STUDY OF PLASMA CHOLINESTERASE ACTIVITY IN HEPATIC DISEASES

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BACKGROUND: Plasma cholinesterase activity (ChE) may vary in some pathological circumstances. We studied the changes in activity of this enzyme according to the type of liver injury, to assess the interest of this parameter in the diagnosis of liver diseases.

METHODS: Our study was performed on 102 patients with different liver diseases and 53 healthy controls. BChE activity was measured by spectrophotometric method on konelab 30® analyser (Thermo Clinical Labsystems), by using butyrylthiocholine as substrate (Elitech diagnostics).

RESULTS: The ChE activity was lower in patients compared to control group (p<0.0001), and more pronounced in cirrhotic patients compared to those suffering from hepatitis. Elevated activities of AST, ALT, GGT and ALP and bilirubinemia, and decreased albuminemia were noted in patients compared to controls (p<0.001). Hypoalbuminemia was significantly important in cirrhotic patients compared to those suffering from cholestasis or hepatitis. A correlation between ChE and bilirubin, albumin and serum protein was found in patients with cirrhosis or those with chronic hepatitis. A significantly lower activity of ChE was found in patients with hepatic insufficiency (HI).

CONCLUSIONS: In case of suspicion of HI, the prescription of ChE activity could guide or confirm the diagnosis of the impairment.
DIAGNOSTIC ROLE OF ANTI-MULLERIAN HORMONE TO DEFINE THE FUNCTIONAL GONADAL EXHAUSTION IN FERTILE HCV POSITIVE WOMEN

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BACKGROUND: Women with Chronic Hepatitis C (HCV) are reported to have anticipated menopause (1). In these women, menopause, especially early, is associated with faster fibrosis progression and marked resistance to antiviral therapy (2). The aim was to try to better define the exact timing of functional gonadal exhaustion in fertile HCV+ women through the use of anti-Müllerian hormone (AMH) (2,3), and to evaluate its influence on antiviral response.

METHODS: AMH was measured in 107 HCV+ women (73 fertile) and 180 HCV- controls (119 fertile) of similar age and tested by ELISA (AMH Gen II ELISA Beckman Coulter Inc, USA), sensitivity 0.08 ng/ml, linearity up to 22.5 ng/ml, CV <6.5%. Data were analyzed with T test, non parametric tests and logistic regression.

RESULTS: Mean age between HCV+ and HCV- women was not significantly different (42.7±11.9; 41.9±6.2, p=0.519). In the fertile group, HCV+ were slightly younger (37.5±8.7 vs. 40.6±5.8 yrs, p=0.034). HCV+ women had significantly lower levels of AMH than HCV:-2.3±3.1 vs. 7.3.0±12.6, p<0.0001. When considering only women in childbearing age, AMH levels were significantly lower in HCV+ (3.2±3.4 vs. 8.7±3.6, p=0.017). The difference between the two groups was extremely evident also in women<30 yrs (i.e. in those at peak estrogenic activity; 6.1±4.1 vs. 34.7±28.7, p<0.0001). Estradiol levels of HCV + women were not different in fertile age and premenopausal (fertile vs. premenopausal: 86±80 ng/mL vs 77±58 ng/mL, p=0.690). At regression analysis for SVR, the independent factors for failure, both for fertile and menopausal women, were genotype and low AMH levels (menopausal: genotype OR 3.004; 95% 1.557-5.797, p=0.001; AMH: OR 5.054, 95% 1.829-13.965, p=0.002; Fertile: genotype OR 2.937; 95% 1.249-6.905, p=0.014; AMH: OR 9.996, 95% 1.666-59.958, p=0.012)

CONCLUSIONS: These results show relevant data: 1) AMH levels are significantly lower in women with HCV+ than in the control group; 2) ovarian exhaustion in HCV+ woman occurs at very early age (<30 yrs). AMH levels of fertile HCV + woman show values comparable to those of pre-menopausal population; 3) AMH level is much more sensitive than estradiol assay for an early diagnosis of initial gonadal insufficiency.
SIGNIFICANCE AND USEFULNESS OF RAPID TEST FOR QUANTITATIVE DETERMINATION OF FECAL CALPROTECTIN

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BACKGROUND: In recent years a new biomarker calprotectin was established for the diagnosis and monitoring of intestinal diseases. Fecal calprotectin (FC) levels are elevated in inflammation of the intestinal mucosa due to a number of diseases, but the most established in the diagnosis and monitoring of chronic inflammatory bowel disease (IBD). FC is useful for monitoring the response to IBD-specific therapy, for IBD relapse detection and separation of active inflammation from complications of non-inflammatory respectively functional disorders.

METHODS: The study group included 232 patients who underwent colonoscopy. Patients were stratified into two groups according to clinical and endoscopic diagnosis. The first group with IBD included 99 patients with Crohn’s disease (CD), 78 with ulcerative colitis (UC) and 4 with indeterminate colitis. In the second group were 46 patients with functional diseases, Irritable Bowel Syndrome (IBS) and others. FC was measured with immunochromatographic Quantum Blue® Calprotectin High Range Rapid Test (BÜHLMANN Laboratories AG) ranged from 100 to 1800 µg/g stool. Differences between median values were evaluated using Mann-Whitney test.

RESULTS: Median FC levels were significantly higher (Mann-Whitney U= 2500.5; P < 0.0001) in first group of patients with organic diseases, IBD (N= 186, median 268 µg/g stool, CI 95%: 203-454 µg/g stool) than the median FC levels found in second group of patients with functional diseases, IBS and others (N= 46, 100 µg/g stool, CI 95%: 100 -114 µg/g stool). The proportion of patients with FC concentration <100 µg/g stool in the first group was 26% compared to 54% in the second group. 20% of patients from IBD group had FC concentration >1800 µg/g stool compared to only 4% in the second group.

CONCLUSIONS: Our study showed that FC levels are statistically significantly higher in patients with diagnosed IBD compared to patients in the second group. Results confirm that FC has a great potential as a diagnostic tool in differentiating IBD from other diseases, especially functional disorders. Therefore invasive endoscopic diagnostics could sometimes be avoided, preventing complications of endoscopic procedures and reducing health care costs, but this has still yet to be confirmed by other studies.
Liver and gastrointestinal diseases

Cod: 1031

NONINVASIVE LIVER FIBROSIS MARKERS IN ALCOHOLICS

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BACKGROUND: An accurate measurements of fibrosis and cirrhosis allow simple noninvasive biomarkers that estimate the liver fibrosis stage on the basis of laboratory blood tests and patient’s data. Among these, FibroTest and Hepascore were validated and patented. Beside the patented tests there are known an non-patented indexes. These involve APRI, Forns index, FIB-4 and GAPRI. The aim of this study was to compare the diagnostic accuracy of these indexes for the assessment of significant fibrosis and cirrhosis in alcohol abusing patients based on the FibroTest scoring system.

METHODS: Diagnostic values (sensitivity, specificity, predictive values, efficiency and accuracy) of serum biomarkers were evaluated in 142 consecutive alcoholics. We calculated these values between the groups with significant fibrosis (F ≥ 2) vs groups without or mild fibrosis (F0, F0-F1, F1-F2), and between the groups with cirrhosis (F3-F4, F4) vs groups without cirrhosis (F0, F0-F1, F1-F2, F2, F3).

RESULTS: The stage of liver fibrosis significantly affects the values of these indexes (ANOVA rank Kruskal-Wallis test: P < 0.001 for all tests). The diagnostic accuracy of APRI, Forns index, FIB-4 and GAPRI in the prediction of significant fibrosis in alcoholics fluctuated between 0.725 and 0.817 (AUC); for GAPRI was higher than for APRI. The sensitivity (39.4%), specificity (98.1%), accuracy (83.8%), positive (83.5%) and negative (86.7%) predictive values were highest for GAPRI (AUC=0.817). The diagnostic accuracy in the prediction of alcoholic cirrhosis for compared tests were between 0.820 and 0.898; the highest was for GAPRI. AUC for FIB-4 (0.885) was significantly higher than that for APRI (0.820). APRI and FIB-4 are accurate to exclusion of cirrhosis in all patients (specificity and NPV were equal of 100%).

CONCLUSIONS: The diagnostic power of APRI, Forns index, FIB-4 and GAPRI for the diagnosis of alcoholic cirrhosis was higher than that for the diagnosis of clinically significant fibrosis. These tests are not useful for the detection of advanced fibrosis and cirrhosis in alcoholics, but for exclusion of these disorders in all patients.
ELF TEST AS A NEW NON INVASIVE DIAGNOSTIC TOOL FOR STAGING LIVER FIBROSIS: VALIDATION IN A COHORT OF PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE

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BACKGROUND: The identification of fibrosis in patients with nonalcoholic fatty liver disease (NAFLD) is important for assessing prognosis and selecting patients candidates for emerging therapeutic interventions. Liver biopsy histological examination is the reference standard for detecting liver fibrosis; however, biopsy can be painful and hazardous, assessment is subjective and prone to sampling error. Recently, the serum Enhanced Liver Fibrosis (ELF) test has been developed for staging liver fibrosis in patients with chronic liver diseases. The aim of our study was to validate the ELF test as a non invasive diagnostic tool for predicting fibrosis stage in an independent adult cohort of NAFLD patients.

METHODS: 74 patients (mean age 44.3 years) with suspected NAFLD underwent percutaneous liver biopsy and serum sampling. Fibrosis was assessed and scored by using the modified Brunt classification (F0=no fibrosis; F1=perisinusoidal/periportal; F2=perisinusoidal and portal/periportal; F3=bridging fibrosis; F4=cirrhosis). The ELF test was determined in all patients by means of an algorithm working on a combination of hyaluronic acid, amino-terminal propeptide of type III collagen and tissue inhibitor of metalloproteinase 1 levels. Diagnostic accuracy was assessed determining the area under receiver operating characteristic curves (AUCs).

RESULTS: The distribution of fibrosis stages in our cohort was as follows: F0 = 8.1% (n=6), F1 = 45.9% (n=34), F2 = 39.2% (n=29), F3 = 5.4% (n=4), F4 = 1.4% (n=1). The ELF test had an AUC of 0.858 (95% Confidence Interval C.I. 0.695-1.021; p = 0.008) for distinguishing severe fibrosis, 0.583 (C.I. 0.452-0.714; p = 0.222) for moderate fibrosis and 0.596 (C.I. 0.307-0.885; p = 0.440) for no fibrosis. ELF scores were significantly higher in patients with severe fibrosis in respect to ones with moderate fibrosis (median 9.93 vs. 8.53; p = 0.005).

CONCLUSIONS: In our cohort of NAFLD patients, the ELF test was able to define liver fibrosis stages with a good diagnostic accuracy. Its ability to discriminate between moderate and severe fibrosis may result in clinically relevant information for the selection of cases with possible progressive fibrogenic outcome suitable for further histopathological analysis and/or therapeutic follow-up.
EFFECT OF AN HIGH-FAT DIET IN SERUM LIPID CONCENTRATION AND GALLSTONES FORMATION PROCESS

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BACKGROUND: Cholelithiasis is considered a multifactorial disease, where the diet appears to play an important role. Studies of the effect of dietary fats on bile composition in humans have yielded controversial results. In most patients with hypertriglyceridemia, generally associated with overweight and insulin resistance, no observed differences in the bile pool size composition, compared with normal subjects. The aim of this study was to investigate the influence of consuming a high-fat diet on serum lipids, and assess their implications in the formation of cholesterol gallstones.

METHODS: We settle for 2 groups of BALB/c mice: one control (n = 10), and the other (n = 10) treated with a high-fat diet (60% fat). Early blood samples were taken for glucose and lipids analysis. After 2 months, the animals were sacrificed and blood samples, tissues and bile were obtained. We determined serum glucose, glucose tolerance curve and the corresponding lipid profiles. In bile samples, cholesterol and phospholipids levels were analyzed, and cholesterol carriers (vesicles and micelles) were separated by gel filtration chromatography.

RESULTS: Treated animals showed: 1) increase by 20% in serum total cholesterol; 2) increase of 50% in HDL-cholesterol, but no change in LDL-cholesterol; 3) increase by 40% in glycemia; 4) increased insulin resistance; 5) no change in biliary lipids (cholesterol: 4.1±0.3 mM and phospholipids: 33.5±0.6 mM); 6) no alteration in biliary cholesterol carriers.

CONCLUSIONS: An high-fat diet significantly alter glycemia and serum lipids, without changing the biliary lipids. Consequently, the intake of a diet rich in fat do not appear to affect the formation of cholesterol gallstones in our experimental model.
STABILITY OF CALPROTECTIN IS AFFECTED BY THE PROTEOLYTIC ENZYME TRYPsin

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BACKGROUND: Calprotectin is a 36 kDa calcium and zinc binding protein. An increased level of calprotectin in faeces is an emerging biomarker for inflammatory bowel disease. However, the biomarker calprotectin shows 14 potential cleavage sites for trypsin. As trypsin activity is quite high in the intestinal lumen, effects of trypsin and alpha1-antitrypsin as potential confounders for fecal calprotectin are investigated in the present study.

METHODS: An in vitro model was created by preparing fecal extracts from routine fecal samples. Calprotectin was added in 1:100 dilution after isolation from white blood cells and subsequently lysis of the cytosolic calprotectin with a 1% Triton X-100 lysis buffer. Trypsin digestion was carried out by adding trypsin solution (1 mg/mL, 1mM HCl). Incubation occurred for 24 h or 48 h at 37°C. To study the influence of alfa1-antitrypsin on trypsin activity, incubation of trypsin with a serum sample was performed at room temperature for 30 min.

RESULTS: In-vitro experiments allowed to monitor the digestion of calprotectin present in a fecal extract matrix by trypsin, leading to loss of immunoreactivity. Trypsin activity showed to be a potential confounder in the interpretation of calprotectin, in particular for proximal lesions, where the exposure of calprotectin to trypsin is prolonged. The more trypsin was added to the mixture the higher the relative calprotectin breakdown. Decreases of calprotectin were higher after 48h of incubation in comparison to 24h of incubation at 37°C. Next to the presence of trypsin, also the occurrence of its endogeneous inhibitor protein, alpha1-antitrypsin after a minor or major bleeding may play a role. Experiments showed a decrease in trypsin activity after adding alpha1-antitrypsin.

CONCLUSIONS: Transit time, trypsin activity and addition of blood as a source of alpha1-antitrypsin may be regarded as potential confounders in the interpretation of calprotectin results. Cut-off values depending on the anatomical localization of the lesions could improve the diagnostic efficiency of calprotectin testing.
IMPORTUNES OF REGISTERING F- CALPROCTECTIN IN PATIENTS WITH INFLAMATORY COLON DISEASES AND WITH SYNDROME OF IRRITATION OF THE COLON

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BACKGROUND: Registering of calprotectin is very sensitive and easy way for differential diagnosis of organic diseases of colon, ulcerous colitis (UC), Chron diseases and irritable bowel syndrome (IBS) and disease function of colon. F-calprotectin is sensitive marker for differential diagnosis of pre-endoscopic screaming intestinal disease.

METHODS: We investigate 123 patients (66 male, 57 female) 68 of them with UC, 22 with Chron diseases and 33 with IBS. All patients were hospitalizing on Clinic for gastroenterology. In all patients the diagnosis of inflammatory diseases were establish with colonoscopy and pathohistology investigation. The diagnosis of IBS was establishing on Rome III criterion, excluded organic disease with colonoscopy F-calproteictin were measure with Buhrmann quantum blue imunoenzyme tests in human fecal samples on bulhlmann quantum blue rider.

RESULTS: The mean values of F - calprotectin was 100,8±96,2 µg/g in IBS, 756,2±639 µg/g in Chron disease and 1457,6±948 µg/g in UC. In all our patients the values of F-calprotectin were high. In patients with IBS the mean value of F-calprotectin were lower, then in other patients.

CONCLUSIONS: Those high values of F-calprotectin might indicate on active inflammation of intestinal tract, witch is establishing with colonoscopy and pathohistology findings. Low values of F-calprotectin exclude the organic colon diseases witch implicate not necessary invasion diagnostic procedures.
SERUM FERITIN, HEMOGLOBIN AT HELICOBACTER PYLORI INFECTION

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BACKGROUND: Helicobacter pylori induces gastric inflammation in host and such gastritis increases the risk of gastric and duodenal ulceration and adenocarcinoma as well. Recently, Helicobacter pylori infection was associated with iron-deficiency anemia. The aim of this study was to examine the relationship between Helicobacter pylori infection, hemoglobin and iron status using serum ferritin as a marker for total body iron.

METHODS: Serum ferritin, hemoglobin and immunoglobulin G (IgG) antibodies against Helicobacter pylori were evaluated at 90 dyspeptic patients. IgG antibodies and ferritin were examined using a chemiluminescent assay, and hemoglobin with Coulter's.

RESULTS: The seroprevalence of Helicobacter pylori infection did not relate to hemoglobin. Serum ferritin levels were significantly lower in men (\(x=40\pm12\) ng/ml) and in menopausal women who were IgG positive (\(x=32\pm8\) ng/ml) than in seronegative patients (\(p<0.05\)). IgG-positive more often had reduced serum ferritin levels than seronegative patients.

CONCLUSIONS: Serum ferritin levels are reduced in patients with increased IgG antibodies to Helicobacter pylori infection affects iron metabolism in humans.
**RED CELL DISTRIBUTION WIDTH (RDW) CORRELATES WITH THE SEVERITY OF ACUTE PANCREATITIS DURING THE EARLY PHASE OF DISEASE**

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**BACKGROUND:** 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).

**CONCLUSIONS:** 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.
CHOICE OF A CONFIRMATORY METHOD AFTER CDT ANALYSIS BY CAPILLARY ELECTROPHORESIS IN CIRRHOTIC PATIENTS

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BACKGROUND: The detection of alcohol abuse and the monitoring of abstinence is an important concern in many countries due to its prevalence and its consequences. In the patients with liver disease of any aetiology, a six month period of alcohol abstinence is required prior their inclusion in a liver transplant program, which can be assessed by repeated medical advice and the absence of biological signs of alcohol abuse. In most patients, the usual biochemical parameters can not be used because of the liver dysfunction. Among the specific markers of sustained alcohol intake, Carbohydrate Deficient Transferrin (CDT) in serum is considered as the best available marker due to its specificity and available automated procedures. Three procedures based on capillary zone electrophoresis (CZE) are marketed. One procedure (Capillarys, Sebia, Lisses, France) is widely distributed and may be used in this purpose. However, the electrophoretic pattern is sometimes altered in cirrhotic patients, which prevents obtaining of a reliable result and this requires a confirmatory method in these patients. This study aimed at evaluating whether HPLC or nephelometric immunoassay can be used as confirmatory methods in these patients.

METHODS: Fifty five patients were included in the study on the basis of clinically proved liver cirrhosis and abnormal profile by CZE. All samples were analyzed by using the HPLC candidate reference method and the N Latex immunoassay (Siemens, Marburg, Germany). The results were divided in three groups: quantitative reliable result (QR), result lower than the upper limit of reference values (SQ), unreliable or absent result (NQ).

RESULTS: Twenty eight patients were classified as QR using the N Latex method versus 17 by HPLC. However, in patients with low (<1.40 g/l) transferrin, HPLC appeared superior to the N Latex method (n=11 vs. n=3). When considering that a SQ result can be useful to ascertain a sustained abstinence, 6 CZE results can be accepted. In the remaining 49 patients, a similar number of acceptable results were given by both confirmatory methods.

CONCLUSIONS: In patients with liver cirrhosis, the N Latex method is more appropriate than HPLC as a confirmatory method when CZE fails, except in patients with low serum transferrin concentrations.
Liver and gastrointestinal diseases

Cod: 1039

PRIMARY CARE REQUEST OF LABORATORY LIVER TESTS IN SPAIN: POTENTIAL SAVINGS IF APPROPRIATENESS INDICATORS TARGETS ARE ACHIEVED


BACKGROUND: To compare the inter-practice and inter-regional variability in liver laboratory tests requested by General Practitioners (GPs) in Spain, according geographic and hospital characteristics, using appropriateness indicators, to try to ascertain the degree in requesting appropriateness.

METHODS: 76 laboratories from diverse regions across Spain filled out the number of alanine aminotransferase (ALT), alkaline phosphatase (AP), aspartate aminotransferase (AST), direct bilirubin (dBIL), gammaglutamil transpeptidase (GGT), lactate dehydrogenase (LDH) and total bilirubin (tBIL) requested by GPs for the year 2012. Two types of appropriateness indicators were calculated: every test requests per 1000 inhabitants and ratios of related tests requests (AST/ALT, GGT/ALT, LDH/ALT and tBIL/ALT). The indicators results obtained in different location, type of management and in three communities were compared.

RESULTS: In total GPs requested 20916780 laboratory liver tests in year 2012 in a Spanish population (17679195 inhabitants) that is almost half of the whole country population. No significant differences were obtained in test requesting by GPs in rural, urban or rural-urban locations. In relation to institution type, LDH and dBIL per 1000 inhabitants indicators results were significantly higher in institutions with private management when compared to public. AST, AP, GGT and tBIL per 1000 inhabitants were lower in Valencia when compared to Castilla-Leon, and AP and tBIL per 1000 inhabitants were also lower when compared to the rest of regions. Andalucía presented GGT and AP per 1000 inhabitants lower values than Castilla-Leon and lower values in GGT per 1000 inhabitants than the rests of regions. Castilla-Leon when compared to the rest of regions had the highest values in AP, AST, dBIL, GGT and tBIL per 1000 inhabitants. 9, 31 and 13 laboratories achieved the AST/ALT (0.25), LDH/ALT (0.05) and tBIL/ALT (0.25) indicators targets respectively. No laboratory achieved the GGT/ALT (0.20) indicator goal.

CONCLUSIONS: The high variability observed is difficult to explain by differences in patient case mix between regions and emphasizes the need to accomplish interventions to improve liver tests appropriate use.
THE CONCENTRATION OF CARBOHYDRATE-DEFICIENT TRANSFERRIN (CDT) IN PANCREATIC DISEASES

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BACKGROUND: The changes of CDT concentration in the course of pancreatic diseases might confirm the existence of alterations in transferrin glycosylation during these disorders. Therefore, the aim of this study was to assess the effect of pancreatic diseases (acute and chronic pancreatitis, and primary pancreatic cancer) on serum CDT concentration.

METHODS: The tested group consisted of 110 patients suffering from pancreatic diseases. The patients were divided into the subgroups according to the diagnosis of pancreatic diseases: 32 patients with acute pancreatitis (AP), 34 patients with chronic pancreatitis (CP) and 44 patients with primary pancreatic cancer (PPC). The control group consisted of 40 healthy subjects recruited from hospital workers. CDT, as an absolute (mg/L) and relative concentration (percentage of total transferrin, %CDT) was assayed by immunonephelometric method using N Latex CDT test on the BN II System.

RESULTS: Both, relative and absolute CDT serum levels, were significantly higher in AP (5.22%; 98.43 mg/L) and CP (4.76%; 103.60 mg/L) when compared to the control group (1.76%; 44.32 mg/L) (P<0.001 for all comparisons). However, there were no significant differences of %CDT and CDT levels in PPC (1.68%; 39.7 mg/L) when compared to the controls (P>0.05). There were significant differences in the serum %CDT and CDT levels between pancreatic diseases (H=68.46, P=0.000; H=46.91, P=0.000; respectively). Further analysis showed that the mean %CDT and CDT levels in AP and CP were significantly higher than that in PPC (P<0.001 for all comparisons). There were no significant differences in the %CDT and absolute CDT concentrations between AP and CP (P>0.05). The highest diagnostic sensitivity was obtained for %CDT in AP (86.7%), and for absolute CDT level in CP (75%). The area under the ROC curve for %CDT was 0.938 in CP and 0.928 in AP, and for absolute CDT concentration it was 0.877 and 0.831, respectively.

CONCLUSIONS: We conclude that the acute and chronic pancreatitis may affect both, relative and absolute CDT concentrations. These results indicate the occurrence of the alterations in the glycosylation of plasma transferrin in patients with pancreatic diseases.
Liver and gastrointestinal diseases

SERUM SIALIC ACID CONCENTRATION AND CONTENT IN APOB-CONTAINING LIPOPROTEINS IN LIVER DISEASES

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BACKGROUND: The synthesis and catabolism of the lipids and lipoproteins occurs in the liver. The great significance for the metabolism of lipoproteins has a composition of carbohydrate chain of apolipoproteins. In this part is located sialic acid (SA). The largest part of the sialic acid has been detected in apolipoprotein B (ApoB) of LDL. The aim of this study was to compare the concentration and content of sialic acid in lipoproteins containing apolipoprotein B.

METHODS: The patients were divided into subgroups according to clinical diagnosis: alcoholic cirrhosis (AC) -53 patients, non-alcoholic cirrhosis (NAC) -32 patients, chronic hepatitis (CH) -13 patients, toxic hepatitis (HT) -17 patients, chronic viral hepatitis (HV) -31 patients and liver cancer (LC) -19 patients. Control group consisted of 50 healthy subject recruited from hospital workers. SA concentration was measured according to the enzymatic method and ApoB by an immunoturbidimetric procedure. The content of SA in ApoB was calculated using adequate formula: SA/ApoB.

RESULTS: The mean SA concentration in ApoB-containing lipoproteins was significantly higher in the HV (mean±SD, 54.28±12.64 µM/L) and HT group (50.19±21.46 µM/L) in comparison to the control group (44.62±13.63 µM/L) (P=0.010; P=0.006, respectively). The serum levels of SA in ApoB-containing lipoproteins appeared to be different between liver diseases (ANOVA: H=17.722, P=0.003). Post-hoc analysis revealed that in AC (51.35±13.91 µM/L) and HV patients these levels appeared to be higher than that in LC (40.58±16.95 µM/L) (P=0.016; P<0.001, respectively). The contents of SA in ApoB-containing lipoproteins in AC (57.50±20.88 µM/L) and HV patients (65.39±25.40 µM/L) were significantly higher than that in the control group (47.63±15.59 µM/L) (P=0.049; P=0.013, respectively), but did not differ between diseases. The serum concentration of ApoB were not significantly different between specific liver diseases (P>0.05 for each comparison).

CONCLUSIONS: We concluded that the SA concentration in ApoB-containing lipoproteins differs between liver diseases, but the content is similar and these findings have a role in the metabolism of lipoproteins.
COMPARISON OF ENHANCED LIVER FIBROSIS TEST AND ACOUSTIC RADIATION FORCE IMPULSE ELASTOGRAPHY WITH LIVER BIOPSY IN PATIENTS WITH AUTOIMMUNE HEPATITIS: PRELIMINARY RESULTS

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**BACKGROUND:** The enhanced liver fibrosis (ELF) test, a noninvasive diagnostic test panel consisting of hyaluronic acid (HA), amino-terminal propeptide of type III collagen (PIIINP), and tissue inhibitor of metalloproteinase 1 (TIMP-1) for the evaluation of liver fibrosis in chronic liver diseases. ARFI elastography is also a noninvasive method based on ultrasound elastography to evaluate viscoelastic properties of tissues. Liver fibrosis is evaluated by this method with increased tissue strain in fibrotic liver. The aim of this study is to compare the performance of ELF test and ARFI elastography with biopsy in detection of liver fibrosis level in patients with autoimmune hepatitis.

**METHODS:** Serum samples were collected from 12 patients with autoimmune hepatitis who have undergone liver biopsy. ELF panel parameters were measured with Siemens ADVIA Centaur XP immunoassay analyzer. ELF score was calculated with an algorithm using HA, PIIINP and TIMP-1 values. Acoustic radiation force impulse (ARFI) elastography is performed with a Siemens Acuson S2000 TM. Liver biopsy materials were evaluated according to METAVIR system and cut-off for advanced fibrosis was determined as \( \geq F3 \). Results of ELF test, ARFI elastography and liver biopsy were evaluated and compared with each other by Spearman’s rank correlation test.

**RESULTS:** The means of tissue stiffness measured by ARFI elastography and ELF scores of all patients were 2.11 m/s and 10.36, respectively. Means of ARFI elastography results of mild to moderate fibrosis (Group 1) and advanced fibrosis (Group 2) were 1.11 m/s and 2.44 m/s. Means of ELF scores of Group 1 and Group 1 were 9.07 and 10.79. Both ELF scores and ARFI elastography results were found to be correlated with liver biopsy results \( p < 0.01 \).

**CONCLUSIONS:** Our results suggest that both ELF score and ARFI elastography can be used as non-invasive methods for the assessment of liver fibrosis in autoimmune hepatitis patients. Both methods are useful in discriminating mild and advanced fibrosis. Further studies are required with more patients especially to compare their diagnostic values – both alone and in combination – with liver biopsy for autoimmune hepatitis.
Background: The aim of this study is to investigate effect of quercetin (Q) on cerulein induced-acute pancreatitis (AP).

Methods: For this reason, rats were randomly divided into four groups: 1. Control group: Only saline (SF) in DMSO (6 times) was given (ip). 2. Cerulein (Cer) group: 4 times cerulein and twice SF-DMSO in 1 hour intervals were given. 3. Q pre-treated (Q+Cer) group: quercetin was given 1 hour before cerulein and the SF-DMSO was given 6 hours after Cer treatment. 4. Q post-treated (Cer+Q) group: The SF-DMSO was given 1 hour before cerulein and quercetin was given 6 hours after cerulein.

Results: The both pretreatment and posttreatment of quercetin treatment attenuated the severity of AP, as shown by the histology of the pancreas, and serum amylase and lipase activities; malondialdehyde (MDA), carbonyl content, myeloperoxidase (MPO) levels, proinflammatory cytokine levels such as, interleukin 6 (IL-6), and tumor necrosis factor-alpha (TNF-α) and oxidized glutathione/reduced glutathione (GSSG/GSH) ratio of pancreatic tissue MDA, carbonyl, MPO, TNF-α and IL-6 levels of cerulein group in pancreatic tissue were significantly higher than the control group (p<0.001, p<0.001, p<0.01 and p<0.05, respectively). MDA, MPO, TNF-α and IL-6 levels of quercetin pretreatment group (Q+Cer) were significantly decreased compared to Cer group (p<0.05, p<0.01, p<0.001, p<0.05, respectively). MDA, MPO, TNF-α and IL-6 levels of quercetin posttreatment groups (Cer+Q) were significantly lower than the Cer group (p<0.001, p<0.001, p<0.05, p<0.05, respectively). GSSG/GSH ratio of cerulein group was significantly higher than the control group (p<0.05), but GSSG/GSH ratio of Ce+Q group was significantly lower than Cer group (p<0.05).

Conclusions: This study shows that quercetin reduced the severity of cerulein- induced acute pancreatitis as reflected by changes in the parameters of pancreatic oxidant and antioxidant.
Liver and gastrointestinal diseases

Cod: 1044

SERUM HEPcidIN LEVELS PREDICT INTESTINAL IRON ABSORPTION IN IBd PATIENTS

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BACKGROUND: Circulating hepcidin is proposed to regulate iron absorption by modulating iron export by ferroportin at the basolateral membrane of the duodenal mucosal cells and/or uptake into the cells at the apical membrane by DMT1. To date, no data have shown a relationship between plasma hepcidin concentrations and iron absorption in IBD patients. In the present study, we used stored samples from a human iron absorption study to further test the hypothesis that plasma hepcidin may explain inter-individual variation in iron absorption in IBD patients.

METHODS: Serum ferritin (SF) and serum markers of inflammation [high-sensitivity C-reactive protein (hsCRP) and IL-6] were measured in stored samples from a human iron absorption study using commercially available immune-assays. Hepcidin-25 concentrations were determined in fasting samples from 71 adult subjects with IBD (31 UC, 40 CD) and 26 healthy controls. Hepcidin was measured by LC-MS/MS.

RESULTS: There was a positive correlation between hepcidin (mean: 2.3; range: 0.1–7.8nmol/L) and hsCRP (p<0.005), but not between hepcidin and serum ferritin (p>0.05). Whereas iron absorption was negatively correlated with serum ferritin only in patients with inactive disease (hsCRP<5md/dl; p< 0.001), a negative correlation was observed with serum hepcidin in both active and inactive disease (p= 0.006), independent of IBD phenotype. Multiple linear regression models showed that serum hepcidin in isolation significantly predicted the inter-individual variation in iron absorption.

CONCLUSIONS: Concentration of serum hepcidin, but not serum ferritin, was highly correlated with intestinal iron absorption in IBD patients.
THE BALANCE OF PRO- AND ANTI-INFLAMMATORY CYTOKINES IN PATIENTS WITH ACUTE PANCREATITIS

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BACKGROUND: Inflammatory mediators play a key role in acute pancreatitis and the resultant multiple organ dysfunction syndrome, which is the primary cause of death in this condition. Recent studies have confirmed the critical role played by inflammatory mediators such as pro- and anti-inflammatory cytokines. Our objective was to evaluate profile of cytokine synthesis in peripheral blood according to the severity of acute pancreatitis.

METHODS: 39 patients with acute pancreatitis have been investigated (18 severe, 21 mild). All of them were admitted to hospital within 24 h from the onset of pain. Severity of acute pancreatitis was assessed by APACHE II, Imrie and Ranson scores. Serum samples for cytokine measurements were collected on admission. Plasma levels of inflammatory cytokines (IL-1beta, IL-6, TNF-alpha, IL-10) determined using the enzyme-linked immunosorbent assay (ELISA).

RESULTS: Plasma levels of cytokines (IL-1beta, IL-6, TNF-alpha) were significantly higher in patients with severe acute pancreatitis (p=0,039, p=0,037, p=0,041). In contrast, IL-10 increased significantly in patients with mild acute pancreatitis (p=0,034).

CONCLUSIONS: In severe acute pancreatitis, there is an imbalance of the inflammatory cascade with hyperproduction of proinflammatory cytokines and reduced production of anti-inflammatory. A reduced functional reserve for the synthesis of IL-10 may be observed in patients with severe acute pancreatitis, which might lead to a worst prognosis. The level of IL-10 can serve as an additional diagnostic criterion for assessing the severity of acute pancreatitis.
**SERUM OSTEOPONTIN CONCENTRATIONS IN EARLY PHASE OF ACUTE PANCREATITIS**

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**BACKGROUND:** Acute pancreatitis (AP) is a self-limiting disease in most patients, but its severe form develops in up to 20-30% of cases. Early diagnosis of severe form of AP has been considered a key determinant of successful therapy and patients’ survival. Osteopontin (OPN), a glycosphosphoprotein belonging to SIBLING (Small Integrin-Binding Ligand, N-linked Glycoprotein) protein family, is an important serum mediator of inflammation released by various immunological cells (e.g. natural killers, macrophages, activated T cells). It is also an important mediator of bone mineralization and it was connected with extraosseous calcification, including the formation of pancreatic ducts’ stones. The aim of this study was to evaluate the diagnostic value of OPN in the early phase of AP.

**METHODS:** The study included 40 patients with AP (28 mild, 12 moderately severe and severe); 24 (60%) males and 16 (40%) females with mean age 47 years admitted to the Surgical Department. Serum OPN concentrations were measured by ELISA. Additionally, levels of interleukin 6 (IL-6), polymorphonuclear elastase (PMN-elastase), soluble receptor of TNF-α (sTNFRII), serum amyloid A (SAA), neopterin, procalcitonin and albumin were determined. In data analysis Mann-Whitney and Spearman tests were used as appropriate; p<0.05 was considered statistically significant.

**RESULTS:** Serum OPN concentrations was significantly higher in severe than in mild AP at the time of admission and the next 48 hours (median: 224.5 vs 140.9 ng/mL (p<0.05); 568.3 vs 129.7 respectively; p<0.01). Statistically significant correlation between OPN levels and concentrations of IL-6 (R=0.540), sTNFRII (R=0.481), SAA (R=0.795), neopterin (R=0.545), procalcitonin (R=0.594), PMN-elastase (R=0.515) and albumin (R=-0.631) were found after admission (p<0.05). After 48 hours, the positive correlations of OPN with IL-6 (R=0.866); sTNFRII (R=0.654); SAA (R=0.871) and PMN-elastase (R=0.655) increased in strength (p<0.01).

**CONCLUSIONS:** Osteopontin levels strongly correlate with early inflammatory mediators during the first 48 hours of AP. Monitoring of OPN levels could be potentially useful in prediction of severity of AP.
SEARCH FOR THE ACUTE-PHASE BIOMARKERS IN THE SERUM AND FAECES OF PATIENTS WITH IRRITABLE BOWEL SYNDROME

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BACKGROUND: Irritable bowel syndrome (IBS) is regarded as a chronic functional bowel disorder. Its pathophysiological mechanisms are unknown and there are no specific markers unique to this disorder. The hypothesis of a subclinical gradual development of local intestinal inflammation which generates IBS remains to be confirmed. The aim of the study was to search for a laboratory parameter or their panel among the acute-phase proteins in the blood and their association with faecal calprotectin as a specific parameter of local intestinal inflammation.

METHODS: The study was conducted in 38 patients with the diagnosis of IBS (Rome II criteria), including 18 patients with IBS with diarrhoea, 12 patients with IBS with constipation and 8 patients with IBS with alternating diarrhoea and constipation. The concentrations of the acute phase proteins alpha-1-antitrypsin, orosomucoid (ORS), transferrin and hs-CRP were measured in the blood (by immunuprecipitation) and of calprotectin in the stool (ELISA-kit, Immunodiagnostik AG).

RESULTS: In all patients with IBS, there was a significant relationship between the serum concentrations of positive acute-phase proteins (alpha-1-antitrypsin, ORS, hsCRP) at p<0.05. The concentrations of faecal calprotectin (µg/g dry faeces) demonstrated a widespread distribution of measurements (range: 0.16-19.83) and a significant correlation with ORS alone (r=0.40, p=0.014). Transferrin (a negative acute-phase protein) was not significantly correlated with any other of the study parameters (p>0.05). In IBS with diarrhoea: (1) the ORS concentrations were significantly elevated compared to IBS with constipation (p=0.016); (2) hs-CRP was significantly increased (p=0.007) in patients with ORS concentrations > 1.0 g/l.

CONCLUSIONS: ORS measured in the serum is the acute-phase protein with the highest specificity for IBS with diarrhoea. A considerable intersubject variability of faecal calprotectin concentrations in IBS with diarrhoea and a significant association between faecal calprotectin and serum ORS suggest the use of the two parameters as a panel to differentiate inflammation in patients with IBS.
THE SERUM ACTIVITY OF ALCOHOL DEHYDROGENASE (ADH) ISOENZYMES AND ALDEHYDE DEHYDROGENASE (ALDH) IN VIRAL HEPATITIS C

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BACKGROUND: Alcohol dehydrogenase (ADH) comprises a family of enzymes that has been grouped into several classes. Class I is the classical liver alcohol dehydrogenase but also detected in the gastrointestinal tract. Class II in humans is found only in the liver whereas class III is present in all examined tissues. Class IV of alcohol dehydrogenase exists mainly in the stomach. Several of aldehyde dehydrogenase (ALDH) isoenzymes are expressed also in the liver. In humans, particularly class I and II alcohol dehydrogenase is considered to be the hepato-specific. Their activity measured in the blood may indicate hepatocellular damage. We have investigated the activities of ADH isoenzymes and ALDH in the sera of patients with viral hepatitis C. These results were also compared with the activities of enzymes which are commonly accepted as liver cell injury markers.

METHODS: Serum samples were taken from 33 patients (20 males, 13 females 35-80 years) suffering from viral hepatitis C. Clinical diagnosis of illness was made on the basis of serological examinations. Class I and II ADH isoenzymes and ALDH were measured by fluorometric method using the specific substrates (4-metoxy-1-naphthaldehyde and 6-metoxy-2-naphtaldehyde respectively). The activity of class III was measured by photometric method with n-octanol and class IV with m-nitrobenzaldehyde as a substrate. Total ADH activity was estimated by the photometric method with p-nitrosodimethylaniline.

RESULTS: We have found that the serum median activity of class I alcohol dehydrogenase isoenzymes was more than twice elevated in viral hepatitis C (3.58±1.95 mU/l) in comparison to the control level (1.65±1.21 mU/l) (p<0.001). The total ADH activity was also significantly higher (23.5 %) among patients with hepatitis C (695±466 mU/l) than healthy ones (522±230 mU/l). The activities of other tested ADH isoenzymes and total ALDH were unchanged. Class I isoenzymes and total ADH activity correlated well with alanine and aspartate aminotransferases.

CONCLUSIONS: These results clearly demonstrate that especially the activity of class I alcohol dehydrogenase isoenzyme measured by a fluorimetric method can be a useful marker of liver cell damage during the course of viral hepatitis C.
GUANOSINE DEAMINASE ACTIVITY IN LIVER TRANSPLANT PATIENTS

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BACKGROUND: Guanosine Deaminase (GDA) catalyses the deamination of guanosine. Amongst human tissues, liver contains the highest guanosine deaminase activity and also detectable in brain and kidney. Increase activity of GDA in serum is considered to be a specific indication of hepatocellular damage and has been shown to have prognostic and diagnostic value in a variety of clinical situations.

METHODS: We estimated the GDA activity in serum by 18 hrs incubation of colorimetric Berthelot reaction method in healthy individuals (n= 20) and 17 patients of liver diseases undergone Liver transplantation. We observed GDA activity in the range of 80-300 mu/L for normals, there was no significant difference between sex and age.

RESULTS: The GDA activity was observed in pre liver transplant of 17 patients with different liver diseases Acute Liver Diseases with Portal hypertension (PHT) 527 ± 88 mu/L (n=5), Chronic Liver Diseases with PHT 779 ± 222 mu/L (n=6), Portal systemic encephalopathy (PSE) with hypothyroidism 410 mu/L (n=1), Non Alcoholic Fatty Liver Diseases 491±35 mu/L (n=3), Auto immune disorder 710 mu/L (n=1) and liver decompensate with hypertension 620 mu/L (n=1) respectively. we also estimated GDA post Liver Transplant (LTx) day 1,5 and 10 day. After liver transplantation the mean GDA activity was gradually decreased and reached to normal range on day 10th. Initial raised activity of GDA may be due to harvesting of organ, storage of organ, anhepatic phase, and reperfusion or may be surgical technique.

CONCLUSIONS: The GDA activity of patients with different diagnosis before LTx showed elevated levels and reached to normal activity after transplantation. So GDA activity in serum is a specific biomarker to estimate hepatic function in post LTx.

Keywords: GDA, LTx

Liver and gastrointestinal diseases

Cod: 1051

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Keywords: GDA, LTx
Non-invasive markers of liver fibrosis in patients with hepatitis C: Evaluation of ELF test and its correlation with acoustic radiation force impulse elastography (ARFI)

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Background: Evaluation of liver fibrosis in patients with hepatitis C is essential for establishing prognosis and indication of treatment. The standard is biopsy but has risks and limitations, so that non-invasive diagnostic tools such as serum biomarkers and imaging methods have been developed in recent years. ARFI is a radiological technique that provides the speed (m/s) at which an acoustic pulse crosses the liver parenchyma. This is a quantitative measure of tissue elasticity and correlates with the degree of fibrosis. ELF is a diagnostic algorithm of liver fibrosis that combines three serum direct markers: hyaluronic acid (HA), procollagen III amino terminal peptide (PIIINP) and tissue inhibitor of metalloproteinase 1 (TIMP-1). The result becomes a score without units that indicates the level of fibrosis. We aimed to evaluate the utility of ELF to discriminate degrees of liver fibrosis obtained by ARFI in a group of patients with hepatitis C and its correlation with it.

Methods: 105 patients with chronic hepatitis C were evaluated with ARFI (Acuson S2000, Siemens®) to determine the degree of liver fibrosis. According to results, they were classified into five groups: F0-F4. In serum of all the patients, ELF (ADVIA Centaur, Siemens®) was determined.

Results: The results obtained in ELF in the different groups were: F0 [9.017±1]; F1 [9.46±0.57]; F2 [10.35±1.18]; F3-F4 [11.32±1.23]. Significant differences between groups (p<0.001) were found. ELF was significantly correlated with ARFI, both the speed expressed in m/s (0.567, p <0.001) as with the degree of fibrosis obtained (0.928, p <0.001). When we analyzed HA, PIIINP and TIMP-1 individually, significant correlation was also observed with the speed (0.533, 0.392 and 0.616, p<0.001 respectively) and with the degree of fibrosis (0.525, 0.393 and 0.637, p <0.001 respectively). To evaluate the effectiveness of ELF in the diagnosis of liver fibrosis, we elaborated ROC curves considering pathological degrees of fibrosis ≥F2. The area under the curve was 0.856, p <0.001.

Conclusions: ELF test difference accurately and correlates with the degree of fibrosis determined by ARFI. It allows stratification and assessment of fibrosis in patients with hepatitis C so could be useful in hospitals where there isn’t this imaging technique.
CORRELATION BETWEEN F-CALPROTECTIN AND CRP AND ESR AS REACTANTS OF ACUTE PHASE

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BACKGROUND: Faecal calprotectin (F-calprotectin) is the best biomarker known so far in diagnosing and/or assessment of inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC) basically, as well as to exclude these conditions for the diagnosis of irritable bowel syndrome (IBS). The aim of this study is to determine the correlation between F-calprotectin and C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) in inflammatory and functional gastrointestinal disorders.

METHODS: 365 patients were included in this study, age range: 2-88.183 males, 182 females. 212 were diagnosed with IBD (115 CD, 81 UC and 16 overlap condition), 153 with IBS, the rest were patients with gastrointestinal disorders included in the differential diagnosis with IBD/IBS. Faecal samples were extracted using the Smart Pep extraction device (Roche Diagnostics) and were measured with the EliA Calprotectin immunoassay (Thermo Fisher Scientific) on ImmunoCAP 250. CRP was determined by a Siemens immunoturbidimetric assay in Advia analyzers. ESR was obtained from BD Sedi 15 analyzer. Data were processed in Excel 2011 tables and statistical tests were performed using SPSS 15.0 and G-Stat 2.0 statistical packages.

RESULTS: According to Shapiro-Wilk's test, a non parametric distribution was found on F-calprotectin, CRP and ESR data, p<0.0001 in all cases. Correlation analysis was statistically significant (p<0.0001) when F-calprotectin was correlated against CRP as well as ESR. Spearman’s Rho statistics were 0.3896 and 0.3451, respectively. Taking into consideration non IBD condition (IBS, principally) for analysing data, non correlation was found between F-calprotectin and CRP (Spearman’s Rho= 0.2012, p=0.0545) and a very week with ESR (Spearman’s Rho=0.1816, p<0.05). On IBD conditions, correlation was found between F-calprotectin and CRP (Spearman’s Rho= 0.3755, p<0.001) and ESR (Spearman’s Rho= 0.3500, p<0.001).

CONCLUSIONS: Significant but weak correlation in both cases was found between with F-calprotectin versus CRP and ESR. Therefore, traditional acute phase reactants not contribute to increase significantly the semiologic value of F-calprotectin on gastrointestinal diseases, although CRP seems to be more reliable for monitoring IBD conditions.
THE USE OF SEROLOGICAL ANTIBODIES IN THE DIAGNOSIS OF INFLAMMATORY BOWEL DISEASE


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BACKGROUND: Despite of all available diagnostic methods, approximately 5-15% of patients with IBD affecting the colon are unclassifiable. The aim of this study is to evaluate the role of antibodies against Saccachomyces Cerevisiae (ASCA), anti-neutrophil cytoplasmic antibodies (ANCA), autoantibodies against goblet cells, glikoproteins CUZD1 and GP2, Lactoferrin bind DNA antibody (LFS) in differential diagnosis of Crohn’s Disease and Ulcerative Colitis.

METHODS: 27 Crohn’s Disease patients (CD), 33 Ulcerative Colitis patients (CU) and 20 healthy matched subjects (CG) were enrolled in the study ANCA, ASCA, GAD, LFS, CUZD1 and GP2 antibodies were measured using indirect immunofluorescence method (Euroimmun, Germany). This study was funded by a National Science Center Grant (number: DEC-2011/01/N/NZ5/000054).

RESULTS: The presence of ASCA (CD=66.7%, CG=0%, p=0.0001), CUZD1 (CD=15.4%, CG=0%, p=0.0276), combination of panel ASCA+ ANCA- (CD=63.0%, CG=0%, p=0.0001), and panel ASCA+ ANCA- CUZD1 GP2 (CD=74.1%, CG=0%, p=0.0001) was significance higher in CD than in CG. What is more, the highest accuracy (85.1%), sensitivity (74.1%), specificity (85.1%) was observed for ASCA+ ANCA- CUZD1 GP2’s panel in diagnosis of CD. Also presence of ASCA (CD=66.7%, CU=0%, p=0.0001), panel ASCA+ ANCA- (CD=63.0%, CU=0%, p=0.0001), and panel ASCA+ ANCA- CUZD1 GP2 (CD=74.1%, CU=12.1%, p=0.0001) was significance higher in CD than in CU. The presence of ANCA (CU=45.5%, CG=10.0%, p=0.0046), combination of panel LFS GAD (CU=18.2%, CG=0%, p=0.0132), panel ASCA- ANCA+ (CU=45.5%, CG=10.0%, p=0.0046), and panel ASCA- ANCA+ LFS GAD (CU=48.5%, CG=10.0%, p=0.0024) were significance higher than in CG. The highest accuracy (64.2%), sensitivity (75.8%), specificity (90.0%) was observed for ANCA ASCA GAD LFS’s panel in diagnosis of CU. The presence of ANCA (CU=45.5%, CD=7.4%, p=0.0006), combination of panel ASCA- ANCA+ (CU=45.5%, CD=3.7%, p=0.0001) and panel ASCA- ANCA+ LFS GAD (CU=48.5%, CG=11.1%, p=0.0013) was significance higher in CU than in CD.

CONCLUSIONS: Differential diagnosis of IBD is most efficient using a combination of antibodies ASCA, ANCA. If LFS, GAD, CUZD1 and GP2 are used in addition the hit rate for the serological diagnosis of inflammatory bowel diseases can be increased significantly.
OLGO SYMPTOMATIC FORM OF COELIAC DISEASE IN CHILDREN, PATIENT CASE

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BACKGROUND: According to the Association of Serbia for coeliac disease, from this disease in Serbia suffers 35000 - 75000 people. Coeliac disease or gluten enteropathy is life long gluten intolerance, malabsorptive syndrome, caused by hypersensitivity of intestinal mucosa to alpha gliadin, gluten extract and proline risk proteins that is found in wheat, barley, rye and oats. It is familial, genetic (HLA B8, D3-7, DQW2), autoimmune mediated disease with chronic inflammation and atrophy of the mucosa of the upper small intestine. Clinical symptoms can be hidden.

Aim: Introduced oligo symptomatic form of coeliac disease. The patient was 12 years old girl. Her parents noticed that the child, in 2 - 3 last years, was complaining about chronic fatigue and weakness. Except frequent respiratory tract infections there were not any other problems. When her brother was 18 years old he got the diagnosis - classic form of coeliac disease. Girl was sent to the University Childrens Hospital Belgrade for diagnosis.

METHODS: General examination, abdominal and thyroid ultrasound and laboratory tests.

RESULTS: Thyroid was hyperechoic, diffused coarse echo structure, hypervascular, no clear nodal changes. She was tall 153 cm, (P 75 - 90), weight 36 kg(- 7.1 kg-16%). Laboratory: SE 1/5, CRP -2 mg/L, Hb 124 g/L, Er 4.9 x10⁴/12/L, MCV 84.5 fl, Leu. 5.6 x 10⁹/L, Tr 273 x 10⁹, Fe 10μmol/L, Feritin 15 ng/ml. Total and direct bilirubin, GGT, AST, ALT, Ca, P, alkaline phosphatase, total protein, albumin, cholesterol, urea and creatinine in normal range, triglycerides 0.42(0.8 - 2) mmol/L. FT4 15.84 pmol/L, TSH 1.81 IU/ml, anti-Tg 29.6IU/ml, anti TPO 0.6IU/ml. Antibody to tissue transglutaminase are: IgG positive, IgA >200(<20)U/ml. Anti-endomysial antibodies positive. IgA 1.38 G/l. HLA typing characteristic of celiac disease.

CONCLUSIONS: According to the results of examination Morbus Coeliacus was diagnosed in oligo symptomatic form. Life long gluten free diet was recommended. Although the diagnosis of coeliac disease biopsy of the small intestine is the gold standard, laboratory diagnosis can greatly assist in the diagnosis and monitoring the patient.
EVALUATION OF AUTOMATED FECAL CALPROTECTIN

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BACKGROUND: Calprotectin is a promising marker to distinguish inflammatory bowel disease from irritable bowel syndrome. This marker could help to reduce invasive tests such as colonoscopy.

METHODS: To investigate the performance of the automated fecal calprotectin analysis on the Alegria analyser (Orgentec/Siemens) and on the ImmunoCAP250 analyser (Thermo Fisher), 25 fecal samples of patients with either irritable bowel syndrome, ulcerative colitis or Crohn’s disease were collected. The performance was evaluated by the analytical accuracy, the analytical imprecision and the handling of the samples. The accuracy was determined by using the alternative method comparison protocol. The imprecision was determined by the CLSI EP10 protocol with calprotectin levels of approximately 60, 150 and 300 mg/kg feces. All samples were kept frozen until extraction. To establish equal preparation conditions, the fecal sample preparation kit of Roche Diagnostics was used for both methods. The only difference was the extraction buffers used. The extracted samples were kept frozen in aliquots until use. Both methods use a cut-off of 50 mg/kg feces.

RESULTS: Twenty-two samples showed equal results. Two samples of patients with irritable bowel syndrome were just above the cut-off with the Alegria (67 and 52 mg/kg), while the ImmunoCAP250 measured below (24 and 35 mg/kg). For one patient with ulcerative colitis in remission, the Alegria gave a high result (620 mg/kg), whereas the ImmunoCAP250 measured just above the cut-off (69 mg/kg). The Alegria showed a total CV of 23.5%, 16.5% and 12.6% at low, intermediate and high levels of fecal calprotectin, whereas the ImmunoCAP250 showed a total CV of 11.9%, 13.2% and 7.1%, respectively. To complete the EP10 protocol with the Alegria, three extra runs were needed because of rejected control measurements (2x) and an outlier (1x). With the ImmunoCAP250 the extracted aliquots appeared to decrease over time.

CONCLUSIONS: Both analysers perform well. The fecal calprotectin analysis on the ImmunoCAP250 appears to resemble the clinical situation better. The Alegria is more suitable for daily testing, whereas the ImmunoCAP250 is more designed for batchwise analysis with a higher amount of tests.
DIAGNOSTIC UTILITY OF FIB-4 FOR THE PREDICTION AND EXCLUSION OF LIVER FIBROSIS IN CHRONIC HBV MONOINFECTED PATIENTS


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BACKGROUND: Diagnosis of the fibrosis stage that provides information about the progression and response to the treatment is currently done by a liver biopsy which has lots of drawbacks and complications like pain, sampling error, bleeding, rupture and even death. Therefore, many studies have recently been led to non-invasive, simple and accurate methods and indexes as surrogates of liver biopsy to assess significant liver fibrosis and cirrhosis. We aimed to evaluate aspartate aminotransferase (AST), alanine transaminase (ALT), FIB-4, AST to Platelet Ratio Index (APRI), AST to ALT Ratio (AAR), Age Platelet Index (API), and platelet count in patients with chronic HBV carriers to figure out the diagnostic performances of these indexes in predicting significant liver fibrosis and cirrhosis.

METHODS: Data of the 205 percutaneous liver biopsy performed and serologically confirmed chronic HBV-monoinfected patients were included in this retrospective study. METAVIR classification was used to determine the stage of liver fibrosis.

RESULTS: Seventy five (36.6%) of patients were with significant liver fibrosis (F2-F4), while 43 (21.0%) were with cirrhosis (F4). AUROC of FIB-4, platelet count, API, APRI and AAR for significant liver fibrosis were 0.857, 0.801, 0.800, 0.755 and 0.703; for cirrhosis were 0.921, 0.847, 0.842, 0.774 and 0.755, respectively. In multivariate logistic regression analysis just FIB-4 had the statistical significance, OR(95%CI) was 6.964(3.485-13.917), p< 0.001 for SF, and was 9.064(4.118-19.952), p< 0.001 for cirrhosis.

CONCLUSIONS: Among these parameters, the most reliable one for the non-invasive prediction and exclusion of significant liver fibrosis and cirrhosis in chronic HBV monoinfected patients was FIB-4.
LABORATORY INDICATORS OF THE EFFICIENCY OF FIBRIN GLUE IN LAPAROSCOPIC SURGERY

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BACKGROUND: Fibrin glue (FG) is a blood-derived tissue adhesive that mimics the natural coagulation process. It consists of two basic components - fibrinogen and thrombin, where activation of fibrinogen and its transformation into fibrin under the action of thrombin is the third phase of blood coagulation. FG is used to promote wound healing, skin grafting, to provide hemostasis in microvascular surgery and parenchymal injury and to serve as a matrix for repair of bone defects. The aim of this study was to analyze laboratory indicators of the metabolic response to surgical trauma, when applying different means of hemostasis during laparoscopic cholecystectomy.

METHODS: The study included a total of 40 experimental pigs in which was performed laparoscopic cholecystectomy and intraoperative artificially damage of gallbladder boxes, which was repaired using FG in animals of experimental group (EG) or using standard means in animals of the control group (CG). FG was homemade, prepared from two components, of which the first was prepared from the cryoprecipitate with the addition of antifibrinolytic agents (aprotinin). The second component was a commercial bovine thrombin with calcium chloride. During 30 days of follow-up we have taken blood samples for following biochemical tests: general laboratory tests (glucose, bilirubin, cholesterol, triglycerides), enzyme markers of hepato-biliary damage (AST, ALT, AP, GGT), parameters of synthetic liver function (total protein and albumin), electrolytes (Na, K).

RESULTS: There is a statistically significant higher levels of AST and ALT in CG (p<0,05), while the level of GGT and AP is less in EG from the fifth to thirtieth day, but without statistical significance. The elevated values of AST and ALT in EG faster return to normal (day 5th in EG vs day 14th in CG). Postoperative concentration of Na+ does not show a statistical difference between groups, while the concentration of K+ in CG is high statistical decreased until the 14th day (3,725±0,386 in CG vs 5,025±1,237 in EG, p<0,0001).

CONCLUSIONS: Application of FG provides less parenchyma destruction and faster liver recovery and thus can be used as efficient hemostatic agent in laparoscopic surgery.
PHOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID EFFECT ON CYTOCHROME P450 2E1 (CYP2E1) ENZYME LEVELS IN EXPERIMENTALLY INDUCED NON ALCOHOLIC STEATOHEPATITIS (NASH)

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BACKGROUND: Non-Alcoholic Steatohepatitis (NASH) is a severe form of Non Alcoholic Fatty Liver Disease (NAFLD) spectrum, which progresses to the end stage liver disease. A common denominator in the pathogenesis of insulin resistance and nonalcoholic steatohepatitis is increased oxidative stress. Hepatic induction of the pro-oxidant enzyme Cytochrome P450 2E1 (CYP2E1) occurs in both NAFLD and type-2 diabetes. In this study, the comparative effect of pioglitazone, quercetin and hydroxy citric acid on liver CYP2E1 enzyme levels in experimentally induced NASH has been studied.

METHODS: The experimental protocol consists of 5 groups viz. Control (n = 6); NASH Induced (n=6); NASH + Pioglitazone (n=6); NASH + Quercetin (n=6); NASH + Hydroxy Citric Acid (n=6). CYP2E1 enzyme levels were detected in liver by immunoblot analysis in all the groups.

RESULTS: CYP2E1 catalytic activity was increased in experimentally induced NASH group compared to control group as evidenced by the Immunoblot analysis. It revealed low levels of CYP2E1 in the experimentally induced NASH, treated with pioglitazone, quercetin and hydroxy citric acid. Mild decrease in the levels of CYP2E1 level was in experimental NASH treated with pioglitazone compared to NASH group. Hydroxy citric acid also showed mild decrease in the levels of CYP2E1 level in experimental NASH treated with hydroxy citric acid. On contrary to the action of pioglitazone and hydroxy citric acid, quercetin showed an approximate 2-fold decrease in the level of CYP2E1 levels in experimental NASH treated with quercetin compared to NASH group.

CONCLUSIONS: By virtue of our findings, it could be concluded that that being a powerful antioxidant, quercetin offers absolute protection to liver against NASH by reducing the levels of CYP2E1 and thereby reducing CYP2E1 mediated oxidative stress, which is believed to be the one of the key factor in the pathogenesis of NASH. On the other hand, pioglitazone and quercetin exerted limited effect on the levels of CYP2E1. This study showed the therapeutic value of quercetin, pioglitazone and hydroxy citric acid.

Keywords: Pioglitazone, Quercetin, Hydroxy citric acid, Cytochrome, CYP2E1, Non-Alcoholic Fatty Liver Disease (NAFLD), Non-Alcoholic Steatohepatitis (NASH)
APOPTOSIS IN LIVER TOXICITY

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BACKGROUND: Apoptosis is a genetically programmed form of cell death that plays a major role in development and tissue homeostasis in addition to pathological processes. The CASP3 protein is a member of the cysteine-aspartic acid protease (caspase) family. The activation of caspases also is a marker for cellular damage in diseases. M30 can also be used as markers of apoptosis. M30 recognizes a neoepitope of cytokeratin 18 that becomes available after cleavage by caspase-3, before nick-end labeling identifies the cell as apoptotic. We explored the use of the active form of caspase-3 and M30 for the detection of apoptotic events.

METHODS: In the study, total of healthy 32 Wistar rats were divided into four groups. Control group (Group I, n=6), Group NSO (Group II, n=6), Group CCl4 (Group III, n=10), Group CCl4+NSO (Group IV, n=10). Group I and III, 0.4mL/kg olive oil (ip) injection was performed daily for 14 days once a day. Group II and IV NSO for 14 days at 0.4 ml/kg (ip) applied. 1 hour after administration 14th day carbon tetrachloride 1ml/kg (ip) applied at III and IV groups. 24 hours after the end of the experimental period blood samples were taken from the hearts of rats with ketamine anesthesia rats were sacrificed and liver tissue samples were fixed in formaldehyde. ALT, AST, LDH, bilirubin levels were measured spectrophotometrically, Caspase-3 activities were examined immunohistochemically and M30 levels measured as biochemical parameter with ELISA, were used to show apoptosis.

RESULTS: The data obtained from the result of study was assessed by Kruskal-Wallis variance analysis. ALT, AST, LDH and bilirubin levels statistically increased in Group III and IV. Accordingly the four groups taken together there was significant difference between groups (p<0.01). For M30 levels, accordingly the four groups taken together there wasn’t significant difference between groups. CASP3 activities increased in Group CCl4 but decreased in Group CCl4+NSO.

CONCLUSIONS: In our study CCl4 hepatotoxicity shown to increase ALT, AST, and LDH levels. Bilirubin level is rising in hepatocellular injury and CASP3 levels decreased in Group CCl4+NSO. So it is show that NSO protects hepatocytes from damage.

Key words: Caspase-3, M30, hepatotoxicity
BASIC LABORATORY TESTS AT PATIENTS WITH DECOMPENSATE HEPATIC CIRRHOSIS

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BACKGROUND: Patients with decompensate hepatic cirrhosis have complications which include ascites and hemorrhage from esophageal varices. Treatment of this complications needs transfusion of Erythrocytes concentrates and plasma products (albumin, fresh frozen plasma).

AIMS: To show the biochemical and haemostatic disorders in patients with advanced disease stadium which were treated in daily transfusion hospital and in the Internal and Infectious diseases ward units in Clinical Hospital Stip.

METHODS: From the eastern part of Macedonia in the past 10 years 162 patients were hospitalized and treated with blood and plasma components. At the same time routine test of liver function were made of them: serum albumin, AST, ALT, serum bilirubin, protrombin time and hematological such as platelet count. They were made on Dimension clinical chemistry system by reagents Siemens.

RESULTS: In all these patients there was a elevated level of ALT in range of 70-215 IE/L (normal range to 65 IE/L), AST elevated in range of 115-673 IE/L (normal range to 37 IE/L), total bilirubin level 25-47 µmol/L (normal range to 17,1 µmol/L), protrombin time (Quick) strongly extended from 25,5-27,5 sec. (INR 2,16-3,5), platelet count in range of 90-115x10⁹ (normal range 150-400x10⁹), normochromic normocytic anemia, leucopenia and albumin level in range 21-28g/L (normal range 34-50 g/L).

CONCLUSIONS: Routine tests of liver function may be quite normal in patients with cirrhosis, but with the advancement of the disease, disorders become more manifested. Especially in patients with cirrhosis caused of hepatitis B and C infections (HBsAg +, anti-HCV +), where level of ALT and AST activity are higher than in patients with alcoholic cirrhosis, and reversal of the ALT/AST ratio in those with hepatitis C infection.
EVALUATION OF SERUM FERRITIN LEVELS AS A MARKER OF ACUTE PHASE RESPONSE IN PATIENTS WITH ULCERATIVE COLITIS

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BACKGROUND: Ferritin is a 24-subunit evolutionarily conserved protein. Ferritin levels represent a good index of iron stores in the body. Generally clinical usefulness of ferritin measurement is in the early diagnosis and management of iron deficiency and iron-overload states. However it can be abnormally elevated as an acute phase reactant in a wide range of diseases including malignancy, infection, liver disease, hematopoietic disease, rheumatoid arthritis, fever, inflammation, and chronic iron-overload syndromes. In the present study we aimed to evaluate serum ferritin levels in patients with ulcerative colitis.

METHODS: We retrospectively analyzed colonoscopy and serum ferritin levels before colonoscopy. Records of outpatients with ulcerative colitis attending Katip Celebi University Atatürk Training and Research Hospital gastroenterology outpatient clinic during the period 2009 - 2012 were evaluated. The present study included a total of 232 ulcerative colitis patients of whom 148 were in active phase and 84 were in remission.

RESULTS: The mean age of patients with active ulcerative colitis was 45.28±15.94 and mean of patients in remission was 46.86±13.4 (P=0.421). Mean ferritin levels were 41.86±44.76 in patients in remission and 60.98±100.4 in patients in active phase. Mean ferritin levels in patients with active ulcerative colitis was significantly higher than in patients in remission (p=0.048). When the upper reference limit for serum ferritin was defined as 200ng/mL for women and 300ng/ml for men only 7(4.7%) of 148 patients in active phase were above upper reference limit. None of the ferritin results of the patients in remission was above reference limit. The number of patients above reference limit was not significantly different between two groups.

CONCLUSIONS: Serum ferritin levels may be elevated as it is an acute phase reactant in many cases characterized by inflammation. We evaluated serum ferritin levels in ulcerative colitis as an inflammatory disease. Since in a very limited number of patients serum ferritin concentrations exceeded upper reference limit it is not appropriate to use ferritin as an acute phase reactant in ulcerative colitis.
REDUCTION OF FALSE POSITIVE BILIRUBIN RESULTS ON THE CLINITEK NOVUS V1.1 ANALYZER

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BACKGROUND: Bilirubin, a waste product of red blood cell breakdown is excreted in feces, although small amounts may be found in urine. Trace or higher amounts may suggest underlying pathology and indicate the need for further clinical investigation. Unfortunately, even the most established methods can generate a high percentage of false positive bilirubin results, often due to interfering substances and/or abnormal urine color. The CLINITEK Novus® v1.1 urine chemistry analyzer employs a unique algorithm to decrease false positive bilirubin results. Utilizing camera-based detection technology, this algorithm allows for the improved detection of true bilirubin, as confirmed by Ictotest® reflex testing. We assessed false positive CLINITEK Novus analyzer bilirubin results versus incidence on the CLINITEK Atlas® analyzer, and correlation of CLINITEK Novus analyzer v1.1 software bilirubin results with results on the CLINITEK Atlas system.

METHODS: An internal Siemens investigation was conducted to assess false positive incidence, 270 clinical and contributed samples were run on three CLINITEK Novus analyzers and one CLINITEK Atlas analyzer with each analyzer using one reagent lot. Ictotest® tablet reflex testing was employed to identify false positive results. Ictotest-negative results (35) were eliminated from further statistical analysis to assess true bilirubin concordance. Results were compiled in frequency tables. Positive Agreement, Negative Agreement, Exact Agreement, and Within One Level Agreement were calculated.

RESULTS: Compared to the CLINITEK Atlas analyzer, the CLINITEK Novus v1.1 analyzer reduced the bilirubin false positive rate by 7% with 0.4% false negatives. CLINITEK Novus analyzer v1.1 software test results correlated well with CLINITEK Atlas analyzer bilirubin results with 95% exact block agreement.

CONCLUSIONS: The CLINITEK Novus v1.1 analyzer showed improved detection of bilirubin. True bilirubin results were concordant with those generated on a reference analyzer. Greater accuracy (fewer false positives with 0.4% false negatives) in routine bilirubin urinalysis with the CLINITEK Novus v1.1 analyzer serves to decrease the time and costs associated with potentially unnecessary follow-up bilirubin testing.