, and it is produced on the surface of various cells including monocytes, macrophages, neutrophils, and B cells in response to bacterial infections. Therefore it was hypothesized that presepsin is a good marker for the diagnosis of sepsis. Sepsis is also associated with oxidative stress, whereas tissue damage produced reactive oxygen and nitrogen species (RONS) which results in severe oxidative stress. The aim of this study was to evaluate the clinical performance of presepsin levels in two different experimental sepsis models in rats. Additionally presepsin levels were correlated with RONS measurements.

METHODS

Sprague-Dawley rats were divided into four groups (n=6). The first group received an operation for short bowel disease; the third group for cecal ligation; the second and fourth groups were as sham operated control groups. After 7 days rats were decapitated, trunk blood was collected for presepsin measurements; liver and intestinal tissues were removed for reactive oxygen and nitrogen species measurements with the chemiluminescence (CL) method. Statistics were performed using GraphPad Instat program, $p<0.05$ was regarded as significant.

RESULTS

Presepsin levels in rats with short bowel disease (0.64±0.17 ng/mL) and cecal ligation model (0.68±0.12 ng/mL) were significantly higher than their sham operated (0.14±0.05 ng/mL; $p<0.05$ vs. 0.25±0.06 ng/mL; $p<0.01$, respectively) control rats. Luminol (hydrogen peroxide, hypochlorus acid, hydroxyl radical specific probe), lucigenin (superoxide radical specific probe) enhanced CL and NO measurements were significantly higher in cecal ligation and short bowel disease treated liver and intestinal tissues, than their sham operated control tissues. A correlation between presepsin levels and RONS was not determined ($P>0.05$)

CONCLUSION

Our results suggested that presepsin levels and RONS release are associated with development of sepsis in experimental cecal ligation and short bowel disease models, in rats.

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W215

NONINVASIVE FIBROSIS MARKERS IN LIVER DISEASES

B. Cylwik[3], A. Panasiuk[2], E. Gruszewska[1], M. Gudowska[1], M. Rogalska[2], R. Flisiak[2], B. Zelazowska-rutkowska[3], M. Szmitkowski[1], L. Chrostek[1]
[1]*Department of Biochemical Diagnostics, MEDICAL UNIVERSITY OF BIALYSTOK*
[2]*Department of Infectious Diseases and Hepatology, MEDICAL UNIVERSITY OF BIALYSTOK*
[3]*Department of Pediatric Laboratory Diagnostics, MEDICAL UNIVERSITY OF BIALYSTOK*

BACKGROUND-AIM

The assessment of liver fibrosis and cirrhosis can be divided into invasive and non-invasive methods. The aim of this study was to compare the values of noninvasive serum fibrosis markers (non-patented indexes): GAPRI, HAPRI, APRI, FIB-4 and Forn's index in liver diseases of alcoholic and non-alcoholic origin.

METHODS

We studied 102 patients aged 26-88 years: 53 had alcoholic cirrhosis (AC), 32 – non-alcoholic cirrhosis (NAC), and 17 – toxic hepatitis (TH). The causes of NAC were HBV (13 patients) or HCV (8) infections, and unidentified factors (11). The causes of TH were acute alcohol (12 patients) and drugs abuse (5). The severity of liver cirrhosis was evaluated according to the Child-Pugh score. The GAPRI, HAPRI, APRI, FIB-4 and Forn's index were calculated using specific formulas based on routine laboratory tests and clinical data.

RESULTS

The mean value (median) of GAPRI, HAPRI, FIB-4 and Forn's index appeared to be different between liver disease (ANOVA rank Kruskal-Wallis test: $H=7.30$, $p=0.026$; $H=14.95$, $p<0.001$; $H=18.91$, $p<0.001$, $H=18.60$, $p<0.001$; respectively). Post-hoc analysis revealed that GAPRI was higher in TH than that in NAC (292.5 vs 89.8, $p=0.043$), HAPRI was higher in AC than that in NAC and TH (47555 vs 21090, $p=0.036$; 47555 vs 16392, $p=0.001$, respectively), Forn's index was higher in AC and NAC than that in TH (9.46 vs 6.53, $p<0.001$; 9.23 vs 6.53, $p=0.001$, respectively), and FIB-4 was higher in AC and NAC than that in TH (7.81 vs 1.84, $p<0.001$; 5.88 vs 1.84, $p=0.007$, respectively). The HAPRI, APRI, FIB-4 and Forn's index in alcoholic liver cirrhosis appeared to vary according to the severity of liver damage ($H=17.11$, $p<0.001$; $H=15.26$, $p<0.001$; $H=16.81$, $p<0.001$, $H=12.99$, $p=0.001$ respectively). Post-hoc analysis showed that HAPRI was higher in score C than that in A ($p<0.001$) and B ($p=0.018$), and was higher in score B than in A ($p=0.037$), but APRI, FIB-4 and Forn's index were higher in score C than that in A ($p=0.002$, $p=0.002$, $p=0.021$, respectively) and B ($p=0.005$, $p=0.002$, $p=0.002$, respectively).

CONCLUSION

We concluded that the values of all noninvasive markers of liver fibrosis assessed in our study differ between liver diseases and are affected by the severity of liver cirrhosis.

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W216

THE VALUE OF PANCREATIC ELASTASE IN PATIENTS WITH CELIAC DISEASE

E. FiŠiĆ[1], V. Šupak SmolČiĆ[1], M. KriŽanec[1]
[1]*CLINICAL DEPARTMENT OF LABORATORY DIAGNOSTICS, CLINICAL HOSPITAL CENTER RIJEKA, CROATIA*

BACKGROUND-AIM

Celiac disease is an autoimmune, genetic predisposed, chronic disease of the small intestine caused by gluten. Exocrine pancreatic insufficiency is common in patients with inadequately treated celiac disease. The aim of study was to determine the value of pancreatic elastase (PE) in the stool of patients with celiac disease and compare them with a control group, and then compare the values of PE in the stool of children with celiac disease with values of PE in the stool of adults.

METHODS

The study included 86 patients (65 adults and 21 children) with clinically, serologically and histologically confirmed diagnosis of celiac disease. There were 12 healthy subjects of both sexes in the control group. The values of PE in the stool were measured with ELISA ScheBo Pancreatic Elastase stool Test (Biotech AG, Giessen, Germany).

RESULTS

Our work has shown that there is no statistically significant difference between the value of pancreatic elastase in the stool between groups of patients with celiac disease and control group (P=0,98), and between groups of adult patients and children (P=0,20). However, in the group of patients with celiac disease 18.6% of cases had exocrine pancreatic insufficiency, and no one in the control group. Comparative values of PA in the stool between the groups were made using Mann-Whitney test for independent samples.

CONCLUSION

In patients with celiac disease the value of the pancreatic elastase needs to be determined for timely replacement of the enzymatic treatment, and to be able to reduce or eliminate exocrine pancreatic insufficiency symptoms.

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W217

CHILD-PUGH VERSUS MELD SCORE TO PREDICT SURVIVAL IN PATIENTS TRANSPLANTED FOR ALCOHOLIC CIRRHOSIS.

I. Legaz[5], E. Navarro-noguera[3], J. Bolarin[4], A. Garcia-narbón[1], E. Osuna[2], M.D. Pérez-cárceles[2], M. Miras[3], A. Luna[2], M. Alvarez-lopez[4], A. Minguela[4]

[1]*Biochemistry Service, Clinic University Hospital ''Virgen de la Arrixaca'', Murcia*
[2]*Department of Legal and Forensic Medicine, University of Murcia. Murcia. Spain.*
[3]*Digestive Medicine Service. Clinic University Hospital Virgen de la Arrixaca, Murcia.*
[4]*Inmunology Service, Clinic University Hospital "Virgen de la Arrixaca". Murcia.*
[5]*Instituto Murciano de Investigación Biosanitaria (IMIB), Murcia, Spain.*

BACKGROUND-AIM

Two models for predicting the survival of patients with alcoholic cirrhosis are used in clinical practice for patient counseling, clinical decision making, and risk stratification. The Child-Turcotte-Pugh score (CPS) is the most widely used system due to its easy handling, and contains five variables including serum bilirubin and albumin, prothrombin time, ascites and encephalopathy. The observed variability in subjective parameters in the CPS classification is amortized using the "model for end-stage liver disease (MELD)" score based only on laboratory values, bilirubin, INR and creatinine allowing predict short term survival rate of patients with end-stage liver disease.The aim of the study was to analyze the validity and predictability of the CPS and MELD on patient's survival in alcoholic cirrhosis patients undergoing liver transplantation evaluating the predictive value of both methods.

METHODS

A total of 281 male patients with terminal alcoholic cirrhosis (viral and nonviral) were analyzed in this study. The mean age of all patients was 53.06 ± 0.45 years. The different variables used to calculate both methods were collected at the time of inclusion of the patient on the waiting list. Both methods were applied to each patient according to the appropriate formulas.

RESULTS

Patients survival analysis with alcoholic cirrhosis classified as CPS-A had a survival rate one year (92%) being slightly theoretical frequency lowers. By contrast, patients with CPS-B showed lower survival rates (84% and 73% at 1-10 years. Furthermore, patients initially classified as CPS-C showed high survival rates at 1-10 years (86.4% and 79.5%, respectively). The same trends were observed in presence and absence of viral preinfections. Most patients were classified MELD values 10-19 (71.2%), while the rest presented values of 9-29 (27.6%). The values of patient survival at 10 years were slightly higher than the theoretical at 3 months and similar in presence and absence of viral preinfections.

CONCLUSION

The analysis of postransplant survival in cirrhosis alcoholic considering prognostic factors as CPS and MELD confirm and support the theoretical validity of both methods, although in our cohort of patients we should reevaluate patients considered with CPS-C.

Liver, pancreas, gastrointestinal diseases, microbiome

W218

THE PROFILE OF TRANSFERRIN ISOFORMS IN PANCREATIC CANCERS

E. Gruszewska[1], L. Matus[3], M. Gudowska[1], B. Cylwik[2], M. Szmitkowski[1], B. Kedra[3], L. Chrostek[1]
[1]*Department of Biochemical Diagnostics, Medical University of Bialystok, Poland*
[2]*Department of Pediatric Laboratory Diagnostics, Medical University of Bialystok, Poland*
[3]*II Department of General and Gastroenterological Surgery, Medical University of Bialystok, Poland*

BACKGROUND-AIM

Glycan structures on serum transferrin are terminated by sialic acid, which plays various important roles. The changes in transferrin sialylation are observed in many diseases, especially in cancers. The aim of this study was to assess the effect of pancreatic cancers on serum profile of transferrin isoforms.

METHODS

Serum samples were obtained from 44 patients suffering from pancreatic cancers and 25 healthy volunteers (controls) recruited from hospital workers. The samples were analyzed by capillary electrophoresis (CE) technology on a MINICAP electrophoresis system (Sebia, France). The normal serum transferrin isoforms were separated into five major fractions according to their sialylation level: asialo-, disialo-, trisialo-, tetrasialo- and pentasialotransferrin.

RESULTS

There were no differences in the relative concentrations of disialo- (mean±SD; 0.63±0.30%) and trisialotranferrin (3.0±1.39%) in patients with pancreatic cancers when compared to the control group (0.70±0.42%, 3.54±1.08%; respectively) (Mann-Whitney U test: $P>0.05$ for both). But the mean concentrations of tetrasialo- (81.06±2.22%) were significantly higher and pentasialotransferrin (15.33±2.72%) were significantly lower in cancer patients than that in the controls (77.98±4.21%, 17.82±4.38%; respectively) ($P<0.001$, $P=0.009$; respectively). There were no differences in the concentrations of transferrin isoforms according to the size of tumor, presence of regional lymph nodes and distant metastasis ($P>0.05$ for all comparisons). The exception was the concentrations of trisialotransferrin isoforms which was significantly higher in patients without (M0) (3.25±1.09%) than in those with presence (M1) (2.04±0.85%) of the distant metastasis ($P=0.008$). The ratio of tetrasialo- to pentasialotransferrin in pancreatic cancers (5.45±0.97) was significantly higher than in the controls (4.66±1.27) (Mann-Whitney U test: $P=0.007$).

CONCLUSION

The electrophoretic visualization of the total transferrin isoforms shows the occurrence of alterations in transferrin sialylation in pancreatic cancers. It is clearly visible that tetrasialylated isoforms were frequently present than high-sialylated (pentasialotransferrin).

Liver, pancreas, gastrointestinal diseases, microbiome

W219

GALECTIN-3 CONCENTRATION IN LIVER DISEASES

M. Gudowska[1], A. Panasiuk[2], E. Gruszewska[1], B. Cylwik[3], R. Flisiak[2], M. Rogalska[2], M. Szmitkowski[1], L. Chrostek[1]
[1]*Department of Biochemical Diagnostics, Medical University of Bialystok, Poland*
[2]*Department of Infectious Diseases and Hepatology, Medical University of Bialystok, Poland*
[3]*Department of Pediatric Laboratory Diagnostics, Medical University of Bialystok, Poland*

BACKGROUND-AIM

Galectin-3 (Gal-3), a β-galactoside-binding lectin, is an important mediator of fibrogenesis through the regulation of the phagocythosis. During this process hepatocytes are differentiating into myofibroblasts. Under the influence of Gal-3 myofibroblasts produce procollagen type I which is irreversibly crosslinks to form collagen. Fibrosis is an irreversible condition present in toxic hepatitis and cirrhosis. Therefore, the aim of this study was to evaluate the changes in serum Gal-3 concentration during liver diseases.

METHODS

The patients were divided into subgroups according to clinical diagnosis: alcoholic cirrhosis (AC) -57 patients, non-alcoholic cirrhosis (NAC) -30 and toxic hepatitis (HT) - 22. Cirrhotic patients were classified into subgroups according to Child-Pugh scale: stage A, B and C (27, 31, 25 subjects, respectively). Control group consisted of 20 healthy people recruited from hospital workers. Gal-3 concentration was measured according to the chemiluminescent microparticle immunoassay (ARCHITECT Galectin-3, Abbott, Germany).

RESULTS

The mean serum Gal-3 concentration was significantly higher in the AC, NAC and HT group (mean±SD, 23.4±11.8, 18.1±7.0, 16.9±5.5 ng/mL, respectively) in comparison to the control group (9.9±2.2 ng/mL) ($P<0.001$ for all comparisons). There were significant differences in the serum Gal-3 levels between liver diseases (ANOVA: $H=8.94$, $P=0.011$). Further analysis showed that the mean Gal-3 concentration in AC was significantly higher than that in HT group ($P=0.034$). There were no significant differences between HT and NAC group ($P=1.000$), and between AC and NAC group ($P=0.065$). There were significant differences in the serum Gal-3 levels between Child-Pugh stages of cirrhosis ($H=12.82$, $P=0.001$). Post-hoc analysis revealed that in Child Pugh stage A (17.2±6.6 ng/mL) Gal-3 concentration was lower in comparison to Child-Pugh B and C (23.7±11.3, 24±11.2 ng/mL; $P=0.010$, $P=0.003$, respectively). The differences between patients with Child-Pugh C and B scores were not statistically significant ($P=1.000$).

CONCLUSION

Gal-3 concentration changes in liver diseases and is affected by the severity of liver cirrhosis. We conclude that galectin-3 may be a good marker which reflects the stage of liver damage.

Liver, pancreas, gastrointestinal diseases, microbiome

W220

MULTIORGAN FAILURE IN AN ONCOLOGIC PATIENT FOLLOWING A TUMOR LYSIS SYNDROME - A CASE REPORT

J. Hidalgo Martínez[1], J.I. Tortuero Posada[1], C. Vázquez García[1], P. Gallego Anguí[1], S. Lapeña García[1], M. Barrionuevo González[1], M.d.C. Tenreiro González-llanos[1], I. Arribas Gómez[2]
[1]*Servicio de Análisis Clínicos, Hospital Universitario Príncipe de Asturias*
[2]*Servicio de Bioquímica Clínica, Hospital Universitario Ramón y Cajal*

BACKGROUND-AIM

Tumor lysis syndrome (TLS) is defined as an oncologic emergency caused by tumor cell lysis with release of potassium, phosphorus and uric acid into the bloodstream. It appears after cytotoxic treatment of malignancies such as lymphomas and acute leukemias. It can also occur spontaneously and in other solid carcinomas. The treatment employed seems to be a related factor in the development of these clinical features. In 2004, Cairo and Bishop established the parameters to be estimated for the determination of TLS and made a distinction between the laboratory and the clinical version. In the laboratory version, two or more parameters changes from the previous three days to seven days after chemotherapy should be produced (uric acid ≥ 8 mg/dL, potassium ≥ 6 mmoL/L, phosphorus ≥ 4,5 mg/dL and calcium ≤ 7 mg/dL). In the clinical version, laboratory abnormalities must coexist beside one or more clinical complications (creatinine more than one and a half times upper limit of reference range, cardiac arrhythmia and/or seizures).

METHODS

A 61-year-old male patient was admitted to Oncology Service with uncontrolled pain, jaundice and hepatomegaly. He was diagnosed of grade 2 colon adenocarcinoma, and received surgery that year. For three and a half years had liver metastases during five times. Cetuximab was administered as an antitumor treatment until three weeks before last admission. With clinical data, the presence of hepatopathy with cholestasis due to spread of primary tumor was suspected.

RESULTS

Complete analysis (Siemens® Advia 1800 Chemistry Systems) was requested [uric acid 10,6 mg/dL (2,3-7,3), potassium 7,6 mmoL/L (3,5-5,3), phosphorus 6,7 mg/dL (2,2-4,9), calcium 7,9 mg/dL (8,5-10,4)]. Liver metastases causes hepatic failure and ascites which lead to renal failure with secondary hyperkalemia, and finally the patient died.

CONCLUSION

This case is a spontaneous TLS without temporal relationship with the cytotoxic treatment based on analytical results. The interest of this case resides in the rarity of this entity in the context of a solid tumor as a colorectal adenocarcinoma with liver metastases.

Liver, pancreas, gastrointestinal diseases, microbiome

W221

ALCOHOL DEHYDROGENASE (ADH) ISOENZYMES AND ALDEHYDE DEHYDROGENASE (ALDH) ACTIVITY IN THE SERA OF PATIENTS WITH HEPATITIS C

W. Jelski[1], A. Strumnik[1], K. Orywal[1], T. Lapinski[2], M. Swiderska[2], M. Szmitkowski[1]
[1]*Department of Biochemical Diagnostics, Medical University, Bialystok, Poland*
[2]*Department of Infectious Diseases and Hepatology, Medical University, Bialystok, Poland*

BACKGROUND-AIM

Approximately 3% of the world population is chronically infected by hepatitis C virus (HCV). The changes of enzyme activity in the hepatocytes in the course of different liver diseases are reflected by increase of the corresponding enzyme activity in the blood. For example the activities of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), which are most abundant in the liver (especially isoenzymes of class I and II ADH), correlate with the severity of the condition during cirrhosis. We have investigated the activity of ADH isoenzymes, and ALDH in the sera of patients with hepatitis C.

METHODS

Serum samples were taken from 70 patients (48 males, 22 females, 32-76 years) suffering from viral hepatitis C and from 80 persons of control. Class I and II ADH isoenzymes and ALDH were measured by flurometric method using the specific substrates (4-metoxy-1-naphtaldehyde and 6-metoxy-2-naphtaldehyde). The activities of class III and IV were measured by photometric method with specific substrates. Total ADH activity was estimated by the photometric method.

RESULTS

The total activity of ADH was significantly higher in patients with hepatitis C than in healthy subjects (about 42%). The total activity of ADH was 1284 mU/l in patients, and 522 mU/l in controls. The comparison of ADH isoenzymes activities showed that the high difference was exhibited by class I and II ADH. The activity of these classes isoenzymes in the hepatitis C group increased respectively about 55% (4.24 vs 1.88 mU/l) and 47% (26.63 vs 14.11 mU/l) in the comparison to the control. There was significant increase in the activity of ADH I isoenzyme (4.90 vs 3.72 mU/l) and ADH total (1760 vs 1042 mU/l) in the sera of patients with high viral load (> 600 000 IU/ml) in comparison to patients with low viral load. We also observed the increasing tendency of ADH I, ADH II and ADH total activity in accordance with the advance of disease. The analysis of ADH and ALDH activities in the sera did not indicate significant differences between patients with hepatitis C virus genotype 1b and 3a.

CONCLUSION

The increase of the activity of total ADH and class I and II ADH in the sera of patients with HCV infection seems to be caused by the release of this isoenzyme from damaged liver cells and depends on the severity of viral infection.

Liver, pancreas, gastrointestinal diseases, microbiome

W222

PLASMA AND TISSUE FIBRONECTIN AND SERUM PROCOLLAGEN III PEPTIDE IN CHRONIC LIVER DISEASE PATIENTS AS RELIABLE BIOMARKERS FOR HEPATIC FIBROGENESIS.

L. Kamel[1], M. Akl[2], N. Badrawy[2], A. Anass[4], T. Abou Shousha[2], I. Mostafa[4], S. Hunter[3]
[1]*Department of Clinical Chemistry, Theodor Bilharz Research Institute, Cairo, Egypt*
[2]*Department of Pathology, Theodor Bilharz Research Institute, Cairo, Egypt*
[3]*Department of Tropical Medicine, Kasr El Aini University Hospital, Cairo, Egypt*
[4]*Department of Tropical Medicine, Theodor Bilharz Research Institute, Cairo, Egypt*

BACKGROUND-AIM

This work was designed to assess the role and distribution of hepatic tissue fibronectin (FN) and both plasma FN and serum procollagen III peptide (PIIINP) in fibrogenesis in chronic liver disease (CLD) of different etiologies and to investigate the correlation of plasma FN and serum PIIINP with the grades of inflammatory activity, stages of hepatic fibrosis and the intensity of expression of hepatic tissue FN (intracellular or extracellular) in the different studied groups of CLD.

METHODS

Eighty five patients with chronic liver disease were enrolled in the study, further subdivided according to the etiological pathogenesis into 6 subgroups: chronic hepatitis C group (HCV) (n= 28), HCV and hepatic schistosomiasis group (HCV +Sch) (n=21), HCV and chronic hepatitis B group (HCV+HBV) (n= 6), chronic HBV group (HBV) (n= 12), HBV and Sch group (HBV+Sch) (n = 9) and Sch group (n=9). Fifteen chronic calcular cholecytitis patients were also included as a control group after exclusion of concomitant liver disease. Plasma fibronectin was measured by a sandwich ELISA, while serum PIIINP was performed using a competitive radioimmunoassay technique. FN expression, localization and intensity were assessed in hepatic unstained tissue sections of all subjects by the indirect immunohistochemistry technique using the polyclonal rabbit anti-human-FN-antibody.

RESULTS

Plasma fibronectin levels showed a significant increase in both HCV and HCV+HBV groups as compared to controls. Serum PIIINP showed significant elevation in HCV, HCV+Sch, HCV+HBV, HBV, HBV+Sch, Sch groups as compared to controls. In CLD group, plasma fibronectin correlated positively with serum PIIINP, the grades of activity and hepatic extracellular fibronectin but not with hepatic intracellular FN. Serum PIIINP in CLD group correlated with the grades of activity, and hepatic fibrosis. On the other hand, the increased expression of extracellular FN in CLD group was found directly correlated with the grades of inflammatory activity and stages of fibrosis.

CONCLUSION

We concluded that hepatic tissue FN, plasma FN and serum PIIINP could be considered reliable biomarkers for the hepatic fibrogenesis in chronic liver diseases.

Liver, pancreas, gastrointestinal diseases, microbiome

W223

ADIPONECTIN, ADIPOCYTE FATTY ACID BINDING PROTEIN AND FIBROBLAST GROWTH FACTOR 21 LEVELS IN ACUTE PANCREATITIS

P. Malina[2], D. Novotný[3], P. Krumpholcová[1], I. Tozzi[1], V. Procházka[1]
[1]*2nd Department of Internal Medicine, University Hospital Olomouc, Olomouc, Czech Republic*
[2]*Department of Clinical Biochemistry, Hospital Písek, Písek, Czech Republic*
[3]*Department of Clinical Biochemistry, University Hospital Olomouc, Olomouc, Czech Republic*

BACKGROUND-AIM

The study aimed to evaluate plasma adiponectin (ADP), adipocyte fatty acid binding protein (A-FABP) and fibroblast growth factor 21 (FGF 21) levels as potential predictors of severity of acute pancreatitis (AP).

METHODS

Simultaneously, the classical proinflammatory markers were analysed as well. Both adipokines and proinflammatory markers were analysed at admission (day 1) and day 4. The study was conducted in subjects with acute pancreatitis (n = 84, 37 females, 47 males). The analyses were performed in the groups according to the mild/severe classification of AP, and partly in the computed-tomography severity index (CTSI) score subgroups.

RESULTS

Adiponectin, fibroblast growth factor 21 and adipocyte fatty acids binding protein showed no ability to predict the severity of acute pancreatitis. Only in fibroblast growth factor there was an apparent trend to discriminate between some categories of severity of acute pancreatitis. However, a significant decrease was observed in the subgroups between day 1 and day 4 after admission (together but independently of the decrease in IL-6). FGF-21 was significantly elevated in day 1, which confirms the results of animal studies. ADP, A-FABP and FGF-21 did not provide any significant result in ROC analysis, suggesting the poor potential for discrimination of severity of the disease on the day of the fourth.
For cut-off value of CRP 100 mg/L we found high NPV (96 %) but positive prediction remains problematic. Unlike the PCT, in our work elevated IL-6 levels were associated with severe forms of AP on day 4. Threshold of IL-6 37 ng/L excluded severe form of AP with NPV of 92%, but PPV was only 29%. From adipokines, only FGF 21 tended to be higher in the severe AP subgroup in day 4. The receiver-operator characteristics (ROC) analysis confirmed that not adipokines, but only CRP and IL-6 were suitable as potential predictors of the disease severity. The cut-off values were established for both parameters: 100 mg/l for CRP and 37 ng/l for IL-6, with negative predictive values (NPV) 96 % and 92 %, and positive predictive values (PPV) 39 % and 29 %, respectively.

CONCLUSION

The role of ADP, A-FABP and FGF 21 is limited in the prediction of the acute pancreatitis severity, while CRP and IL-6 might be useful to exclude a severe AP. As compared to inflammatory markers, adipokines can not yet be used for the prediction of the severity of AP.

Liver, pancreas, gastrointestinal diseases, microbiome

W224

CALPROTECTIN IN PEDIATRIC DISEASE

A.I. Sánchez Bermúdez [1], N. Lopez Riquelme[1], L. Albert Botella[1], M.C. Ramirez Ruiz[1], J.C. Del Rey Jimenez[1], F. Aviles Plaza[1], M. Martinez-villanueva[1], J.A. Noguera Velasco[1]
[1]*University Hospital Virgen de la Arrixaca, Murcia*

BACKGROUND-AIM

There is growing evidence showing the importance of the fecal calprotectin assay in differentiating organic from functional gastrointestinal disease. It is a simple, non-invasive biomarker that is especially useful in children, who may require general anesthesia for colonoscopy. The aim of this study was to assess the use of fecal calprotectin (FCP) in pediatric patients with signs and symptoms of Inflammatory Bowel Disease (IBD) and to distinguish between IBD (ulcerative colitis and Crohn´s disease), or non-IBD (organic and functional gastrointestinal pathology).

METHODS

We selected 145 patients from Pediatrics Gastroenterology Department, who had values above 50 µg/g. Patients were stratified according to the diagnosis: Inflammatory Bowel Disease (ulcerative colitis and Crohn´s disease), intolerances, cystic fibrosis, gastroesophageal reflux, infectious enteritis or undetermined. We used ROC analysis to describe the differences between the diagnostic groups. For that, we classified the patients in two groups, IBD disease or non-IBD disease. Calprotectin concentrations were measured by enzyme immunoassay Calprest® (Eurospital).

RESULTS

The median FCP in each group were: IBD: 168 µg/g (interquartile range: 56-535); intolerances: 107µg/g (51-528); cystic fibrosis: 149(65-561), gastroesophageal reflux: 101(52-213); infectious enteritis: 101(52-499); and undetermined 80(51-445). All the group had different median FCP: Kruskal–Wallis test $p<0.003$. Children with IBD had higher FPC (168 µg/g) than children with non-IBD disease 100 µg/g (51-561) ($p<0.002$). The area under the curve (AUC) was 0.678 ($p<0.002$, CI 95%;0.561-0.795), a FCP of 121 µg/g had a sensitivity of 75%, specificity of 65%. For a value of calprotectin over 121 µg/g an OR= 4.3 ($p<0.05$; CI 95%:1.5-10.2) was obtained.

CONCLUSION

FCP may be useful as a marker of inflammatory disease activity and could, therefore, be implicated in the diagnosis and treatment of a variety of inflammatory and other pathological conditions in pediatric patients. More specifically, according to our study, selecting a suitable cutting value, the FCP may help stratify the risk of a pediatric patient suffering from IBD.

Liver, pancreas, gastrointestinal diseases, microbiome

W225

C-REACTIVE PROTEIN FOR DIAGNOSIS OF SEVERE ACUTE PANCREATITIS

J.D. Santotoribio[1], C. Cañavate-solano[1], A. Garcia-de La Torre[1], F. Arce-matute[1], S. Perez-ramos[1]
[1]*Clinical Biochemistry Laboratory, Puerto Real University Hospital, Cadiz, Spain*

BACKGROUND-AIM

Severe acute pancreatitis (SAP) is related to high mortality rates. Elevated serum CRP levels in patients with acute pancreatitis have been proposed as a prognostic marker of the disease. The aim of this study was to assess the quantification of CRP in serum of patients with acute pancreatitis, through its monitoring during the first 72 hours and determine their relationship with SAP.

METHODS

We studied patients with acute pancreatitis. Serum CRP levels were measured initial, 24, 48 and 72 hours after admission in the emergency room. Acute pancreatitis was established in patients with symptoms of acute abdominal pain and amylasemia > 300 IU/L (reference values: < 100 IU/L). Serum CRP was determined by turbidimetric immunoassay in Dimension EXL (Siemens®) (reference values: < 0.5 mg/dL). Patients were classified into two groups according to the type of acute pancreatitis: edematous acute pancreatitis (EAP) and SAP. Statistical analysis was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC) using the software MEDCALC®.

RESULTS

We studied 17 patients with ages between 17 and 86 years old (mean = 56.4), 7 women and 10 men. Twelve patients were EAP and 5 were SAP. No statistically significant differences were found between EAP and SAP according to the serum CRP levels initial and 24 hours ($p>0.05$). The AUC of serum 48 hours CRP levels for diagnosis of SAP was 0.955 ($p <0.0001$), optimal cut-off value was 3.3 mg/dL exhibiting 100% sensitivity and 81.8% specificity. The AUC of serum 72 hours CRP levels was 0.970 ($p <0.0001$), optimal cut-off value was 3.3 mg/dL exhibiting 100% sensitivity and 90.9% specificity.

CONCLUSION

Serum CRP levels measured after 48 hours of evolution of the disease showed high diagnosis efficacy to predict whether an acute pancreatitis is edematous or severe.

Liver, pancreas, gastrointestinal diseases, microbiome

W226

ANALYSIS OF BIOMARKERS ACCORDING TO BALTHAZAR SCORE IN THE MANEGEMENT OF ACUTE PANCREATITIS

G.M. Varo Sánchez[1], E. Salas Herrero[3], A. Cayuela[4], M. Muñoz Calero[3], M.V. Romero Ruíz[2], M.C. Montilla López[3]
[1]*Departament of Oncology. Hospital Universitario Reina Sofía de Córdoba, Spain*
[2]*Department of Quality. Hospital San Juan de Dios del Aljarafe, Seville, Spain*
[3]*Unit of Laboratory, Hospital San Juan de Dios del Aljarafe, Sevilla, Spain*
[4]*Unit of Public Health. Hospital de Valme, Sevilla, Spain*

BACKGROUND-AIM

The Balthazar Score (BS) is a computer tomography (CT) severity index that combines the grade of pancreatitis (A: normal pancreas; B: enlargement of pancreas; C: inflammatory changes in pancreas and peripancreatic fat; D: ill-defined single fluid collection; E: 2 or more poorly defined fluid collections) with the extent of necrosis (none; ≤30%; >30-50%; >50%) as stratification of pancreatitis severity.
The aim is to determine the correlation between biomarkers and the BS, for analyzing whether laboratory findings are helpful in predicting severity.

METHODS

A retrospective analysis of 110 patients who were admitted at our Hospital between June 2013 and June 2014 with acute pancreatitis was performed. We studied the following parameters: s-amylase, s-lipase, glutamyl oxaloacetic transaminase (GOT), glutamyl pyruvic transaminase (GPT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), white blood cell (WBC) count and neutrophils (N). All the tests were determined two times: (1) when the patient was admitted and (2) 48 hours later. The BS and contrast-enhanced CT were assessed as imaging variables. Descriptive statistics were used for all the variables studied. One-Way ANOVA test followed by Bonferroni post-hoc tests was used.

RESULTS

The mean age was 60.2 years (SD:17.4). According to the BS, the patients were classified: 26(23.6%) grade A, 8(7.3%) grade B, 48(43.6%) grade C, 25(22.7%) grade D and 3(2.8%) grade E.
The results of ANOVA were statiscally significant for LDH2, CPK2, N1 y 2, WBC1 y 2 ($p<0.05$). Bonferroni showed that the followings multiple comparisons: LDH2 and CPK2 (grade E vs A to D), WBC1 (E vs A-B, C vs A and D) WBC2 (D vs A-B) and N2 (A vs C-D) were statiscally significant ($p<0.05$).
The area under the ROC curve, considering BS grade A as negative and grades B to C as positive, was 0.667 [95%confidence interval(CI):0.536-0.798] for WBC2, and was 0.707 [95%CI:0.588-0.826] for N2, both were statistically significant ($p<0.05$).

CONCLUSION

Having elected the convenient group of BS, this study concludes that parameters as LDH, CPK, WBC and N may represent a useful and inexpensive non-invasive tool for the classification of acute pancreatitis. In addition, WBC and neutrophils may be used as predictive factors of severity as complements for BS.

Liver, pancreas, gastrointestinal diseases, microbiome

W227

SERUM IGG4 AS A MARKER OF AUTOIMMUNE PANCREATITIS IN INDIAN POPULATION

D. Gujjarlapudi[1]
[1]*Asian Institute Gasteroenterology, Hyderabad, India.*

BACKGROUND-AIM

AutoImmune Pancreatitis (AIP) is increasingly recognized form of Chronic Pancreatitis that is characterized by lymphoplasmacytic Infiltrate, Storiform Fibrosis, Obliterative Phlebitis, and increased IgG4+ plasma cells in Pancreas. High Serum ImmnoglobulinG4 concentrations have been observed in 90% of AIP patients. Serum IgG4 concentrations are over 10-fold higher in these patients, which will significantly reduce with Corticosteroid therapy.

METHODS

This study was done starting from January 2011 till December 2014 at Asian Institute of Gastroenterology, Hyderabad, India. We measured IgG4 levels in adult age groups between 20-60 years, GroupA: 200 normal healthy control group and GroupB: 824 Chronic Pancreatits cases, out of which SubGroupB1: 106 are AIP cases, SubGroupB2: 222 are pancreatic cancer cases, and the remaining SubGroupB3: 474 cases were having other pancreatic disorders. Serum IgG4 levels were measured by MINI NEPH Binding Site on Nepholometry.

RESULTS

StudyI: The GroupA normal control Mean (530.5+274.6) v/s GroupB Chronic Pancreatitis Mean (602.0+364.6) shows a low statistical significance(p 0.0097). StudyII: The GroupA normal control Mean (530.5+274.6) v/s SubGroupB1 AIP Mean (1012.8+837.6) shows a high statistical significance (p<0.0001). StudyIII: GroupA normal control Mean(530.5+274.6) v/s Sub GroupB2 Mean(784.5+301.8) shows significance (p 0.001). Compared with AIP, pancreatic cancer patients were more likely to have CA19-9 levels of >100 U/l. StudyIV: GroupA normal control Mean(530.5+274.6) v/s Sub GroupB3 Mean(564.5+299.7) shows no significance (p 0.1711). Sensitivity, specificity, and positive predictive values for elevated serum IgG4 (>1400 mg/L) for diagnosis of AIP were 82%, 92%, and 36%, respectively.

CONCLUSION

This study infers that, elevated IgG4 is a sensitive and specific marker of AIP. Serum IgG4 concentration is therefore a reliable marker for the diagnosis of AIP and should be included in various diagnostic criteria for AIP. It also helps in differentiating between AIP and Pancreatic cancer.

Liver, pancreas, gastrointestinal diseases, microbiome

W228

BACTERIAL OVERGROWTH SYNDROME IN MARGINALISED ROMA POPULATION

B. Hubková[2], J. Mašlanková[2], P. Jarčuška[1], J. Guzy[2], O. Rácz[3], M. Mareková[2]
[1]*1st Department of Internal Medicine, Faculty of Medicine, P. J. Š. University in Košice, Slovakia*
[2]*Department of Medical and Clinical Biochemistry, Faculty of Medicine, P. J. Š. University in Košice, Slovakia*
[3]*Department of Pathological Physiology, Faculty of Medicine, P. J. Š. University in Košice, Slovakia*

BACKGROUND-AIM

Data documenting the length of hospitalisation pointed to an extended period of treating the same disease in people with poor socio-economic status as compared with majority population. Bacterial overgrowth syndrome (BOS) is one of such complication responsible for prolonged hospital stay. The aim of the present work is to compare the urinary markers of BOS in marginalised Roma and in majority population. The study was carried out as part of the HEPA-META project, mapping the prevalence of hepatitis B/C, metabolic syndrome and other health problems in adult Roma population from segregated settlements (R = 422; age 18 to 55 years). Majority people (M = 348) comprised the control group.

METHODS

Urinary 4-hydroxyphenylacetic acid (4HPAA) and 3-indoleacetic acid (3IAA) were determined by RP - HPLC (Shimadzu JP). Semi quantitative analysis of leukocytes (Leu) and proteins (Prot) was carried out in first morning urine sample by urine dipsticks (Dekaphan Leuko CZ).

RESULTS

The results showed a skewed distribution of investigated metabolite values and therefore medians (IQR) are presented as mg/g creatinine: 4HPAA (R) = 5606 (2928 – 10648); 4HPAA (M) = 4150 (2218 – 7686); 3IAA (R) = 2817 (0 – 5988); 3IAA (M) = 1191 (0 – 3228). Leukocyturia and proteinuria was observed in 25 % (R), 14 % (M) and in 37 % (R), 26 % (M), respectively. The results of the chemical analyses showed significant association with the results of the semi quantitative analysis. Friedman nonparametric repeated measures ANOVA with a Dunn's multiple comparison post test showed significant difference of 4HPAA and 3IAA levels as well in urinary leukocytes and protein levels between R and M ($P<0.001$, both).

CONCLUSION

We found statistically significant increased levels of urinary 4HPAA and 3IAA in marginalised Roma population which correlated with a high incidence of leukocyturia and proteinuria in this population. The results clearly point to the need to establish objective laboratory tests, as well as the cut off values for BOS metabolites to detect this type of complication as a prerequisite of their elimination leading to the shortening of the overall treatment period and of the hospital stay.
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Liver, pancreas, gastrointestinal diseases, microbiome

W229

CONTRIBUTION OF SOLUBLE TRANSFERIN RECEPTOR IN DIAGNOSIS OF IRON DEFICIENCY IN CROHN'S DISEASE

j. Khira[1]
[1]*laboraory of clinical bioloy, faculty of medecine, hospital La Rabta, Tunis*

BACKGROUND-AIM

Anemia is a frequent complication of Crohn's disease (CD). The main incriminated mechanisms were the iron deficiency and the chronic inflammation. Iron deficiency in patient with inflammatory bowel disease is difficult to establish because of the inflammatory nature of the disease. The aim of this study was to determine the prevalence of anemia in CD patients and to examine the role of soluble transferrin receptor (sTfR) in diagnosis of iron deficiency in CD subjects.

METHODS

A prospective study was carried out between june 1st 2012 and march 12th 2013 on a batch of 86 CD patients diagnosed in the Gastroenterology and Hepatology unit of hospital La Rabta in Tunisia. The CD diagnosis was based on clinical, biological, endoscopic and histological criteria. The anemia diagnosis was based on the World Health Organization criteria. the serum iron level was measured using colorimetric method (Koné 30 Konelab), serum ferritin level using immunoassay method (Axsym, Abbott) and serum sRTf level using immunoturbidimetric method (Cobas Integra, Roche).

RESULTS

The Population studied included 45 men and 41 women, with a sex ratio of 1.097. The average age was 34.97 ± 12.43 years, ranging from 16 to 67 years. Anemia was present in 66,3% of CD subject (57 patients). Anemia was microcytic in 29.8% of cases and normocytic in 70.2% of cases (no case of macrocytic anemia was noted). The main cause identified was mixed type anemia, followed by iron deficiency and inflammation. the analysis of biological parameters showed that blood levels of ferritin was significantly lower in iron deficiency anemia than in inflammatory anemia (16μg / l ± 20 μg / l versus 58,11μg / l ± 45,23μg / l , p <0.001). But the mean rate of sTfR was significantly higher in iron deficiency anemia than in inflammatory anemia (8±6 mg/l vs 3±1 mg/l, p <0.001). All patients with microcytic anemia have higher levels of sTfR than nonanemic patients and in case of normocytic anemia.

CONCLUSION

Anemia remains a frequently associated pathology to the inflammatory bowel disease that requires specific management. The sTfR has obvious interest in diagnosis of iron deficiency in CD subjects. It helps to understand the physiopathologic mechanisms of anemia and to guide the therapeutic approach.

Liver, pancreas, gastrointestinal diseases, microbiome

W230

CYTOKINES IN ALCOHOL LIVER DISEASE: COMPARISON OF DIFFERENT STAGES OF ALCOHOL INTAKE AND LIVER DAMAGE

I. Korita[1], S. Degandt[2], A. Bulo[1], M. Langlois[2], V. Blaton[2]
[1]*Laboratory Department, Faculty of Medicine, University Center Mother Tereza Tirana Albania*
[2]*Laboratory of Medicine AZ Sint-Jan Av Brugge, KU Leuven and UGent Belgium*

BACKGROUND-AIM

Alcohol consumption may cause excessive cytokine production in the liver, leading to inflammatory alcoholic liver disease (ALD). TNF-α is known to induce liver injury, whereas IL-6 and IL-10 have a protective role in ALD. IL-1β is a potent proinflammatory cytokine and is increased in case of ALD.
We aimed to establish the relationship of cytokines and alcohol intake in the development of liver cell necrosis associated with ALD.

METHODS

We recruited 160 males divided in five groups: (1) no alcohol intake (< 20 g ethanol/day); (2) low alcohol intake (20-40 g ethanol/day); (3) high alcohol intake (> 40 g ethanol/day) without liver necrosis; (4) high alcohol intake with liver necrosis; (5) high alcohol intake and proven liver cirrhosis.
Cytokines (TNF-α, IL-6, IL-10 and IL-1β) were analyzed with Immulite 1000® (Siemens). Statistical tests were performed using MedCalc® 12.6.1.0. Baseline characteristics and cytokine values between groups were compared using one-way ANOVA, followed by a post-hoc Tukey-Kramer test. Kolmogorov-Smirnov tests were used to test normality of all parameters before conducting ANOVA tests. Multivariate analysis was used to confirm significant associations independent of other baseline characteristics. All p-values <0.050 were considered statistical significant.

RESULTS

The characteristics of the study population groups were not entirely equally randomized between study groups, as was expected. Multiple regression for each cytokine showed no dependency with covariates.
There was no significant difference between all groups for IL-10. IL-1β was significantly higher in the liver cirrhosis group only. TNF-α and IL-6 showed significantly higher values in group 4 and 5 only. For the liver cirrhosis group all measured cytokines were higher, except for IL-10.

CONCLUSION

Serum cytokine values varied according to level of alcohol intake and liver damage. High alcohol intake without liver necrosis showed no significant rise in cytokine levels. We demonstrated that TNF-α and IL-6 concentrations in alcoholics rise only when liver damage occurs. Contrary to previous publications, we found no elevated IL-10 and IL-1β concentrations when liver damage occurs.

Liver, pancreas, gastrointestinal diseases, microbiome

W231

SEVERE HYPERCHOLESTEROLAEMIA MEDIATED BY LIPOPROTEIN X IN A PATIENT WITH GRANULOMATOUS TUBERCULOUS HEPATITIS

D. Phatlhane[1], A. Zemlin[1], R. Erasmus[1]
[1]*DEPARTMENT OF CHEMICAL PATHOLOGY, NATIONAL HEALTH LABORATORY SERVICE (NHLS), TYGERBERG HOSPITAL, STELLENBOSCH UNIVERSITY, PAROW.*

BACKGROUND-AIM

Lipoprotein X (LpX) is an abnormal lipoprotein that is associated with cholestasis. It is formed from bile lipoprotein that refluxes into the circulation where it binds to albumin. Unlike LDL cholesterol, LpX does not usually require therapy as it disappears after the cholestasis is relieved. This case highlights the significance of LpX as a cause of severe hypercholesterolaemia in a patient with cholestasis.

METHODS

We report a case of a 46 year old HIV positive female patient on anti-retroviral therapy (ART). She was referred from her local clinic to Tygerberg Hospital for persistently elevated liver enzymes.

RESULTS

The liver function tests, from the initial presentation at Tygerberg Hospital, demonstrated a cholestatic picture with no hyperbilirubinaemia. The lipogram at that stage was essentially normal except for a triglyceride value of 1.9 mmol/l. Over the next 3 months the liver enzymes progressively worsened with development of hyperbilirubinaemia. A liver biopsy revealed a granulomatous hepatitis with focal necrotising granulomas and portal tract fibrosis. A severe hypercholesterolaemia was detected with a total cholesterol value of 32.3 mmol/l. LpX was found to be the cause of this hypercholesterolaemia and was detected on gradient gel electrophoresis. Anti-tuberculous therapy was initiated and the liver functions improved with normalisation of the lipid profile.

CONCLUSION

This case highlights the importance of determination of LpX in patients presenting with severe hypercholesterolaemia in the setting of cholestasis.

Liver, pancreas, gastrointestinal diseases, microbiome

W232

SERUM CYTOKINE LEVELS IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

B. Alicja[1], H. Andrzej[2]
[1]*Department of Biochemistry and Molecular Biology, Medical Centre of Postgraduate Education, Warsaw, Poland*
[2]*Department of Gastroenterology, Hepatology and Clinical Oncology, Medical Centre of Postgraduate Education, Warsaw, Poland*

BACKGROUND-AIM

Primary biliary cirrhosis (PBC) is a slowly progressing, cholestatic autoimmune liver disease characterized by specific serum anti-mitochondrial (AMA) and anti-nuclear (ANAs) autoantibodies.
We determined the cytokine pattern characterizing PBC. Interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α) and interferon-γ (INF- γ) belong to pro-inflammatory cytokines, playing a role in inflammatory diseases. The aim of the study was to evaluate serum IL-6, IL-8, TNF-α and INF- γ concentration in patients with PBC and to determine a correlation with specific autoantibodies.

METHODS

We studied sera from 96 patients with PBC and 25 controls. Cytokine levels were evaluated using commercial Elisa assay: IL-6 and IL-8 (Sanquin, The Netherlands), TNF-α and INF- γ (BioLegend, USA).

RESULTS

Elevated levels of IL-6 and IL-8 were measured in 43% and 58% patients with PBC, respectively. They were significantly higher compared with controls: for IL-8 92.3 ± 20.4 vs. 4.7 ± 0.5 pg/ml, P = 0.0076, for IL-6 78.6 ± 12.0 vs. 3.9 ± 0.9 pg/ml, P < 0.0001. In AMA-positive PBC group we found 44% of patients with higher levels of IL-6 and 64 % with higher values of IL-8. Similarly results we obtained in ANAs positive PBC patients. Elevated levels of IL-6 presented 25% of the AMA-negative PBC patients. Concentration of IL-8 in this group was compared with controls. Elevated levels of TNF-α was measured in 63% patients with PBC – 5.08 ± 1.23 vs. 0.21 ± 0.08, P = 0.0024. In patients with primary biliary cirrhosis, high serum TNF-a is accompanied by severe liver fibrosis, as graded by liver biopsy. Median INF- γ levels were increased in patients with PBC compared with healthy controls (40 vs 15 pg/ml; P < 0.01)

CONCLUSION

PBC sera manifest higher levels of cytokine. There was a correlation between concentration of IL-6, IL-8 and TNF-α and specific autoantibodies. Through the pro-inflammatory effects, IL-6, IL-8 and TNF-α may be an important factors in liver pathology in patients with PBC, especially in the development of the inflammatory process. Interesting results indicate that serum TNF-a might be a candidate marker for prediction of the degree of liver fibrosis.
This research was supported by grant 501-1-25-01-14 from the Medical Centre of Postgraduate Education, Warsaw, Poland

Liver, pancreas, gastrointestinal diseases, microbiome

W233

PLASMA AND TISSUE PHOSPHOLIPID FATTY ACID PROFILE IN PATIENT WITH INFLAMMATORY BOWEL DISEASES

K. Sztefko[1], J. Bugajska[1], M. Zwolińska-wcisło [2], J. Berska[1], T. Mach[2]
[1]*Department of Clinical Biochemistry, Polish American Institute of Pediatrics, Jagiellonian University Medical College, Krakow, Poland*
[2]*Department of Gastroenterology, Hepatology and Infectious Diseases, Jagiellonian University Medical College, Krakow, Poland*

BACKGROUND-AIM

Fatty acids (FA) are involved in the development of inflammation. It is known that omega-3 FA and their derivates have a potential anti-inflammatory effect. In contrast, omega-6 FA are mainly pro-inflammatory compounds. There are some indications that patients with inflammatory bowel disease are at risk of essentials fatty acid insufficiency (EFAI) and, in severe cases, of essentials fatty acid deficiency (EFAD).
Aim: to find out the relation between the percentage of individual FA of phospholipids (PL) fraction in serum and in normal as well as in inflamed colon tissue in patients with inflammatory bowel diseases.

METHODS

The study group included 14 patients with inflammatory bowel diseases (12 patients with ulcerative colitis, 2 patients with Crohn's diseases; patients group; M/F 7/7, mean age 49±19 years). 16 patients without colorectal pathology (M/F 4/12, mean age 48±12 years) served as control group. After proper lipids extraction, serum and tissue FA of PL were measured by GC-FID. The results of individual FA were expressed as percentage of total FA.

RESULTS

Significantly lower the mean serum percentage of total for C18:3 (ω-3), C20:2 (ω-6) as compared to control group were noted ($p<0.05$ in both cases). Conversely, C16 concentration was significantly higher in patient group than in control group ($p<0.006$). The percentage of saturated FA (SFA) was significantly higher in patient group, whereas polyunsaturated FA (PUFA) and ratio of PUFA/noPUFA were significantly lower in patient group than in control ($p<0.002$, $p<0.008$, $p<0.002$; respectively). Only the mean percentage of C20:4 (ω-6) was significantly higher in inflamed tissue as compared to normal colonic tissue ($p<0.04$).

CONCLUSION

a) The fatty acid profile of serum phospholipids fraction in patients with inflammatory bowel diseases is characteristic for Essential Fatty Acids Insufficiency (EFAI). b) Increased percentage of C20:4 in colon tissue is an indicator of developed inflammation.

Liver, pancreas, gastrointestinal diseases, microbiome

W234

REGULATORY T CELL SUBPOPULATIONS IN LIVER TRANSPLANT PATIENTS. POTENTIAL ROLE IN REJECTION.

A. De Bejar Almira[2], B. Revilla-nuin[1], L.F. Saenz Mateos[1], J.A. Pons Miñano[1], A. Baroja-mazo[1]
[1]*Digestive Surgery and Abdominal Organ Transplantation Group. Murcia´s Biohealth Research Institute-Virgen de la Arrixaca, Murcia (Spain).*
[2]*Hospital General Universitario Santa Lucia, Cartagena, (Murcia, Spain)*

BACKGROUND-AIM

Regulatory T cells (CD4+Foxp3+,Tregs) are central to the maintenance of self-tolerance and the control of immune homeostasis. Tregs are frequently associated with rejection; however, this does not preclude their protective role and importance in tolerance induction. The aim of this study was to identify the differential expression of markers for Treg subpopulations, during a rejection episode.

METHODS

Our study group was composed of 11 liver transplant patients under submitted to a monitorized immunosuppression withdrawal trial. Blood samples were obtained by venipuncture at the begining of the study (M1) and at the time of rejection (M2). Liver enzymes analysis was carried out in an authomatic analyzer Cobas 8000 Roche. Peripheral blood mononuclear cells (PBMCs) were isoslate by Ficoll density gradient centrifugation (Histopaque, Sigma Aldrich), and the differential expression of Treg markers was analyzed by flow cytometry (FASCAria, BD Bioscience).
Statistical analysis was performed using the statistical software SPSSv 20.0. For groups comparison a Mann-Whitney U test and Spearman rho test were used. A p value of less than 0.05 was considered statistically significant.

RESULTS

The median levels for biochemical liver markers were:M1 group; AST (17.0(IR: 7), ALT (17.0(IR: 10), AP (75.0(IR: 91) and GGT (23.0(IR: 62). M2 group; AST (109.50(IR: 59), ALT (185.0(IR: 126), AP (121.5(IR: 138) and GGT (144.0(IR: 333). There are significant differences between groups for all biochemical markers studied ($p<0.05$). Expression of Latency-Associated Peptide (LAP) in Tregs was significantly higher in M2 group (median 4.97(IR:20.4) than M1 group (median 2.88(IR: 2.98)($p<0.05$). Also observed a positive but not significant association between LAP and AST (rho:0.316, p :0.089).

CONCLUSION

We found that blood LAP+ Tregs are significantly increased at the time of rejection. LAP has been described as unique cell-surface marker that distinguish activated Tregs from induced Tregs. This increased frequency of LAP# Tregs in PBMCs during rejection and its positive correlation with the liver damage indicator AST, could suggest a potential role of this Treg subpopulation in controlling immune response to transplant rejection.

Liver, pancreas, gastrointestinal diseases, microbiome

W236

SMALL INTESTINAL BACTERIAL OVERGROWTH MAY INCREASE THE LIKELIHOOD OF LACTOSE INTOLERANCE FALSE POSITIVE DIAGNOSIS

T.T. Perets [2], D. Hamouda [2], E. Shporn [2], O. Ashorov[2], Y. Niv[1], R. Dickman[1]
[1]*Gastroenterology Department - Rabin Medical Center*
[2]*Gastroenterology Laboratory - Rabin Medical Center*

BACKGROUND-AIM

Small intestinal bacterial overgrowth (SIBO) is defined by the presence of an excessive concentration of bacteria in the small intestine. Lactose intolerance (LI) and SIBO share many gastro-intestinal (GI) symptoms usually attributed to patients diagnosed with irritable bowel syndrome (IBS). Our aim was to evaluate the role and effect of SIBO in the formation of LI symptoms in affected patients.

METHODS

A total of 348 patients with suspected IBS underwent SIBO and LI diagnosis by hydrogen breath test (HBT). 15 gr of lactulose dissolved in 50 ml of water and 50 gr of lactose dissolved in 250 ml of water were used for SIBO and LI HBT respectively. The test result was considered positive when hydrogen concentration acceded 10 PPM for SIBO and 20 PPM for LI above baseline.

RESULTS

Out of the 348 patients, 90 (26%) were negative for both SIBO and LI, 59 (17%) were positive for SIBO and negative for LI, 98 (28%) were negative for SIBO and positive for LI and finally, 101 (29%) were positive for both SIBO and LI. Out of the 101 SIBO and LI positive patients, 82 (81%) had an increase of hydrogen measurement above threshold between 30-90 minutes during their LI-HBT, implying SIBO.

CONCLUSION

The fermentation of lactose in the small bowel due to SIBO may increase the likelihood of LI incorrect diagnosis. We suggest that all symptomatic patients will undergo SIBO testing and eradication if diagnosed positive, prior to LI HBT evaluation.

Liver, pancreas, gastrointestinal diseases, microbiome

W237

STUDY OF THE CONCENTRATION OF CIRCULATING TUMOR CELLS AND BIOCHEMICAL, HEMATOLOGICAL AND COAGULATION MARKERS LEVELS IN PATIENTS WITH HEPATOCELLULAR CARCINOMA AWAITING LIVER TRANSPLANTATION.

M.I. Sánchez Lorencio[2], L.F. Sáenz Mateos[2], S. Veganzones De Castro[1], A. Baroja Mazo[2], B. Revilla Nuin[2], B. Mediero Valeros[1], M.L. Maestro De Las Casas[1], E. Díaz-rubio García[1], J.A. Noguera Velasco[2], P. Ramírez Romero[2]
[1]*Clinical Hospital San Carlos. Madrid*
[2]*IMIB-FFIS. Clinical University Hospital Virgen de la Arrixaca. Murcia*

BACKGROUND-AIM

Hepatocellular carcinoma (HCC) is one of the most causes of cancer mortality. Circulating tumor cells (CTCs) has been associated with the progression of tumor disease and metastasis or recurrence. Isoflux System is an immunomagnetic isolation system for CTCs in peripheral blood. This study aimed to correlate CTCs levels in HCC patients listed for liver transplant with hematologic, biochemical and coagulation parameters necessary in the HCC management.

METHODS

17 HCC patients waiting liver transplantation according to Milan criteria, were included. CTCs were isolated using Isoflux System with immunomagnetic beads coated with antiEpCAM antibodies. Cell count was performed in a fluorescence microscope.
Biochemical, hematologic, and coagulation tests were performed in COBAS 8000 Roche, Sysmex xe5000 and ACLTOP 300 respectively.
Spearman's Rho and the Mann-Whitney U test were estimated to verify the correlation between CTCs and analyzed markers (SPSS 22.0).

RESULTS

CTCs median was 27 (IR 133.5-14.5). There were statistically significant positive correlation between CTCs and bilirubin (ρ=0.505 p=0.039) and cholesterol levels (ρ=0.556 p=0.021). There was statistically significant negative correlation between CTCs and glucose levels (ρ=-0.688 p=0.002). There weren't statistically significant correlation between CTCs levels and the other parameters we studied (p>0.05).
CTCs levels weren't significantly different between pathological and non-pathological bilirubin (U=18 p=0.149). The level of CTCs was statistically significant between pathological and non-pathological cholesterol (U=11 p=0.048) and between pathological and non-pathological glucose (U=10 p=0.020).

CONCLUSION

HCC patients with higher count of CTCs could have low blood glucose due to a large tumor energy metabolism and secretion of insulin-like substances. Increased cholesterol level could be a result of an aberrant lipid metabolism in these patients. Most markers studied for the HCC management don't provide us relevant data about CTCs. Because the CTC has been associated with metastasis and tumor recurrence, we need to expand our study to evaluate the importance of direct measurement of CTC in the control and prioritization of these patients.
Supported by Instituto de Salud Carlos III (FIS PI13/01195)

Liver, pancreas, gastrointestinal diseases, microbiome

W238

GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTOR-1 IN CHILDREN WITH HEREDITARY DISEASE OF HEPATOBILIARY SYSTEM

O.P. Shevchenko[1], O.E. Gichkun[1], O.M. Tsirulnikova[1], R.M. Kurabekova[1], G.A. Olefirenko[1], I.E. Tsirulnikova[1], O.V. Silina[1], S.V. Gautier[1]
[1]*Academician V.I.Shumakov Federal Research Center of Transplantology and Artificial Organs, Moscow*

BACKGROUND-AIM

End stage liver disease (ESLD) is often associated with growth retardation in children with hepatobiliary disease. Insulin-like growth factor-1 (IGF-1) is a hormone produced mainly by the liver in response to the growth hormone (GH) stimulus. Living-donor liver transplantation (LDLT) is the accepted treatment for pediatric patients with ESLD.

METHODS

The study included 52 children with ESLD aged 14±6 (4-36) months before and after living donor liver transplantation (LDLT). The procedures in donors included left lateral sectorectomy, in recipients - hepatectomy, orthotopic implantation of left lateral sector, biliary reconstruction by hepaticojejunostomy. All recipients received 2- or 3-drug immunosuppressive therapy including tacrolimus. Plasma concentrations of GH and IFR-1 were measured by ELISA.

RESULTS

Plasma level of IGF-1 (21.0 ± 29.5 μg/l) was significantly lower in patients with ESLD than in healthy children (52.2 ± 26.3 μg/l, p<0,001). Concentration of GF in children with liver cirrhosis was higher 3.32±7.7 ng/ml vs. 1.16±1.46 ng/ml in healthy children (p=0.01).
There were GH positive and IGF-1 negative correlations with PELD (pediatric end-stage liver disease) score (r=0.79 and r=-0.66, p<0.001). Up to one month after transplantation plasma levels of IGF-I are significantly increased and GH are reduced. There was significant correlation between GH concentration and height (r=0.80, p=0.01) in a year after transplantation.

CONCLUSION

Low level of IGF-I and high GH are associated with growth retardation in patients with ESLD. Investigation of IGF-1 and GH levels in children with liver cirrhosis and their evolution after liver transplantation may be important objective criterion of recovery of physical development regulation and as an additional parameter, which correlates with severity of end-stage liver disease.

Liver, pancreas, gastrointestinal diseases, microbiome

W239

THE CD34/CD45+CELL NUMBER IN THE PERIPHERAL BLOOD OF PEDIATRIC RECIPIENTS CORRELATES WITH INFLAMMATION BIOMARKERS PLASMA LEVELS AND THE OUTCOME AFTER LIVING DONOR LIVER TRANSPLANTATION

O.P. Shevchenko[1], O.M. Tsirulnikova[1], R.M. Kurabekova[1], O.E. Gichkun[1], S.A. Lugovskaya[2], E.V. Naumova[2], I.E. Tsirulnikova[1], V.V. Dolgov[2]

[1]*Academician V.I.Shumakov Federal Research Center of Transplantology and Artificial Organs, Moscow*

[2]*Russian medical academy of postgraduate education, Moscow*

BACKGROUND-AIM

It has been proposed that circulating hematopoietic stem cells (HSCs) play a role in graft survival and immune modulation after liver transplantation. The aim of this study was to analyze the relationship between the number of circulating HSCs before and after living donor liver transplantation (LDLT), plasma levels of immune biomarkers and clinical outcomes in the early posttransplant period in pediatric patients.

METHODS

We studied 15 pairs of adult healthy liver donors (29±5 years, 6 males) and pediatric recipients (age median – 8 months, range 4 - 60 months, 8 boys) with end-stage liver disease (ESLD). The recipients underwent transplantation of the left lateral sector. The CD34/CD45+ cell number was measured in the blood via flow cytometry. Plasma levels of immune biomarkers like C-reactive protein (CRP), anti-human leukocyte antigen class I and II antibodies (HLA I and II ab), soluble CD40 ligand (sCD40L), soluble CD30 (sCD30), and neopterin were measured via ELISA.

RESULTS

The CD34/CD45+ cell number in the peripheral blood of pediatric recipients decreased within the first week after LDLT. The cell number before LDLT negatively correlated with the plasma levels of C-reactive protein and the development graft dysfunction in the early posttransplant period: it was significantly lower in patients who developed graft dysfunction than in those without dysfunction. After LDLT, the CD34/CD45+ cell number positively correlated with the pretransplant plasma level of sCD40L, a T-cell activation marker. There were no significant correlations the cell number with HLA I and II ab, sCD30 and neopterin levels in the recipient`s plasma. In adult liver donors, the cell number did not change within the first week after liver resection and was lower than in pediatric recipients.

CONCLUSION

The results suggest that in pediatric recipients, the HSC number in the peripheral blood and plasma level of immune (inflammation) biomarkers may be associated with graft function and could be regarded as potential predictors of the clinical outcome after LDLT.

Liver, pancreas, gastrointestinal diseases, microbiome

W240

EFFECT OF FASTING ON PLASMA LEVELS OF 7-ALPHA-HYDROXY-4-CHOLESTEN-3-ONE – A MARKER FOR BILE ACID SYNTHESIS IN HUMANS

M. Axelson[1], K. Brismar[2]
[1]*Department of Clinical Chemistry, Karolinska University Laboratory, Karolinska University Hospital, Stockholm, Sweden*
[2]*Department of Endocrinology and Diabetology, Karolinska University Hospital, Stockholm, Sweden*

BACKGROUND-AIM

Background
We have previously shown that plasma levels of 7-alpha-hydroxy-4-cholesten-3-one (C4) reflect rates of bile acid synthesis in humans. Clinically, analysis of C4 has mainly been used to diagnose patients with chronic diarrhoea caused by bile acid malabsorption.
Aim
To characterize different factors and conditions that can affect plasma levels of C4 (bile acid production) in patients in addition to bile acid malabsorption. In this case the effect of fasting was studied.

METHODS

Methods
Diurnal rates of bile acid production under normal and fasting conditions in seven healthy human subjects have been studied. Blood samples were collected with 2-hour intervals during 24-hour periods prior to, during and after a 3-day fast and C4 was analyzed.

RESULTS

Results
Under normal conditions the plasma levels of C4 were relatively constant or mildly fluctuating throughout the day, and the median level of C4 was about 14 ng/mL.
Fasting for 2-3 days resulted in a marked decrease of C4 in plasma (by more than 75%), consistent with a significant reduction of bile acid production. Two days after the fast had ended, the plasma levels of C4 remained low, whereas those of bile acids were elevated. At this time cholesterol production, hormone levels and the metabolism were apparently normalized.

CONCLUSION

Conclusion
The results show that fasting reduces bile acid production and suggest that there is a delayed normalization of this after a period of fasting. This may be considered when C4 analysis is used for diagnosing bile acid malabsorption in patients.

Liver, pancreas, gastrointestinal diseases, microbiome

W241

A MULTIPLEX SERUM BIOCHIP ALLOWS SCREENING FOR PANCREATIC CANCER AT EARLY TUMOR STAGES

S. Bunger[4], S. Freitag-wolf[1], T. Gemoll[4], M. Kelly[2], A. Chacko[3], P. Lowry[3], S. Fitzgerald[3], H. Bruch[4], U. Roblick[4], T. Keck[4], J. Habermann[4]

[1]*Institute of Medical Informatics and Statistics, University Clinic Schleswig-Holstein, Kiel, Germany*
[2]*Randox Laboratories GmbH, Wilhelmstr, Wulfrath, Germany. Randox Laboratories Limited, Crumlin, United Kingdom*
[3]*Randox Laboratories GmbH, Wilhelmstr, Wulfrath, Germany. Randox Laboratories Limited, Crumlin, United Kingdom*
[4]*Section for Translational Surgical Oncology and Biobanking, Department of Surgery, University of Lubeck, Germany*

BACKGROUND-AIM

Pancreatic cancer is one of the most lethal malignancies worldwide. Detection of pancreatic cancer at early stages is crucial because successful surgery at early tumour stages is the only curative therapy today. The persistent delay in diagnosis and the associated high mortality are attributable to the lack of symptoms at early tumour stages combined with a high biological aggressiveness of the tumour and limited treatment options. Therefore, improved screening for earlier diagnosis is essential in order to increase the rate of curatively resectable carcinomas thereby ameliorating patients' prognosis. A relatively non-invasive, cost-efficient possibility could be provided by the measurement of disease specific markers in peripheral blood. This study reports a novel biochip array for the multiplex detection of the serum proteins carcinoembryonic antigen (CEA), interleukin-8 (IL-8), vascular endothelial growth factor (VEGF), macrophage colony-stimulating factor (M-CSF), S100A11, C3adesArg, CD26 and C-reactive protein (CRP) and its application to the screening of pancreatic cancer at early stages.

METHODS

Simultaneous chemiluminescent immunoassays, defining discrete test sites on a biochip surface were employed. Highly standardized preserved serum samples (n=201) reflecting healthy controls, pancreatic adenomas, and pancreatic carcinomas were assessed with this methodology.

RESULTS

Serum levels of CEA, VEGF, S100A11, MCSF, CD26, and CRP showed significant differences between cancer cases and controls. An independent quartile-based predictive model showed a clinical performance for detecting pancreatic carcinomas using a combination of MCS-F, S100A11, C3adesArg and CD26 with 70% sensitivity at 90% specificity (AUC = 0.9015). At 90% specificity, even early carcinomas were detected with 69% sensitivity.

CONCLUSION

CEA, VEGF, S100A11, MCSF, CD26, and CRP show a high potential for early detection of pancreatic cancer, and could thus aid early detection for curative treatment in a clinical setting.

Liver, pancreas, gastrointestinal diseases, microbiome

W242

SCREENING FOR THE IDENTIFICATION OF AUTOIMMUNE OR LYMPHOPROLIFERATIVE ONSET IN PATIENTS NAïVE TO HCV ANTIVIRAL TREATMENT.

F. Gulli[1], U. Basile[2], L. Colacicco[2], L. Miele[1], N. De Matthaeis[1], P. Cattani[3], G.L. Rapaccini[1]

[1]*Department of Internal Medicine, Istitute of Internal Medicine and Geriatry- Catholic University of the Sacred Heart, Rome, Italy*

[2]*Department of Laboratory Medicine, Institute of Biochemistry -Catholic University of the Sacred Heart, Rome, Italy*

[3]*Department of Laboratory Medicine, Institute of Microbiology -Catholic University of the Sacred Heart, Rome, Italy*

BACKGROUND-AIM

Hepatitis C virus(HCV) may be responsible of extra-hepatic manifestations. A chronic infection of immunocompetent cells is most likely at the origin of a benign mono-oligoclonal B lymphocyte proliferation, typically observed in mixed cryoglobulinemia(10% showing late B-NHL).The aim of this study is to identify early markers of autoimmune lymphoproliferative disease onset in a group of antiviral treatment-naïve patients infected by HCV that could identify the transition between a state of silent autoimmune and lymphoproliferative conditions and frank disease.

METHODS

Fourty patients were recruited. Antinuclear antibodies(ANA) were detected by indirect immunofluorescence. Autoantibody detection of IgG directed against M2,gp210,SP100,LKM1,LC1,SLA,Factin antigens were performed by Immunodot analysis. Free light chain(FLC) detection were carried out by turbidimetric assay. Cryoglobulin and cryofibrinogen analysis was carried out following the guidelines of the SIBIOC.

RESULTS

Our results show an 84% prevalence of cryoglobulinemia in samples collected from HCVinfected patients. Of these, 27% showed ANA positivity a negligible percentage of autoantibody liver disease and absence of positivity of cryofibrinogen. The most significant result concerns the finding of high doses of FLC in 73% of patients, of which 21% showing an abnormally elevated k/l ratio. Statistical analysis suggests that patients presenting cryoglobulinemia and FLCratio above 1.6 are also ANA positive.

CONCLUSION

ANA positivity is indicative of the presence of a persistent antigenic stimulus by the virus and the activation of any autoimmune clones.The presence of cryoglobulinemia suggests a continuous lymphocyte stimulation. Interestingly, our results suggest a possible role for the presence of high levels of FLCs and their use to identify the transition between a silent state of probable autoimmune lymphoproliferative disease or a frank illness, using k/l ratio as a cut-off value. The presence of a subpopulation of HCVpositive patients may open new scenarios to targeted therapeutic treatment strategies in subclinical phases. Our study is a contribution to presenting a panel of potential predictive markers of disease progression.

Liver, pancreas, gastrointestinal diseases, microbiome

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ENDOTHELIAL SPECIFIC MICRORNA EXPRESSION PROFILE IN EARLY PHASE OF ACUTE PANCREATITIS.

B. Kusnierz-cabala[5], E.Ł. Stepień[3], E. Nowak[1], M. Kapusta[5], M. Sporek[2], A. Kowalik[1], M. Kuzniewski[4], B. Solnica[5]
[1]*Department for Molecular Diagnostics, Holycross Cancer Center, Kielce, Poland*
[2]*Department of Anatomy, Jagiellonian University Medical College, Krakow, Poland*
[3]*Department of Physics, M. Smoluchowski Institute of Physics, Jagiellonian University, Krakow, Poland*
[4]*Dept. of Nephrology, Jagiellonian University Medical College, Krakow, Poland*
[5]*Diagnostic Dept. Chair of Clinical Biochemistry Jagiellonian University Medical College, Krakow, Poland*

BACKGROUND-AIM

Acute pancreatitis (AP) is a self-limiting disease in most patients, but its severe form develops in up to 20-30% of cases. Early diagnose of severe form of AP has been considered a key determinant of successful therapy and patients' survival. Vascular dysfunction is a severe complication that can cause organ ischemia and damage during AP (acute pulmonary edema, cerebral edema, abdominal compartment syndrome). Laboratory assessment of AP is based on several routine parameters and does not reflect directly endothelial dysfunction or organ injury. Recently, small non-protein-coding RNAs (miRNAS) have been introduced to laboratory diagnostics as a new biomarkers or predictive parameters. Candidate organ and endothelial miRNAs (has-miR-16-5p, -103a-3p, 122-5p, -126-5p, 148a-5p, -216a-5p, -148a-5p, 216a-5p, -375 and -551b-5b) were selected to check their possible clinical application in stratification of patients with mild and severe AP.

METHODS

The study included 64 patients with mild (MAP) and 26 with moderate and severe (SAP) form of AP, mean age 53±16.8 years. The severity of AP was classified according to revised Atlanta Classification 2013. Control group consisted of 10 age and sex matched subjects. Circulating miRNAs were analyzed in serum using quantitative real PCR method (q-RT-PCR) by means of LNA primers. As a control, miR-103a-3- was selected.

RESULTS

In SAP patients, the significant increase of most selected miRNAs was observed (miR-126-5p,-148a-3p, -216a-5p) including those of pancreatic origin: specific miR-375 and -551b-5p. In MAP patients only 3 miRNAs were highly and significantly overexpressed: endothelial specific miR-216a-5p, -551b-5p and pancreatic miR-375. ROC analysis showed that miR-126-p and miR-551b-5p may be useful in prediction of AP severity (AUC 0.748, sensitivity 60.0%, specificity 87.1%; $p<0.001$) and (AUC 0.716, 69.2%and 72.6%; p.001) respectively.

CONCLUSION

A pancreatic miRNA signature can be useful for assessment of pancreas injury during early phase of AP. Early identification of patients with potential endothelial dysfunction during AP can be reflected by specific circulating miRNA levels and may help in the use of appropriate therapy.

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STABILITY OF 13CO2 BREATH TESTS SAMPLES OVER TIME IN THE DIAGNOSIS OF HELICOBACTER PYLORI

T.T. Perets[2], E. Shporn[2], R. Dickman[1], Y. Niv[1]
[1]*Gastroenterology Department - Rabin Medical Center*
[2]*Gastroenterology Laboratory - Rabin Medical Center*

BACKGROUND-AIM

The accuracy and repeatability of breath test in the diagnosis of Helicobacter pylori (HP) infection is debatable. Although it has been shown that storage for long periods does not affect the analysis results, no data are available, on the effect of repetitive testing. Our aim was to evaluate the repeatability of the analysis of the breath samples.

METHODS

A total of 202 breath samples were collected in duplicates, before and after administration of 75 mg urea-13C dissolved in 50 ml of orange juice and the results were expressed as delta 13CO2 (d13CO2). The cut-off value was 3.5 parts per thousand. Each sample was analyzed in a mass spectrometer 7 days after collection and in intervals of 7 days for the duration of additional 3 weeks. The precision calculation was based on the comparison of the d13CO2 obtained in the three consecutive weeks following the first run to the d13CO2 obtained in the first run. The samples were stored at room temperature.

RESULTS

In the second run, 200 out of the 202 (99%) samples were tested positive for HP and the precision of the d13CO2 was 98.6%. In the third run, 197 out of the 202 (97.52%) samples tested positive and the precision was 99.2%. In the fourth and final run 196 out of the 202 (97%) samples tested positive and the precision was 96.7%.

CONCLUSION

We conclude that short term storage of 1 month, does not affect sample stability and the results of HP diagnosis for up to three consecutive repeats.

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STUDY OF THE RELATIONSHIP BETWEEN CIRCULATING TUMOR CELLS CONCENTRATION IN PERIPHERAL BLOOD OF CIRRHOTIC PATIENTS WITH HEPATOCELLULAR CARCINOMA AND WAITING TIME FOR A TRANSPLANT.

L.F. Sáenz Mateos[2], M.I. Sánchez Lorencio[2], V. De La Orden García[1], A. Baroja Mazo[2], B. Revilla Nuin[2], P. García Vigueras[2], M.L. Maestro De Las Casas[1], M.d.R. González Sánchez[2], J.A. Noguera Velasco[2], E. Díaz-rubio García[1], P. Parrilla Paricio[2], P. Ramírez Romero[2]
[1]*Clinical Hospital San Carlos. Madrid*
[2]*Clinical University Hospital Virgen de la Arrixaca IMIB-FFIS. Murcia*

BACKGROUND-AIM

The Barcelona Clinic Liver Cancer (BCLC) system allows the classification of patients with hepatocellular carcinoma (HCC) according to the tumor characteristics and liver disease. This system facilitates the assignment of therapeutic attitudes. Liver transplantation is a relevant curative alternative for patients in early stages (A-B). Detection of circulating tumor cells (CTCs) in peripheral blood indicates progression of neoplastic disease and could play an important role in the BCLC classification system. The aim of this study was to determine the correlation between CTCs and time in the waiting list for liver transplantation in patients with cirrhosis and HCC.

METHODS

We studied 18 patients with cirrhosis and HCC included in the transplant waiting list (stage A-B, BCLC system). The isolation of CTCs was performed by isoflux™ system and cell counting was performed by fluorescence microscopy. To check the relationship between CTCs and time on the transplant list, Spearman Rho and Mann-Whitney tests were performed by SPSS 17.0 software.

RESULTS

Median of CTCs in peripheral blood was 420 CTCs/dL (Min= 40, Max=7187 and IR=1570-203). The mean number of days waiting for a transplant was 200.6 days (CI95%=122.3-278.9 and SD=157.4). The Shapiro Wilk test for time on the waiting list was P=0.096 and for CTCs was P=0.001. Spearman's Rho coefficient was 0.188 (P= 0.455). Median of CTCs in patients with ≤200 days on the waiting list was 320 CTCs/dL (Min=107, Max=2413 and IR=1193.5) and >200 days was 707 CTCs/dL (Min=40, Max=7187 and IR=3666.5-287). The Mann-Whitney test presented a P=0.233 in the comparison of both groups.

CONCLUSION

We conclude that there is no statistically significant association between the concentration of CTCs in peripheral blood and time on the waiting list for liver transplantation in patients with HCC (P>0.05). We observed a weak positive relationship wich could become significant increasing the number of patients. We found no significant differences in CTCs number between the ≤200 days and >200 days group (P>0.05). The information derived from this developing work could play in the future an important role in the prioritization criteria for transplant patients.
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EVALUATION OF THE ELF™ SCORE FOR THE DIAGNOSIS OF LIVER FIBROSIS IN A POPULATION OF HEPATITIS C PATIENTS

C. Trocmé[1], B. Toussaint[1], D. Guergour[5], J. Bosson[3], J. Zarski[4], P. Faure[2]
[1]*Biochimie des Enzymes et des Protéines, Institut de Biologie et de Pathologie, CHU Grenoble, France*
[2]*Biologie Hormonale et Nutritionnelle, Institut de Biologie et de Pathologie, CHU Grenoble, France*
[3]*Centre d'Investigation Clinique INSERM CIC003, CHU Grenoble, France*
[4]*Clinique Universitaire d'Hépato-Gastroentérologie, CHU Grenoble, France*
[5]*Plateforme Commune de Biologie, Institut de Biologie et de Pathologie, CHU Grenoble, France*

BACKGROUND-AIM

Evaluation of liver fibrosis, previously only measured by anatomo-pathologic analysis, has increased over the last years by the emergence of several serum diagnostic tests, combining four to nine blood parameters. The main are the Fibrotest®, the Fibrometer® and the Hepascore® and all three have been recently recognised by the French Social Security as efficient tools for diagnosis of liver fibrosis in hepatitis B and C. More recently, another seric test ELF™ (Enhanced Liver Fibrosis) combining three parameters, procollagen III N-Terminal propeptide (PIIINP), hyaluronic acid (HA) and type 1 inhibitor of matrix metalloproteinases (TIMP-1) was commercialized by the firm Siemens. This score characterized an absence or a low liver fibrosis stage for values below 7.7 and cirrhosis when above 9.8, whereas moderate fibrosis was reported for values between those two limits. Our aim was to compare the diagnostic performance on this new score to the three other tests and to the echographic method Fibroscan®.

METHODS

The three parameters of the ELF™ score were evaluated on serum samples of the Fibrostar cohort comprising 432 hepatitis C patients. The performance diagnostic of the ELF™ score was compared to the three accredited tests on this population. A second analysis was performed on a subset of 331 patients whom fibrosis was also evaluated by Fibroscan®. New thresholds corresponding to the diagnostic performance aimed by Siemens for the score were determined on this population.

RESULTS

On the whole population, the ELF™ score was as powerful as Fibrotest® for the determination of liver fibrosis. However Fibrometer® and Hepascore® were shown to be statistically more accurate than ELF™ for the evaluation of fibrosis stage. On the subset of the cohort, all tests as well as Fibroscan® were demonstrated to identify more specifically fibrosis stage than ELF™. The two thresholds of 7.7 and 9.8 of the ELF™ test was re-calculated on our cohort at 8.41 and 10.16 to achieve the same specificity and sensibility than those seeking by Siemens.

CONCLUSION

Our study showed that the ELF™ score measured may be a powerful tool for the diagnosis of liver fibrosis, but that the thresholds initially determined may be re-evaluated for a better performance of the test.

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DETECTION OF CHYLE IN FLUIDS BY A NOVEL SENSITIVE ETHER TREATMENT METHOD

A. Basak[1]
[1]*J.N.MEDICAL COLLEGE,WARDHA,MAHARASHTRA,INDIA*

BACKGROUND-AIM

Filariasis is common in countries like India, Bangladesh, Africa, etc. Adult parasites of W. banchrofti and B. malayi block lymphatic channels, cause inflammation followed by fibrosis. Sometimes lymphatic channels rupture lymph with chylomicron particles to urinary tract (chyluria - commonest), pleural cavity (chylothorax) and peritoneal cavity (chyloperitonium).Aim of this study is to develop a sensitive method to detect chylomicron in faint white fluids where Routine Method pose problem.

METHODS

over period of 5years 132 white fluids sample suspected for chyle is analyzed Routine method: Sample is mixed with one drop of Phenolphthalein in a small porcelain basin, Sodium bicarbonate (5 gm/dL) is mixed till faint pink colour develops, Pinch of commercial pancreatic lipase and bile salt are mixed, basin is kept at 37°C, disappearance of faint pink colour after 5 minutes or 10 minutes is diagnostic of presence of chylomicron. Our method: In a small glass tube 1.0 ml of sample and 1.0 ml of diethyl ether are shaken vigorously with for one minute by hand. Tube is kept in stand vertically for 05 minutes for separation of phases. At the junction of two liquids, white coloured precipitate appears 10 uL of white precipitate from the junction is seen Under microscope plenty of refractile fat grobules are seen, then presence of chylomicrons is confirmed. We call our procedure as ether treatment method. If the sample is faint white coloured, increasing sample volume procedure is followed to get distinct white precipitate.

RESULTS

Out of 113 chyluria sample, 106 were positive and 07 were negative (04 due to Phosphate) by Routine Method; but all the 109 positive samples were detected by our method. Out of 13 chyloperitonium samples, 09 were positive by Routine Method; but 12 were positive by our method. Out of 06 chylothorax samples, 03 were positive by Routine Method as compared to 04 samples by our method

CONCLUSION

Fat globules are seen under microscope by our method is due to stripping of phospholipid monolayer membrane of chylomicrons. Advantage of our method in faint white samples, fat globules can be extracted more increasing sample volume and this increases sensitivity as compared to the routine method. Surprisingly, chloroform-methanol mixture does not work.

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AMMONIA LEVEL CHANGES IN ICTERIC SAMPLES

G. Fressl Juros[1], A. Ceri[1], I. Rako[1], D. Rogic[1]
[1]*Emergency laboratory, Department for Laboratory Diagnostics,Universitiy Hospital Centre Zagreb*

BACKGROUND-AIM

Emergency laboratory often receives icteric samples for ammonia measurement. Reagent manufacturer declares that there is no interference of unconjugated bilirubin up to the concentration of 513 μmol/L but not whether the interference is negative or positive. So we investigated the influence of bilirubin on ammonia measurement.

METHODS

Three EDTA plasma pool samples with low (43 μmol/L), medium (76 μmol/L) and high (132 μmol/L) ammonia concentrations were spiked with unconjugated bilirubin stock solution to get five different bilirubin concentrations, approx. 70, 130, 190, 250 and 300 μmol/L. Ammonia and bilirubin were measured on Roche Cobas c501. The percentage of difference between ammonia concentration in samples with normal bilirubin level (non-icteric) and samples with high bilirubin concentration (icteric) was calculated using the following formula: (ammonia icteric – ammonia nonicteric)/ammonia nonicteric*100. Statistical analysis was performed using paired t-test.

RESULTS

In the pool with low ammonia concentration, the difference between icteric and non-icteric samples at bilirubin level of 75, 140, 194, 255 and 308 μmol/L were -4%, -15%, -22%, -39% and -32%, respectively. In the pool with medium ammonia level, the difference between icteric and non-icteric samples at bilirubin concentration of 73, 128, 190, 246 and 303 μmol/L were -8%, -8%, -15%, -22% and -36%, respectively. In the pool with high ammonia concentration, the difference between icteric and non-icteric samples at bilirubin level of 69, 118, 166, 211 and 258 μmol/L were -4%, -7%, -7%, -10% and -18%, recpectively. Paired t-test showed statistically significant difference ($p<0.05$) in ammonia level between non-icteric and icteric samples.

CONCLUSION

Our investigation has shown that icteria causes negative interference on ammonia measurement. We observed that the higher bilirubin concentration the stronger negative interference, particulary at the low ammonia level.