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Gastrointestinal diseases

Cod: T160

## EFFECT OF NEUTRAL SPHINGOMYELINASE INHIBITION ON ER STRESS AND APOPTOSIS IN LIVER ISCHEMIA–REPERFUSION INJURY

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BACKGROUND: Previous studies have revealed the activation of neutral sphingomyelinase (N-SMase)/ceramide pathway in hepatic tissue following warm liver ischemia reperfusion (IR) injury. Excessive ceramide accumulation is known to potentiate apoptotic stimuli and a link between apoptosis and endoplasmic reticulum (ER) stress has been established in hepatic IR injury. Thus, this study determined the role of selective N-SMase inhibition on ER stress and apoptotic markers in a rat model of liver IR injury.
METHODS: Selective N-SMase inhibitor was administered via intraperitoneal injections. Liver IR injury was created by clamping blood vessels supplying the median and left lateral hepatic lobes for 60 min, followed by 60 min reperfusion. Levels of sphingmyelin and ceramide in liver tissue were determined by an optimized multiple reaction monitoring (MRM) method using ultra fast-liquid chromatography (UFLC) coupled with tandem mass spectrometry (MS/MS). Electron microscopic immunohistochemistry and quantitative PCR analysis was performed to evaluate ER stress markers while TUNEL analysis and immunoassay was used to examine apoptosis.
RESULTS: Spingomyelin levels were significantly increased in all IR groups compared to controls. Treatment with a specific N-SMase inhibitor significantly decreased all measured ceramides in IR injury. A significant increase was observed in ER stress markers C/EBP-homologous protein (CHOP) and 78 kDa glucose-regulated protein (GRP78) in IR injury, which was not significantly altered by N-SMase inhibition. Inhibition of N-SMase caused a significant reduction in phospho-NF-kB levels, hepatic TUNEL staining, cytosolic cytochrome c and caspase-3, -8 and -9 activities which were significantly increased in IR injury.
CONCLUSIONS: Data herein confirm the role of ceramide in increased apoptotic cell death and highlight the protective effect of N-SMase inhibition in down-regulation of apoptotic stimuli responses occurring in hepatic IR injury.

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Gastrointestinal diseases

Cod: T161

**FIT PERFORMANCE IN SURVEILLANCE AND SYMPTOMATIC PATIENTS. THE SAME TEST BUT DIFFERENT INTERPRETATION.**

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BACKGROUND
The Faecal Immunochemical Test (FIT) for haemoglobin is widely used in screening of colorectal cancer (CRC) but presently there are just few studies regarding its usefulness in symptomatic patients and in surveillance. The different characteristics of populations: screening, surveillance and symptomatic, recommends evaluate the performance of FIT in this different groups of patients. This study evaluates FIT performance in two group of patients. A cohort of patients in surveillance for familiar history of CRC or history of adenomas or CRC with regular colonoscopies and a cohort of symptomatic patients with an indication of diagnostic or therapeutic colonoscopy.

METHODS
We have evaluated 967 faecal samples from 484 patients. 433 samples corresponding to patients in surveillance, mean age was 61 years (32-88 years) and the 58.2% of samples corresponding to women and 534 samples corresponding to symptomatic patients, mean age was 61 years (21-94 years) and the 58.8% of samples corresponding to women. The faecal haemoglobin concentration was assessed using SENTiFIT® 270 analyser using FOB Gold® latex reagent and SENTiFIT® pierceTube (Sentinel Diagnostics, Italy – Sysmex, Spain).

RESULTS
No significant statistical differences were observed in faecal haemoglobin concentration between the two groups but a clear trend with higher haemoglobin concentration was observed in the symptomatic group when compared with the surveillance group. Using the recommended manufacturer cut-off (17 μg haemoglobin / g faeces) the positivity rate in symptomatic and surveillance group was 18.5% and 14.5%, respectively. The positive predictive value for advanced neoplasia (advanced adenoma and CRC) in symptomatic and surveillance group was 39.4% and 28.6%, respectively and the negative predictive value for advanced neoplasia was 90.3% and 88.7%, respectively; decreaing to 24.6% and 23.2%, respectively when the whole colorectal pathology was included.

CONCLUSIONS
The characteristics of the different population makes the FIT interpretation different, in general there are not significant differences in faecal haemoglobin concentration, but as expected the positivity rate, the positive predictive values as well as the distribution of faecal haemoglobin are different between the two studied populations, however it is important to mention that the negative predictive value is similar in both groups, remarking mainly the FIT utility as rule out test.

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Gastrointestinal diseases

Cod: T162

**USEFULNESS OF DIRECT SERUM BIOMARKERS FOR LIVER FIBROSIS (ELF® SCORE) IN PATIENTS WITH HEPATITIS C**

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INTRODUCTION
Liver cirrhosis is a leading cause of death in developed countries and is mostly originated from an initial liver fibrosis (LF). Current LF assessment includes imaging techniques, elastography, biopsy and the measurement of indirect serum biomarkers. Transient elastography (FibroScan®) is based on liver elasticity, and shows an excellent correlation with liver biopsy.

Direct serum biomarkers for LF include hialuronic acid, tissue inhibitor of metalloproteinase (TIMP1) and the N-terminal propeptide of type III collagen (PIIINP), whose combination in the ELF® algorithm (Enhanced Liver Fibrosis) is able to classify up to 65% of patients without the need of biopsy. The combination of ELF® and FibroScan® raises both positive and negative predictive values in the diagnosis of LF and cirrhosis.

AIM
We aimed to assess the concordance between the ELF® score, and the conventional screening methods using FibroScan® and biopsy, in patients with Hepatitis C (HCV).

METHODS
Between March and September 2014, up to 28 LF patients with HCV under follow-up by FibroScan® were recruited. Direct serum biomarkers were measured (direct chemiluminescence, Advia Centaur CP, Siemens), and the ELF® score was calculated by:
ELF® score=2,494+0,846 ln[AH]+0,735 ln[PIIINP]+0,391 ln[TIMP1]
Patients were classified depending on liver biopsy and FibroScan® results: ≤7,5 kPa (mild), 7.5-14 kPa (moderate), >14 kPa (severe).
The ELF® algorithm yields a qualitative interpretation of the degree of fibrosis (mild, moderate, severe). Frequencies of the qualitative variables were compared using the Chi square and exact Fischer's tests. Statistical significance was set at 0.05.

RESULTS
Concordance between ELF® and FibroScan was 83% in patients with FibroScan >7.5kPa, while it reached 100% in patients with severe fibrosis (FibroScan >14kPa) ($p<0.001$).
Among patients with discordant results (FibroScan <7.5 kPa, and positive biopsy), ELF® score was concordant with biopsy in 86% of cases ($p<0.001$). The global overestimation in the degree of fibrosis by ELF® was 3.6%.

CONCLUSIONS
ELF® score may be a very useful diagnostic tool for the screening of moderate-severe LF in patients with high risk, and for the optimization of biopsy indication in more than 80% of patients with HCV.

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Gastrointestinal diseases

Cod: T163

**MORPHOLOGICAL LIVER STRUCTURE (LIGHT AND MICROSCOPIC OBSERVATION) AND TGF-BETA1, IFN-GAMMA AND TNF-ALPHA CONCENTRATION IN SERUM DURING DISTURBED LYMPH FLOW FROM THE LIVER IN RATS.**

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Background
Hepatic lymph flow - example of regulating the lymphatic microenvironment on the cellular and functional relationship with the nervous and endocrine system, is still interesting and unexplained the object of studies. Cytokines play a major role in the development of hepatic fibrosis, the wound-healing response of the liver to chronic injury. Cytokines in hepatic fibrogenesis may be pro- or antifibrogenic.
Methods
Male albino Wistar rats (162 animals) weighing between 200 and 230 grams were selected for the experiment. The animals were kept in stable condition and were fed a standard diet with no fluid restriction. The animals were divided into 9 experimental groups. In each experimental group were 3 subgroups: Group K was the control group – sham operated rats. Group B consisted of rats with disturbed lymph flow from a liver. Group 0 – animals not subjected to any surgery, but were placed under pentobarbital anaesthesia. Each subgroup consisted of 6 rats. Lymphostasis was induced in group B rats by tying two silk ligatures passed behind the hepatic trunk just distal to the juncture of the hepatic ducts
The rats were sacrificed for experiment in 1, 3, 7, 14, 21, 28, 35, 56 and 103 day after operation to obtain blood samples directly from the heart. Serum concentration of Transforming growth factor-beta1 - TGF-beta1 (ELISA, R&D Systems), Interferon-gamma -IFN-gamma and Tumor Necrosis Factor-alpha - TNF-alpha (ELISA, BENDER Med System) was measured. Differences between groups were assessed by ANOVA and RIR Tukey test, and were considered significant when $p \leq 0.05$.
Liver tissue was studied for light and electron microscopy.
Results
Our results showed appearance of collagenous fibres in heatocytes. We observed an increase of TGF-beta1 and TNF-alpha and decrease of IFN-gamma concentration in serum.
Conclusions
Disturbed lymph flow from a liver contributes to liver fibrogenesis and probably to hepatocirrhosis. During disturbed lymph flow from the liver TGF-beta1 and TNF-alpha plays probably an antifibrogenic role in liver fibrogenesis. IFN-gamma is profibrogenic cytokine in this process.

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Gastrointestinal diseases

Cod: T164

## THE WAY TO TOTAL AUTOMATION OF CALPROTECTIN MEASUREMENT IN FAECES WITH BÜHLMANN FCAL® TURBO

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Calprotectin, an important marker to detect and monitor inflammatory processes in the gastrointestinal tract, has been well established during the last few years. As a consequence the amount of Calprotectin determinations in the laboratory has increased rapidly. Calprotectin has to be extracted from faecal specimens. Due to the nature and inherent inhomogeneity of this specimen the work load in the laboratory increased over time and reduction of hands-on-time is needed by simplification and automation.
In our laboratory we evaluated the user-friendly CALEX® Cap Extraction device to speed up and streamline the cumbersome and labour-intensive extraction procedure. As a second step we established and validated the new turbidimetric test fCAL turbo on our Roche Cobas® c501 analyzer. The fCAL turbo allows random access sample handling with a time to result of 12 min and with a measuring range of 20 – 8’000 µg/g. The time saving on hands-on-time was 70% and the total turn-around-time decreased to 20 minutes. The combination of the CALEX® Cap extraction device together with the new turbidimetric assay fCAL turbo (PETIA; particle enhanced turbidimetric immuno assay) is a paradigm-shift to total automation of Calprotectin quantification in faeces. Moreover the CALEX® Cap, with its unique stability of 3 days at room temperature, would allow extraction by the patient at home increasing efficacy even more.

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Gastrointestinal diseases

Cod: T165

## COMPARISON SERUM HE4 LEVELS WITH COLON CANCER STAGES

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### Introduction
Human Epididymis Protein 4 (HE4) is a secretory protein originally identified in the distal human epididymis. Serum levels of HE4 have been investigated in patients with ovarian and endometrial cancer. Only a few have described the role of HE4 in different stages of colorectal cancer.

### Material and methods
89 healthy individuals (37 men and 52 women) were recruited. 10 diagnosed colorectal cancer stages I/II (4 men and 6 women), 22 diagnosed colorectal cancer stage III (13 men and 9 women) and 39 diagnosed colorectal cancer stage IV (23 men and 16 women) patients were selected. No other affections were found in the whole groups.
Ca19.9 and CEA were performed using ADVIA Centaur XP System (Siemens®). HE4 was analyzed in the Cobas Elecsys E411 (Roche®).
Statistical analysis was performed with SPSS. Marker comparison test between case and control groups were made using U of Mann-Whitney. Student's t-test was made for Age comparison and Chi-squared for gender comparison.

### Results
HE4 median was 70,2 (44,5)pmol/L in stage I/II, 85,2 (36,4)pmol/L in stage III and 87,0 (43,2)pmol/L in stage IV. HE4 median in control group was 50.1 (19)pmol/L. Ca19,9 median was 9,00 (12,0)U/ml stage I/II, 13,0 (18,8)U/ml stage III and 33,0 (165)U/ml stage IV with 9,23 (11,0)U/ml in control group. CEA median was 1,10 (2,48)ng/ml stage I/II, 1,90 (2,80)ng/ml stage III and 4,45 (52,9)ng/ml stage IV with 0,76 (0,93)ng/ml in control group. We found an increasing statistically significant differences in HE4 adjusting by age ($p<0.001$) considering those stages ($p=0.035$ stage I/II; $p<0.001$ stage III and $p<0.001$ stage IV), ca19.9 was only significant in stage IV ($p=0.017$); CEA was significant in stages III and IV ($p=0.006$ and $p<0.001$ respectively). ROC analysis showed and increasing in the prediction value in HE4 with the different stages (AUC 0.85, 0.89 and 0.93 respectively). HE4 sensitivity and specificity we calculated was 70% and 89,9% in stage I/II cut-off point of 67.1pmol/L; 68.2% and 93.3% in stage III cut-off point of 72.2pmol/L, 92.3% and 87,6% in stage IV cut-off point of 64.9pmol/L.

### Conclusion
HE4 showed an excellent prediction value in colorectal cancer increasing its prediction considering the different stages of the disease. The sensitivity and specificity found was excellent, especially in stage IV. HE4 could be a potential marker in colorectal cancer. More studies are needed in order to elucidate the role of HE4 in colorectal cancer.

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Gastrointestinal diseases

Cod: T166

## LIVER FIBROSIS AND IL28B GENOTYPE IN PATIENTS WITH CHRONIC HEPATITIS C

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Background: Interleukin (IL)28B polymorphisms are strongly associated with spontaneous clearance of hepatitis C virus (HCV) infection and response to therapy, but whether IL28B genotype affects liver fibrosis severity is unclear. Our aim was to study the relationship between IL28B genotype and markers of liver fibrosis.

Methods: We studied a population of 107 chronic hepatitis C patients. Liver fibrosis was assessed by shear wave velocity (Vc) determined by Arfi technique. In all patients was determined IL28B polymorphism, biomarkers of fibrosis hyaluronic acid (HA), procollagen III amino terminal peptide (PIIINP-1), tissue inhibitor of metalloproteinase 1 (TIMP-1), platelets, AST and ALT. Moreover algorithms for estimating the degree of fibrosis ELF, Apri, Forns, Fibrotest, Fibrometer, Fib-4, Fibro-Q and Hepascore were calculated. An abdominal ultrasound was also performed to evaluate liver disease.

Results: Patients had a mean age of 47 years and 60% were men. Prevalent viral genotype was 1 (77%) and distribution of IL28B genotypes was as follows: 24.6% CC, 62.3% CT, and 13.1% TT. Patients with CT genotype had significantly lower concentrations of PIIINP ($p=0.008$), TIMP-1 ($p=0.037$) and higher platelet count ($p=0.003$) compared to those with CC and TT. Apri, Forns, Fibrotest, Fibrometer, Fib-4 and Fibro-Q also were significantly lower in CT patients ($p<0.05$). AST, ALT, ELF and Hepascore were also lower in CT patients but differences were not significant. Furthermore CT patients have lower degree of liver fibrosis according to a lower Vc ($p=0.049$) and lower presence of ultrasound findings of liver disease ($p=0.007$).

Several authors have suggested that CC genotipe is associated with a state of enhanced immunity that can promote viral clearance but alternately can increase the liver damage. Other authors have noted that T allele is more prevalent in patients who develop cirrhosis. According to this and based on our results, the combination of the two alleles (CT) could be the most favorable option in relation to the severity of fibrosis.

Conclusions: The genotype CT of IL28B is associated with a lower degree of liver fibrosis determined by biomarkers, imaging techniques (ultrasound) and elastography techniques (Arfi).

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Gastrointestinal diseases

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## TOTAL SIALIC ACID (TSA) IN CHRONIC HEPATITIS B AND C

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BACKGROUND: The synthesis, catabolism and attaching of sialic acid to the oligosaccharide chains of proteins and lipids take place in the liver. So, the estimation of serum sialic acid level may be helpful as a biomarker of liver diseases. The aim of this study was to evaluate the effect of the activity of chronic hepatitis B and C on the serum concentration of total sialic acid (TSA).
METHODS: Serum samples were obtained from 90 patients (62 males and 28 females) (mean age: 33.17 years; range: 19-71) suffering from liver diseases divided into 2 subgroups: 50 with hepatitis B (35 males and 15 females) (mean age: 33.2 years; range: 19-71) and 40 with hepatitis C (27 males and 13 females) (mean age: 41.7 years; range: 19-67). The control group consisted of 30 healthy subjects (16 males and 14 females) (mean age: 29.5 years; range: 21-54). The TSA concentration in the serum was measured according to the enzymatic method (BioAssay System, Hayward, USA) using the colorimetric procedure.
RESULTS: The mean TSA concentration in patients with chronic hepatitis C (mean±SD: 1.72±0.33 mmoL/L) was significantly higher (P=0.025) than the mean level in the control group (1.50±0.32 mmoL/L), while in patients with chronic hepatitis B (1.32±0.30 mmoL/L) was significantly lower (P=0.047) than that in the controls. The TSA concentration was significantly higher in patients with chronic hepatitis C than that in patients with chronic hepatitis B (P=0.005). Taking into consideration the grade of portal/periportal activity, the grade of lobular activity and the stage of fibrosis, there were no significant differences in TSA concentrations between both patients with chronic hepatitis B and C (ANOVA rank Kruskal-Wallis test: P>0.005 for all comparisons).
CONCLUSIONS: We conclude that chronic hepatitis affect the total serum sialic acid concentrations. Moreover, the concentration of TSA may be useful marker to differentiating chronic hepatitis B from C, but is not useful for evaluation of the progression of chronic hepatitis.

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Gastrointestinal diseases

Cod: T168

## GASTROPANEL: THE "SEROLOGIC BIOPSY" IN DYSPEPTIC PATIENTS

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Background. Gastric cancer is the second cause of cancer-related death in the world that develops from pre-cancerous lesions and Helicobacter Pylori infection is clearly correlate with its carcinogenesis. Although the gold-standard for diagnosis is the gastrointestinal endoscopy, many studies have included the use of serum markers to study the gastric functinality for the diagnosis and the follow-up of patients with pre-cancerous lesions. These findings include serological panel called Gastropanel® that measures: pepsinogen I, pesinogen II, gastrin-17 and anti-Helicobacter Pylori IgG antibodies serum levels.
We developed a prospective and observational study to optimize the approach to dyspeptic patients through the use of serological test Gastropanel®.
Methods. We enrolled 80 patients with dyspeptic disorders and sera were analysed for Gastropanel®, anti-parietal cells antibodies and anti-intrinsic factor antibodies for discriminating autoimmune gastritis.
Results. Our results show the presence of anti-Helicobacter Pylori antibodies in 25% of patients and it is associated with alterations of gastrin-17 and pepsinogens levels. These findings suggest the presence of different gastritis and correlate with the biopsy results.
Conclusion. Gastropanel® is confirmed to be a quick, reliable, non invasive and useful tests for the menagment of dyspeptic disorders. After other studies our goal is the development of diagnostic algorithm that use Gastropanel® as a screening and help the clinicians to discriminate patients with high risk of cancer and need biopsy.

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Gastrointestinal diseases

Cod: T169

## MARKERS IN PATIENTS WITH LIVER CIRRHOSIS

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Cirrhosis is a late stage of fibrosis of the liver caused by many forms of liver disease and condition, such as hepatitis and chronic alcoholism. The liver carries out several necessary functions, including detoxifying harmful substances in your body, cleaning your blood and making vital nutrients. Serum albumin, retinol binding protein (RbP) and cholinesterase (ChE) levels reflect the synthetic function of the liver.
Serum albumin, RbP and ChE levels were measured and compared between patients with liver cirrhosis and healthy persons (blood donors). The study included 31 patients (22 males and 9 females) with cirrhosis and 28 healthy persons (19 males and 9 females). Determination of albumin concentration and ChE activity was provided by spectrophotometry on Architect c8000, Abbott, USA and RbP by immunonephelometry on BNII, Siemens, Germany.
The values of serum albumin were statistically significantly lower for patients with cirrhosis compared to controls (28.45±o.49 g/L versus 42.79±5.87 g/L, $t=14.4$, $p<0.01$). The same conclusion was obtained for RbP (0.014±0.004 g/L for patients, versus 0.038±0.005 g/L for controls, $t=23.3$, $p<0.01$) and ChE activity (3054.2±825.3 U/L for patients versus 9481.9±1921.6 U/L for controls, $t=18.1$, $p<0.01$).
Serum albumin, RbP levels and ChE activity may be considered as the important diagnostic and monitoring markers in patients with liver cirrhosis.

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Gastrointestinal diseases

Cod: T170

**HELICOBACTER PYLORI AND PROBIOTICS**

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BACKGROUND: The main cause of chronic gastritis and peptic ulcer disease and a risk factor for gastric malignancy is infection with Helicobacter Pylori. Eradication of Helicobacter Pylori is successful in more than 90% of the cases by applying antibiotic therapy. The antibiotics can cause side effects and resistance to antibiotics. Therefore the application of probiotics can be a successfull alternative that would reduce or prevent the colonization of Helicobacter Pylori.
METHOD: Chemiluminescence (two-step method) of immunochemical analyzer Immulite 2000.
RESULTS: In our study we included 2 groups of patients where we had proven the presence of Helicobacter Pylori infection. The first group of patients was treated with standard antibiotic therapy, while the second group of patients despite the antibiotic therapy also received probiotic. The percentage of eradication of Helicobacter Pylori infection in the probiotic group is 84% which is higher compared to the group with standard antibiotic therapy which is 72%. Also, the side effects of antibiotic therapy in the probiotic group were 21% in comparison with 51% in the standard antibiotic therapy.
CONCLUSION: The use of probiotics has a positive effect on the Helicobacter Pylori infection. The eradication is faster and more efficient while also the risk of developing diseases associated with the gastrointestinal tract is reduced.

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Gastrointestinal diseases

Cod: T171

## THE PROFILE OF TRANSFERRIN ISOFORMS IN CHRONIC HEPATITIS

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BACKGROUND: The occurrence of alterations in proteins glycosylation in liver diseases is well known, also during the course of chronic hepatitis. The aim of this study was to evaluate the effect of chronic hepatitis on the serum profile of transferrin isoforms.
METHODS: Serum samples were obtained from 160 patients (95 males and 65 females) with chronic hepatitis (mean age: 39.7 years; range: 19-73) and 30 healthy subjects (16 males and 14 females) (mean age: 29.5 years; range: 21-54). The samples were analyzed by capillary electrophoresis on MINICAP electrophoretic system (Sebia, France). The normal serum transferrin isoforms were separated into five major fractions according to their sialylation level.
RESULTS: In patients with chronic hepatitis tetrasialotransferrin level (mean±SD; 80.89±4.45%) was increased (P=0.002) and pentasialotransferrin (14.90±4.53%) was decreased (P=0.009) in comparison to the controls (76.84±5.62%; 18.61±6.03%; respectively). ANOVA rank Kruskal-Wallis test showed that only trisialotransferrin level was significantly different according to the grade of portal/periportal activity (P=0.009), the grade of lobular activity (P=0.004) and the stage of fibrosis (P=0.022). Post-hoc analysis revealed that trisialotransferrin was higher in grade 4 of portal/periportal activity (severe piecemeal necrosis) (5.24±2.49%) than that in grade 3 (moderate piecemeal necrosis) (3.01±1.17%) (P=0.024). The trisialotransferrin was higher in grade 4 of lobular activity (severe piecemeal necrosis) (7.23±2.08%) than that in grade 2 (mild piecemeal necrosis) (3.09±1.27%) (P=0.014). In stage 4 of fibrosis (definite cirrhosis) (6.75±1.96%) the trisialotransferrin was higher than that in stage 2 (periportal or portal-portal septa, but intact architecture) (3.25±1.01%) and stage 1 (enlarged, fibrotic portal tracts) (3.40±1.38%) (P=0.014 and P=0.016; respectively). There were no differences in tetrasialotransferrin and pentasialotransferrin according to the advancement of hepatitis activity and the stage of fibrosis (P>0.05 for all comparisons).
CONCLUSIONS: We conclude that chronic hepatitis affect the serum profile of transferrin isoforms, but only trisialotransferrin level might be useful in determining progression of chronic hepatitis and the stage of fibrosis.

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Gastrointestinal diseases

Cod: T172

**SERUM LEVEL OF INTERLEUKIN-6 (IL-6) AND N-TERMINAL PROPEPTIDE OF PROCOLLAGEN TYPE I (PINP) IN LIVER DISEASES**

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BACKGROUND: The aim of this study was to evaluate the effect of liver diseases of different etiologies and clinical severity of liver disease on the serum level of N-terminal propeptide of procollagen type I and interleukin-6.
METHODS: Serum samples were obtained from 82 patients with liver diseases: 31 with alcoholic cirrhosis (AC), 28 - non-alcoholic cirrhosis (NAC) and 23 - toxic hepatitis (HT). The severity of liver cirrhosis was evaluated according to the Child-Pugh score (22 patients in class A, 18 in class B, and 19 in class C). Control group consisted of 30 healthy volunteers. IL-6 and PINP were determined according to the electrochemiluminescence immunoassay (Cobas e411, Roche Diagnostics).
RESULTS: The mean serum IL-6 concentration was significantly higher in alcoholic cirrhosis (mean±SD: 21.52±15.01), non-alcoholic cirrhosis (20.07±32.12) and toxic hepatitis (15.14±17.18) when compared to the control group (C) (1.67±0.42) (Mann-Whitney U test: $P<0.001$ for all comparisons). The mean serum PINP concentration was significantly higher in patients with AC (104.32±54.50) in comparison with the control group (54.70±19.83, $P<0.001$). The mean values of IL-6 and PINP significantly differ between liver diseases (ANOVA rank Kurskal-Wallis test: $P=0.020$, $P<0.001$, respectively). Post-hoc analysis revealed that the serum levels of IL-6 and PINP were significantly higher in patients with AC than that in NAC ($P<0.001$, $P=0.022$, respectively). IL-6 and PINP concentrations appeared to vary according to the severity of liver damage ($P<0.001$ for both). The concentrations of IL-6 and PINP were significantly higher in class C (31.88±21.51; 132.73±65.63, respectively) compared to the class A (6.12±9.00; 57.32±28.85, respectively) ($P<0.001$ for both). There were also significant differences in the IL-6 concentration between Child-Pugh class B (27.88±24.45) and class A ($P<0.001$).
CONCLUSIONS: In conclusion, serum concentration of interleukin-6 and N-terminal propeptide of procollagen type I show the alterations in liver diseases. The changes of IL-6 and PINP in liver diseases reflect the severity of liver disease.

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Gastrointestinal diseases

Cod: T173

## DETERMINATION OF COMPLEMENT FACTORS IN EARLY PHASE OF ACUTE PANCREATITIS

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Background: Complement activation occurs in patients with acute pancreatitis (AP) and may contribute to the development of organ failure. In acute pancreatitis, local as well as, systemic organ complications are mediated by activation of various inflammatory cascades. AP is a self-limiting disease in most patients, but its severe form develops in up 20-30% of cases. Assessment of the severity of AP has been considered a key determinant of successful therapy and patients survival. The aim of this study was to determine the level of complement activation in early phase of acute pancreatitis.
Methods: We enrolled 49 patients with AP (23 mild and 16 with moderate and severe form of AP), mean age 59,83±18,67 years, admitted to Surgery Department. The severity of AP was classified according to revised Atlanta Classification 2012. Serum complement C3 and C4 concentrations were measured by ELISA (Assaypro LLC). In addition, we determined concentrations of CRP using the nephelometric method (Dade Behring Nephelometer BNII, Siemens), Neutrophil gelatinase-associated lipocalin (NGAL) and Angiopoietin-2 (ANG-2) were measured using ELISA (R&D System).
Results: Serum C3 and C4 concentrations were significantly lower in severe and moderate than in mild AP at the time of admission and on 2nd and 3rd day after admission (median: 0,85 vs 1,21; 0,72 vs 1,01; 0,62 vs 0,92, $p<0,05$) and (median: 0,39 vs 0,57; 0,38 vs 0,62; 0,34 vs 0,58 respectively, $p<0,05$). Statistically significant negative correlation between C3 levels and concentrations of ANG-2 ($R=-0,42$; $R=-0,41$) and NGAL ($R=-0,28$; $R=-0,31$, respectively) were found on 2nd and 3rd day after admission, ($p<0,05$).
Conclusions: The magnitude of complement activation depends on the severity of the disease. Monitoring of complement factors C3 and C4 could be potentially useful in prediction of severity of AP. C3 correlate with ANG-2 and can be considered for assessment of endothelial dysfunction and pancreas injury during early phase of AP.

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Gastrointestinal diseases

Cod: T174

## DEVELOPMENT OF A NEW BIOCHIP ASSAY FOR THE QUANTITATIVE DETECTION OF ANTIBODIES TO HELICOBACTER PYLORI

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**Background.** Helicobacter pylori (H. pylori) infection is the most common cause of atrophic gastritis and is associated with a higher risk of developing gastric carcinoma. Effective monitoring is a challenge as the majority of patients positive for H. pylori are asymptomatic. Therefore, the development of a fast, non-invasive screening tool that provides an accurate profile on the condition of the patient's stomach mucosa is essential.
Enzyme-linked immunosorbent assays (ELISA) have been developed for the individual detection of H. pylori antibodies, Gastrin-17 (G17), Pepsinogen I and II (PGI & PGII) in plasma (Biohit Oyj, Helsinki, Finland). In a collaborative study, the application of Biochip Array Technology (BAT) to the multiplex determination of PGI, PGII and G17 from a single sample was reported. With the aim to provide a comprehensive profile of the stomach mucosa using BAT, the present collaborative study reports the development of a new biochip assay for the quantitative detection of H. pylori antibodies.
**Methods.** H. pylori antigen was immobilised on the biochip surface defining a discrete test site. An indirect sandwich chemiluminescent immunoassay, applied to the biochip analyser Evidence Investigator, was used for detection of H. pylori antibodies. A correlation study was carried out on a cohort of 338 plasma samples between this biochip assay and the ELISA (Biohit Oyj, Helsinki, Finland).
**Results.** The assay presented a functional sensitivity value of 9IU/mL (assay range 0-1100IU/mL). Both inter and intra assay precision showed CV <12%. Assessment of 338 plasma samples using the biochip assay and the ELISA, indicated a percentage agreement of 95% and the regression analysis showed an R value of 0.85.
**Conclusions.** Results show optimal analytical performance of the newly developed biochip assay for the quantitative determination of H. pylori antibodies from plasma samples. Thus offering a new non-invasive screening tool for atrophic gastritis and those at risk of gastric cancer. When used in combination with the previously reported three-plex Gastropanel (PGI, PGII and G17) will provide a comprehensive profile on the stomach mucosa. Further application to the automated random access analyser Evidence Evolution will ensure reliable high throughput analysis.

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Gastrointestinal diseases

Cod: T175

## QUANTITATIVE FAECAL HAEMOGLOBIN TEST (FIT) IN CHILDREN WITH GASTROENTEROLOGICAL DIAGNOSIS

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**Introduction**
Quantitative analysis of haemoglobin (Hb) level in stool samples (FIT) using automated analysers is routinely used in colorectal screening in adults, but never been described in children diagnostics. The aim of this study is analysis of faecal Hb level in children samples in years 2010 - 2016 tested by FIT faecal blood detection OC-Sensor (Eiken).
**Methods**
From the total number of FIT samples analysed (n=2488) in children (age 0,1-18 years, mean 7.65, males 1232/females 1256) samples with Hb level > 100 ng/ml, i.e. 20 μg/g of stool (n=236; 9.5%) were selected. By retrospective analyse of the possible causes of FIT positivity we identified (n=170; 72%) cases with confirmed gastroenterological diagnoses. Six groups according to the most widely represented gastroenterological diagnoses were created – IBD (n=66, 38.8% of the total number of gastroenterological causes), acute gastroenteritis (n=17; 10%), gastroesophageal reflux (n=17; 10%), cow's milk protein intolerance (CMPI) (n=27; 15.8%) and anal fissures (n=17; 10%); the sixth group consists of those gastroenterological causes not elsewhere classified, marked as others (n=26; 15,3%).
**Results**
The median values of faecal Hb levels were in the group of IBD 102.3 μg/g (range 20.4 - 858.6 μg/g); in acute gastroenteritis 220.8 μg/g (range 23.6 - 558.8 μg/g); in CMPI 110.4 μg/g (range 22.2 - 517.6 μg/g); in gastroesophageal reflux 48.6 μg/g (range 21.6 - 291.2 μg/g); in anal fissures 370.8 μg/g (range 29.8 - 1756 μg/g); in the group of others 48.9 μg/g (range 20.4 - 396.4 μg/g).
**Conclusions**
Quantitative analysis of haemoglobin (Hb) level in stool samples we described in children most widely represented gastroenterological diagnoses. Evaluation of cut-off criteria values for future diagnostic procedures will follow in future studies.
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Gastrointestinal diseases

Cod: T176

**EVALUATION OF A TURBIDIMETRIC IMMUNOASSAY FOR FECAL CALPROTECTIN OPTIMIZED FOR DEDICATED CHEMISTRY ANALYZERS**

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BACKGROUND
Fecal calprotectin assays are widely used in diagnosis and monitoring of inflammatory bowel disease (IBD) in patients with suspected IBD. Turbidimetric assays for analysis of fecal calprotectin can significantly reduce turnaround time. Many laboratories may be reluctant to run fecal samples on their large chemistry analyzers. The aim of this study was to evaluate fecal calprotectin PETIA testing on smaller chemistry analyzers that could be dedicated for fecal samples.
METHODS
The BÜHLMANN fCAL® turbo assay was validated on two table top chemistry analyzers, Mindray BS-200E and cobas® c111.
RESULTS
The assay was linear in the range between 20 and 1,900 µg/g, with a limit of quantification around 20 µg/g. The total coefficient of variation was <7% in the range between 50 and 1,300 µg/g. No antigen excess hook effect was observed up to 18,000 µg/g on the Mindray BS-200E. The BÜHLMANN fCAL® turbo assay showed a high correlation with the BÜHLMANN fCAL® ELISA.
CONCLUSIONS
The BÜHLMANN fCAL® turbo reagent in combination with Mindray BS-200E or cobas® c111 chemistry analyzers can provide rapid test results without exposing large routine chemistry analyzers to stool samples.

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Gastrointestinal diseases

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**LACTOSE TOLERANCE TEST: 60 MINUTES AND BEYOND?**

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INTRODUCTION: Lactose malabsorption (LM) assumes a variable prevalence worldwide according to ethnicity and geographic location, with high prevalence in Mediterranean populations. The lactose tolerance test (LTT) is an affordable test requested worldwide for its diagnosis, due to its low cost and simplicity. The purposes of our study was to consider a shortening the PTL, through suppression of the determination of blood glucose at 120 min, without changing the final outcome, and to evaluate the benefits from such a reduction.

METHODS: An observational study of consecutive patients who underwent LTT for suspected LM during 2015 was conducted. Patients had received 50 g of lactose dissolved in 400 mL of water, following a fasting period of 8 hours, at least. Blood samples were collected at 30, 60, and 120 minutes after lactose ingestion. Glucose levels at the different time points were compared between the tolerant and the intolerant individuals through the Student's t test for comparison for independent mean values. Differences between the classic and an eventually shortened LTT were analyzed using the McNemar test. A value of p 0.005 < was considered statistically significant.

RESULTS: The study included 199 patients (75,4% females) with a mean age of 46.18± 16 years. 63,3% of the tests were requested by the gastroenterology department. LTT was normal for 97 patients (48.7%) and abnormal for 102 (51.3%). There were no statistically significant differences in baseline glucose level between patients with normal and abnormal LTT results ($p = 0.198$). Conversely, such differences were found at subsequent time points ($p < 0.001$). Suppressing the measurement of glucose at 120 min didn't alter the LTT kappa index of 0.99 (95% CI) ($p < 0.001$).

CONCLUSION: The 120 min LTT time point does not add new information to previously obtained levels and its suppression does not alter the final results. A shortened LTT would reduce patient's waiting time as it would save financial, material and human resources.

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Gastrointestinal diseases

Cod: T178

## DO NOT FORGET ABOUT PRE-ANALYTICS IN FAECAL CALPROTECTIN TESTING

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Introduction
Calprotectin is an acute-phase protein used in the assessment of inflammatory bowel diseases. Prior to measurement, extraction of calprotectin from faeces using a method-dependent extraction buffer is necessary. We aimed to study the influence of extraction device, buffer and different storage conditions as pre-analytical variables in faecal calprotectin determinations.
Methods
The imprecision and accuracy of two commercially available extraction devices (Roche Smart Prep and the novel Inova QUANTA Flash®) for faecal calprotectin extraction were evaluated by extracting three patient samples with low, medium and high calprotectin concentrations at ten different days and by parallel extraction of 17 samples with known positive calprotectin concentration.
Secondly, we compared the stability of faecal calprotectin at three different storage conditions (refrigerator (2-8°C), freeze-thaw (3x) and freezer (-20°C)) for six extraction buffers (EliA Calprotectin 2, Diasorin Calprotectin, Inova QUANTA Flash®, Bühlmann fCAL Turbo, Euroimmun and Orgentec calprotectin extraction buffers) using the Roche Smart Prep extraction device. Calprotectin measurement was performed with the respective immunoassay.
Results
The extraction imprecision was found to be higher for the Inova QUANTA Flash® device (range: 16.8-22.8%) compared to the Roche Smart Prep device (6.0-13.8%), thereby contributing more to the overall imprecision of faecal calprotectin determinations. Regarding accuracy, 17,6% of the samples statistically and clinically significant lower faecal calprotectin concentrations were found for the Inova QUANTA Flash® device, which were independent of the sample consistency.
Significant degradation of extracted faecal calprotectin was found when stored in the refrigerator (up to 7 days) for EliA (up to 17.0%), Inova (up to 22.8%), Euroimmun (up to 37.1%) and Orgentec (up to 41.7%) extractions. At -20°C, faecal calprotectin was found to be stable for all the assays, except for the Euroimmun (up to 40.5% after 2 freeze-thaw cycles) and Orgentec assay (up to 23.7% after 2 freeze-thaw cycles and up to 23.7% after 2 months in the freezer), for which degradation was found.
Conclusion
As pre-analytics can have a substantial influence on the calprotectin measurement, depending on the used method and storage conditions, clinical laboratories need to be aware of these variables and validate the whole procedure, including extraction device and extraction buffer stability.

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Gastrointestinal diseases

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## QUANTIFICATION OF SERUM HEPCIDIN IN INFLAMMATORY BOWEL DISEASES

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Background. Inflammatory bowel disease (IBD) includes various intestinal pathologies, the most common of which are ulcerative colitis (UC) and Crohn's disease (CD). Anemia is one of the most common symptoms of inflammatory bowel disease. Hepcidin is a major mediator of anemia and plays a central role in the homeostasis of the iron metabolism. It regulates the absorption of iron and release of the element from the cells by blocking the action of ferroportin, which is the only known iron exporter from macrophages, hepatocytes and duodenal enterocytes.
Methods. 21 Crohn's disease and 27 ulcerative colitis patients were enrolled in our study. They were evaluated for serum iron and hepcidin levels. Interleukin-6 (IL-6) and C-reactive protein (CRP) were measured as inflammation markers. Hepcidin and IL-6 were measured by ELISA methods. AAS was used for quantification of serum Fe. CRP was quantified by nephelometric method. The results form IBD patients were compared to age and gender matched healthy controls. Statistical analysis of established results was performed using Pearson's correlation and Student's paired t-test.
Results. We found statistically significant elevated serum iron results in CD and UC patients (42.5 vs. 41.8 vs. 19.9 μmol/l; $P<0.001$). Hepcidin concentrations were increased in CD and UC cases compared to controls (53.6 vs. 58.9 vs.24.8 μg/l; $P<0.001$). IL-6 and CRP levels were elevated in both Crohn's disease and ulcerative colitis vs. normal values in healthy controls (IL-6: 10.9 vs. 11.4 vs.4.95 pg/ml; CRP: 10.1 vs. 11.9 vs. 0.9 mg/l; $P<0.005$).
Conclusions. Evaluation of serum hepcidin in IBD patients may become a key element in the diagnosis and treatment of anemia in the near future. The study of hepcidin has potential role in diagnostic algorithms for differentiation between iron deficiency anemia (IDA) and anemia of chronic diseases (ACD) and the combination of IDA/ACD.

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Gastrointestinal diseases

Cod: T180

# F-CALPROTECTIN HELPS REDUCING COSTS AND RISKS FOR PATIENTS IN THE DIAGNOSIS OF COLONIC PATHOLOGY: A PROSPECTIVE REAL-LIFE STUDY FROM THE CLINICAL HOSPITAL OF ZARAGOZA

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BACKGROUND
Colonoscopy represents the gold standard in case of suspected colonic pathology. However, availability is limited and it brings about avoidable risks for the patients and important costs. F-Calprotectin is a fecal marker of intestine inflammation capable to differentiate between organic and functional intestinal disorder and could therefore potentially, be used as a pre-endoscopic tool to identify patients that could potentially avoid a colonoscopy. The purpose of this observational prospective study was to quantify in a Secondary Care (SC) setting in Zaragoza (Spain) the burden of colonoscopy in 87 consecutive unselected patients referred to colonoscopy either by Primary Care (PC) or SC doctors (gastroenterologists, or other specialists), and to evaluate the economic impact associated with the pre-endoscopic usage of F-Calprotectin.
METHODS
Diagnosis was established by colonoscopic investigation, and F-Calprotectin levels were evaluated by means of EliA Calprotectin 2 at both the recommended 50 mcg/g, and at the optimal 234.5 mcg/g cut-offs (sensitivity=69%, specificity=87%). Real-life data (including diagnosis, costs, colonoscopy-related complications, and resource utilization) were prospectively collected. Three scenarios (S) were compared: the actual situation (S1) and two simulations (S2=considering patients sent to colonoscopy by PC doctors only, S3=all patients) in which F-Calprotectin is used to select which patients require further investigations.
RESULTS
In S1, 71 patients (81.6%) were declared healthy after colonoscopy. Using the optimal cut-off, the actual total cost for visits and procedures was 75875€ (average cost/patient 872€); 4.6% of the patients experienced colonoscopy-related complications, which accounted for 7.9% of the total costs. At the optimal threshold, F-Calprotectin reduces the average cost/patient by 250€ (29%) in S2, and by 427€ (49.0%) in S3. At the recommended threshold (50 mcg/g), 24 (27.6%) and 40 (46.0%) colonoscopies are avoided in S2 and S3, while at the optimal threshold 34 (39.1%) and 62 (71.3%); respectively, 3 (3.5%) and 5 (5.8%) sick patients are missed by using F-Calprotectin alone.
CONCLUSIONS
Results show that the usage of F-Calprotectin as pre-endoscopic diagnostic tool is associated with less colonoscopies, less complications, and important cost savings ascribable to reduced resource utilization.

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Gastrointestinal diseases

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**THE RELATIONSHIP BETWEEN SELECTED CIRCULATING MICROPARTICLES IN THE PLASMA AND THE ACTIVITY OF CROHN'S DISEASE**

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Background:
Circulating micropaticles may play a role in crohn's disease pathomechanism . The aim of the study was to evaluate the association of selected microparticles derived from platelet, neutrophils and lymphocytes and clinical activity of Crohn's disease expressed by CDAI (Chrohn's disease activity index).
Method:
49 patients with Crohn's disease (CD) were enrolled in the study. The CD patients were divided into active phase of disease (CDAI >150, 33 patients) and inactive phase of disease(CDAI <150, 16 patients) according to CDAI index (assessing amount of defecation per day and systemic disorders). The number of micropaticles was calculated by flow cytometry. Annexin V was used as a marker of all microparticles, CD 41+ as a marker of palettes, CD 66b as marker of neutriphils and CD62l as a marker of limfocytes.
Results:
Total Annexin (+) microparticles were statistically higher in patients with active CD then inactive CD (5688/µl (2877/µl - 29006/µl) vs 1624/µl (256/µl - 6167/µl), p< 0.0001). Also, it was a significantly increased number of Annexin (+) CD41+ microparticles in patients with active than in inactive phase of disease (2362/µl (217/µl - 15919/µl vs 563/µl (99/µl-4258/µl), p=0.0002). CD patients with active disease displayed elevated Annexin (+) CD62L+ microparticles, compared with those in remission (120/µl (34/µl-1732/µl) vs 44,2/µl (11/µl-189/µl), p=0.00119). What is more, Annexin (+) CD66b + microparticles were significantly elevated in active phase of disease compared to inactive phase (231/µl (55/µl - 3375/µl) vs 86/µl (25/µl – 403/µl), p=0.00192). It was also observed correlation between total microparticles, all subgroup micropaticles and CDAI index (range for the assessed groups :R= 0,45 – 0,51, p<0.001)
Conclusions:
It was observed that the total amount of microparticles and platelet-derived microparticles, lymphocytic and neutrophilic are elevated in patients with active CD. Also noted a correlation between the index and the total CDAI microparticles and chosen microparticles subgrups. This may indicate a share of these indicators in the pathogenesis of exacerbations in patients with CD.

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Gastrointestinal diseases

Cod: T182

**POLYMORPHISM ASP299GLY TLR4 GENE AND THE PRESENCE OF THE SELECTED ANTIBODIES IN PATIENTS WITH PARENTERAL COMPLICATIONS OF INFLAMMATORY BOWEL DISEASE.**

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Background:
The aim of this study was to assess the association between the occurrence of parenteral complications in patients with Crohn's disease (CD) and ulcerative colitis (UC) and polymorphism of TLR-4 Asp299Gly (rs4986790) and antibodies to Saccachomyces cerevisiae (ASCA), anti-neutrophil cytoplasmic antibodies (ANCA), autoantibodies to globelet cells (GAD), glycoprotein CUZD1 and GP2, DNA-bound lactoferrin (LFS).
Method:
120 patients with IBD (80 with CD and 40 with UC) were included in the study. Based on data from medical history, they were divided into those patients presenting with parenteral complications and those in whom such complications were not observed. A total of 32 (40.0%) of CD patients and in 21 (52.5%) of patients with UC parenteral complications were observed. ANCA,ASCA,GAD,LFS,CUZD1 and GP2 antibodies were detected using indirect immunofluorescence. Genomic DNA was extracted from peripheral blood lymphocytes . Allele-specific polymerase chain reactions (PCRs) were used to detect TLR-4 Asp299Gly polymorphisms in DNA. This study was funded by a National Science Center Grant (number:DEC-2011/01/N/NZ5/000054).
Results:
Based on the results obtained in patients with parenteral complications statistically significant differences in the distribution of the AA genotype as compared to the AG genotype ($p = 0.042$). There were no significant differences in the distribution of allele frequencies in the study group. Threre were observed in CD patients with parenteral complications lower incidence of ASCA antibody or panel of ANCA- ASCA + antibodies($P<0.0001$). In contrast, it was shown that CD patients with parenteral complications, significantly a higher incidence CUZD1 antibodies($P=0.042$) or panel CUZD1 + GP2 + ($P= 0.028$). In CD patients assessing of all antibodies panel (ASCA + panel of antibodies and ANCA- and / or CUZD1 + and / or GP2 +) significance disappeared. In patients with UC, there was no association of the test results from parenteral complications.
Conclusion:
The study show a relationship between parenteral complications of Crohn's disease and incidenceof TLR-4 polimorfism, CUZD1 antibody and panel GP2 and / or CUZD1 presence and a lower incidence ASCA antibodies.

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Gastrointestinal diseases

Cod: T183

## ISOLATION & CHARACTERIZATION OF A COMPOUND FROM THE LEAVES OF AGERATUM CONYZOIDES LINN RESPONSIBLE FOR ANTI-GASTRIC ULCER ACTIVITY IN ALBINO RATS

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Background: Ageratum conyzoides Linn (A. conyzoides L.) is a medicinal plant under the family asteraceae. Studies have claimed that A. conyzoides L. has antibacterial and anti-tumor activity as well as wound healing effect. We have noted anti gastric ulcer activity of the leaves of A. conyzoides L. in indomethacin induced gastric ulcer in albino rats. The objective of this study was to isolate and characterize the active ingredient from the leaves of A. conyzoides L. responsible for anti-gastric ulcer activity in albino rats.

Methods: A compound was isolated from the leaves of A. conyzoides L. by solvent extractions, acid hydrolysis, chromatography followed by crystallization. To note anti gastric ulcer activity of the isolated compound, Sprague Dawley male rats were divided into four groups: Control, Indomethacin (10 mg/kg) treated, Indomethacin (10 mg/kg) along with isolated compound (50 mg/kg), Indomethacin (10 mg/kg) along with Ranitidine (50 mg/kg). After the completion of experiment, rats were sacrificed. Stomachs were taken out and examined for ulcers for evaluation of ulcer index. Ulcer Index was evaluated by the method of Szelenyi and Thiemer. Characterization of the compound was done by infrared spectroscopy, mass spectroscopy and nuclear magnetic resonance studies.

Results: Compound isolated from Ageratum conyzoides L significantly reduced ulcer index in rats induced by indomethacin. Ulcer index was 30.8 ± 1.32 in indomethacin treated group vs 10.6 ± 1.19 in group which received both indomethacin and isolated compound. Results were comparable to that of ranitidine (ulcer index 8.8 ± 1.01), a standard anti-ulcer drug. Analysis of spectral data revealed that the isolated compound was 1,3,5-trihydroxy-7-methylanthracene-9,10-dione.

Conclusions: 1,3,5-trihydroxy-7-methylanthracene-9,10-dione present in the leaves of A. conyzoides L. was responsible for anti-gastric ulcer activity in indomethacin induced gastric ulcer in albino rats.

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Gastrointestinal diseases

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## AN NEW ENZYMATIC CYCLING METHOD FOR DETERMINATION OF TOTAL BILE ACIDS IN SERUM

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Objective
Determination of total bile acids in serum is a sensitive indicator of liver function and can be used in diagnosis and prognosis of liver disease e. g. viral hepatitis, intrahepatic cholestasis in pregnancy, liver cancer or toxic hepatic damage. Available assays show limitations regarding the detection on various bile acids with identical sensitivity. The objective of this study was to compare the new DiaSys total bile acids reagent to other tests in the market, in particular with regard to the recognition of diagnostically relevant primary and secondary bile acids and their conjugates.

Methodology
The kinetic DiaSys total bile acids test is based on a cycling reaction which reversibly changes bile acids to an oxidized form, catalyzed by a specific 3α-hydroxysteroid-dehydrogenase. In this reaction Thio-NAD is reduced to Thio-NADH. In a second coupled reaction step oxidized bile acids are reduced by the same enzyme with subsequent reduction of NADH to NAD. The rate of formation of Thio-NADH is determined by measuring specific change of absorbance at 405/415 nm which is directly proportional to the concentration of bile acids in sample material.

Results
The new DiaSys reagent is a liquid stable, 2-component reagent with a sample reagent ratio of 4:270:90 µL (on Hitachi 917). The measuring wavelength of the bile acids test is 405/415 nm. Linearity is given up to180 µmol/L. The reagent shows good correlation for sera in comparison studies with a commercially available method: y = 1.019x – 0.026 µmol/L, r = 0.9997; n= 37 samples. The recovery of diagnostically relevant bile acids is in the range of +/-11.6%.

Conclusion
All results clearly show that the new DiaSys total bile acids test meets the requirements of a sensitive marker to analyze all stages of impaired liver function. The performance of the test is comparable to competitor products, diagnostically relevant primary and secondary bile acids and their conjugates are reliably recovered.

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Gastrointestinal diseases

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# DEVELOPMENT OF THE ENZYMATIC METHOD FOR MALTOSE PERMEABILITY TEST OF GASTRIC MUCOSA USING THE ORAL GLUCOSE TOLERANCE SAMPLES

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## BACKGROUND

Sucrose of two monosaccharide is not absorbed in the normal stomach; however, it is absorbed directly from the part of gastric mucosa injury and penetrates in the blood. Maltose permeability has been suggested as a simple and non-invasive marker of gastric mucosal damage. We here report on the sensitive enzymatic assay for maltose of glucose tolerance tests (GTT) as substitution of sucrose using glucosidase-glucose oxidase-peroxidase (POD) method.

## METHODS

Principle of assay
Endogenous glucose is changed to glucose-6-phosphate by hexokinase (HK), then, HK activity is prevented by EDTA. Maltose changed to glucose by glucosidase, and glucose measured by mutarotase, glucose oxidase, POD using TPM-PS color reagent, and it was detected by 660nm.

Reagents
1) Reagent-1; 0.1 mol/L pH 8.0 Tris-HCl buffer, HK, ATP, Mg, POD
2) Reagent2; 0.5 mol/L pH 5.5 MES buffer#EDTA-2Na, α- glucosidase, GOD,TPM-PS
3) Standard solution#500 mg/dL glucose, 100 μmol/L maltose
4) TRELAN-G75 (oligosaccharide, 250ml)
5) Sample: Blood was taken two times, before load with TRELAN-G75 and 60 minutes after.

Assay method
For the maltose measurements, take 3 μL serum to 177μL R1, after five minutes adding R2 80μL and leave it at 37°Cfor five minutes. It is measured by the 2 points end assay with wavelength 660nm using the HITACHI 7070 (Hitachi, Tokyo, Japan).

## RESULTS

Linearity of maltose measurement was up to 200μmol/L. The intra-assay imprecision of the method was determined by 20 times of 200μmol/L maltose analysis, and its CV was 0.4%. The inter-assay CV of 200μmol/L maltose solution was 2.3%. The recovery rate was 93% using 100μmol/L maltose solution added to serum.
The maltose amount of fasting drawing blood was 30~75 μmol /L in 22 to 64 year-old healthy subject (n=10). Six healthy subjects admitted the rise of maltose amount after GTT, and the increase rate was 124%. One subject, 24 years old female, admitted a rise of 228% and was diagnosed as gastritis by an endoscope check.

## CONCLUSIONS

Since the maltose permeability test has the potential to be a screening test of gastric disorders, rapid determination of maltose with high sensitivity applicable to a large number of serum samples is warranted. It became clear that measurement of maltose could detect the gastric mucosa disorder by using this analysis method.

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Gastrointestinal diseases

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**PERFORMANCE EVALUATION OF LUMIPULSE G1200 FOR THE MEASUREMENT OF PIVKA-II IN HEPATOCELLULAR CARCINOMA**

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Background
Hepatocellular carcinoma (HCC) is the most frequent primary hepatic tumor and is the eight most common type of solid cancer in incidence worldwide. The early diagnosis of HCC is challenging since early curative resection or liver transplantation give the patients the best chance of long-term survival?. Thus, HCC screening is recommended in patients at high risk (i.e. patients with cirrhosis), and isbased on ultrasonography that remains the gold standard. Serum Alpha-foetoprotein (AFP) is no longer used in this context since this biomarker is elevated only in 10 to 20 % of early stage HCC patients. Many studies from Asian countries suggest that Protein Induced by Vitamin K Absence or Antagonist-II (PIVKA-II), an autologous mitogen produced by liver cancer cells, could be a promising alternative to AFP. The objective of this study was to evaluate the analytical performances of Lumipulse G1200 for the detection of PIVKA-II.
Methods
PIVKAII levels were measured on a Lumipulse G1200 (Fujirebio-Europe, Belgium), using a chemiluminescent enzyme immunoassay (CLEIA) technology. Precision, linearity, limit of detection (LOD) limit of quantification (LOQ) and stability were evaluated. Clinical performances were checked in patients with very early and early HCC (n=52) and cirrhotic patients without HCC (n=38).
Results
Within-run and between-run reproducibility were estimated to 3.7 and 6.2 %, respectively, on the lower level of quality control (mean: 35 mAU/mL). The LOD was found to be 1.16 mAU/mL. The LOQ was estimated to 3.9 mAU/mL. The linearity was obtained until 60 000 mAU/mL. Stability at 4°C was excellent.
As previously published, we confirmed the diagnosis value of this marker in patients with both early and very early HCC. In cirrhotic patients without HCC, the usual values were ranging from 7 to 160 mAU/mL, with a mean of 40 mAU/mL. In patients with HCC, the values were ranging from 4 to 4187 mAU/mL, with a mean of 468 mAU/mL.
Conclusion
The CLEIA method for PIVKAII measurement represents an excellent alternative to radioimmunological assays previously used. Further studies in European populations will be needed to ascertain the place of this marker in the screening of HCC.

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Gastrointestinal diseases

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## DIAGNOSTIC PERFORMANCES OF FECAL CALPROTECTIN ACCORDING TO CLINICAL CONTEXT

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Background
Inflammatory bowel diseases (IBD) include Crohn's disease (CD), ulcerative colitis (UC) and inflammatory bowel disease unclassified. The current diagnosis of IBD is multidisciplinary, based on both clinical and biological criteria. Fecal calprotectin (fCal) is increased in patients with IBD. Its concentration in stools correlates with the infiltration of the intestinal mucosa by polynuclear neutrophils. This marker is used in clinical practice in both diagnostic and follow-up of patients. However, the best threshold according to the clinical context remains debated and appears variable according to the method.
Methods
Within the frame of this retrospective monocentric study, we examined fCal results obtained by a chemiluminescent assay (Diasorin), and clinical data of 378 patients referred to Beaujon Hospital Gastroenterology Department over a two months period and for whom a diagnosis was obtained.
Results
Among this cohort, 75 patients were diagnosed as non-IBD, with a mean fCal at 65 μg/g (range: 5-684 μg/g, this latter suffering from an adenocarcinoma of the rectum), whereas all other patients were IBD patients. Among them, only 54 IBD patients were untreated, 9 with UC (mean fCal: 767 μg/g) and 45 with CD (mean fCal: 463 μg/g). Most of the IBD patients (n=249) were chronically treated, displaying a wide range of fCal values according to the efficacy of the treatment.
Endoscopy results were available for 85 patients. As previously demonstrated, fCal was strongly associated with the presence of endoscopic lesions in IBD, with a mean of 2926 μg/g in treated patients with ulcerations.
Conclusion
The fCal determination is a simple, cheap, non-invasive, easy tool to avoid many colonoscopies. Some international studies have now to be undertaken to ensure a better standardization of assays, and to allow the determination of reliable thresholds.

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Gastrointestinal diseases

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## NEED FOR EARLY AND ACCURATE DIAGNOSIS OF LOCAL CMV REACTIVATION FOR THE PROPER TREATMENT OF INFLAMMATORY BOWEL DISEASE

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BACKGROUND: Management of severe or steroid-resistant inflammatory bowel disease (IBD) still presents difficulties to clinicians. Unrecognized local human cytomegalovirus (CMV) reactivation may be responsible for some patients' relapse. As CMV infection of the inflamed colonic mucosa cannot be recognized solely by endoscopy, histology or serology, quick CMV diagnosis from biopsy samples seems essential so that adequate treatment is applied to the patients.
METHODS: Virus reactivation can only be detected in tissue samples obtained from the inflamed colonic mucosa by CMV specific polymerase chain reaction (PCR). Our quantitative PCR method helps monitoring not only the progress of the CMV infection, but the efficacy of therapy, too.
RESULTS: A total of 354 different clinical samples (blood, colon-, rectum-, sigma-bowel biopsy tissues and urine) obtained from 159 patients (age 41±14 years, sex ratio M/F:0,93, diagnosed with colitis ulcerosa (93; 58,9%), Crohn's disease (47; 29,5%), undefined IBD (17;10,7%) and other diseases (2; 1,25%) were analysed with PCR. Patients were treated in accordance with the current guidelines. CMV-DNA was detected in 61 intestinal biopsy tissues representing 64.8% of the 94 CMV positive samples found entirely in all analysed samples (26.5%). In 61 CMV positive biopsies taken from the inflamed intestine patients CMV DNA copies distributed as follows: <100 copies/mg 33 cases (54%); 100-1000 copies/mg 16 cases (26.2%); >1000 copies/mg 12 cases (19.7%). 36 CMV-DNA positive cases were treated with gancyclovir resulting in an improvement of the clinical symptoms in 26 cases. In some IBD patients also presenting intestinal CMV-DNA positivity, anti-viral treatment even resulted in a dramatic recovery from the disease. It was also noticed that 7 IBD patients with persisting or repeatedly identified CMV-DNA low-positivity were among the cases who were subjected to colectomy.
CONCLUSIONS: Rapid and exact detection of CMV from inflamed colonic mucosa seems essential for choosing the right treatment in severe or steroid-refractory IBD. Additionally, tissue PCR or immunhistochemistry are also recommended by the European Crohn's and Colitis Organisation for the diagnosis of CMV colitis in IBD. This laboratory assistance could present a strong support for the clinician in applying the most appropriate treatment to IBD patients that might well pay off in economical terms, as well.

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Gastrointestinal diseases

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## IDENTIFICATION OF HEPATITIS C VIRUS 2K/1B INTERGENOTYPIC RECOMBINANTS IN GEORGIA

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Background and Aim: Hepatitis C virus (HCV) is highly diverse and is divided into six major genotypes (GTs). Recombinant strains have beenidentified,predominantly in Eastern Europe and Asia. Though only the intergenotype recombinant form 2k/1b (CRF1_2k/1b), described in 2002 has been shown to be actively circulating in the population, other single recombinant isolates of genotypes 2/5, 2b/1b, 2b/1a, and 2i/6p have also been described. In 2011, we detected the recombinant form of a HCV CRF1_2k/1b in Georgia. About 70% of GT2 and almost 20% of all HCV infected patients were suspected to have CRF1_2k/1 by partially gene sequencing of the core- and the NS5A region. The aim of this study was to determine recombination breakpoint of Georgian patients' samples by HCV full genome sequencing and to evaluate genetic relationship to previously described recombinant HCV viruses.
Methods: We randomly selected the eight Georgian patients' samples, which were CRF1_2k/1 by partially gene sequencing of the core- and the NS5A region. Afterwards those samples were sequenced using full genome sequencing. RNA was reverse transcribed and amplified using the Ovation RNA-Seq V2 system (NuGEN, San Carlos, CA, USA). Library preparation, multiplexing, and deep sequencing using the MiSeq platform were performed at DDL Diagnostic Laboratory (Rijswijk, Netherlands).
Results: HCV full genome sequences were generated from 7 of 8 patient samples; sequencing failed for
1 of 8 samples. Alignment of the consensus sequences confirmed that all 7 patients were infected with GT2k/1b HCV recombinant virus with the recombination breakpoint located within 73 to 77 amino acids before the NS2-NS3 junction, which is consistent with the breakpoint previously described for CRF1_2k/1b recombinants. Phylogenetic analysis showed that the 2k/1b recombinant viruses from Georgia formed a monophyletic cluster with previously described CRF1_ 2k/1b sequences.
Conclusions: Phylogentic analysis revealed clustering of the highly prevalent 2k/1b recombinant HCV virus from Georgia with CRF1_2k/1b, suggesting that they are genetically related and not individual recombination events.

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Gastrointestinal diseases

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**EMERGENCE OF HEPATITIS C VIRUS GENOTYPE RECOMBINANT FORMS 2K/1B IN GEORGIA**

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Background:Hepatitis C virus(HCV) evolution is thought to proceed by mutations within the six major genotypes(GT). Studies of HCV recombinant genotypes in different parts of the world have been initiated recently. Only a few cases of recombination have been identified worldwide, predominantly in Eastern Europe and Asia. Though only the intergenotype recombinant form 2k/1b (CRF1_2k/1b), described in 2002 has been shown to be actively circulating in the population, other single recombinant isolates of genotypes 2/5, 2b/1b, 2b/1a, and 2i/6p have been also described.
In 2011 we detected the recombinant form of a HCV CRF1_2k/1b in Georgia. About 70% of GT2 and almost 20% of all HCV infected patients were suspected to have CRF1_2k/1b by partially gene sequencing of the core- and the NS5A region.
Methods: The eight Georgian patient samples suspected to be RF 2k/1b were amplified and sequenced using full genome sequencing. RNA was reverse transcribed and amplified using the Ovation RNA-Seq V2 system(NuGEN,San Carlos,CA,USA). Amplified products were fragmented using the Covaris system (Covaris,Inc.,Woburn,MA), and paired-end libraries were created for each sample using Ovation Ultralow DR Multiplex Systems (NuGEN). Libraries were subjected to Illumina MiSeq deep sequencing (at DDL Diagnostic Laboratory, Rijswijk, Netherlands).
Results: Seven of eight samples were successfully sequenced and consensus sequence was generated. Alignment of the consensus sequences confirmed that these sequences were GT2k/1b recombinants. In all seven patients the recombination breakpoint was identical and located within 73 to 77 amino acids before the NS2-NS3 junction, which is consistent with the breakpoint previously described for CRF1_2k/1b recombinants.
The phylogenetic analysis showed that the 2k/1b recombinant viruses from Georgia formed a monophyletic cluster with previously described CRF1_ 2k/1b sequences.
Conclusions: Taken together, seven of eight Georgian HCV patient samples were successfully sequenced. All samples were HCV intergenotypic recombinant genotype 2k/1b viruses with recombination breakpoint located close to the NS2/NS3 junction, which is the same as previously described for CRF1_2k/1b recombinant strain. Phylogentic analysis revealed clustering of the Georgian sequences with CRF1_2k/1b, suggesting that they are likely to be genetically related to the CRF1_2k/1b recombinant virus and not individual recombination events.

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Gastrointestinal diseases

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## ANALYTICAL PERFORMANCE AND DIAGNOSTIC ACCURACY OF SIX DIFFERENT FAECAL CALPROTECTIN ASSAYS IN INFLAMMATORY BOWEL DISEASE

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Background
We evaluated the analytical performance of six different faecal calprotectin immunoassays together with their diagnostic accuracy in the discrimination between functional and organic bowel disorders.

Materials and methods
Measurement of faecal calprotectin was performed using Thermo Fischer EliA Calprotectin 2 assay on the ImmunoCAP 250, Diasorin Calprotectin assay on the Liaison, Inova QUANTA Flash® Calprotectin on the Inova BIO-FLASH instrument, Bühlmann fCAL Turbo on the Roche Cobas c501, Euroimmun Calprotectin assay and Orgentec Calprotectin assay on the Allegria instrument.
The faecal samples were obtained from Inflammatory Bowel Disease patients (n=27) at the time of diagnosis (Crohn's Disease (n=15), Colitis Ulcerosa (n=12)), gastroenterologic disease control patients (n=52) and rheumatologic disease control patients (n=26). All individuals included in the study underwent a concurrent ileocolonoscopy. Analytical performance (imprecision, accuracy, carry-over, correlation and agreement) and diagnostic accuracy (sensitivity, specificity, likelihood ratios) of the different assays were evaluated.

Results
All methods demonstrated acceptable analytical sensitivity: within-run and total imprecision varied from 0.6% to 19.7% and 1.5 to 23.3%, respectively, and no significant carry-over was detected (<0.03% for all methods). The results of the eQC-samples (Instand® Quality control scheme) were qualitatively correctly interpreted (i.e. positive or negative) by all the assays and at the manufacturer's cut-offs. Using Passing and Bablok and Bland-Altman analyses, low quantitative agreement was observed between the assays.
All the assays showed excellent diagnostic accuracy, with areas under the receiver operating curves ranging from 0.974 to 0.998. The areas were not statistically significantly different among the assays ($P > 0.05$). Diagnostic sensitivity at the cut-off at a fixed specificity of 75% ranged from 95.2-100%.
Introduction of positive likelihood ratio's for IBD for multiple test result intervals (<1x Upper Limit of Normal (ULN), 1-3 x ULN, 3-10 x ULN and >10 x ULN) increased the clinical interpretation of all the assays.

Conclusion
Analytical and diagnostic performance of the evaluated faecal calprotectin assays is good, but numerical values differ substantially between the assays. Introduction of multiple test result intervals increased the diagnostic performance of all the assays, aiding in clinical decision-making.

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Gastrointestinal diseases

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## ASSESSMENT OF IRON DEFICIENCY STATUS BASED ON TRANSFERRIN SATURATION IN PATIENTS WITH ENDOSCOPICALLY ASCERTAINED LARGE INTESTINE POLYPOSIS

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**Background:** The presence of polyps and cancer in the large intestine has been tightly associated with anemia, entailing an iron (Fe) deficiency. Moreover, low levels of transferrin saturation (TSAT) have been closely associated with the progress of colon neoplasia. **Aim:** Correlation of serum Fe and transferrin (TRF) levels, TIBC and TSAT values in patients (ptns) with endoscopically documented colorectal polyposis and concomitant diseases of the digestive tract or other localization. **Methods:** Fe (NR 55-140μg/dl) and TRF (NR 200-360mg/dl) serum levels were evaluated in 180 ptns, according to an immunoturbidimetric method to Modular P Roche automatic analyser. TSAT% values fluctuate by gender (men: 20-50%, women: 15-50%). T-test was applied for the statistical analysis. Criteria of classification: A. Gender (male: N=113, female: N=67), B. Age [<50: N=32, (50-65): N=85, ≥66: N=63], C. Number of polyps [P0: N=60, P1: N=45, P2: N=24, P3-9: N=41, P≥10: N=10], D. Combination of (C) with concomitant benign or malignant diseases, creating broader groups: 1. Inflammation (IF: N=24), 2. Benign Tumors (BT: N=138), 3. Cancer (CA: N=18). Division of P0 and IF into non-specific (IBD) and specific inflammatory diseases (SID): P0-IBD: N=12, P0-SID: N=48, IF-IBD: N=9, IF-SID: N=15. **Results:** MV Fe: P0 (77.6) to P2 (97.6) [p=0.026], P0-IBD (61.0) to: P1 (90.3) [p=0.022], P2 (97.6) [p=0.017], P3-9 (87.9) [p=0.016], IF (68.6) to BT (91.7) [p=0.0034], BT to CA (63.8) [p=0.0024], IF-IBD (60.3) to BT (91.7) [p=0.012]. MV TRF: P0 (274.5) to P≥10 (303.1) [p=0.082]. MV TSAT%: P0 (21.1%) to P1 (25.2%) [p=0.061] and P2 (26.3%) [p=0.065], P0-IBD (17.3%) to P1 (25.2%) [p=0.037], IF (18.2%) to BT (24.9%) [p=0.0052], BT to CA (17.3%) [p=0.0062], IF-IBD (16.9%) to BT (24.9%) [p=0.035], IF-SID (19%) to BT [p=0.046]. **Conclusions:**1.There is no statistically significant difference (level 5-10%) for each of the three defined parameters, regarding age and gender. 2. Level of statistical significance 5% is achieved for Fe, when comparing P0 to P2 ptns. Level of statistical significance 10% is found for serum Fe in P1 ptns for TRF in P≥10 ptns and TSAT% in the P1 and P2 groups, compared to P0 ptns. In the latter group, Fe deficiency is attributed primarily to GI inflammation (IBD /SID), cardiac disease, recent surgical GI operations and medication intake. Concerning P0 ptns with low Fe - TSAT%, the combination with high TIBC/TRF appears in 5%, while the combination with increased TIBC appears in 23%. Nearly 16% of ptns with polyposis have reduced TSAT% - Fe values and increased TIBC, while 2.5% of them simultaneously appear with high levels of TRF. Anemia with low TSAT% is indicated to 47.6% and 48.6% to the P0 and P + group respectively. 3. Serum Fe proved a useful parameter in the discrimination of IBD ptns and the P1, P2, P3-9 groups. TSAT% discriminates ptns with IBD and those with 1 to 9 polyps to ~5% statistical significance. 4. The comparison of serum iron levels and TSAT%, either between IF exclusively and BT, or between BT and CA reaches a level of statistical significance 5%. Iron deficiency with high levels of TRF is inferred to 8.3% of IF patients, 8.1% with BT and 5.6% with CA. The corresponding percentages, for each group of this classification, which combine low TSAT% - Fe levels and increased TIBC values, are 33.3%, 59.5% and 27.8% respectively. Anemia with low TSAT% is demonstrated to 66.7% of CA, while IF and BT groups approach a level close to 40%. (VI) Discrimination between IBD and BT ptns is feasible by measuring serum iron levels and calculating TSAT%, at a statistically significant level ~5%.