Gastrointestinal diseases, including hepatic and pancreatic diseases

**VALIDATION OF THE CALPROTECTIN QUANTITATIVE TEST USING THE OC-SENSOR PLEDIA INSTRUMENT**

T. Stam ², D. Orolovsky ³, G. Prahagrod ¹

¹Meuhedet Health Maintenance Organization, Laboratory Management
²Meuhedet Health Maintenance Organization, North Laboratory, Nesher
³Meuhedet Health Organization, North Laboratory, Nesher

**BACKGROUND-AIM**

Measurement of FCa assists clinicians in discriminating inflammatory bowel diseases (IBD) from irritable bowel syndrome (IBS). FCa has a high negative predictive value in ruling out IBD in undiagnosed, symptomatic patients and a high sensitivity for diagnosing the disease.

Here, we report the performance of FCa measurements collected by the novel latex agglutination turbidimetric immunoassay that utilizes the EIKENs OC-SENSOR PLEDIA instrument. This study goals were first, to evaluate the performance of the novel FCa assay (OC-FCa-LATIA) with OC-SENSOR PLEDIA instrument. Secondly, to compare the results of the novel FCa assay with the current existing assay: The LIAISON Calprotectin Stool Test.

**METHODS**

252 frozen Fecal Specimens were collected (124 Males, 128 Female ages 3-90 years).

A Correlation analysis was applied on test results collected from both instruments. The calculated correlation factor between the OC-FCa-LATIA and LIASION immunoassays was done by applying a linear regression model to both assay’s results.

**RESULTS**

Neg/Pos Test Results Analysis. Concordance values of: 74%, 82% and 84% were observed when cut-off values of: 50ug/g, 150ug/g and 200ug/g were applied for negative test results, respectively. Concordance values of: 96%, 94% and 97% were observed when cut-off values of: 50ug/g, 150ug/g and 200ug/g were applied for positive test results, respectively. Furthermore, an ideal concordance between the two methods was observed at the higher cut-off values. PLEDIA OC-FCa-LATIA and LIAISON Immunoassays Correlation Analysis. We have found that the results from LIAISON instrument were lower than the results from the PLEDIA instrument mainly at the high levels of result. OC-SENSOR PLADIA Accuracy Analysis. Accuracy evaluation was performed by conducting Inter/Intra-days repeatability assays utilizing the PLEDIA’s instrument. 100% of the inter-days sample population exhibited CV% values<10 and 83.3% of the intra-days sample population exhibited CV% values<10, both results showed high precision levels.

**CONCLUSIONS**

To conclude, Eiken’s OC-SENSOR PLEDIA LATIA method performance is to the very least equal to that of the method we currently use: CLIA, DIASORIN LIAISON XL. In some cases, the PLEDIA platform exhibited higher sensitivity compared to the LIAISON XL.
Gastrointestinal diseases, including hepatic and pancreatic diseases

MOLECULAR CHARACTERIZATION OF ANTIBODY BINDING PROTEINS IN THE HUMAN INTESTINAL MICROBIOTA

C. Drees 2, M.T. Borowska 1, E.J. Adams 1, A. Bendelac 2
1Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637, USA.
2Department of Pathology, University of Chicago, Chicago, IL 60637, USA.

BACKGROUND-AIM
Ruminococcus gnavus (R. gnavus) is a prevalent member of the human intestinal microbiota and local expansion of this species has been associated with inflammatory bowel disease (IBD). R. gnavus expresses the membrane-bound protein homologs immunoglobulin-binding protein (Ibp) A and Ibp B which bind human VH3 and mouse VH5/6/7 variable regions with high affinity. Supraclonal antibody binding and Ibp's protein structure are reminiscent of Protein A, a well described virulence factor and B cell superantigen expressed by Staphylococcus aureus (S. aureus). However, whether Ibp – immunoglobulin (Ig) interactions also involve similar molecular mechanisms remains unknown.

METHODS
Here we provide detailed functional and molecular studies revealing the basis of Ibp/Ig interactions.

RESULTS
We take advantage of fluorescently labeled Ibp and FACS-purified human and mouse B cells to determine the repertoire of Ibp reactive antibodies in detail. In addition, labeling studies and in vitro B cell activation assays demonstrate Ibps BCR cross-linking capabilities. Furthermore, we present the crystal structure of a truncated Ibp A construct in complex with mouse VH5 antigen binding fragments (Fab) demonstrating non-canonical binding of heavy chain framework residues to Ibp heavy chain binding domain (HCBD), a conserved C-terminal domain outside of Ibp A repeat regions. To uncover the molecular basis of how a broad but VH-restricted immunoglobulin repertoire is recognized by Ibp, we use targeted mutagenesis of contact residues and utilize wild-type Ibp A and individual Ibp A domains for affinity measurements and labeling experiments.

CONCLUSIONS
Altogether, we dissect multiple layers of Ibp-immunoglobulin interactions and clarify how Ibp proteins bind entire families of immunoglobulins via their heavy chains. The association of R. gnavus blooms and IBD suggests that intentional IgA coating might be advantageous for certain taxa, particularly under inflammatory conditions. Furthermore, since R. gnavus represents a genetically heterogeneous species, further studies should assess polymorphisms of Ibp genes and their respective roles for the maintenance of host/microbiome homeostasis.
Fecal elastase-1 in the diagnosis of exocrine pancreatic insufficiency

M. Arnaldos Carrillo 1, L. Arenas Vicent 2, M. Martínez Villanueva 1, F. Avilés Plaza 1, Y. Mestre Terkemani 1, M. Caparrós Guerrero 1, M. Expósito García 1, J.A. Noguera Velasco 1

1Hospital Clínico Universitario Virgen de la Arrixaca
2Universidad de Murcia

BACKGROUND-AIM
Exocrine pancreatic insufficiency (EPI) is a disease characterized by poor digestion of macronutrients and micronutrients as a result of inadequate release of pancreatic enzymes. It causes numerous intestinal ailments and severe health problems such as malnutrition. The clinical manifestations, unspecific and common to other gastrointestinal diseases, together with the delay in the symptom manifestation, are a great inconvenience when recognizing and diagnosing the pathology.

The main aim of this study is to evaluate the diagnostic performance of faecal elastase-1 in exocrine pancreatic insufficiency (EPI)

METHODS
A database of 399 patients with pathological levels of elastase (<200 µg/g) measured between May 2020 and May 2021 was selected. Clinical and analytical data related with exocrine pancreatic insufficiency were collected and the statistical analysis were performed using IBM SPSS Statistics v.22 and MedCal 20.008. Elastase was measured with an Enzyme-Linked ImmunoSorbent Assay (Pancreatic Elastase 1™, ScheBo®).

RESULTS
60% of the patients with <200 µg/g levels of elastase were male (N=239), however, the incidence of EPI between each group were similar, 50% for women and 51% for men. The main cause of the development of EPI in children was cystic fibrosis and chronic pancreatitis in adults, being this group where this pathology manifested itself the most, with a mean of 53.4 years and median of 55 years at the moment of diagnosis. An AUC (Area under ROC curve) of 0.610 for elastase was obtained (IC: 0.560-0.658), an optimal cut-off point of 117 µg/g was found, with a sensitivity of 76.62% and a specificity of 40.91%. The Odd ratio of suffering EPI with an elastase <15 µg/g were 2.15 (p=0.0019).

CONCLUSIONS
IPE manifest itself mainly in adulthood, with a similar incident in men and women, being its main cause chronic pancreatitis, while in children is cystic fibrosis. Patients with elastase levels <15 µg/g have 2.15 higher probabilities of being diagnosed with IPE. AUC ROC result for elastase shows that the test has discriminatory capacity, but not enough to diagnose a patient with IPE.
Gastrointestinal diseases, including hepatic and pancreatic diseases

**SHORTEN LACTOSE INTOLERANCE TEST AS A SAFER ALTERNATIVE TO HYDROGEN BREATH TEST FOR THE DIAGNOSIS OF LACTOSE MALABSORPTION**

M. Muñoz-Calero, D. Rodríguez Cano, M.P. Pinel Julian, J. Caballero Villarraso, F. Rodríguez Cantalejo

1Clinical Biochemistry Department, Reina Sofia University Hospital

**BACKGROUND-AIM**

Nowadays the recommended test for Lactose Malabsorption diagnosis is the Lactose Breath Hydrogen Test (LBHT), but, in the context of covid-19, the exhalation of droplets and aerosols may constitute a high infectional risk. Lactose Tolerance Test (LTT) emerges as a safer alternative.

The LTT consists in an oral administration of Lactose and monitoring glucose (GLU) at 0, 30, 60, 90 and 120 minutes (LTT 120). Failure of blood GLU to rise by ≤20 mg/dL above the basal level is diagnostic of abnormal LTT. The increasing demand for this test could be challenging in labs because of the lengthy development for each test.

Recent studies have reported that duration of this test could be shortened only in two points (Basal and 30 minutes, LTT 30) without affecting the accuracy of it.

**METHODS**

A retrospective observational study of LTT carried out in laboratory from 2015 to 2020. Patients were subjected to an overload of 50 grams of oral lactose. Blood GLU was measured fasting (Basal), at 30, 60, 90 and 120 minutes. GLU was measured in an Advia Centaur XP (Siemens Healthcare). Increases of GLU under 20 mg/dL were reported as abnormal LTT.

The objective of the study was to evaluate the closeness of agreement between traditional LTT (LTT 120, 5 points) with the shortened test (LTT 30, 2 points). For this purpose, the Kappa Index was applied (SPSS statistical program).

**RESULTS**

The study involves 3,658 patients who were submitted to LTT. With classical LTT, 1,644 patients (45%) had an abnormal LTT while LTT 30 had 1,705 (47%). Shortened LTT modified the interpretation in 61 patients (1.7%), where the GLU levels increased after 60 minutes.

Results of Kappa Index (0.966, p<0.001) demonstrated a good concordance between LTT 120 and LTT 30.

**CONCLUSIONS**

Reducing the LTT in only two points does not affect diagnostic accuracy and offers multiple advantages. This shortened test could be easily implemented in daily routine in laboratory as an alternative to LBHT during Covid-19 pandemic. In the context for optimizing resources, it also carries important cost saving on staff, reactive and fungible materials.
Gastrointestinal diseases, including hepatic and pancreatic diseases

EVALUATION OF ANALYTICAL PERFORMANCE OF AN ASSAY FOR THE MEASUREMENT OF LIPASE

L. Rami, S. Fernandez, I. López, R. Pérez, M. Sánchez

1BioSystems S.A., Barcelona, Spain

BACKGROUND-AIM

Lipase is an enzyme found primarily in the pancreas and hydrolyzes glycerol esters of long-chain fatty acids, being responsible for fat digestion. Clinical serum lipase determination is useful as an aid in the evaluation of pancreatic disorders. Lipase activity increases after an attack of acute pancreatitis, together with amylase, but the elevation of lipase persists for longer time.

There are many spectrophotometric commercial methods for measuring lipase activity; some of them based in different measurement principles, which generate a lack of specificity in lipase results. Although there is no primary reference procedure described yet, Biosystems have developed a lipase reagent based on one of the candidate reference methods, which the International Federation of Clinical Chemistry has been working on. A synthetic substrate, 1,2-O-dilauryl-rac-glycerol-3-glutaric acid-(6’-methylresorufin)-ester, is hydrolyzed by lipase and the catalytic concentration is determined by the rate of the red dye formation measured at 560 nm. The aim of this study is to evaluate the analytical performance of the new Biosystems assay in combination with associated reference materials.

METHODS

The assay has been applied to both A25 and BA400 automated BioSystems analyzers. Detectability, linearity, precision and interferences were evaluated according to the Clinical and Laboratory Standards Institute guidelines. Correlation with a commercially available method was determined by Passing-Bablok regression analysis.

RESULTS

The obtained measuring range of the assay was (9.84 – 250 U/L) for A25 and (8.52 – 250 U/L) for BA400. Total precision (CV) was < 5.40 %. Hemoglobin (500 mg/dL), bilirubin (30 mg/dL), triglycerides (330 mg/dL) did not interfere at the indicated concentrations. High correlation was obtained in the comparative studies with 81 serum samples ($y = 0.984 x + 6.15; r = 0.985$) and with 57 plasma samples ($y = 0.964 x + 5.59; r = 0.996$).

CONCLUSIONS

The results of analytical performance features meet the established requirements to ensure an optimal clinical functionality of the Biosystems Lipase measurement procedure. Although the method comparison studies show a constant error, it is not considered significant due to the references values for serum lipase (13 - 60 U/L).
Gastrointestinal diseases, including hepatic and pancreatic diseases

ENKEPHALIN AND DYNORPHIN IN THE COURSE OF CROHN’S DISEASE

A. Martyniak 1, A. Wędrychowicz 2, P. Tomasik 1
1Jagiellonian University Medical College, Faculty of Medicine, Pediatric Institute, Department of Clinical Biochemistry, Krakow, Poland
2Jagiellonian University Medical College, Faculty of Medicine, Pediatric Institute, Department of Pediatrics, Gastroenterology and Nutrition, Krakow, Poland

BACKGROUND-AIM
Crohn’s disease (CD) is severe condition, recently affected an increasing number of children and teenagers. Abdominal pain and weight loss associated with diarrhea and diminished appetite are typical in CD. Opioids are frequently used in the CD treatment as a painkiller and antidiarrheals. However, little is known about secretion of endogenous opioids (endoopioids) in the course of IBD. Therefore, the aim of this study was to assess enkephalin and dynorphin, members of endoopioid family, concentration in children suffered from IBD.

METHODS
We have studied 38 children with CD, mean age 12.8 ys ± 2.9. The blood was collected three times – in active phase of the disease (during admission to the hospital, before treatment), 2-4 weeks later during consolidation of medical treatment, and month to 6 months later during remission. As a control group served a group of 34 age-matched healthy children. In all cases, fasting samples were taken in the morning. The enkephalin and dynorphin were measured in the serum using EIA kits (Fine Test; Wuhan, China).

RESULTS
In the study group before treatment median concentration of enkephalin was 0.725 ng/ml (0.344 - 1.010). During treatment it diminish to 0.425 ng/ml (0.176 - 0.993), and in remission concentration was 0.448 ng/ml (0.204 - 0.905). All these values were significantly lower as compare to values observed in control group 1.777 ng / ml (1.146 - 2.736), (p<0.0001 in all cases).
Dynorphin median concentration in study group was stable - acute phase 29.906 pg/ml (17.064 - 42.102), during treatment 22.817 pg/ml (11.955 - 40.914); remission 28.316 pg/ml (17.181 - 35.373). These concentrations were significantly lower than values observed in control group 38.174 pg/ml (27.369 - 55.767); (respectively p=0.0705; p=0.0033; p=0.0356).

CONCLUSIONS
The lower concentrations of enkephalin and dynorphin in the CD children than in controls suggest some factors negatively affecting endoopioids concentration in the course of CD in children. Probably, endoopioids system do not counteract pain as well diminished appetite in CD children.
Gastrointestinal diseases, including hepatic and pancreatic diseases

**CALPROTECTIN STABILITY IN FAECES AND THE OC-SENSOR COLLECTION DEVICE.**

S. O'Driscoll 1, C. Piggott 1, S. Benton 1

1Bowel Cancer Screening Programme, Royal Surrey Foundation Trust, Guildford

**BACKGROUND-AIM**

Faecal calprotectin (f-cal) is often used to aid the diagnosis of inflammatory bowel disease (IBD) over irritable bowel syndrome (IBS) and to monitor ongoing prognosis. Patients send faecal samples in ‘poo-pots’ to laboratories for analysis. Although f-cal is often thought to be stable in a stool for up to 7 days at room temperature (RT), evidence shows it may be less. Eiken Chemical Co Ltd (Japan) have developed a calprotectin method (OC-FCa) using the same faecal immunochemical test (FIT) collection device and analyser (OC-SENSOR PLEDIA) used for faecal haemoglobin, including for bowel cancer screening programmes.

This study aimed to investigate stability of f-cal in faeces at RT and 4°C, and to compare this to stability in the OC-SENSOR collection device.

**METHODS**

Excess portions of faeces less than 48 hours old sent for routine calprotectin analysis (n=30) were each homogenised for 2mins and loaded into 6 OC collection devices. The devices were mixed by inversion and incubated for 3 hours before pooling the buffer and analysing (day 0). 2 mL aliquots of pooled buffer were stored at RT or 4°C. The remaining faecal sample was aliquotted into plain containers and stored in the same conditions. Samples were re-analysed on day 2, 3 or 4, day 7 and day 14. Prior to analysis all samples were equilibrated to RT for 1 hour.

**RESULTS**

The percentage change from the day 0 result was calculated for each sample and the median change for each time point calculated. Wilcoxon signed rank test was used to test statistical significance. The median percentage changes at day 2, 3 or 4, day 7 and day 14 were -8.9%, -16.6% and -27.7% for samples in buffer at RT; -16.7%, -27.1%, and -27.6% for plain samples at RT; -3%, -4.7% and -8.2% for samples in buffer at 4°C; and -9.5%, -8.8% and -11.2% for plain samples at 4°C. Samples stored in buffer at RT were significantly different by day 7 (z=-2.53, p<0.05). Samples stored in buffer at 4°C had significant difference at day 14 (z=-2.14, p<0.05). Faecal samples were significantly different by day 2, 3 or 4 at RT (z=-3.24, p<0.05) and 4°C (z=-2.13, p<0.05).

**CONCLUSIONS**

Calprotectin in faeces is not stable at RT for up to 7 days. It is recommended to extract calprotectin from samples as soon as possible. If storage is necessary, 4°C is advisable.
Gastrointestinal diseases, including hepatic and pancreatic diseases

ENDORPHIN AND ENDOGENOUS CANNABINOIDS IN CHILDREN AND ADOLESCENT WITH CROHN’S DISEASE

A. Martyniak 1, A. Wędrychowicz 2, P. Tomasik 1

1 Jagiellonian University Medical College, Faculty of Medicine, Pediatric Institute, Department of Clinical Biochemistry, Krakow, Poland
2 Jagiellonian University Medical College, Faculty of Medicine, Pediatric Institute, Department of Pediatrics, Gastroenterology and Nutrition, Krakow, Poland

BACKGROUND-AIM

Crohn’s disease (CD) is one of Inflammatory Bowel Disease (IBD). CD is severe condition, recently affected an increasing number of children and teenagers. Abdominal pain and weight loss associated with diarrhea and diminished appetite are typical in IBD. Opioids are frequently used in the CD treatment and recently few papers described also a positive effect of medical cannabis during IBD recurrence. Their effect is based on antinociceptive and enhancing appetite action. However, little is known about the secretion of endogenous opioids like endorphins and endogenous cannabinoids in the course of IBD. Therefore, this study aimed to assess endorphin and 2-Arachidonoylglycerol (2-AG; member of endocannabinoid family) concentration in children who suffered from IBD.

METHODS

We have studied 38 children with CD, mean age of 12.8 ys ± 2.9. The blood was collected three times – in active phase of the disease (during admission to the hospital, before treatment), 2-4 weeks later during the consolidation of medical treatment, and month to 6 months later during remission. As a control group served 34 age-matched healthy children. In all cases, fasting samples were taken in the morning. The endorphin and 2AG were measured in the serum using EIA kits (respectively Fine Test; Wuhan, China, and Abclonal Technology; Wuhan, China).

RESULTS

In the study group before treatment median concentration of endorphin was 496.27 pg/ml (389.68 - 722.15). During treatment it diminish to 414.01 pg/ml (311.79 - 584.76), and in remission concentration was 390.26 pg/ml (331.56 - 587.60). All these values were significantly lower as compare to values observed in control group - 725.83 pg/ml (516.60 - 881.53); (p=0.0234, p<0.0001, p<0.0001 respectively).

2-AG mean concentration in study group was stable - acute phase 272.24 ng/ml (115.81 – 388.27), during treatment 214.53 ng/ml (127.83 – 376.75); remission 297.33 ng/ml (123.96 – 490.85) and similar to the values observed in control group 228.55 ng/ml (148.43 – 449.10); (p>0.9999 in all cases).

CONCLUSIONS

The lower concentrations of endorphin in these children than in controls suggest some factors negatively affecting endorphin concentration in the course of IBD in children. Endocannabinoids are not disturbed in children with IBD. Probably, the endorphins do not act against the pain as well as diminished appetite in IBD children.
Gastrointestinal diseases, including hepatic and pancreatic diseases

EVALUATION OF A FAECAL CALPROTECTIN METHOD USING THE OC-SENSOR PLEDIA

S. O’Driscoll 1, C. Piggott 1, S. Benton 1

1Bowel Cancer Screening Programme, Royal Surrey Foundation Trust, Guildford

BACKGROUND-AIM

The National Institute for Health and Care Excellence (NICE) recommends faecal calprotectin to help differentiate inflammatory bowel diseases from irritable bowel syndrome. Currently patients send ‘poo pots’ to laboratories, where calprotectin is extracted before analysis. Eiken Chemical Co Ltd (Japan) have produced an immunoturbidimetric method using the same collection device for faecal haemoglobin, whereby patients collect samples at home. No extraction step is needed.

This study aimed to perform an analytical evaluation of the Eiken OC-FCa using the OC-SENSOR PLEDIA.

METHODS

Using calprotectin solutions provided by Eiken, the evaluation followed CLSI guidelines where available. Limit of detection (LOD), limit of quantification (LOQ) (CLSI EP17-A2), within-run imprecision (2 concentrations, n=20), between-run imprecision (3 concentrations over 20 days, n=80), linearity (20 concentrations over the analytical range 20-2720 µg calprotectin/g faeces (µg/g)), prozone 6 dilutions of 1 sample, expected concentrations 1563-50,016 µg/g), recovery (2 series, volume replacement of low concentration sample (70 µg/g) with high concentration sample (1013 µg/g) or buffer, n=24), and carryover (Broughton method) were assessed. A method comparison against the BÜHLMANN fCAL® turbo (BÜHLMANN Laboratories AG, Switzerland) was performed using patient samples (n=39) and EQA (n=6).

RESULTS

LOD was 7 µg/g, LOQ 19 µg/g. Imprecision was <5% for all samples within and between-batch (mean within-batch 247, 516 and between-batch 49, 98, 992 µg/g); linearity was good (R²>0.99); prozone was appropriately reported as ‘PRC’ in 2 samples >37,000 µg/g, 4 results from 3000-25000 µg/g gave ‘Over Range’ without a numerical result. Recovery was 99.6%; carryover k=-0.3-0.2%. Eiken method showed a strong positive bias compared with BÜHLMANN fCAL® turbo, mean difference for patient samples 161.4% and EQA 42.6%.

CONCLUSIONS

The OC-FCa method performed well. It shows positive bias compared with the BÜHLMANN fCAL turbo®. There is currently no standardisation for calprotectin and a clinical study should be performed to evaluate the impact of this bias.
Gastrointestinal diseases, including hepatic and pancreatic diseases

REDEFINING THE LIVER TESTS UPPER LIMIT OF NORMAL

R. Mondejar, C. Cañavate Solano, M. Mayor Reyes, L. Diez Herran, J.D. Santotoribio

1Department of Laboratory Medicine, Puerto Real University Hospital, Cádiz, Spain

BACKGROUND-AIM

The American College of Gastroenterology (ACG) published in 2016 a clinical guideline for the evaluation of liver tests, where the upper limit of normal (ULN) was debated. More than a third of the laboratories used the manufacturer’s recommendations to define their ULN, as in our laboratory. Recently, a study showed that ULN from liver tests is age-dependent. We aimed to evaluate ULN using local healthy controls, taking into account sex and age.

METHODS

Consecutive blood samples from Primary Care were retrospectively analysed from June to September 2019. All blood samples were considered in which ALT and AST were measured on the Alinity platform (Abbott Laboratories). The ULN for ALT was 55 U/L and for AST was 32 U/L for females and 40 U/L for males. Age was categorized for decades (15-24, etc). We defined “healthy” controls as patients without diabetes, dyslipidemia, and without known hepatitis and oncological history. We also excluded haemolysed samples. Statistical analysis was performed using MedCalc.

RESULTS

A total of 15,633 samples were derived for ULN analysis. The ALT 95th percentile (p95) for males reached 65 U/L at 45-54 years and fell to below 35 U/L by the age 75. In females, p95 reached 40 U/L at 45-64 years and falling to below 22 U/L by the age 85. Independent of the age, p95 was 51 U/L for males and 34 U/L for females. AST was less age-dependent, especially in females (p95 26-33 U/L). In males, p95 reached 47 U/L at 45-54 years and fell to below 35 U/L by the age 75. Independent of the age, p95 was 39 U/L for males and 30 U/L for females, similar to the manufacturer’s recommendations.

CONCLUSIONS

Our data strongly support that sex and age adaptation for ALT reference ranges should be considered in our population. Although the ACG guideline is flexible with liver test reference ranges, our ULN for ALT is far from what they proposed (29-33 U/L for males and 19-25 U/L for females). We need to take care modifying reference values because lower ULN for ALT would have implications by defining more patients as having abnormal ALT levels.
Gastrointestinal diseases, including hepatic and pancreatic diseases

**PERFORMANCE EVALUATION OF FPELA® TURBO ASSAY FOR PANCREATIC ELASTASE: INTRA-ASSAY PRECISION AND METHOD COMPARISON WITH LIAISON ELASTASE-1®**

D. Pohlers 1, G. Stamminger 1

1Zentrum für Diagnostik am Klinikum Chemnitz - Labor Chemnitz

**BACKGROUND-AIM**

Endocrine pancreatic insufficiency (EPI) is characterized by inadequate production of enzymes resulting in poor digestion of food ingredients, which leads to gastrointestinal symptoms and malnutrition. Diagnosis of EPI will be supported by determination of pancreatic elastase in stool, which reflects the secreting capacity of the pancreas. Recently, fully automated assays were introduced, leading to faster and more flexible processing than manual methods. The technical performance of the turbidimetric test FPELA turbo was proved on ROCHE cobas c502 laboratory analyser and the results compared to another automated assay (Liaison Elastase-1 on Diasorin LIAISON XL®).

**METHODS**

The evaluation included the determination of the intra-assay precision with two levels of quality control material over a period of 21 days and a method comparison with n = 84 routine fecal samples. Determination of coefficients of variation (CV), of Passing-Bablok regression (intercept and slope), Spearman rank correlation (r), and the reliability of clinical classification of both assays with the calculation of Cohen’s kappa (κ) were employed.

**RESULTS**

The precision study resulted in total CVs of 2.0-2.3 % for control materials (150 and 400 mg/kg). Comparison of elastase levels by FPELA turbo® with the Liaison Elastase-1® assay in 84 routine fecal samples (0.2 – 800* mg/kg) resulted in r = 0.931 intercept value of 0.968 mg/kg, and slope of 0.9715, respectively. Calculation of Cohens’s kappa for inter-rater reliability of both assays for clinical classification resulted in κ = 0.719 and an overall agreement of 84 %.

*primary upper measuring range for Liaison Elastase-1 assay

**CONCLUSIONS**

The evaluation generated excellent performance data and demonstrated a high degree of comparability to other commercially available automated Elastase assay.
Gastrointestinal diseases, including hepatic and pancreatic diseases

PERFORMANCE EVALUATION OF THE SECOND GENERATION CALiAGOLD®, A NEW IMMUNOTURBIDIMETRIC TEST FOR THE QUANTIFICATION OF HUMAN CALPROTECTIN IN STOOL

C. De Cunto 2, A.I. Leuci 1, G. Grammatico 1, C. Roveta 1, G. Inzaina 1, F.E.O. Ferrara 1, M. Pirovano 2, F. Magro 2

1CDI Centro Diagnostico Italiano, Via Saint Bon, 20, 20147 Milano, Italy
2Sentinel Diagnostics, Via Robert Koch, 2, 20152 Milano, Italy

BACKGROUND-AIM

Calprotectin is the main protein expressed by neutrophil cells, and it is considered as one of the most interesting biomarkers of inflammation. Due to its district specificity, the measurement of calprotectin in stool is widely used to detect inflammatory process in intestinal tract. It is mainly used to distinguish inflammatory bowel diseases (IBD), such as Crohn disease and ulcerative colitis, from non-inflammatory disorder like the irritable bowel syndrome (IBS).

METHODS

Sentinel Diagnostics has developed the second generation CALiaGOLD®, a particle enhanced turbidimetric immunoassay (PETIA) for the quantification of calprotectin in fecal extracts. Aim of the study was to evaluate the performances in an external clinical lab and under routine conditions on chemistry platform SENTiFIT® 270. CLSI derived evaluation protocols and an acceptance criteria of +/- 10% bias at clinical decision level have been adopted.

RESULTS

The second generation CALiaGOLD® showed a limit of detection of 6.1 µg/g and a limit of quantification of 18.6 µg/g. The measuring range of the assay was between 20 – 2200 µg/g; concentrations up to 22000 µg/g could be measured in automatic rerun mode. No prozone effect was found up to 6000 µg/g. Calprotectin stability in the extraction buffer after sampling was 3 days at room temperature up to 37 °C and 14 days at 2-8 °C.

The method comparison between the first generation (routine method) and the second generation of CALiaGOLD® assays showed a bias lower than 10% on routine stool samples. The sampling procedure was done with both old and new sample collection tube on the same sample. Each tube was analyzed on SENTiFIT® 270 with their own respective reagents. A total of 306 samples were included in the study with a range of concentrations ranging between < 22 and 7800 µg/g. Passing-Bablok fit gave a slope of 0.96 (0.90 – 1.02), an intercept of 1.04 (-4.84 – 7.51) and R= 0.93 (0.91-0.94).

CONCLUSIONS

The outcomes of the study proved that the 2nd Gen. CaliaGold met the requirements for its use as IVD-MD and offers a valid alternative solution for in clinical laboratory.
Gastrointestinal diseases, including hepatic and pancreatic diseases

T053

NEW BILIRUBIN TOTAL ASSAY FOR THERMO SCIENTIFIC INDIKO AND KONELAB CLINICAL CHEMISTRY ANALYZERS

L. Virtanen 1, H. Laitinen 1

1Thermo Fisher Scientific

BACKGROUND-AIM

Bilirubin is a degradation product of hemoglobin. Total bilirubin in serum is composed of three fractions: Unconjugated bilirubin ($B_u$), which is extremely apolar and practically insoluble in water at physiologic pH and body temperature; conjugated bilirubin ($B_c$), bound to sugar, is water soluble and δ-bilirubin. A number of inherited and acquired diseases affect one or more of the steps involved in the production, uptake, storage, metabolism and excretion of bilirubin. Depending on the disorder, unconjugated bilirubin, conjugated bilirubin, or both, are major contributors to hyperbilirubinemia. The Bilirubin Total assay applied on Thermo Scientific™ Indiko™ and Konelab™ clinical chemistry analyzers from Thermo Fisher Scientific is indicated to be used in conjunction with clinical evaluation for aid to diagnosis and monitoring of hyperbilirubinemia and hepatobiliary diseases. World Gastroenterology Organization recommends testing bilirubin from all patients with underlying chronic viral hepatitis and symptomatic COVID-19 infection. In addition, IFCC recommends monitoring bilirubin during COVID-19 drug treatment of patients with hepatotoxic medications, and in those with pre-existing liver disease.

METHODS

Direct bilirubin forms a red colored azocompound with diazotized 2,4-dichloroaniline (DCA) in acidic solution. A specific mixture of detergents enables a safe determination of total bilirubin. This new two-reagent liquid assay contains Triton X free reagents and is less sensitive to lipemia interference compared to the NBD chemistry-based method.

RESULTS

The assay measuring range is 1.0-500 µmol/l (0.06-29 mg/dl) extended with automatic dilution to 1.0-2000 µmol/l (0.06-117 mg/dl). The repeatability (within-run precision) is 0.3–2.3 % (CV; n=80). The within device (total) precision is 1.0–3.3 % (CV; n=80). Open on-board stability is 30 days. A comparison study was performed on Indiko analyzer using Bilirubin Total (NBD) method as the reference. Linear regression was $y=1.003x -0.95$, $r=0.9997$ (n=141).

CONCLUSIONS

With this ready-to-use system reagent, Bilirubin total analysis on Indiko and Konelab analyzers is quick and accurate with excellent open on-board stability.
Gastrointestinal diseases, including hepatic and pancreatic diseases

EVALUATION OF THE POTENTIAL INTERFERENCE OF MICROORGANISMS ON THE PERFORMANCE OF THE IDS CALPROTECTIN ASSAY

M. Berodes 1, E. Cavalier 1, L. Lutteri 1, P. Lukas 1, V. Castiglione 1
1Department of Clinical Chemistry, University of Liège, CHU Sart-Tilman, 4000 Liège, Belgium

BACKGROUND-AIM

IDS Calprotectin assay is an in vitro diagnostic test intended for the quantitative determination of calprotectin. Calprotectin is a marker of intestinal mucosal inflammation, released by neutrophils indicating their abnormal presence in stools. Calprotectin dosage is a non-invasive assay that is useful for the follow-up, the diagnosis and the differentiation between patients with IBD or patients with IBS. The goal of this study is to determine if interfering microorganisms potentially affect IDS calprotectin concentration.

METHODS

Four stool specimens were used, covering the medical relevant range (negative ≤50µg/g, equivocal 50-120µg/g and positive ≥120µg/g). Microorganisms were added to each stool sample at 10% of the total specimen volume. Control sample was made by adding solvent (10% of the total specimen volume) used to constitute the interferent microorganisms solution. All samples were extracted using the IDS Calprotectin Extraction device and measured on the IDS IS-6000. The microorganisms tested are Citrobacter freundii, Escherichia coli, Klebsiella pneumoniae, Salmonella enterica, Shigella flexneri and Yersinia enterocolitica.

RESULTS

The percentage of recovery obtained varied from 93.9% to 104.2%, within the acceptance criteria of 80 to 120%. There was no change in the interpretation of the class of the tested sample compared to the control sample.

CONCLUSIONS

The presence of these interferents microorganisms did not affect the quantification of the human fecal calprotectin by using the IDS calprotectin test kit. This study meets the acceptance criteria for all microorganisms tested.
Gastrointestinal diseases, including hepatic and pancreatic diseases

**VALIDATION OF THE IDS CALPROTECTIN IMMUNOASSAY AND COMPARISON WITH THE DIASORIN LIAISON CALPROTECTIN.**

M. Berodes, E. Cavalier, L. Lutteri, P. Lukas, V. Castiglione

1Department of Clinical Chemistry, University of Liège, CHU Sart-Tilman, 4000 Liège, Belgium

**BACKGROUND-AIM**

Concentration of faecal calprotectin reflects the number of neutrophils present in the stool and therefore provides an indication of the severity of the inflammation of the gut. Currently, although this test is not specific, noninvasive measurement of fecal calprotectin is considered a useful screening and monitoring tool to differentiate inflammatory bowel disease (IBD) from irritable bowel symptom (IBS). The aim of this study was the analytical validation of the IDS Calprotectin immunoassay and to compare the results of this new kit with the Diasorin Liaison assay on a clinically defined population of patients.

**METHODS**

Precision was evaluated on 5 native samples measured 5 days in pentaplicate. Specificity and sensitivity have been done using the concordance between clinic and IDS values. A lot to lot comparison was performed. 229 Calprotectin extracts from patients with IBD or not, across the range, have been used to compare IDS calprotectin and DiaSorin Liaison Calprotectin. Stability at 2-8°C was evaluated for 7 days and stability at -20°C for 14 weeks.

**RESULTS**

Inter-assay coefficients of variation (CV) were 1.9%, 2.9-3.0% and 1.8-2.9% negative, equivocal and positive patients, respectively. Intra-assay CV was 2.6%, 3.5-3.8%, 2.5-4.1% for negative, equivocal and positive patients, respectively. The clinical performance study met the acceptance criteria for the diagnostic sensitivity $\geq 85\%$, and specificity $\geq 80\%$. Lot to lot comparison was $\text{Lot1} = 3.4 + 1.0 \times \text{Lot2}$ ($p=0.02$). The regression equation for comparison was $\text{IDS} = -5.6 + 1.09 \times \text{DiaSorin}$ ($p=0.08$). Chi-Carre test was performed to know if patients classification (equivocal, positive, negative) changed, IDS detected more positive patients than Diasorin ($p<0.0001$). Calprotectin was stable up to 4 days at 2-8°C and up to 4 weeks at -20°C.

**CONCLUSIONS**

IDS Calprotectin showed excellent performance and very good concordance with Diasorin Calprotectin, with no significant deviation. IDS Calprotectin detected significantly more positive patients than Diasorin Calprotectin.
Gastrointestinal diseases, including hepatic and pancreatic diseases

EFFECT OF DIFFERENT DIETARY FRUCTOSE CONCENTRATIONS ON GALLSTONE FORMATION IN MICE

R. Del Pozo 1, L. Mardones 1, M. Villagran 1, K. Muñoz 1, M. Muñoz 1

1Department of Biological Science, Faculty of Medicine, Universidad Catolica de la Santisima Concepcion, Concepcion, Chile.

BACKGROUND-AIM

Little information is available on the effect of fructose on bile lipids. Biliary cholesterol is transported mainly by vesicles and micelles. The first stage in the formation of gallstones corresponds to biliary cholesterol crystallization, derived from the vesicular transporters. The aim of this study was to investigate the influence of consuming different fructose concentrations diets on serum lipids, and assess their implications in gallstones formation.

METHODS

The experimental design was quantitative. We use BALB/c mice. Control groups and treated with different fructose concentrations (10%, 30%, 50% or 70%) and for different periods (1, 2 or 5 months) were arranged. After animal treatment, the animals were sacrificed, and blood, liver and bile samples were obtained. We determined serum glucose and the corresponding lipid profiles. In bile samples, cholesterol and phospholipids levels were analyzed, and cholesterol transporters (vesicles and micelles) were separated by gel filtration chromatography.

RESULTS

Treated animals showed: 1) increases in body weights similar to the control group; 2) a significant decrease in plasma triglycerides only at very high fructose concentrations; 3) a significant increase in total serum cholesterol in the treatment for 1 month, but they were normalised after 2 months treatments at very high fructose concentrations; 4) no variations in HDL-cholesterol concentrations at low and high fructose concentrations; 5) a significant increase in serum glucose only at very high fructose concentrations in the second month of treatment; 6) no differences in the plasma alanine-aminotransferase activity; 7) a significant increase in liver triglyceride levels only at very high fructose concentrations; 8) no change in biliary lipids; 9) no change in vesicular and micellar phospholipids.

CONCLUSIONS

Diets with low or medium fructose concentrations did not alter body weight, plasma lipid concentrations, glycemia, liver lipid composition, or biliary lipid concentrations and biliary cholesterol transporters. Changes in plasma, liver and bile lipids were only observed at very high fructose concentrations diets. We conclude that fructose apparently does not alter the gallstone formation process in our experimental model.
Gastrointestinal diseases, including hepatic and pancreatic diseases
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ANALYSIS OF CRITICAL TRIGLYCERIDE VALUES AND ACUTE PANCREATITIS IN A COHORT OF SPANISH PATIENTS
M. Díez Blanco 1, C. Pérez Barrios 1, M. Valle González 2, E. Donoso Navarro 1, F.A. Bernabeu Andreu 1, N.M. García Simón 1, I. Ortiz Zafra 1, A.M. Roldán Cabanillas 1, M. Marín Martínez 1, J. Vega Benjumea 1, I. Del Águila Barrado 1
1Clinical Biochemistry Laboratory, Hospital Universitario Puerta de Hierro, Majadahonda, Spain
2GMPH Student, Imperial College, London, United Kingdom

BACKGROUND-AIM
Elevated triglycerides are deemed the third cause of acute pancreatitis after biliary lithiasis and alcohol intake, increasing the risk of acute pancreatitis from 1% to 14%. The pathophysiology of acute pancreatitis attributed to hypertriglyceridemia is unclear but the main purported mechanism is pancreatic damage caused by high free triglycerides levels due to albumin saturation. According to the guidelines of the Spanish Society of Laboratory Medicine (SEQC-ML), followed by our center, triglyceride values over 880 mg/dL can be critical and should be reported to the clinician to ensure that appropriate actions are taken.

The aim of this observational retrospective study is to describe patients triglyceride values over the critical threshold (≥ 880mg/dL) retrieved between January 2019 and December 2019 as well as describing how triglyceride levels are distributed according to different related factors, such as age, sex and associated pathologies.

The secondary aim of this study is to evaluate the distribution and frequency of pancreatitis in this cohort of patients, to compare them with data from previous studies.

METHODS
For this observational retrospective study, patient's records with critical serum triglyceride levels collected and measured during 2019 using spectrophotometry were retrieved from Servolab software. Duplicates were removed and remaining patient electronic health records were collated to create a data set. Statistical analysis was performed using MedCalc 8.0.2.0.

RESULTS
A total of 111 patients with critical triglyceride values were observed during 2019. Within this cohort, 73 patients received primary care and 25 received specialist care, being predominant in the latter group oncology and rheumatology services (5 patients and 4 patients respectively). The remaining 13 patients were hospitalized. Most patients were male (78.38%; n=87) within age group 50 to 60 (35.14%; n=39) and with a median age of 54 years (IQR=62-48 years).

Regarding triglyceride values, ranged from 881 to 4062 mg/dL, with a median of 1129.5 mg/dL (IQR=1432.5-973.5). The associated pathologies with the highest proportion of elevated triglycerides in our cohort included type 2 diabetes mellitus (DM2) (n=43), alcohol abuse (n=28), obesity (n=16) and to a lesser extent kidney failure (n=5), acute hepatitis (n=2), kidney transplant (n=1) and lupus erythematosus (n=1). In terms of diagnosis, 45.95% of all patients (n=51) were diagnosed with hypertriglyceridemia, 26.13% (n=29) with dyslipidemia, 18.92% (n=21) with mixed dyslipidemia and 2.70% (n=3) with only hypercholesterolemia.

With respect to blood tests, it could be observed that 88.28% of the patients (n=91) had concomitant hypercholesterolemia. Furthermore, 50.45% of the men had low levels of HDL and only 15.31% of women. It is important to note that LDL could not be calculated using the Friedewald formula, due to the high triglyceride content. Regarding treatment, almost half of all patients were treated with fibrates (49.55%), 29.73% with statins, 9.01% with omega-3 acids and 7.21% with ezetimibe.

To conclude, six patients suffered from pancreatitis in the last few years (5.40%). Interestingly, all of them were men, and none of them died due to this illness.

CONCLUSIONS
Critical triglycerides values are mainly observed amongst men, 50 to 60 years old, presenting hypercholesterolemia, as well as associated pathologies such as DM2, obesity and alcoholism, retrieved mainly from primary care records. The estimated frequency of pancreatitis among the sample of individuals with hypertriglyceridemia in our study was 5.40%. Although the sampling method was not probabilistic, results obtained are quite consistent with previous studies (S Ian Gan et al.; 2006, Andres Geruld et al.; 2021, Søren Schou Olsen et al.; 2021), showing that despite the potential biases associated with our methodology, it appears that the sample is quite representative.
Gastrointestinal diseases, including hepatic and pancreatic diseases

ABSENCE OF LOW VIRAL LOAD STAGES QUANTIFICATION IS A CONTINGENCY IN HCV PATIENTS

N. Funel 1, C. Fornai 1, P. Isola 1, I. Lanini 1, P. Petricci 1, A. Buriani 1, V. Lattaro 1, G. Lombardi 1, E. Stenner 1

1Dipartimento delle Diagnostiche, Laboratorio Analisi Chimico Cliniche, Ospedale di Livorno, Italy

BACKGROUND-AIM

The quantifications of hepatitis C virus (HCV) is performed routinely in clinical pathology laboratories through well established automated procedures. The request of clinicians is to quantify the viral load (VL) of patients, in order to guide their clinical management. Indeed, to assess the VL during the management of clinical treatment (i.e. antiviral therapy), represents the gold standard point of medical treatment. Nevertheless, the success of antiviral treatment is demonstrated by absence VL.

HCV patients with complete clinical response (CCR) seem to be the 95% of them. So far, very low percentage of patients with low viral load stages in diagnostic tests is observed.

To understand if the lower limit of quantification (LLoQ) might represent the cornerstone between the performance of the instrumentation and the reality of the clinical management of the HCV patient, we compared two methods of Real-Time PCR for quantification of VL in HCV patients.

METHODS

Sixty-six plasma samples from HCV patients were analyzed. This HCV cohort included 20 negative (virus not detected) cases. A lower Log10 series were obtained by 1:10 dilution from a pool of samples showing the highest HCV quantification 7.50 Log10(IU/ml), was use as referral point. The Cobas AmpliPREP (CAP/CTM; Roche) and COBAS 6800 (C6800; Roche) platforms were used in this study. Deming regression and Paired T-test analyses were performed in order to compare the results.

RESULTS

All sample were analyzed in both platform. No significant differences were observed comparing the two systems (p=0.0001). The VL quantification of HCV samples was detected in less than 30% of samples. Looking in the positive samples, the mean value of VL was 6.59 Log10 (IU/ml) (CI 5.27 Log10 IU/ml – 7.38 Log10 IU/ml) and no one VL was observed under 5.00 Log10 (IU/ml).

CONCLUSIONS

The therapeutic approach against HCV infection showed a great performance in clinical practice. However, this positive feedback opens the question of whether the laboratory platform has shown the real clinical situation or fails in its systematic quantification. Probably the comparison between different methodologies could rely to the following question: How do I do it?
TRANSFERRIN ISOFORMS IN THE DIAGNOSIS OF ACUTE PANCREATITIS

L. Chrostek 1, M. Zaczek 3, E. Gruszewska 1, B. Cylwik 4, A. Panasiuk 2

1Department of Biochemical Diagnostics, Medical University of Bialystok
2Department of Clinical Medicine, Medical University of Bialystok and Department of Gastroenterology, Hepatology and Internal Diseases with the Center for Diagnostics and Endoscopic Treatment at the Provincial Welded Hospital in Bialystok
3Department of Gastroenterology, Hepatology and Internal Diseases with the Center for Diagnostics and Endoscopic Treatment at the Provincial Welded Hospital in Bialystok
4Department of Pediatric Laboratory Diagnostics, Medical University of Bialystok

BACKGROUND-AIM

The aim of the study was to assess the changes in the profile of transferrin isoforms in acute pancreatitis (AP) and the possibility of their use in diagnostics.

METHODS

The study group consisted of 20 patients with acute pancreatitis, aged 30 to 77 years. The patients were admitted to the Department of Gastroenterology, Hepatology and Internal Diseases with the Center for Diagnostics and Endoscopic Treatment. The control group consisted of 20 healthy subjects from the Occupational Medicine Clinic, aged 21-54. Capillary electrophoresis was used to determine the profile of transferrin isoforms.

RESULTS

A significant decrease in the concentration of total transferrin (median in the control group - 2.91 g/l and median in the tested group - 1.79 g/l, P<0.001) and the changes in its isoforms profile have been demonstrated. The concentration of tetrasialotransferrin was significantly higher (median: 81.85%, range: 74.6-84.3) and pentasialotransferrin lower in the study group (median: 12.55%, range: 11.2-19.9) compared to the control group (median for tetrasialotransferrin: 78.15%, range: 65-84.7 and median for pentasialotransferrin: 17.2%, range: 11.1-32.8)(P=0.004 for both comparisons). There were no significant differences in the concentration of carbohydrate deficient transferrin (CDT). The CDT threshold value (1.6%), considered an indicator of chronic alcohol abuse, was not exceeded. This could indicate abstinence for more than 2 weeks (the half-life of CDT was 14-17 days) or a cause of the disease other than alcohol abuse. This conclusion is also supported by the lack of correlation between CDT and triglycerides, which are considered as one of the possible mechanisms of pancreatic damage in alcohol abuse. There was also no increase in the concentration of bile acids. It is known that an increase in circulating bile acids occurs in extrahepatic jaundice. Noteworthy is the increase in bilirubin concentration, which could indicate a cholestatic etiology of the disease. Summarizing, the analysis of biochemical parameters allows to direct the diagnosis towards cholestatic etiology with intrahepatic jaundice.

CONCLUSIONS

The obtained results indicate that the measurement of transferrin isoform along with other laboratory tests may be useful in the diagnosis of acute pancreatitis.
Gastrointestinal diseases, including hepatic and pancreatic diseases

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DIAGNOSTIC POWER OF SERUM ALKALINE PHOSPHATASE ISOENZYMES IN PATIENTS WITH PRIMARY BILIARY CHOLANGITIS

B. Cylwik 3, A. Bauer 2, E. Gruszewska 1, A. Grytczuk 3, B. Zelazowska-Rutkowska 3, L. Chrostek 1

1Department of Biochemical Diagnostics, Medical University of Bialystok
2Department of Clinical Biochemistry and Molecular Biology, Center of Postgraduate Medical Education, Warsaw
3Department of Pediatric Laboratory Diagnostics, Medical University of Bialystok

BACKGROUND-AIM

Primary biliary cholangitis (PBC) is a chronic inflammatory autoimmune cholestatic liver disease. The majority of patients are asymptomatic or present characteristic but not specific clinical symptoms, therefore PBC is often suspected because of abnormal laboratory findings, particularly elevated serum alkaline phosphatase (ALP) activity. The aim of the study was to evaluate the diagnostic power of serum total ALP and its two isoenzymes - liver (ALP-L) and bone (ALP-B) in PBC. We hypothesized that ALP isoenzymes in patients would be more effective for diagnosed as PBC.

METHODS

To test our hypothesis, isoenzymes of ALP were separated by agarose electrophoresis using an INTERLAB G26 Easy Fix analyzer and receiver operating characteristic (ROC) analysis was applied to assess their diagnostic sensitivity, specificity and prognostic values in 65 PBC patients and 38 healthy controls.

RESULTS

We found that the mean total ALP activity and percentage of ALP-L isoenzyme were significantly elevated, whereas the percentage of ALP-B fraction was decreased in PBC patients in comparison to the control group (172 vs. 58 IU/L, p < 0.001; 83.7 vs. 45.3%, p < 0.001; 15.2 vs. 54.7%, p < 0.001, respectively). Both ALP-L and ALP-B correlated with PBC histological stage (r = 0.321, p < 0.001 and r = -0.310, p = 0.011). ALP-L had a lower diagnostic sensitivity in the detection of PBC than total ALP activity (80.0 vs. 87.3%), but its specificity was higher (94.7 vs. 92.7%). ROC analysis revealed that the area under curves (AUCs) for total ALP activity and ALP-L were comparable to each other and had excellent diagnostic power (0.923, 0.952, respectively).

CONCLUSIONS

The ALP-L fraction was found to have higher diagnostic power and specificity than total ALP activity and to be a better diagnostic tool for PBC in some asymptomatic patients, especially those with normal total ALP activity.
ROLE OF PANCREATIC STONE PROTEIN AS AN EARLY BIOMARKER FOR RISK STRATIFICATION OF ACUTE PANCREATITIS

C. Rodríguez-Rojas, L. García De Guadiana-Romualdo, S. Morán Sánchez, J. Prazak, V. Algara Soriano, Y. Que, R. Benninga, M.D. Albaladejo Otón

1Abionic SA
2Department of Intensive Care Medicine, Inselspital; Bern University Hospital, University of Bern
3Gastroenterology Department, Hospital Universitario Santa Lucía
4Laboratory Medicine Department, Hospital Universitario Santa Lucía, Cartagena, Spain.

BACKGROUND-AIM

Acute pancreatitis (AP), the most common gastrointestinal diagnosis leading to hospitalization, is associated with high mortality and morbidity rates, especially when associated with severe local and organ dysfunction. Early intensive management of acute pancreatitis has been shown to improve outcomes, requiring an early and accurate identification of patients at risk of developing severe complications. Several laboratory tests (C-reactive protein (CRP), urea, procalcitonin (PCT) and hematocrit) and multifactorial scoring systems, combining clinical and analytical findings, have been proposed and are currently used for this purpose. However, laboratory tests and scoring systems have demonstrated suboptimal accuracies in terms of early prediction of complications so far and new predictors tools are needed.

We aimed to evaluate the performance of PSP, a protein secreted by the pancreas in response to stress induced by systemic infection and sepsis, in predicting the severity of acute pancreatitis, in comparison to traditional biomarkers and severity scores recommended for this purpose.

METHODS

This was a prospective, observational and single-center study enrolling consecutive patients ≥ 14 years admitted to the ED within 72 h from onset of symptoms for a suspicion of acute pancreatitis. Final diagnosis was established according to national recommendations.

On serum sample collected upon Emergency Department admission, biomarker levels, including PSP, were measured and severity scores (SOFA, PANC-3 and BISAP) were computed.

According to Determinant-Based Classification (DBC) criteria patients were classified into two groups: non-severe (mild and moderate AP) and severe AP (SAP).

Area under the curve (AUC) and regression analysis were used to analyze the discrimination abilities and the association of biomarkers and scores with severity.

RESULTS

Study population included finally 268 patients (median age: 69 years; Interquartile range [IQR]: 52–78, 51.9% (n = 139) male). Thirty-three patients (12.3%) were classified as SAP. The biliary AP was the most common etiology (78.4%).

PSP levels were increased in patients with severe AP (Median [IQR]: 890 µg/L [559–1142] vs. 279 µg/L [141–496]; p < 0.001) and it was the best predictor (ROC AUC: 0.827 [95% CI 0.776–0.870]; p < 0.001) for SAP. No significant differences were found for other tested biomarkers (CRP, procalcitonin, and hematocrit). A cut-off point of 344 µg/L achieved a sensitivity and specificity of 87.9% and 67.2%, respectively. In multivariable regression analysis and after adjusting for confounders, urea (Odd ratio [OR]: 1.022 [95% CI 1.009–1.036]; p = 0.001) and PSP levels (OR = 1.000 [95% CI 1.000–1.001]; p = 0.056) were the only biomarkers independently associated with SAP. A model combining them both (“biomarker model”) showed an AUC of 0.841 (95% CI 0.791–0.882) for prediction of SAP, showing a trend to be superior (p = 0.072) in comparison with BISAP (0.755; 95% CI 0.699–0.805). Both PANC-3 and SOFA scores achieved significantly lower performance than the “biomarker model,” with ROC AUCs of 0.655 and 0.582, respectively.

CONCLUSIONS

The present study suggests that PSP, in combination with urea, is a promising biomarker to predict early the severity of AP. PSP could be incorporated in an early management protocol to identify patients who might require more aggressive management.
THE PROFILE OF SERUM TRANSFERRIN ISOFORMS IN LIVER CANCER

E. Gruszewska, M. Żaczek, B. Cylwik, A. Panasiuk, B. Mroczko, L. Chrostek

1Department of Biochemical Diagnostics, Medical University of Bialystok
2Department of Biochemical Diagnostics, Medical University of Bialystok; Department of Neurodegeneration Diagnostics, Medical University of Bialystok
3Department of Clinical Medicine, Medical University of Bialystok; Department of Gastroenterology, Hepatology and Internal Diseases with Center for Endoscopic Diagnostics and Treatment, Voivodeship Hospital in Bialystok
4Department of Gastroenterology, Hepatology and Internal Diseases with Center for Endoscopic Diagnostics and Treatment, Voivodeship Hospital in Bialystok
5Department of Pediatric Laboratory Diagnostics, Medical University of Bialystok

BACKGROUND-AIM
The occurrence of alterations in proteins glycosylation in liver diseases is well known, also in liver cancer. The aim of this study was to assess the changes in the profile of serum transferrin isoforms in liver cancer and the possibility of their use in diagnostics.

METHODS
Serum samples were obtained from 30 patients with liver cancer (LC) (11 females and 19 males) (mean age: 67±10.7 years), 25 patients with non-alcoholic cirrhosis (NAC) (16 females and 9 males) (mean age: 61.1±17.4 years), as an example of non-cancerous liver disease, and 30 healthy subjects (14 females and 16 males) (mean age: 29.5±10 years). Capillary electrophoresis method was used to determine the profile of transferrin isoforms, while total transferrin concentration was determined by immunoturbidimetric method.

RESULTS
Serum concentration of total transferrin in patients with LC (median: 1.9 g/l, range: 1.06-3.19) was significantly lower in comparison to healthy subjects (median: 2.49 g/l, range: 1.68-3.26) (P<0.001). There was a significant increase in the concentration of tetrasialotransferrin (median: 82.1%, range: 71.7-85.5), and significant decrease in the pentasialotransferrin (median: 14.65%, range: 11.8-22.1) in LC patients in comparison both with healthy subjects (median for 4sialo-TRF: 78.15% range: 65-84.7; median for 5sialo-TRF: 17.2%, range:11.1-32.8) (P=0.005 and P=0.036; respectively) and patients with NAC (median for 4sialo-TRF: 78.65%, range: 70.3-83; median for 5sialo-TRF: 16.5%, range: 12.2-26) (P=0.001 and P=0.040; respectively). Moreover, the concentration of trisialotransferrin was significantly higher in NAC patients (median: 4.5% range: 1-9.4) than in the LC patients (median: 2.5%, range: 0.8-12.1) (P=0.003) as well as to healthy subject (median: 3.65%, range: 1.6-5.6) (P=0.027). There were no significant changes in the disialotransferrin concentration in liver cancer patients.

CONCLUSIONS
We conclude that profile of transferrin isoforms in liver cancer is altered and specific. Our data indicate that analysis of serum transferrin isoform profile may be useful tool in the diagnosis of patients with liver cancer.