ABSTRACTS

Poster presentation

PP-1. Case Report: Alpha-fetoprotein-producing lung adenocarcinoma

Bauça JM, Robles J, Morell D, Fuego L, García A, Barceló A

BACKGROUND Alpha-fetoprotein (AFP) is usually elevated in patients with liver cancer or gonadal germ cell tumors. Some cases of extrahepatic tumors able to produce AFP have been reported, such as ovarian and gastric. However, AFP-producing lung malignancies are rare. CASE PRESENTATION: We present a 60-year-old woman, active smoker, who was referred to the oncologist for neoadjuvant chemotherapy. After biochemical testing yielded an AFP of 9796 ng/ml, whereas all other magnitudes were within normal ranges. Thoracic CT showed a mass at the superior lobule of the right lung. Subsequent bronchial biopsy confirmed the diagnosis of an extensively necrotized undifferentiated carcinoma (T3/4N0M0). The patient was dismissed surgical procedure. Instead, chemotherapy was changed. No further AFP analyses were performed beyond this point. After several weeks, and 2484 ng/ml after 5 weeks. Control CT showed a reduction of the lung mass. Two months later, an upturn of AFP levels (4256 ng/ml) together with TC images compatible with mediastinum affectation, dismissed surgical procedure. Instead, chemotherapy was changed. No further AFP analyses were performed beyond this point. After several episodes of respiratory failure, asthenia and great worsening of general condition, palliative care was started. The patient finally died. DISCUSSION: Serum alpha-fetoprotein is a useful tumor marker in patients with hepatocarcinoma. Due to scarce bibliography, pathophysiological and clinical features of AFP-producing lung tumors are not well defined yet. As is the case for AFP-producing gastric cancers, this type of lung cancers might show higher propensity for metastasizing to the liver and lymph nodes, as well as poorer prognosis compared to non-AFP-producing cancers.

Further laboratory analysis showed a plasma ACTH of 309 pg/mL (normal 1–46), serum cortisol 41.2 μg/dL (normal 3.7–19.4) and urine cortisol 5327 μg/24h (normal 176), but normal aldosterone (68 pg/mL; normal 40–300). All these results agreed with an enlargement of the adrenal glands, also described in the X-ray. All measured tumor markers (CEA, CA 125, CA 19-9, alpha-fetoprotein, NSE, SCC) were normal. One large retrospective study found that 1.7% SCLC patients develop paraneoplastic syndromes due to ectopic secretion of ACTH, which was associated with a low response rate to chemotherapy and a worse overall survival. In this case, the altered ACTH-cortisol profile was the only laboratory result pointing towards this cancer, as no tumor marker was altered enough to reveal the disease.

PP-3. EGFR mutations in lung carcinoma: Real time PCR method

Muñoz-Colmenero A1 (pocahontas_j@hotmail.com), Ocaña-Pérez E1, Dueñas-García R2, Fernández-Núñez A3

1 UGC Laboratorio. Complejo Hospitalario de Jaén. Spain; 2 UGC Oncología. Complejo Hospitalario de Jaén. Spain; 3 Área de Biotecnología. Agencia Sanitaria Alto Guadalquivir Andújar Spain.

INTRODUCTION: EGFR, the epidermal growth factor receptor, is a cellular transmembrane receptor. This receptor is expressed across a number of different tumour types, including non-small cell lung cancer (NSCLC). Mutations in the tyrosine kinase domain of the EGFR gene increase the activity of pro-survival intracellular signalling cascades. EGFR mutation testing in advanced NSCLC patients helps physicians to prescribe the most appropriate treatment for each patient. EGFR mutations are found in exons 18 to 21 of the EGFR gene, which is part of the gene coding for the tyrosine kinase domain of the EGFR protein. MATERIALS AND METHODS: Sixty-six formalin-fixed paraffin-embedded tumours from patients diagnosed with NSCLC were analyzed by Cobas EGFR Mutation Test. All patients had been tested as part of standard clinical practice. Patient and tumour characteristics, such as gender, smoking status, histology were analyzed. DNA was isolated from paraffin-embedded samples with Cobas® DNA kit Sample Preparation. The cobas® EGFR Mutation Test kit is a Taq-Melt™-based PCR assay to detect somatic mutation in exon 18, 19, 20 and 21. RESULTS: Sixty-six patients diagnosed with NSCLC were included in the study. Of 66 patients, 8 were female and 58 were male (6 non-smokers, 15 smokers, 24 former smokers, 21 unknown habit). The histological diagnosis was adenocarcinoma in 31 patients (49.9%), squamous cell carcinoma in 17 patients (25.7%) and large cell carcinoma in 6 patients (9.1%). In 12 cases (18.2%), the histological diagnosis was unknown. EGFR mutations were detected in 61% of samples. CONCLUSION: The prevalence of mutations in EGFR identified a positivity rate of 6.1%, which was lower to that described in earlier studies conducted on advanced NSCLCs. The lowest percentage of mutated cases found in the present study may be due to the low number of women as well as the low percentage of non-smokers patients and the high percentage of “non adenocarcinoma” histology NSCLCs included in our study.
PP-5. Selective small molecule AXL inhibitor BGB324 overcomes acquired drug resistance in non-small cell lung carcinoma models

Wnuk-Lipinska K, Gausdal G, Sandal T, Frink RE, Hinz S, Hellerosy M, Ahmed L, Haugen H, Liang X, Blo M, Micklem DR (david.micklem@bergenbio.com), Minna JD, Brekenra R, Zhou L, Lorens JB

AXL is a member of the TAM (Tyro3, Axl and Mer) family of receptor tyrosine kinases that regulate multiple cellular responses including cell survival, proliferation, and migration. Axl expression is associated with a variety of human cancers including non-small cell lung carcinoma (NSCLC), and is predictive of poor patient overall survival. Axl is induced by the epithelial-to-mesenchymal transition (EMT) gene program in cancer cells. Axl signaling is required to maintain EMT-associated features including invasiveness, metastasis, and can confer resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), as well as other chemotherapeutic agents. BGB324 is a potent, reversible and selective small molecule inhibitor of Axl that has recently entered into phase I clinical trials. In this study, we evaluated the effects of BGB324 on NSCLC cells in vivo using 3D assays and in mouse xenograft models, in combination with targeted and chemotherapeutic agents. BGB324 in combination with EGFR TKI erlotinib demonstrated a synergistic anti-proliferation effect on NCI-H1299 (mesenchymal, EGFR wild-type, erlotinib-resistant) human NSCLC cells in 3D culture.

In mouse xenograft models using NCI-H1299 cells BGB324 treatment significantly enhanced the antitumor activity of Docetaxel. Similarly, in HCC827 (EGFR mutant, erlotinib-sensitive) human NSCLC xenograft model, addition of BGB324 treatment strikingly delayed the emergence of acquired resistance to erlotinib. Taken together, these data suggest the first-in-class selective Axl inhibitor BGB324 can overcome acquired resistance in vivo models of NSCLC. Studies in human NSCLC patient-derived xenograft models and mechanisms of action are in progress.

PP-6. Novel Multiplex Cancer Biomarker assay in gastrointestinal cancers

Dressen K1, Hermann N1, Standop J2, Walgenbach-Bruenagel G1, Schildberg FA3, Hettwer K4, Uhlig S4, Kalff JC2, Holdenrieder S1

1Institute of Clinical Chemistry and Clinical Pharmacology, 2Department of Surgery, 3Institutes of Molecular Medicine and Experimental Immunology, University Hospital Bonn and 4QuoData Statistics, Dresden, Germany

Background: A novel multiplex cancer biomarker assay that enables the parallel assessment of 24 tumor, cell death, immunological and angiogenesis serum biomarkers was evaluated on its diagnostic performance for gastrointestinal cancer detection.

Materials and Methods: Serum samples of 231 individuals comprising 107 patients with gastrointestinal cancers (20 gastroesophageal, 35 colorectal, 19 liver, 14 pancreatic, 19 cholangiocellular), 73 with respective benign gastrointestinal diseases, and 51 healthy controls were analyzed by the Milliplex® MAP Human Circulating Cancer Biomarker Magnetic Bead Panel run on the Bio-Plex® 200 System. Prior to the clinical evaluation, intra- and interassay imprecision and preanalytical robustness of the method were assessed.

Results: Methodical quality was found to be good for most markers (with intra- and interassay imprecisions <20%). In the clinical evaluation, many biomarkers discriminated significantly between healthy persons and malignant diseases while only a few - mostly the established tumor markers - were able to significantly distinguish malignancies from the corresponding group of benign diseases. Particularly the immunological and cell death markers were elevated in benign diseases as well. When comparing malignant and benign diseases for differential diagnosis, best discriminating markers in colorectal cancer were CEA, CA 19-9, CA 125, CYFRA 21-1, IL-8 and OPN reaching AUCs in ROC curves of 0.86, 0.74, 0.71, 0.74, 0.74, and 0.70 respectively. In liver cancer, AFP (0.81) clearly achieved the best diagnostic performance, in pancreatic cancer CA 19-9 (0.82) and in cholangiocellular cancer CA 19-9 (0.80) and OPN (0.83). In gastroesophageal cancer, no marker was able to distinguish malignant from benign lesions.

Conclusions: Biomarker multiplexing enables the parallel analysis of different biomarker classes providing a pattern of established and new parameters for cancer detection.
Materials and Methods: Prospective, observational study on patients between January and March 2013. There were collected epidemiological variables, family history, values of CEA and FOBT.

Results: There were collected serum and faeces samples from 111 patients, av. age 64.2 ± 1.37, 53% women. Neg. values: CEA < 3.5 ng/mL. FOBT < 50 ng/mL. 8.1% of patients had, as a definitive diagnosis, CRC, located in the rectum (n=6), in the descending colon (n=2), and in the ascending colon (n=1); only 5 of them adenocarcinoma.

Colonoscopy was available in 66.6% of patients with CRC, 39 patients with negative CRC diagnosis, whereas 63 were still awaiting a colonoscopy.

In patients with CRC, high CEA levels (>3.5 ng/mL) were detected in 44.4% of them, whereas high FOBT levels (>50 ng/mL) were detected in 77.8%. In these, average values of CEA (3.80 vs 6.14 ng/mL) and FOBT (360.1 vs 545.2 ng/mL) were lesser in women.

In absence of CRC, high values of FOBT and CEA were due to haemorrhoids (25% and 41.7%), polyps (42.9% and 57.1%) and diverticula (28.6% and 14.3%).

Conclusions: High FOBT values (>50 ng/mL) suggest CRC in 3 out of 4 patients, whereas high CEA levels (>3.5 ng/mL) only differentiates 1 out of 2 in case of confirmatory colonoscopy.

Pathological levels of CEA and FOBT are related to CRC, but further studies are needed, by increasing the sample size in order to establish the real value from which they can become combined markers with high sensitivity and specificity.

PP-8. 25-hydroxyvitamin D deficiency is associated with increased interleukin-6 in patients with suspicion of colorectal cancer

Fernández-Suárez A1, Fatela Cantillo D1, Elorza Maza G3, Ocaña Pérez E2, Muñoz Colmenero A3, Díaz Iglesias JM1.

1Department of Biotechnology, Hospital Alto Guadalquivir; Andújar (Jaén), Spain; 2 Unidad de Gestión de Laboratorio y Alergia, Área de Inmunología, Complejo Hospitalario de Jaén, Spain; 3 Laboratory, Hospital de Alta Resolución Sierra de Segura, Puente de Génave (Jaén), Spain.

Background: Vitamin D has the ability to inhibit cell proliferation, to stimulate transcription of the tumour suppressor gene E-cadherin and reduce the transcriptional activity of proto-oncogene β-catenin. 25-hydroxyvitamin D [25(OH)D] is the precursor of active form of vitamin D. Interleukin-6 (IL-6) is a pleiotropic inflammatory cytokine, with a role in growth stimulation, metastases, and angiogenesis in a variety of malignancies.

Objective: The main aim of this study was to investigate the relationship between 25(OH)D and IL-6 in patients with suspected colorectal cancer (CRC). Secondary objectives were: to determine the 25(OH)D potential usefulness to calculate the CRC risk in a symptomatic patients cohort, and the CRC patients prognosis (survival).

Material and Methods: The study included all consecutive patients [April 2008 to July 2010; n=182, mean age 62.1 years (20-93), 51.6% women] undergoing diagnostic colonoscopy with a high CRC clinical suspicion. The mean follow-up was 23.91 months [standard deviation (SD) 14.09]. 25(OH)D (ng/mL) and IL-6 (pg/mL) were measured by ECLIA (cobas® 8000, e601 module; Roche Diagnostics). 25(OH)D deficiency was defined like concentrations less than 20 ng/mL.

Results: 33 polyps, 66 other benign diseases, 5 other non-CRC cancers and 36 patients did not demonstrate any remarkable pathology. There were no significant seasonal differences (p=0.294) in the 25(OH)D concentration. The lowest levels of 25(OH)D were found in the CRC group (mean±SD: 10.75±6.71). 25(OH)D correlated significantly with IL-6 (Spearman’s rho=-0.213, p=0.004). The estimated risk of developing CRC when the 25(OH)D<20 ng/mL was 3.416 (95%CI, 1.308-7.570, p<0.004). In the univariate Cox analysis was obtained an RR (survival) of 0.340 (95%CI, 0.101-1.143, p=0.081).

Conclusions: The 25(OH)D plasma levels are inversely associated with serum IL-6. The 25(OH)D deficiency increase the risk of CRC.

PP-9. Clinical value of the measurement of carbohydrate antigen CA19-9 in biliopancreatic cancer in the presence of cholestasis

Gaspar Blázquez MJ1, Arévalo-Serrano J1, Esteban G2, Diez Alonso M2, Coca C2, Trapú J3.

1 Clinical Biochemistry and 2 Internal Medicine and Surgery Departments. University Hospital Getafe. Príncipe de Asturias University Hospital. Alcalá de Henares. Madrid, Spain. 3Athaia Xarxa Asistencial Universitaria de Manresa Barcelona (Spain)

Introduction: Carbohydrate antigen CA 19.9 is a complex glycoprotein. It is of use clinically in the study of gastrointestinal neoplasias and is the marker of choice in cancer of the pancreas and biliary tree. A rise in CA 19.9 concentrations can be detected principally in the serum of patients with liver diseases associated with cholestasis and in patients with pancreatitis.

Objective: The aim of present study was to analyze the diagnostic value of CA19.9 in patients with carcinoma of the biliopancreatic cancer with obstructive cholestasis.

Material and methods: 128 patients with obstructive cholestasis (Total Bilirubin > 3 mg/dl) were included in the study all of whom had neoplastic infiltration of the biliary drainage causing cholestasis; 76 had cancer of the biliary tree or pancreatic cancer and 52 patients had benign bilohepatic diseases associated with cholestasis. Simultaneous measurement of CA 19.9, total bilirubin (TB) and direct bilirubin (DB) were performed. All samples were processed in the Biochemistry Laboratory of the Príncipe de Asturias Hospital. An electrochemiluminescence immunoassay performed on the Immulite 2000 analyser was used to measure the CA 19.9 concentration. The statistical analysis was performed using non-parametric tests as the CA 19.9, TB, and DB results were not normally distributed.

Results: Medium serum levels (and interquartile range) for the three parameters were : CA19.9 - 204 (194) UI/L, TB - 8.2 (9.6) mg/dl, DB - 4.6 (5) mg/dl for those with malignant conditions and CA 19.9 66.3 (128) UI/L, TB 5.5 (6.3) mg/dl, DB 2.6 (3.7) mg/dl for those with benign conditions. All parameters were significantly higher in patients with malignant disease, CA19.9 (p=0.010), TB (p=0.034), DB (p=0.001). No statistically significant correlation were detected between levels of CA 19.9 and BT or between CA 19.9 and BD in patients with or without neoplasia. As a diagnostic test of malignancy CA19.9 showed an area under ROC curve of 0.634 (IC 95% 0.539-0.730). At a cut-off value of 75 UI/L, CA19.9 showed a sensitivity of 62% and specificity 64%.

Conclusions: The diagnostic value of serum CA19.9 in patients with biliopancreatic cancer and obstructive cholestasis is low.
Introduction Hepatocellular carcinoma (HCC) represents 80-90% of primary malignant liver tumors. Many cases arise from the evolution of viral hepatitis or cirrhosis.AFP is the most common tumor marker, but its diagnostic utility is limited due to its low specificity. Therefore AFP is not able to distinguish HCC from other high-risk liver diseases (chronic hepatitis and cirrhosis) because in all of them, AFP could be elevated. In order to improve the diagnosis, two new biomarkers have been incorporated, α-fetoprotein-L3 (AFP-L3) and des-γ-carboxypromubin (DCP). Objectives: To assess whether the incorporation of combined biomarkers: AFP, AFP-L3 and DCP improves the diagnosis of HCC compared to the unique determination of AFP. Methods: AFP, AFP-L3 and DCP were analyzed in 90 patients, who were attending the Department of Hepatology: 29 with HCC, 21 with chronic hepatitis, 23 who suffered from cirrhosis and 17 with other diseases. In all of them, the combined analysis by immunoassay in microfluidic Wako autoanalyzer was carried out. Results: Sensitivities and specificities are shown in the following table: AFP ALP-L3 DCP FPF +AFP-L3 AFP + ALP-L3 DCP Sensitivity 63% 64% 53% 65% 65% Specificity 89% 88% 81% 83% 75% Plasma levels (ng/mL) of AFP-L3 and DCP ALP in different groups are: AFP 63% 65% 65% 65% Specificity 89% 88% 81% 83% 75% Plasma levels (ng/mL) of AFP-L3 and DCP ALP in different groups are: AFP

Objectives To assess whether the incorporation of combined biomarkers: AFP, AFP-L3 and DCP improves the diagnosis of HCC compared to the unique determination of AFP. Methods: AFP, AFP-L3 and DCP were analyzed in 90 patients, who were attending the Department of Hepatology: 29 with HCC, 21 with chronic hepatitis, 23 who suffered from cirrhosis and 17 with other diseases. In all of them, the combined analysis by immunoassay in microfluidic Wako autoanalyzer was carried out. Results: Sensitivities and specificities are shown in the following table: AFP ALP-L3 DCP FPF +AFP-L3 AFP + ALP-L3 DCP Sensitivity 63% 64% 53% 65% 65% Specificity 89% 88% 81% 83% 75% Plasma levels (ng/mL) of AFP-L3 and DCP ALP in different groups are: AFP 63% 65% 65% 65% Specificity 89% 88% 81% 83% 75% Plasma levels (ng/mL) of AFP-L3 and DCP ALP in different groups are: AFP

Methods: We examined Wester blot, flow cytometric and immunocytochemical analysis to investigate the regulation of fibroblast growth factor receptor 1 (FGFR1) expression by interferon-a/b in several human hepatocarcinoma cell lines. In addition, we studied the efficacy of combined treatment with anti-FGFR1 monoclonal antibody and interferon-a/b in a murine xenograft model of human HCC. Results: Interferon-a/b induces the expression of FGFR1 and a treatment with a combination of interferon-a/b and an anti-FGFR1 monoclonal antibody suppresses HCC cell growth in vitro and in vivo. Conclusions: A combined treatment with an anti-FGFR1 antibody and interferon-a/b is a promising approach to the therapy of HCC.

PP.11. A combination of the anti-fibroblast growth factor receptor 1 monoclonal antibody and interferon-a/b suppresses human hepatic cancer cells in vitro and in vivo

Kato Y (kato@koukai.or.jp), Hiromi H, Iujisaki M, Matsune T, Sasaki S, Hinoda Y, Shinomura Y, Imai K

1.Sapporo Shirakabadi Hospital, Sapporo, Japan; 2.Hirahe Hiromi Clinic, Hakodate, Japan; 3.Inishi Hospital, Sapporo, Japan; 4.Shirakaba Pharmacy, Sapporo, Japan; 5.Sapporo Medical University, Sapporo, Japan and 6.Yamaguchi University, Ube, Japan.

Background: Hepatocellular carcinoma (HCC) is the most commonly occurring primary liver cancer and ranks as the third leading all-cancer mortality. Thus a development of new treatments that improve the prognosis of HCC patients would be highly desirable. Targeting cell surface molecules using monoclonal antibodies is an emerging strategy in cancer therapy. However, to date, only a few pilot studies examining expression of HCC-associated antigens have been carried out. Our aim in this present study is to identify a novel target for antibody therapy against HCC.

Methods: We examined Wester blot, flow cytometric and immunocytochemical analysis to investigate the regulation of fibroblast growth factor receptor 1 (FGFR1) expression by interferon-a/b in several human hepatocarcinoma cell lines. In addition, we studied the efficacy of combined treatment with anti-FGFR1 monoclonal antibody and interferon-a/b in a murine xenograft model of human HCC. Results: Interferon-a/b induces the expression of FGFR1 and a treatment with a combination of interferon-a/b and an anti-FGFR1 monoclonal antibody suppresses HCC cell growth in vitro and in vivo. Conclusions: A combined treatment with an anti-FGFR1 antibody and interferon-a/b is a promising approach to the therapy of HCC.

Introduction: Given the limited impact of conventional factors in colon cancer (CC), it is necessary to identify new prognostic biomarkers. The expression of N-Glycolyl GM3 (NeuGcGM3) ganglioside has been found in a variety of human malignancies suggesting its possible role on the oncogenic process, tumor growth and progression. Aim: To assess the prognostic role of NeuGcGM3 ganglioside, detecting with 14F7 Mab, in patients with colon cancer (CC) and to evaluate the relationship among its expression and clinicopathological features. Methods: Paraffin-embedded specimens were retrospectively collected from 50 patients with CC operated between 2004 and 2008 at National Institute of Oncology, Havana, Cuba. NeuGcGM3 expression was determined by immunohistochemistry technique, using 14F7 Mab, a murine IgG1 highly specific produced by the Center of Molecular Immunology in Havana. The relation of with survival and clinicopathologic features was evaluated. Results: NeuGcGM3 ganglioside expression was detected in all cases. Most cases had high level of immunostaining (70%) that showed statistical correlation with TNM stage (p=0.025). In univariate survival analysis, level of NeuGcGM3 expression (p=0.0078), TNM Stage (p=0.0007) and lymphovascular invasion (0.027) were significant prognostic factors for Overall Survival. Among these variables, level of NeuGcGM3 immunostaining (HR= 0.268; 95% IC 0.078-0.920; p=0.036) and TNM stage (HR= 0.249; 95% IC 0.066-0.932; p=0.039) were independent prognostic factors on multivariate analysis. Conclusions: This study is the first approach on the prognostic significance of NeuGcGM3 expression in patients with colon adenocarcinoma. The assessment of NeuGcGM3 expression might be used in the prognostic estimate of CC, although further studies will be required to validate these findings.

PP.12. Detection of N-Glycolyl GM3 Ganglioside using 14F7 Mab and its role as prognostic biomarker in colon cancer

Labera T (tani.labera@infomed.sld.cl), Calvo A, Torres G, Rengifo CE, Quintero S, Arango MC, Danta D, Vázquez JM, Escobar X and Carr A.

INTRODUCTION: CA 19-9 is commonly used in diagnosis of pancreatic and biliary malignancies because it is a very sensitive and specific marker of these pathologies. However, increased levels in benign conditions has also been reported. This case illustrates an elevated CA 19-9 due to causes other than malignancy.

CASE REPORT: A 69-year-old man came to emergency service with an intensive episode of colic pain and jaundice. For the previous 2 weeks he experienced intermittent abdominal pain with sickness. He reported a weight loss of 12 kg in 3 months. He was previously diagnosed with prostate adenocarcinoma without metastasis in November 2012, which was treated with radiotherapy. Also, he suffers from dyslipemia and hypertension. An abdominal computerized tomography revealed dilatation of all biliary ducts. Medical imaging of the common hepatic duct revealed an image resembling a stone. Multiple stones in the gallbladder were discovered. With a CA 19-9 of 15,000 U/mL it is necessary to make a differential diagnosis between cholelithiasis and ampulloma. An endoscopic retrograde cholangiopancreatography (ERCP) showed no relevant results: mild biliary dilatation and choledocholithiasis. The results of endoscopic sphincterotomy were not suspicious. The patient was reviewed one month later, with his tests results within the normal range: AST= 21 U/L, ALT= 25 U/L, GGT=
PP-14. Analytical performance of two real time PCR assay for the detection of KRAS mutations in tissue samples of colorectal carcinoma

Ocaña-Pérez E (pocahontas_j@hotmail.com)\(^1\), Muñoz-Colmenero A\(^1\), Peña-Casas AM\(^1\), Dueñas-García R\(^2\), Fernández-Suárez A\(^3\).

\(^1\)UGC Laboratorio. Complejo Hospitalario de Jaén. Spain. \(^2\)UGC Oncología. Complejo Hospitalario de Jaén. Spain. \(^3\)Área de Biotecnología. Agencia Sanitaria Alto Guadalquivir. Andújar. Spain.

Introduction: KRAS mutation testing is mandatory before prescribing anti-epidermal growth factor monoclonal antibodies in the treatment of advanced colorectal cancer (CRC). The presence of activating mutations has been shown in randomized clinical trials to play a crucial role in predicting non-responsiveness to anti-EGFR monoclonal antibodies. Mutations in the KRAS gene that lead to its non-responsiveness in patients with advanced CRC who receive the anti-EGFR monoclonal antibodies have been identified in 24% of CRC tumors. Several methods for KRAS testing currently exist, from commercially available CE-marked test kits to laboratory-developed tests but there are limited data to support the performance of individual methods over others.

Materials and methods: A total of 188 samples were analyzed by TherSaScreen® KRAS kit and 111 samples were studied with Cobas® KRAS Mutation Test. DNA was isolated from paraffin-embedded samples with the QIAamp® DNA FFPE Tissue and Cobas® DNA kit Sample Preparation. The TherSaScreen® KRAS kit is a real time PCR assay based on Scorpion technology to detect seven mutations in codons 12 and 13 of the K-RAS oncogene. The cobas® KRAS Mutation Test kit is a Taq-Melt™-based PCR assay to detect somatic mutation in codons 12, 13 and 61. Results: As expected, the percentage of the DNA samples in which mutations were detected varied depending on the method of detection used. KRAS mutations were detected in 27, 12% of samples by TherSaScreen® KRAS kit. The cobas® KRAS Mutation Test detected 48, 64% of samples with KRAS mutations. In both methods, the most frequent mutations were found in codon 12 of KRAS, followed by those found in codon 13. In this study, two mutations were also detected in codon 61, although these mutations could only have been detected in the cobas® KRAS Mutation Test. The percentage of mutated samples found with Cobas KRAS Mutation test is similar to described in the literature.

PP-15. Novel Multiplex Cancer Biomarker assay in gynecological cancers

Hermann N\(^1\), Dressen K\(^1\), Schroeder L\(^1\), Debald M\(^2\), Rudlowski C\(^2\), Walgenbach-Bruenagel G\(^1\), Schildberg FA\(^1\), Hettwer K\(^4\), Uhlig S\(^3\), Kuhn W\(^5\), Holdenrieder S\(^5\).

\(^1\)Institute of Clinical Chemistry and Clinical Pharmacology, \(^2\)Department of Gynecology and Obstetrics, \(^3\)Institutes of Molecular Medicine and Experimental Immunology, University Hospital Bonn, \(^4\)QuoData Statistics, Dresden, Germany

Background: A novel multiplex cancer biomarker assay that enables parallel assessment of 24 tumor, cell death, immunological and angiogenesis serum biomarkers was evaluated on its diagnostic performance for gynecological cancer detection.

Materials and Methods: Serum samples of 353 females comprising 181 patients with gynecological cancers (77 breast, 49 ovarian, 16 endometrial, 22 cervical, 17 vulva), 110 with respective benign gynecological diseases, 26 with precancerous lesions, and 36 healthy controls were analyzed by the Milliplex® MAP Human Circulating Cancer Biomarker Magnetic Bead Panel run on the Bio-Plex® 200 System. Prior to the clinical evaluation, intra- and interassay imprecision and preanalytical robustness of the method were assessed.

Results: Methodological quality was found to be good with for most markers (with intra- and interassay imprecisions <20%). In the clinical evaluation, many biomarkers discriminated significantly between healthy persons and malignant diseases while only a few - mostly the established tumor markers - were able to significantly distinguish malignancies from the corresponding group of benign diseases. Particularly the immunological and cell death markers were elevated in benign diseases as well. When comparing malignant and benign diseases for differential diagnosis, best discriminating markers in breast cancer were CA 15-3, CEA reaching AUCs in ROC curves of 0.71 and 0.64 In ovarian cancer CA 125 (0.84), HE4 (0.71), CA 15-3 (0.78), CYFRA 21-1 (0.71), OPN (0.81) and IL-8 (0.75) achieved the best diagnostic performance. For cervical cancer, endometrial cancer and vulva cancer, no marker was able to distinguish malignant from benign lesions.

Conclusions: Biomarker multiplexing enables the parallel analysis of different biomarker classes providing a pattern of established and new parameters for cancer detection.
PP-17. An Evaluation of CA 125 and HE4 for the Classification of Ovarian Masses

Ruiz Escalera JF1, Martínez Diez M2, Rodríguez Espinosa M3, Casero Ariza C1, Lillo Muñoz JA1, Espejo Martínez AB2, Pérez Valero V1

1 U.G.C. Laboratory. Hospital Carlos Haya. Málaga 2 U.G.C. Obstetric and Gynecologic. Hospital Carlos Haya. Málaga

INTRODUCTION: The CA 125 tumor marker is used to help predict the presence of benign and malignant ovarian masses. Elevated CA 125 levels occur in benign disease. We evaluated the performance of HE4 to distinguish between benign and malignant pelvis masses.

PATIENTS AND METHODS: Serum levels of CA 125 and HE4 were analyzed in women who had been diagnosed through imaging and were compared to final pathology results. All included women underwent surgery or an imaging-guided biopsy; one blood sample was collected before the procedure. Cutoff values were recommended by the manufacturer. The sensitivity, specificity, positive predictive value, negative predictive value, and ROC curves and area under the curve (AUC) values were calculated to compare the accuracy of each method for predicting malignant ovarian masses.

RESULTS: We enrolled 33 patients presenting a suspicious pelvic mass. 23 had benign diseases and 10 epithelial ovarian cancer; 14 were premenopausal. In patients with benign diseases, abnormal serum levels of HE4 and CA 125 were found in 20% (4 of 20) and 40% (8 of 20) of patients, respectively. Significantly higher serum concentrations of CA 125 (p=0.01) and HE4 (p=0.005) were found in patients with cancer than in those with benign diseases. HE4 had higher sensitivity, specificity, positive and negative predictive value (76.9%, 70%, 62.5% and 82.4%) than CA 125 (69.23%, 80%, 52.9% and 82.35%). HE4 showed higher efficacy (72.7% vs. 63.63%), sensitivity and specificity in premenopausal and post-menopausal women. The greatest AUC was associated with CA 125 (0.79 IC95% 0.64-0.94) as compared to HE4 (0.77 IC95% 0.61-0.92). No differences were observed in AUC of CA 125 and HE4 (p=0.58).

CONCLUSION: HE4 best evaluated the malignant ovarian masses and the differential diagnosis of benign gynecologic disease. Our results show that the use of HE4 may be important in the differential diagnosis of pelvic masses in women, including those who are premenopausal.

PP-18. CA19.9 in combination with CA125 for diagnosis of ovarian mucinous cancer

Santotoribio JD (jdsantotoribio@gmail.com), Garcia-de la Torre A, Cañavate-Solano C, Arce-Matute F and Perez-Ramos S.

Clinical Biochemistry Laboratory, Puerto Real University Hospital, Cadiz, Spain.

Introduction: Serum concentration of cancer antigen 125 (CA 125) is the most widely studied biochemical method of screening for ovarian cancer. Preoperative elevated cancer antigen 19.9 (CA 19.9) levels could be related to a higher probability of ovarian mucinous cancer (OMC). The aim of this study was to determine the accuracy of CA 19.9 in combination with CA 125 for diagnosis of OMC. Materials and methods: Samples were collected preoperatively from patients for ovarian mucinous tumour: We measured the serum concentrations of CA 125 and CA 19.9 by electrochemiluminescence immunoassay in MODULAR E-170 (ROCHE DIAGNOSTIC®). Patients were classified into two groups according to the diagnosis of ovarian biopsy: NOT OMC (ovarian mucinous cystadenomas and ovarian mucinous borderline tumors) and OMC. The accuracy for diagnosis of OMC was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC). Results: We studied 104 patients with ages between 15 and 80 years old (median = 43.9 years old). Ninety-two patients were NOT OMC (78 ovarian mucinous cystadenomas and 14 ovarian mucinous borderline tumors) and 12 were OMC. We defined the following multivariable score (S): S = A + B, where A and B are coefficients of CA 125 and CA 19.9 respectively, A and B were obtained according to deciles of both variables. AUC values was 0.83 (p=0.0016) for CA 125, 0.82 (p=0.0024) for CA 19.9 and 0.87 (p=0.0001) for S. Optimal cut off value was 59.08 U/ml (66.7% sensitivity and 88.9% specificity), 37.21 U/ml (83.3% sensitivity and 79.5% specificity) and 1.0 (100.0% sensitivity and 76.4% specificity) for CA 125, CA 19.9 and S respectively. Conclusion: Using the proposed S improved accuracy for diagnosis of OMC, compared with using only CA 19.9 or CA 125. Preoperative CA 19.9 in combination with CA 125 levels showed high diagnosis efficacy to predict whether a suspected ovarian mucinous tumour is benign or malignant.

PP-19. The comparison of CA125 and HE4 dynamics in ovarian cancer patients’ monitoring

Sergeeva NS (nin.mars@mail.ru), Marshutina NV, Alentov II, Solokhina MP, Korneeva IA, Novikova EG

Moscow Herzen Research Oncological Institute

Background: CA 125 as the tumor marker traditionally used for monitoring of patients with ovarian cancer (OC). Some patients however do not exhibit an increase on levels of CA 125 in clinical relapse. One of possible additional marker to CA 125 may be HE4.

Study Aim: To compare CA 125 and HE4 dynamics in monitoring patients with OC.

Methods and Patients: the levels of CA 125 and HE4 were measured in the serum of 76 OC patients on the ARCHITECT system (ABBOTT Diagnostics, US). The mean monitoring duration was 25.6 months (range :18-64 months). As a discriminative level (DL) for CA 125 values >35 U/ml were used to identify positive samples. For HE4 age-depended DLs were used: for women younger 40 years cut-off > 70 pmol/l were used, for women aged 40-50 cut offs > 100 pmol/l were used, and for women over 50 cut offs > 120 pmol/l. The scheme of OC patients treatment included: neoadjuvant chemotherapy (NCh), excluding I-II st, surgery, adjuvant chemotherapy (ACH), chemo-therapy (Ch) of proved relapse.

Results: The diagnostic sensitivity of CA 125 and HE4 at the start of treatment was high (96.2% in CA125 and 92.3% in HE4). Before treatment the multiple of DL for CA 125 was equal 0.34. No differences were observed in AUC of CA 125 and HE4 (p=0.06, in remission –0.34). The dynamics of change in CA125 and HE4 levels during NCh was similar. After 6 courses of Ach CA125 levels remained high in 11.5% cases and HE4 – in 3.8% cases.

In patients with a clinically proven relapse CA125 stayed within normal limits in 20.5% cases and HE4 – in 3.8% cases. In patients however do not exhibit an increase on levels of CA 125 in clinical relapse. One of possible additional marker to CA 125 may be HE4.

Conclusions: Measurement of HE4 levels can, in some cases, give additional (to CA125) information about tumor progress an so may add to monitoring regimens for OC patients.
PP-20. Relationship between T infiltrating lymphocytes and regulatory T cells changes in tissue specimens and peripheral blood before and after neoadjuvant chemotherapy in breast cancer patients with pathological complete response

Barco Sánchez A (barcoantonios@hotmail.com),3 de la Cruz Merino L1, Ibáñez Martínez J1, Brgulj M2, Martínez Peinado A1, Sánchez Jiménez F1, Vallejo Benítez A1, Lobo Acosta MA1, Henao Carrasco F1, Nogales Fernández E1, Sánchez Margalda V2, Nieto García A4.

1 Clinical Oncology Department, Hospital Universitario Virgen Macarena, Seville, Spain; 2 Biochemistry Department, Hospital Universitario Virgen Macarena, Seville, Spain; 3 Pathology Department.

BACKGROUND: Some clinical trials in breast cancer have reported outcomes related to laboratory immune findings in neoadjuvant setting. Tumor infiltrating lymphocytes (TILs) and regulatory T cells (Tregs) in tissue specimens and in peripheral blood are being tested as two emerging prognostic and predictive factors. We designed a protocol to analyze TILs in blood and tissue from breast cancer patients before, during and after neoadjuvant chemotherapy (QT), and to analyze their eventual relationship with pathological complete response (pCR).

METHODS: From March 2011 to November 2013, 53 patients with breast carcinoma treated with neoadjuvant chemotherapy in the Breast Cancer Unit of the Hospital Universitario Virgen Macarena, were included in the study protocol. CD3+, CD8+, CD8-16-56+ and FoxP3+ cell infiltrates were detected by immunohistochemistry before and after the end of neoadjuvant QT in tissue specimens. Blood samples were collected in EDTA-K3 tubes before every cycle of QT to determine the immunophenotype and regulatory cell profile. Cell populations were determined by flow cytometry analysis of whole blood, including the study of CD3-CD4-CD25low and CD3-CD4-CD25high (Tregs).

CONCLUSIONS: Neoadjuvant CT decreases Tregs immunosuppressive infiltrates remain stable during treatment.

PP-21. Circulating microRNAs in Patients with Breast Cancer During Neoadjuvant Chemotherapy

Gezer Ü (ugurdu@istanbul.edu.tr), Keskin S2, Icgi A1, Tikenmez M1, Tiryakioğlu D1, Çetinkaya M1, Dici R2, Dalay N1, Kralp V2

1 Department of Basic Oncology and 2 Clinical Oncology, Oncology Institute, 3 Department of General Surgery, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

BACKGROUND: Recent data have revealed aberrant expression of many microRNAs (miRNAs) in blood circulation of patients with breast cancer (BC). In addition to the role of miRNAs on tumor progression and prognosis, the utility of circulating miRNAs in predicting the efficacy of treatment is also of interest. Our aim was to assess the effect of neoadjuvant chemotherapy on the levels of a panel of BC-associated miRNAs, which are either relatively low (let-7, miR-10b, miR-34, miR-155, miR-200c, miR-205) or abundant (miR-21, miR-195 and miR-221) in circulation.

PATIENTS AND METHODS: Patients with primary operable or locally advanced BC were enrolled in the study. Plasma levels of miRNAs at baseline and at the 4th cycle of treatment were compared by quantitative PCR.

RESULTS: Patients with stage II disease had higher basal miRNAs levels than those with higher stages. The difference was most evident for miR-155 and miR-21 (p=0.05). From basal to 4th cycle of chemotherapy, miRNA levels changed substantially. In samples in which miRNAs generally declined, a remarkable decrease (up to 15.500-fold) was evident for abundant miRNAs. Notably, the events with a decrease were more frequent in patients with smaller tumor sizes (p 0.05 for miR-21 and miR-195).

CONCLUSION: Our findings reveal that highly expressed miRNAs are mostly affected by chemotherapy.

PP-22. Profile of expression of MSH2 and MSH6 in breast cancer patients during chemotherapy

Lira D1, de Sousa Gehlke F1, Alves B1, Aajimee Azzalis L2, Campos Junqueira VB2, Corazinni R1, Afonso Fonseca FL1,2

1 Oncology/Hematology Discipline, FMABC, Santo André, São Paulo, Brazil; 2 Institute of Chemical and Pharmaceutical Sciences, UNIFESP, Diadema, São Paulo, Brazil

BACKGROUND: The genomic integrity is under constant threat of a large number of mutations. There is a complex system of genomic repair proteins (DNA repair genes), among them the hMSH2 and hMSH6. Changes in these genes may be related to susceptibility to the development of secondary leukemia patients undergoing treatment.

Study Aims: Evaluate the expression of hMSH2 and hMSH6 in patients with breast cancer.

Patients and Methods: In the study, 45 patients with breast cancer were included in different stages of chemotherapy. Assessments were made diagnosis, which served as a control sample, and the remaining sample every three months up to one year of treatment. After obtaining mRNA, the expression of hMSH2 and hMSH6 was performed by quantitative RT-PCR in real time.

Results: Significant variation was observed over time to repair hMSH2 gene (p = 0.02) but no significant variation was observed for hMSH6 (p = 0.15) gene. Both showed a moderate correlation at 0, 3 and 10.5 months (p < 0.001) and a weak correlation in the period of 6 months (0.34, p = 0.01). There was no statistically significant association between gene expression and clinical pathological covariates.

Conclusions: The expression of hMSH2 gene varies over chemotherapy and is not dependent on the expression of hMSH6 gene. The modification of DNA repair system appears to be transient.

Keywords: breast cancer, mononuclear fraction, mismatch repair system

PP-23. Paraneoplastic cerebellar degeneration with an occult breast cancer associated with anti-yo antibody: a case report

Ocaña-Pérez E (ecocanap@gmail.com), Muñoz-Colmenero A, Aceituno-Azaustre MI, Peña-Casas AM, Carrero Lérida MJ, Fernández-Suarez A

INTRODUCTION: Antibodies against antigens found in the central nervous system have been detected in neurological diseases. The most well-known are associated with paraneoplastic neurological diseases (Anti-Hu, Yo, Ri, Tr, CV2, Ta and amphiphysin antibodies). Some of these antibodies are specific for certain types of cancer or neurological syndromes and are useful diagnostic tools for the clinician. Anti-Yo-related paraneoplastic cerebellar degeneration (PCD) is most commonly found in women with gynecological and breast cancers. We report a case
INTRODUCCIÓN: Obesity is a well known risk factor for breast cancer development in postmenopausal women. High insulin levels, together with other hormones, positively modulate the growth of these tumor cells through signaling cascades. Sam68 protein is a member of the (STAR) family of RNA-binding proteins that can interact both with RNA and signaling proteins. We have previously described the role of Sam68 in the insulin and leptin signaling pathways as a receptor substrate, participating in proliferation, cellular growth and antiapoptotic effects mediated by these hormones in different cellular types. OBJECTIVE: To study the expression and phosphorylation of Sam68 upon insulin and leptin stimulation, seeking for a possible role of Sam68 in leptin and insulin receptor signaling in human breast adenocarcinoma cells. METHODS: We studied leptin and insulin-mediated Sam68 phosphorylation and Sam68 RNA binding capacity by immunoprecipitation, polyU affinity precipitation and immunoblot in MCF-7 cells. QRT-PCR and immunoblot were used to study the effect of leptin and insulin on Sam68 expression. siRNA was used to downregulate Sam68 expression. RESULTS: Sam68 protein quantity and gene expression were found to be increased under leptin and insulin stimulation. Moreover, both hormones promoted an increase in Sam68 tyrosine phosphorylation in MCF7 cells and negatively regulated RNA binding of Sam68, as previously observed in other systems. Sam68 downregulation resulted in lower activation of MAPK and PI3K pathways under both hormones stimulation. Sam68 was necessary for the complete leptin and insulin phosphorylation of the main proteins of these pathways. CONCLUSION: These results suggest the participation of Sam68 in both leptin and insulin receptor signaling in MCF7 cells, where Sam68 could mediate the trophic effects of these hormones. Thus, Sam68 could be also considered as a future prognostic marker or therapeutic target in this kind of non genetic breast cancer.
performed which influence metabolism of analogs of 5-FU. We used PCR amplification with subsequent reverse hybridization on strips 5-FU and FGGR SripAssay (Pentagen; ViennaLab) and the evaluation was performed using a scanner. Based on established data we managed to determine in patients with amplification of Her2/neu gene their genetic profile which enable selection of suitable candidates for oncological therapy. Acknowledgement: Our work was supported by Reserch Projekt of The Ministry of Hesly RVO-VFN64165

PP-27. Comparison of Tumor Marker Assays for Determination of AFR, CEA, and CA 15-3

Welle-Scharmann H (hwelle-scharmann@diasorin.de), Schlett R, Markowitz G, Schell D

Objectives: The aim of this study was to evaluate the comparability of tumor marker (TM) measurements of AFR, CEA, and CA 15-3 obtained by different analyzers, the DiaSorin LIAISON® XL system, the Siemens ADVIA Centaur® XP and the Roche MODULAR® E170. Methods: A minimum of 40 samples was collected and tested in parallel on the LIAISON® XL, the ADVIA Centaur® XP, and the MODULAR® E170 within the same week. Dose correlation was calculated by regression analysis. Agreement of patient classification was assessed by 4-field-plots. Results: Regression analysis revealed good comparability for all assays on all platforms ranging from r=0.982 to r=0.999. Agreement of patient classification varied in an assay dependent manner with 1 and 2 discrepant results for AFR (98% and 96% concordance), 5 and 6 discrepant results for CA 15-3 (88% and 87% concordance), and 5 and 1 discrepant result for CEA (88% and 98% concordance) respectively. Conclusion: Patient classification revealed excellent agreement for AFR on all systems and satisfactory agreement for CEA whereas patient classification for CA 15-3 showed some discrepant results around the cut-off. Measurement of AFR, CEA, and CA 15-3 obtained by the LIAISON® XL system correlated well with the results of the other analyzers, demonstrating the reliability and accuracy of the LIAISON® AFP, LIAISON® CEA, and LIAISON® CA 15-3 assay on the LIAISON® XL system and is therefore suitable to be used in routine TM measurements.

PP-28. Novel anti-human Axl monoclonal antibodies for improved patient biomarker studies

Ahmed L1,2,3, Hodneland Nilsson L1, Haugen H1, Nalwoga R1,2,3, Lorens JB1,2,3, Aksen La1,2,3, Micklem D4,1

BerGenBio AS, Bergen, 2Department of Clinical Medicine, 3Centre for Cancer Biomarkers(CCBIO), 4Department of Biomedicine University of Bergen, Bergen, Norway

Axl, a member of the TAM receptor tyrosine kinase family, has been associated with poor outcome in a wide variety of cancers including brain, breast, prostate, lung, pancreatic and myeloid cancers. Axl is upregulated during tumour epithelial-mesenchymal transition (EMT) and is associated with drug resistance and metastasis. In order to improve detection of Axl protein in patient biopsies, we developed a panel of monoclonal antibodies (MABs) directed against the extracellular domains of human Axl. We screened these MABs for sensitivity and selectivity in formalin fixed paraffin embedded (FFPE) immunohistochemistry, immunofluorescence, western blotting and sandwich-ELISA assays. One of these, MAB1H12 showed improved sensitivity and reduced background staining in immunohistochemistry applications on FFPE sections compared to existing commercially available antibodies. This antibody also compared favorably to existing antibodies in western blotting applications. We developed a sensitive sandwich-ELISA assay to detect Axl using MAB1H12 as a capture antibody and a second detection antibody from the Axl antibody panel. Hence, MAB1H12 represents a new anti-human Axl monoclonal antibody for improved patient biomarker studies.

PP-29. Evaluation of the Analytical and Clinical Performance of Seven tumor markers on the LUMIPULSE® GI200 Immunoassay System from Fujirebio

Escudero JM (jmescude@clinic.ub.es), Foj L, Augé JM, Filella X, Molina R.

Laboratory of Biochemistry (Oncobiology Unit), School of Medicine, Hospital Clinic, Barcelona, Spain.

AIM: Tumor markers are widely used in clinical practice. Tumor marker concentrations measured by different analyzers vary according to assay methods or epitopes for antibodies used. The aim of this study is to evaluate analytical and clinical performance of the new automated analyzer LUMIPULSE® GI200 in measuring seven tumor markers in comparison with those from conventional analyzers.

MATERIAL and METHODS: Precision performance of the seven tumor markers was evaluated. Clinical performance of LUMIPULSE® GI200 was evaluated by comparing levels of CEA, CA 19-9, CYFRA 21-1 and tPSA with Elecsys® e411 and CA 15-3, CA 125 and AFP with Centaur®, in 250 patients for each marker: 50 healthy people, 100 patients with benign diseases and 100 patients with metastatic cancer (50 were selected to include tumors where each tumor marker is commonly used and other 50 cancers from different origin).

RESULTS: The imprecision of each tumor marker was less than 10% CV. The results from LUMIPULSE® GI200 were highly correlated with those from other analyzers (range from slope=0.778 of CYFRA 21-1 to 1.458 of CA 125). There were no significant statistical differences in medians of groups of patients in comparison with other analyzers. Cut-off was selected from P95 of healthy people, and there were no statistical differences in sensitivity and specificity compared to the others.

CONCLUSION: Our results demonstrate that LUMIPULSE® GI200 has good precision and correlation with other analyzers, even when we analyze different concentration ranges separately. Clinical performance of this platform is good and results are interchangeable with those of other analyzers.

PP-30. Serum levels of tumor markers in diabetic patients

Garcia-de la Torre A, Santotoribio JD, Cañavate-Solano C, Arce-Matute F and Perez-Ramos S.

Clinical Biochemistry Laboratory, Puerto Real University Hospital, Cadiz, Spain.

INTRODUCTION: Carcinoembryonic antigen (CEA), cancer antigen 15.3 (CA 15.3), CA 125 are glycoproteins and carbohydrate antigen 19.9 (CA 19.9) is high molecular weight glycolipid, formed by altered glycosylation. They all have been widely used as tumor biomarkers. Our aim was to investigate the reference values of these tumor markers in diabetic patients and compare them with non-diabetic patients. MATERIAL and METHODS: The study was carried out at in Puerto Real Hospital (retrospective). A total of 1718 consecutive participants were enrolled in the study. Subjects with prior diagnosis of any cancer and any pathology that increase these levels were excluded. All serum tumor markers levels were determined by electrochemiluminescence immunoassay (ECLIA) in MODULAR E-170 (ROCHE DIAGNOSTIC®). Statistical analysis was performed using the software SPSS16®.

RESULTS: A total of 1718 patients enrolled in the study, and 1156 (67.28%) subjects were diagnosed as having diabetes, both groups were similar in the terms of age and gender.
Within normal range, serum CEA, CA15.3 and Ca19.9 levels were significantly associated with HbA1C levels. The Mann-Whitney U test is used to compare differences between the medians of both groups, levels of CEA (1.93, interquartile range (IQR):1.75 vs. 2.53, IQR: 2.11 ng/ml), CA 15.3 (9.29, IQR: 11.8 vs. 12.65, IQR: 13.8 U/ml) and CA 19.9 (15.87, IQR: 8.56 vs. 18.73 IQR: 11.53 U/ml) were significant higher in diabetic patients (all p<0.001), while levels of CA 125 showed no difference. CONCLUSION: Serum CEA, CA15.3 and Ca19.9 levels are increased in diabetic regarding non-diabetic patients.

PP-31. Descriptive study of requested tests for tumor markers and parameters performed by the central laboratory of the university hospital of Guadalajara (Spain) over 10 years (2003-2013)
López de calle pi H (hlopezd@sescam.jccm.es), Universidad hospital of Guadalajara, Guadalajara, Spain.

OBJECTIVES: Estimate the number of requests for blood tests and other tests relating the direct/indirect study of tumor markers made by different units of a Health Area. - Describe the socio-demographic profile of the patients tested over the study period. - Identify the test results above the reference range, with relation to the total number of requests and to the successive requests over time. MATERIAL AND METHODS: -Study of 104,451 requests for one or more tests performed during the July 2003 - June 2013 period. The tests chosen to be studied are: creatinine (CRE), ALT, AST, GGT, LDH, ALP, TBI, ALB and AFP; and the tumor markers CEA, CA-125, CA15-3, CA19-9. - A descriptive analysis is carried out based on the Exploratory Data Analysis (EDA) Model, with particular reference to the age/sex of the patient, the date (month/quarter/year) of the request and the results obtained. - The statistical analysis was carried out using R statistics package. - The results are presented in multiple graphical formats. RESULTS AND CONCLUSIONS: - The number of tests requested increased over the 8-year period between 2003 and 2011 (median: 6.94%), and decreased from 2011 to 2013 (-6.13%). The increase of tumor marker tests between 2003 and 2011 was the following: CEA 12.9%, CA-125 13.7%, CA15-3 12.7% and CA19-9 14.3%. They decreased between 2011 and 2013. - The age of patients increased slightly, from 58.05 years in 2003 to 61.98 years in 2013. - The proportion of men increased slightly (from 34% in 2003 to 40% in 2013). - Requests made by Primary Health Care AP varied (...). - Positive results decreased over the years, while the number of requests increased (...). - Repeated requests for the same patient show a small increase. SUPPORT BIBLIOGRAPHY: - Review of at least 8 accredited documentary references.

PP-32. Evaluation of new assay for thyroglobulin
Krhin B (bkrhin@onko-i.si), CukPasanku V, Mozina B

Background. After removal of differentiated thyroid carcinoma (DTC), serum thyroglobulin (Tg) can indicate persistent or recurrent disease. We describe evaluation of new, more sensitive assay for Tg (Tg-II) with comparison to our current assay (Tg-I), both from Roche. Methods. Our evaluation has been focused on low Tg concentrations only, since some evaluations have been done by Roche already. 120 serum samples from 118 DTC patients, with serum Tg below 40 μg/L, were enrolled. Both assays (Tg-II and Tg-I) were performed on the automated Modular (Roche) platform. Precision (25 replicate readings over a 5-day period) was measured with 2 serum pool samples, pool A (Tg=0.85 μg/L; Tg-Ab negative) and pool B (Tg=1.5 μg/L; Tg-Ab positive). Results. Correlation between the Tg-II and Tg-I assay gave a following Passing-Bablok linear regression fit: Tg-II = 0.755 Tg-I – 0.051 (n=60) for concentrations between 0 and 2 μg/L and Tg-II = 0.879 Tg-I – 0.370 (n=60) for concentrations between 2 and 40 μg/L. Using Bland-Altman difference calculation and compared to Tg-I assay, the Tg-II assay gave lower results as follows: -0.3 μg/L (0.5-1 μg/L range), -0.4 μg/L (1-2 μg/L range), -0.8 μg/L (5-10 μg/L range), -1.3 μg/L (10-20 μg/L range) and -2.6 μg/L (20-40 μg/L range). There was no significant difference between Tg-II and Tg-I assay in 0-0.5 μg/L range. Precision was satisfactory with an intra-assay precision 1.4% and 1.7% for pool A and pool B, respectively and inter-assay precision 1.8% and 3.1% for pool A and pool B, respectively. Conclusions. The new Tg-II (Roche) shows good correlation and improved precision compared to our current assay Tg-I (Roche). The new assay is acceptable and shows additional advantage for long term follow-up of DTC patients, however small negative bias should be considered when switched from one to another assay.

PP-33. Plasma levels of tumour M2-pyruvate kinase in different types of cancer
Muñoz Colmenero A1, Fernández-Suárez A2, Fatela Cantillo D2, Ocaña Pérez E1, de la Torre Calzada MJ3, Domínguez Jiménez JL3, Díaz Iglesias JM1

1Laboratorio, Hospital de Alta Resolución Sierra de Segura, Puente de Génave (Jaén), Spain; 2Departments of Biotechnology and Digestive Diseases, Hospital Alto Guadalquivir, Andújar (Jaén), Spain; 3Unidad de Gestión de Laboratorio y Alergia, Área de Inmunología, Complejo Hospitalario de Jaén, Spain.

Background: Tumour M2-pyruvate kinase (M2-PK) is the dimeric isoform of pyruvate kinase, which is up-regulated in proliferating tissues. It has been shown that M2-PK is detectable and quantifiable in the stool and plasma of colorectal cancer (CRC) patients. M2-PK has been extensively studied in gastrointestinal tumours but its role in other types of cancer has not yet been evaluated.

Objective: To determine and to compare the plasma levels of M2-PK in different types of cancer.

Methods and Materials: All patients undergoing monitoring for cancer in our hospital during 2011 were included in the study (n=139). Plasma M2-PK concentration was analysed by an enzyme-linked immunosorbent assay (ScheBo Biotech AG, Giessen, Germany). We consider 20 U/mL as the cut-off value for cancer detection.

Results: The following types of cancer were included in the study: colorectal 60, breast 43, lung 8, prostate 5, ovarian 4 and the remaining 19 cases were others uncommon tumors in our hospital. Most cancers showed high concentrations (median U/mL; percentile 25- percentile 75) of M2-PK: colorectal (31.40; 16.70-53.00), breast (41.00; 26.25-63.00), lung (60.75; 38.25-132.63), ovarian (63.12; 35.12-78.00), thyroid (80.50; 72.50-88.50). By contrast, only prostate (20.00; 11.45-36.60), pharynx (18.00; 9.00-23.00) and testis tumours (20.3; 15.05-34.05), showed M2-PK levels lower or near cut-off.

Conclusions: Plasma M2-PK is not specific for CRC and is elevated in many others cancers, including breast, lung, ovary, and thyroid. Small amounts are found in prostate, larynx and testis tumours.

PP-34. Stability of Human Serum Analyte HE4 Determined with an Immunoassay
Radwan RR1, Fermer C2, Lundin M2, Raju S1, Jones S1, Hall C1, Dickson D3, Kettlely TR1, Li ZQ1 (zqli@fulil.com)

1FujirebioDiagnostics, Inc., Malvern, PA, USA; 2FujirebioDiagnostics, AB, Gothenburg, Sweden.

The purpose of this study was to determine the stability of serum analyte HE4 under frozen condition. HE4 has become an important biomarker for
ovarian cancer (OC) (Hellstrom I, 2003). Furthermore, the combination of HE4 and CA125 has become a more accurate predictor of OC (Moore RM, 2008). METHODS: This is a retrospective study utilizing banked serum samples collected in an OC clinical trial (NCT00315692). The patients were women greater than or equal to 18 years of age, who were to undergo laparotomy or laparoscopy procedure based on the finding of a pelvic mass. The serum samples were collected in red top tubes or serum separator tubes (SST) in the United States under an IRB-approved protocol. The serum samples were stored at ≤-70°C since the time of collection and underwent no more than five freeze/thaw cycles prior to June of 2013. Sample testing was performed using an HE4 ELIA (Fujirebio Diagnostics, Inc.). The initial testing was performed in July to August of 2007 and the results were retrieved from the clinical trial dataset. The stability testing was performed in June of 2013. A total of 100 available serum samples from the clinical trial were tested. Among them, 84 were from women with benign disease, 11 from women with low malignant potential tumors, 4 from women with OC, and 1 from a woman with other gynecological cancer. RESULTS: The linear correlation coefficient between the two testings was 0.986 (95% CI: 0.979 to 0.991). Weighted Deming regression gave an intercept 2.76 (95% CI: -0.54 to 6.07); and a slope 1.033 (95% CI: 0.970 to 1.095). The intercept and slope were not significantly different from 0 and 1 respectively. Passing-Bablok regression produced a similar intercept 3.06 (95% CI: -1.26 to 6.35) and slope 1.031 (95% CI: 0.965 to 1.108). CONCLUSION: The human serum HE4 slope 1.033 (95% CI: 0.970 to 1.095). The intercept and slope were not

PP-35. p53 regulates negatively the expression of the nuclear export protein XPO1, establishing a positive feedback loop
Sasaki Y, Tamura M, Kobayashi T, Idogawa M, Suzuki H, Shinomura Y, Tokino T

Background: Exportin 1 (XPO1) is involved in signal-dependent nuclear export and overexpressed in several types of cancer, leading to the mislocalization of the nuclear factors including tumor suppressor proteins within cells. We found that XPO1 is a critical target for transcriptional repression by the p53 tumor suppressor.

Aims: The purpose of this study was to characterize the functional relevance of p53-mediated repression of XPO1 in human cancer.

Methods: The effect of p53 on the transcription of XPO1 was investigated using Northern blots, immunoblotting and luciferase assays. Subcellular localization of p53 was analyzed by fluorescent microscopy and immunoblot after cell fractionation. The relationship between XPO1 expression and p53 mutation status in tumor tissues was examined using the Oncomine utility.

Results: Endogenous XPO1 expression was decreased in several cancer cells when wild-type p53 was introduced. We found in human and rodent cells that the expression of XPO1 is down-regulated by DNA damage in a p53-dependent manner. Combining miRNA microarray with in silico miRNA target prediction, p53 induced the expression of miR-142-3p which, in turn, reduced the level of XPO1 through a miR-142-3p-binding site within the 3’-UTR of XPO1. miR-142-3p reduced XPO1 levels and caused nuclear retention of p53, and cell cycle arrest, whereas a miR-142-3p-specific inhibitor prevented the nuclear import of p53. Importantly, exogenous XPO1 rescued miR-142-3p-mediated nuclear import of p53. Interestingly, XPO1 was overexpressed in primary breast cancer tissues harboring mutant p53 genes. We also found that high expression of XPO1 may serve as a poor prognostic factor for cancer patients of the breast and colon.

Conclusions: We uncovered a previously undetected mechanism by which p53 localization is regulated by miRNA and it appears that p53, miR-142 and XPO1 form a positive feedback loop.

PP-36. Z-scan method as a new tool to determine the free circulating DNA: Pilot Study in Patients with Bladder Cancer
Alves S1 (sarah.alves@gmail.com), Oliveira Delgado P2, Fonseca FA1,2

1 Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo, Diadema, SP, Brasil;
2 Laboratório de Análises Clínicas, Faculdade de Medicina do ABC, Santo André, SP, Brasil

Introduction: Bladder cancer is the most common malignancy of the urinary tract, excluding prostate cancer and one of the most common cancers in men, which usually appears in the sixth and seventh decade of life. More recently, it was shown that elevated levels of free circulating DNA in plasma frequently occur in several types of cancer and can be used to differentiate patients with malignancies from healthy patients. Objectives: Using the Z-Scan technique to determine the free circulating DNA in patients with bladder cancer. Patients and Methods: Plasma samples from patients with bladder cancer at diagnosis and during treatment were collected by peripheral venipuncture. The DNA was extracted from plasma and measured by spectrophotometry. Aliquots of extracted DNA were added to the plasma with ethidium bromide. This mixture was verified in nonlinear optical method called Z-Scan technique. For comparative purposes, samples of patients free of disease were tested as controls of the results. Results: In the samples from healthy individuals were observed lower values of the amplitudes peak-valley of the Z-Scan technique, in comparison by the value obtained of the samples from patients with bladder cancer. In addition, these samples had different detections over time and the treatment proposed by the clinician. These differences may be related to changes in compliance of the DNA molecule during treatment. Conclusions: The nonlinear optical method can assist in verifying changes of free circulating DNA molecule and such changes can be standardized and assist oncology clinical response to treatment. Keywords: Z-Scan, nonlinear optic method, free DNA circulating.

PP-37. Significance of FLT3 mutation for clinical evaluation of VEGF in serum of AML patients
Jurisic V1 (vdvd@mailcity.com), Colovic N2, Pavlovic S3, Colovic M4

1 University of Kragujevac, Faculty of Medical Science, Kragujevac; 2 Institute of Hematology, University of Belgrade, School of Medicine, Belgrade; 3 Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Serbia

Human leukemia cells secrete vascular endothelial growth factor (VEGF), which can act in a paracrine manner within the bone marrow microenvironment to promote leukemia cell survival, proliferation, migration and angiogenesis. The FLT-3 receptor tyrosine kinase plays an essential role in regulating normal hematopoiesis, but its constitutive activation via mutation in AML patients is mostly associated with poor outcome. In our study, VEGF was analyzed with respect to FAB classification, classical cytogenetics, presence bone marrow blast cells, peripheral blast count, LDH values, and other clinical parameters. Using our new cytogenetic classification we categorized the AML patients as: favorable, intermediate-I, intermediate-II and adverse prognosis. Results show that the VEGF values using the recommended classifications - mean values for each respective group were 48.75; 57; 104 and 101 pg/ml - had more significant difference (Mann-Whitney-U test, p<0.01) as compared to
classical cytogenetics and the other parameters investigated. Our data indicate that molecular classification based on gene mutation simultaneous with cytokines can be very valuable in disease prognosis for AML patients.

PP-38. Development of a novel screening system for diagnosis of urothelial cancer by detection of urinary monomeric laminin-γ2 (Ln-γ2)

Koshikawa N (nkoshi@me.com), Kamada M, Minegishi T, Kawada C, Shuin T and Seki T.

1Division of Cancer Cell Research, Institute of Medical Science, University of Tokyo; 2Department of Urology, Kochi Medical School; 3Integrated Center for Advanced Medical Technologies, Kochi Medical School.

Laminin-5 is a major component of epithelial basement membranes and plays a crucial role in maintaining normal epithelial structures. It is a heterotrimeric molecule consisting of laminin-α3, -β3, and -γ2 chains. In contrast, laminin-γ2 (Ln-γ2) is expressed as a monomer form in malignant tumors, including those of the urothelium, and its level of expression is correlated with the grade of tumor malignancy. Therefore, we hypothesized that monomeric Ln-γ2 acts as a biomarker for urothelial cancer diagnosis. First, we analyzed monomeric Ln-γ2 in urine by Western blot analysis. Specimens were obtained from 46 urinary cancers and 61 non-cancer patients. We detected monomeric Ln-γ2 (130 kDa), its oligomers (260 and 390 kDa), and a processed form (110 kDa). Interestingly, monomeric Ln-γ2 and its processed forms were positive in about 55% of urothelial cancer specimens. To further confirm the expression of monomeric Ln-γ2 in urothelial cancer tissues, immunohistochemical (IHC) analyses were conducted using anti-laminin-α3 and -γ2 monoclonal antibodies. IHC analysis showed that Ln-γ2, but not Ln-α3, was detected in the cancerous tissues obtained from patients whose urine was Ln-γ2 positive. Next, we measured monomeric Ln-γ2 in urine specimens by sandwich ELISA assay. We found the positivity of monomeric Ln-γ2 in urine was higher in urothelial cancer patients compared to non-cancer patients. The diagnostic value of monomeric Ln-γ2 is better than those of conventional biomarkers such as NMP-22 and BTA in urothelial cancer urine specimens. The sensitivity of monomeric Ln-γ2 was 98%, and the specificity was 46%. The diagnostic accuracy of monomeric Ln-γ2 was higher than that of NMP22 or BTA. Taken together, our results indicate the possibility that monomeric Ln-γ2 is a novel tumor marker in urine specimens for diagnosis of urothelial cancer.

PP-39. Human Endogenous Retroviruses-K (HERV-K) expression correlates with stemness features in melanoma cells

Matteucci C, Denboba A, Balestrieri E (balestrieri@med.uniroma2.it), Sorrentino R, Bucci I, Cipriani C, Spadafora C, Garaci E, SinibaldiVallebona P.

Due to their resistance to current therapies, melanoma remains a significant cause of mortality and one of the most aggressive human cancers. Tumour consists of heterogeneous populations whose properties remain poorly characterized. However, more recently, studies on cancer stem cells have opened new perspectives in the understanding of cancer biology. Indeed, mounting evidences suggest that cancer may arise from a transformed stem cell able to self-renew, differentiate into diverse progenies and drive continuous growth. Moreover, current studies support the idea that retroelements and HERVs play an important role in cell plasticity, transformation and tumor progression. In a previous work, we described how a highly heterogenous melanoma cell line (TVM-A12) with unusual plasticity, under stress culture conditions, changed its characteristic of growth by acquiring a more aggressive phenotype and invasive capacity. These phenotypic changes were associated with activation, expression and production of HERV-K viral particles. TVM-A12 cultured in a stem cell serum-free medium, showed a modification of cell growth and morphology from an adherent culture towards the formation of sphere-like cellular aggregates, characterized by the induction of the expression of HERV-K with the appearance of the stem cell marker CD133, and the loss of the expression of cellular markers important for the immunological escape of tumor (MHC-I, Melan-A/MART-1). The specific inhibition of HERV-K expression by RNA interference, abolished the generation of the CD133+ population in the TVM-A12 cultured in serum free medium, demonstrating the association between the expression of HERV-K and CD133 sub-population. These results provide new molecular basis for understanding the biological features of melanoma, but also useful resources for future development of new therapeutic and diagnostic biomarkers.

PP-40. Clinical and laboratory correlates of elevated 24-hour urinary 5-hydroxyindoleacetic acid in patients with possible carcinoid tumors

Morell-Garcia D (daniel.morell@ssib.es), Baüca, JM; Robles Bauza, J; Fueyo Ramirez, L; Barceló Bennasar A; Pérez Esteban, G.

BACKGROUND Carcinoid tumors derive from enterochromaffin cells, which are found in the gastrointestinal system and bronchi, and have the ability to secrete serotonin. 5-hydroxyindoleacetic acid (5-HIAA), a degradation product of serotonin, is accepted as a good biomarker for carcinoid syndrome. METHODS Measurement by HPLC of 5-HIAA was performed in 24h urine of 148 patients with a possible carcinoid syndrome (55 men and 93 women; median age, 55 years). Reference range was 3.6–42.8 μmol/24h. In patients with a history of carcinoid, serum chromogranin-A (CgA) was additionally quantified (normal < 100ng/mL). No patients consumed interfering substances 24 hours before urine pickup. We retrospectively analyzed the association between symptoms, 5-HIAA levels and the presence of carcinoid, established by imaging studies and histopathological findings. RESULTS Flushing, fluttering and diarrhea were the most common symptoms (71%) for clinical suspicion of carcinoid syndrome. 5-HIAA was increased in 11.5% of cases (median, 130.5 μmol/24h) and the most prominent features were diarrhea and abdominal pain (41%). Six carcinoids were found and CgA was higher in 83% of these cases (median, 2265ng/mL). Two patients showed liver metastasis. For them, 5-HIAA was significantly higher than for the rest: 565μmol/24h against 71.6μmol/24h (p < 0.01). We found 4 carcinoids in patients whose 5-HIAA levels were within normal ranges. For these, CgA was above normal (median, 422ng/mL). For 24h urinary 5-HIAA, our method showed a sensitivity of 60% and a specificity of 92% for carcinoid disease. Together, CgA and 5-HIAA showed a sensitivity of 90% and a specificity of 88%. CONCLUSIONS 24h urinary 5-HIAA has low sensitivity in the diagnosis of carcinoid tumors. In patients with 5-HIAA above 500μmol/24h, an association with metastatic disease may exist, thus representing a factor of poor prognosis. CgA is a promising marker for the detection and management of carcinoid tumors and increase sensitivity of 5-HIAA.
PP-41. Isocitrate Dehydrogenase-1 Mutations and Their Prognostic Role in Glioblastoma Multiforme Patients in West Bohemia

Polivkajr J\(^1,2\) (polivkajr@gmail.com), Polivkova J\(^3\), Repik T\(^1,2\), Pesta M\(^4\), Pitule P\(^1,2\), SadilKOVA P\(^1\), Topolcan O\(^5\).

\(^1\)Department of Histology and Embryology and \(^2\)Biomedical Centre, Faculty of Medicine in Plzen, Charles University in Prague.
\(^3\)Department of Neurology, Faculty of Medicine in Plzen, Charles University in Prague and Faculty Hospital Plzen.
\(^4\)Department of Biology, Faculty of Medicine in Plzen, Charles University in Prague.
\(^5\)Central Imunoanalytical Laboratory, Faculty Hospital Plzen.

Background: Glioblastomamultiforme (GBM) is the most malignant primary brain tumor in adults. Standard therapy has only limited effectiveness and the median survival of patient with GBM is 12.1 – 14.6 months. Recent GBM whole-genome studies revealed novel prognostic and predictive biomarker, recurrent mutations in metabolic enzyme IDH - Isocitrate dehydrogenase (isoforms IDH1 and IDH2). The distinctive mutation IDH1 R132H was uncovered to be a strong prognostic biomarker for glioma patients. Therefore we investigated the prognostic role of IDH1 R132 mutation in our GBM patient cohort.

Methods: The IDH1 R132H mutation status was assessed in the Formalin-Fixed Paraffin-Embedded (FFPE) tumor samples from 44 GBM patients treated in the Faculty Hospital Plzen between 2008 and 2013. The real-time PCR with TaqMan\(^\text{®}\) mutation detection assays and TaqMan\(^\text{®}\) mutation detection IPC reagent kit was used. The IDH1 R132H mutation status was correlated with the progression free survival (PFS) and overall survival (OS) of patients using Kaplan-Meier survival analysis and Wilcoxon test.

Results: The IDH1 R132H mutation was identified in 20 from 44 GBM tumor samples (45.4%). The majority of mutated tumors were secondary GBMs (16 in 18, 89.9%). Low frequency of IDH1 mutations was observed in primary GBMs (4 in 26, 15.3%). Patients with IDH1 R132H mutation had longer PFS - 136 vs. 51 days (P < 0.021) as well as OS - 270 vs. 95 days (P = 0.024).

Conclusion: The prognostic role of IDH1 R132 mutation in our GBM patient cohort is significant, which suggests one more factor contributing to the selection of patients for adjuvant therapy (radio- and chemotherapy).

Supported by MH CZ - DRO (Faculty Hospital Plzen – FNPI, 00698006) and by the project E02.1.00/03.0076 from European Regional Development Fund.

PP-42. New molecular genetics insights to the treatment of oligodendrogiomas

Polivkajr J\(^1,2\) (polivkajr@fnplzen.cz), Polivkova J\(^3,4\), Rohan V\(^1,2\), Repik T\(^1,2\), SadilKOVA P\(^1\), Topolcan O\(^5\).

\(^1\)Department of Neurology, Faculty of Medicine in Plzen, Charles University in Prague and \(^2\)Faculty Hospital Plzen, Czech Republic
\(^3\)Department of Histology and Embryology and \(^4\)Biomedical Centre, Faculty of Medicine in Plzen, Charles University in Prague, Czech Republic
\(^5\)Central Imunoanalytical Laboratory, Faculty Hospital in Plzen, Czech Republic.

Oligodendrogiomas (OT) represent 5% of primary brain malignancies. OT are characteristic for the frequent co-deletion of chromosome 1p and 19q. This genetic aberration was the first identified biomarker in neuro-oncology. 1p/19q co-deletion means the loss of genetic material from the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q). The mechanism is the unbalanced translocation t(1;19)(q10;p10). It appears almost exclusively in OT with frequency of 80% - 90% for grade II oligodendrogliomas and 50%-70% for anaplastic oligodendrogliomas. Recently mutations in two important tumor suppressor genes - CIC (a homolog of the Drosophila gene capicua) located on 19q13.2 and FUBP-1 (far upstream element binding protein) on the 1p chromosome – were discovered in the majority of oligodendrogliomas with 1p/19q co-deletion. Mutations in these genes are implicated in the formation and progression of oligodendrogliomas. The long-term follow up of phase III clinical trials - RTOG 9402 and EORTC 26951 have recently proved favorable effect of combined radiotherapy and chemotherapy (procarbazine, lomustine, vincristine - PCV) in patients with anaplastic oligodendrogliomas and anaplastic oligoastrocytomas carrying the co-deletion 1p/19q. The 1p/19q co-deletion serves as an important diagnostic, prognostic and predictive biomarker in OT with high sensitivity to radio- and chemotherapy in comparison with other malignant gliomas. The role of other molecular biomarkers (mutations in Isocitrat dehydrogenase – IDH 1/2, O6-methylguanine DNA methyltransferase – MGMT gene promoter methylator or glioma genome cytosine-phosphate-guanine islands methylator phenotype - G-CIMP) is an important part of the management of OT. Supported by MH CZ-DRO (Faculty Hospital Plzen—FNPI, 00698006) and the project E02.1.00/03.0076 from European Regional Development Fund.

PP-43. Study of tumor marker HE4 sensitivity in serum of patients with renal cancer

Sergeeva NS(nin.mars@mail.ru), Alentov II, Alekseev BYa, Krashenninikov AA, Marshutina NV

Moscow Herzen Research Oncological Institute.

Background. Measurement of HE4 (human epididymis protein 4) has a diagnostic significance (in addition to CA125) for ovarian and endometrial cancer patients, but data about sensitivity of this antigen for malignant tumors at other localizations are rare.

Study aim: To investigate HE4 levels in serum of patients with renal cell carcinoma (RCC).

Methods and patients: 60 patients (male) with primary renal cancer (I-IV stages) were included in the study. The mean patients age was 61.2 years (range 42-86 years). According to final (after surgery) histological report, 42 patients had clear cell histological type of cancer, 9 patients had chromophobe RCC and 9 patients – papillary cancer. HE4 levels were measured on the automatic immunoanalyzer ARCHITECT i1000SR (Abbott Diagnostics, USA). Cut-off for positivity was set at 70 pmol/l. Serum creatinine levels were also measured.

Results: The HE4 mean level for all RCC patients (n=60) was 101.1 ±9.5 pmol/l (median 70.6pmol/l), and the marker was found to be elevated in 51.7% cases (31 of 60 patients). The share of cases with elevated HE4 among patients with clear cell histological type tumors was 45.2% (19 of 42 patients). Patients who had papillar (n=9) and chromophobe RCC and 9 patients – papillary cancer. HE4 levels were measured on the automatic immunoanalyzer ARCHITECT i1000SR (Abbott Diagnostics, USA). Cut-off for positivity was set at 70 pmol/l. Serum creatinine levels were also measured.

Results: The HE4 mean level for all RCC patients (n=60) was 101.1 ±9.5 pmol/l (median 70.6pmol/l), and the marker was found to be elevated in 51.7% cases (31 of 60 patients). The share of cases with elevated HE4 among patients with clear cell histological type tumors was 45.2% (19 of 42 patients). Patients who had papillar (n=9) and chromophobe RCC and 9 patients – papillary cancer. HE4 levels were measured on the automatic immunoanalyzer ARCHITECT i1000SR (Abbott Diagnostics, USA). Cut-off for positivity was set at 70 pmol/l. Serum creatinine levels were also measured.

Conclusion: The prognostic role of IDH1 R132 mutation in our GBM patient cohort is significant, which suggests one more factor contributing to the selection of patients for adjuvant therapy (radio- and chemotherapy).

Supported by MH CZ - DRO (Faculty Hospital Plzen - FNPI, 00698006) and by the project E02.1.00/03.0076 from the European Regional Development Fund.
Conclusion. For patients with RCC serum HE4 levels may provide an additional predictive factor, and a useful tool for monitoring disease although more data is needed.

PP-44. A Comparison of Tumor Marker Assays for determination of PSA and fPSA
Welle-Scharmann H (hwelle-scharmann@diason.de), Schle R, Markowitz G, Schell D

Objectives: The purpose of this study was to assess the comparability of Tumor Marker results for PSA and fPSA obtained by the LIAISON® XL analyzer with the results obtained by Beckman UniCel® DxI 800. Methods: A minimum of 40 samples spanning the assay range was collected. Samples were tested for PSA and fPSA on the LIAISON® XL system and the Beckman UniCel® DxI 800 analyzer with assays calibrated based on WHO standard. Dose correlation was calculated by regression analysis. Classification of patients was assessed by 4-field-plots and free- and total PSA ratios (f/t-PSA ratio)were compared. Results: Regression analysis showed good comparability of results with r=0.989 for PSA and r=0.994 for fPSA. 4-field-plots revealed 100% agreement for PSA cut-off and 95% for grey zone classification. F/t-PSA ratio illustrated excellent agreement with only 1 sample classified high risk with LIAISON® XL and equivocal risk with Beckman UniCel® DxI 800 assays. Conclusion: PSA and fPSA showed consistent results over the whole assay range with LIAISON® XL vs UniCel® DxI 800. Classification above the cut-off for PSA was concordant, grey zone classification revealed 2 discordant samples near grey zone upper limit. F/t-PSA ratios demonstrated comparable risk stratification. The LIAISON® PSA and LIAISON® fPSA assays are well suited for PSA screening and show good comparability with the Access Hybritech PSA and Access HybritechfPSA assays.

PP-45. Expression of centrosomal genes during the course of chronic myeloid leukemia
Smahelova J (smahel@uhkt.cz), Tachezy R, Machova K, Klamova H, Zemanova H, Smahel M.

Institute of Hematology and Blood Transfusion, Prague, Czech Republic

Centrosomes are cellular organelles that are necessary for the separation of chromosomes to daughter cells. The development of human malignancies is associated with centrosomal aberrations that result in genetic instability. Increased production of centrosomal proteins can be responsible for centrosomal aberrations. As the extent of centrosomal abnormalities in chronic myeloid leukemia (CML) correlates with disease stage and karyotype alterations, abnormal expression of centrosomal genes might be an early prognostic marker of disease progression. When analysing available datasets from microarray transcriptional-profiling studies, we found 12 centrosomal genes upregulated in all stem/progenitor subpopulations of CML. From these genes, we chose AURKA, ESPL1, HMMR, and PLK1 for our examinations. We tested their expression by RT-qPCR in samples from peripheral blood of healthy controls and CML patients at diagnosis and during the course of this disease. The expression of all genes was significantly enhanced at diagnosis and decreased to the basal level in most patients treated with imatinib for three months. This decrease was mostly accompanied by the reduction of BCR-ABL1 expression, but in some patients BCR-ABL1 expression was preserved. Furthermore, in some patients with persisting high expression of BCR-ABL1, the decrease of the centrosomal gene expression was slow and the basal level was achieved after about 9-12 months. These data suggest various expression patterns of centrosomal genes and BCR-ABL1 which could be utilized in CML prognosis. This project was supported by grant NT13862-4/2012 from the Czech Ministry of Health.

PP-46. Molecular Analysis of Tumor Markers of Primary and Secondary Glioblastoma Multiforme (GBM)
Stanek L (stanek.libor@seznam.cz), Vajtir D, Dundr P, Lisova S, Springer D, Petruzela L, Zima T.

1Institute of Pathology, 1st Faculty of Medicine and General Teaching Hospital in Prague, Czech Republic; 2Department of Oncology, 1st Faculty of Medicine and General Teaching Hospital in Prague, Czech Republic; 3Institute of Medical Biochemistry and Laboratory diagnostics, 1st Faculty of Medicine and General Teaching Hospital in Prague, Czech Republic; 4Institute of Forensic Medicine and Toxicology, 1st Faculty

BACKGROUND: Glioblastoma multiforme is one of the most common tumor of the brain. Histologically the tumors are derived from astrocytes and usually arise within cerebral hemispheres.

MATERIAL AND METHODS: We performed molecular cyto genetic analysis of samples from 3 patients with primary and 4 patients with secondary glioblastoma multiforme. We analyzed signaling pathways of HER1, HER2 (EGFR, KRAF, BRAF, HER2/neu); angiogenic factors (VEGF); apoptotic pathways (p53) and gene for tyrosin-fobsites PTEN.

RESULTS: Immunohistochemically, we proved overexpression of p53, expression of VEGF (50%) and high expression of neuronal NOS (80%) in primary glioblastomas. Cyogenetically, we demonstrated amplification of TP53 gene and sporadic amplification of HER2/neu gene. We found no polysomy of CEP 17. We found the loss of function of PTEN gene. Molecular analysis showed increased expression (10× higher) of VEGF compared with secondary glioblastomas. Mutation of EGFR gene was detected (insertion in exon 18; deletion in exon 19), mutation of KRAF gene was found in one case of primary glioblastoma (Gly12Cys (GGT>TGT)). Mutation of BRAF gene (V600E) was not detected.

Immunohistochemically, we detected low expression of p53 (<10%), neuronal NOS expression (70%), higher expression of VEGF (20%) and expression of nestin in secondary glioblastomas. Cyogtenetically, amplification of TP53 gene and sporadic amplification of HER2/neu gene. We found no polysomy of CEP17 was confirmed. Molecular analysis showed lower expression of VEGFA compared to primary glioblastomas. Mutation of EGFR, KRAS and BRAF genes have not been detected.

CONCLUSIONS: In our study we confirmed that primary and secondary glioblastomas from the point of view of molecular genetics are two entities with different biologic activities which may have clinical and therapeutic importance.

Acknowledgement: Our work was supported by Research Projekt of The Ministry of Hesly RVO-VFN64165

PP-47. Comparison of 6 automated total PSA assays with PSA AccesHybritech assay
Foj I, Filella X, Augé JM, Escudero JM, Molina R.

Laboratory of Biochemistry, Hospital Clinic. Barcelona, Spain

Prostate-specific antigen (PSA) is widely used in detection and monitoring of patients with prostate cancer. The variability between different assays for the measurement of PSA has created problems in the interpretation of PSA concentrations. The variability of total PSA results among commercial assays has been suggested to be decreased by calibration to WHO reference materials. We compare 7 commercially available automated assays for tPSA concentrations by using patient samples that
would minimize matrix effects to determine the degree of assay-dependent bias in patient results at several critical cut-offs. We tested 167 samples with tPSA concentrations of 0 to 20 μg/L using 7 PSA automated commercial assays, including Access (Beckman Coulter), ARCHITECT i2000 (Abbott), ADVIA Centaur XP (Siemens), IMMULITE 2000 (Siemens), Elecsys (Roche) and Lumipulse G1200 (Fujirebio). tPSA was measured in Access using Hybritech and WHO calibrators. Passing-Bablok analysis was performed to compare assays using the Hybritech-calibrated Access as comparison as assay. For tPSA, relative differences were more than 10% at 0.2 μg/L for ARCHITECT i2000. No assay exceeded the relative difference of 10% at the critical concentration of 0.4μg/L. At the critical concentrations of 3, 4, and 10 μg/L, the relative difference of 10% was exceeded by ADVIA Centaur XP and WHO-calibrated Access. Maximum differences in tPSA results were observed between WHO-calibrated Access and IMMULITE 2000 assays. The slope was 1.339, being the y-intercept −0.162. Differences between PSA results measured using Hybritech-calibrated Access and IMMULITE 2000 assays were higher than 20% in 74% of patients and higher than 40% in 41% of patients. We have shown significant discordances between assays included in this study despite advances in standardization conducted in last years. Further harmonization efforts are required to obtain a complete clinical concordance.

PP-48. The role of F/T(%) ratio for differential diagnosis between benign prostatic hyperplasia and prostatic carcinoma in patient at the Clinical Center of Kragujevac in Serbia

Ljesevic S1, Joksimovic D2, Stojadinovic D2 and Nedeljkovic T1
(drtomislav.nedeljkovic@gmail.com).

1 Clinical Center Kragujevac, Service for laboratory diagnostics, Kragujevac, Serbia; 2 Clinical Center Kragujevac, Urological Clinic, Kragujevac, Serbia.

Patients with benign prostatic hyperplasia(BPH) and prostatic carcinoma(PCa) were investigated for total prostatic specific antigen (tPSA), free PSA(tPSA) and ratio tPSA/(tPSA/F/T) (%) of values of tPSA in patients with BPH and PCa was not significantly different (t-test, p=0.037). The values of tPSA between the above mentioned, patients were indicated marked significant difference (t-test, p=0.0001). The aims of our investigation was analysis of F/T ratio in patients with BPH and PCa. Analysis of F/T ratio was marked significant difference (t-test, p=0.0001). In group of patients with BPH middle value was 37.40%±9.08% and in group of patients with PCa was 18.0±8.14.

We discuss, that ratio F/T (%) can be of greater help than tPSA and free PSA determination, respectively. For patients with BPH, we found neither results of F/T < 20% and indicate that could be index <20% (< 18% in literature) to serve like value for patients with PCa and indicate nexts clinical investigations (invasive methods like transurethral resections, biopsy etc.).

In conclusions, we show that F/T ratio (%) could be introduced into the diagnostic protocol for early dignostics of PCa.

PP-49. LDH and %fPSA for diagnosis of prostate cancer

Santotoribio JD (jdsantotoribio@gmail.com), Garcia-de la Torre A, Cañavate-Solano C, Arce-Matute F and Perez-Ramos S.

Clinical Biochemistry Laboratory, Puerto Real University Hospital, Cadiz, Spain.

INTRODUCTION: The free-to-total serum prostate specific antigen ratio (%fPSA) has been proposed to diagnosis of prostate cancer (PC) in men with serum total prostate specific antigen (PSA) levels 4 to 10 ng/mL. Serum lactate dehydrogenase (LDH) was suggested to be prognostic indicator in PC patients. The aim of this study was to evaluate the utility of LDH in combination with %fPSA, for diagnosis of PC in men with PSA levels between 4 and 10 ng/mL. METHODS: We studied men with PSA levels from 4 to 10 ng/mL, who underwent 12-core transrectal ultrasound guided prostate biopsy. The following variables were analysed: PSA, %fPSA, LDH and diagnosis after a prostate biopsy. PSA and free-PSA were determined by ECLIA in MODULAR E-170 (ROCHE DIAGNOSTIC®) and LDH was measured by enzymatic colorimetric method in MODULAR COBAS 711 (ROCHE DIAGNOSTIC®). %fPSA was calculated using the following formula: free-PSA / PSA × 100 (%). Two categories of patients were included in the analysis: NOT PC and PC. Statistical analysis was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC). RESULTS: We studied 134 patients with a mean age of 69.9 (range: 37-87) years. One hundred-twelve were NOT PC and 22 were PC. We defined the following multivariable score (S): S = A + B, where A and B are coefficients of LDH and %fPSA respectively, A and B were obtained according to deciles of both variables. AUC values was 0.719 (p=0.0036) for %fPSA, 0.749 (p=0.0082) for LDH and 0.816 (p=0.0001) for S. Optimal cut off value were 21.0% (100.0% sensitivity and 32.1% specificity), 441 U/L (54.5% sensitivity and 96.4% specificity) and 0.8 (100.0% sensitivity and 50.0% specificity) for %fPSA, LDH and S respectively. Using the proposed S increases by 18% specificity compare only with %fPSA parameter. CONCLUSIONS: The proposed score S, LDH and %fPSA combination, improves diagnostic performance for detection of PC compared to using only %fPSA.

PP-50. MCJ predicts immunotherapy response in T1 high grade bladder cancer

Murillo V1, Pompas N1, Moya P1, Gonzalez-Peramato P2, Palou J3, Rodríguez O3, Algaba F1, Sánchez-Carbayo M1.

1 Bladder Cancer Group, Proteomics Unit, Molecular Oncology Program, CIC bioGUNE, Bilbao, Spain; 2 Pathology Department, Hospital Universitario La Paz, Madrid, Spain; 3Urology Department, Fundación Puigvert, Barcelona, Spain.

INTRODUCTION: Methylation-controlled J protein (MCJ) is a member of the DnaJ family of cochaperones. The DNAJ proteins participate in processes such as protein folding and translocation, cell cycle control by DNA tumor viruses, and regulaton of protein kinases. To our knowledge, MCJ has not been evaluated in bladder cancer to date.

OBJECTIVE: In this report we aimed at evaluating the potential relevance of MCJ in bladder cancer progression and in the response to Bacille Calmette-Guerin therapy in high-risk T1 high-grade bladder cancer.

RESULTS: MCJ was frequently methylated in bladder cancer cells (n=14), bladder tumors (n=150). The epigenetic silencing of MCJ by hypermethylation was tested in bladder cancer cells before and after azacitidine treatment, a demethylating agent. MCJ expression patterns were analyzed by immunohistochemistry on tissue arrays containing bladder tumors for which MCJ methylation was assessed. MCJ expression was significantly associated with tumor stage (p<0.001) and tumor grade (p<0.05). In a series of patients treated with BCG, the presence of a low MCJ expression alone, or combined with MCJ methylation, were associated with poor BCG response in terms of muscle-invasive progression (p<0.05).
CONCLUSIONS: MCJ was identified epigenetically modified in bladder cancer. MCJ methylation and protein expression patterns associated with cancer progression and clinical outcome and BCG response. This data suggests further assessment of the utility of MCJ methylation and protein evaluations for the clinical management of patients affected by uroepithelial neoplasias.

**PP-51. Levels of homocysteine and its cofactors regarding metalloproteinase 13 and e-cadherin in patients with prostate adenocarcinomas during chemotherapy**

Gascón Belardo TM; Schindler F; de Brito Luz MC; Lambiasi Sant’Anna AV; Azzalis LA; Campos Junqueira VB; Pereira EC; Affonso Fonseca FL. 1 Oncology/Hematology Discipline, FMABC, Santo André, São Paulo, Brazil. 2 Institute of Chemical and Pharmaceutical Sciences, UNIFESP Diadema, São Paulo, Brazil

INTRODUCTION: Prostate adenocarcinoma, loss expression of E-cadherin consists of amendments that characterize invasive phenotype, being interpreted many times as a tumor suppressor gene. As collagenase-3, metalloproteinase-13 is family member of matrix metalloproteinase with broad substrate specificity and potential role in tumor invasion and metastasis. Folate, Vitamin B12 and homocysteine are essential for metabolism of methyl group, therefore, also for DNA methylation.

OBJECTIVE: This research assessed levels of E-cadherin, MMP-13, homocysteine and its cofactors, and to correlate the results of indicators prognosis in patients with prostate cancer undergoing chemotherapy.

MATERIAL AND METHODS: The 59 patients studied were from Faculty of Medicine ABC with prostate adenocarcinoma. Samples were collected at diagnosis, after 3 and 6 months of treatment. Measured Homocysteine (and their co-factors), E-cadherin, MMP-13, Free and Total PSA, Free and Total Testosterone.

RESULTS: An increase concentration of E-cadherin (p = 0.02), reduced of PSA (p <0.001), total testosterone (p <0.001) and free (p = 0.002) throughout the study without significant changes in other variables in different times (zero, three and six months).

From zero to three months, there was a negative correlation of changes in homocysteine with variations of folate (rs = -0.46, p = 0.02) and free testosteron (rs = -0.55, p =0.01). From start to end of study (from zero to six months), there was only a correlation positive variation between homocysteine and total testosterone (r = 0.39, p = 0.04).

CONCLUSION: There were no interaction between biochemical recurrence and homocysteine concentrations, MMP-13 and E-cadherin over time. It is suggested that dosage of E-cadherin in solid tumor cancer at diagnosis and during treatment, assisting physician in traceability in time. It is suggested that dosage of E-cadherin in solid tumor cancer at diagnosis and during treatment, assisting physician in traceability in time.

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**PP-52. The first clinical experience with cabazitaxel application in the IIInd line of castration-resistant prostate cancer treatment**

Holubec L (holubec@fnplzen.cz), Katolicka J, Finek J

Biomedical Centre, Faculty of Medicine in Plzen, Charles University in Prague, Plzen; Department of Oncology, Faculty Hospital and Medical Faculty Pilsen; Department of Oncology and Surgery, Faculty Hospital at Saint Ann, Brno,Czech Republic

Background: Docetaxel represents standard treatment in the IInd line of castration-resistant prostate cancer (CRPC), and results of studies of the IIInd phase recommend application of several drugs for the IIInd line of CRPC. The exact profile of a patient for whom the drugs are meant, as well as the sequence which would bring the best possible results, have not been defined so far. Methods and Patients: The group includes 16 patients of 56-84 years of age with diagnosis of prostate adenocarcinoma, castration-resistant disease with castration level of testosterone, who were treated with docetaxel in the first line of CRPC therapy. Docetaxel was applied for the period of 3-26 months within the first line, and 75% of the patients were treated with docetaxel for longer than 6 months (3-24 months). Doses of 25mg/m² of cabazitaxel were applied intravenously every three weeks. Results: The only undesirable effects of the grade III-IV recorded were hematological toxicity, leucopenia of GIII and neutropenia of GIII without any infection complications, and non-hematological toxicity was minimum. Gleason Score (GS) of 8-9 was assessed histologically with 9 patients, and all these patients’ response to docetaxel applied in the IInd line for the period longer than 6 months (6-11months) was good. The follow-up therapy with cabazitaxel has taken place in this subgroup for 3-15 months so far, and PSA has dropped by more that 50%, which means partial remission with 5 patients. Only 3 patients out of the total number of 16 patients died within the course of assessment, and in case of both of them visceral organs and lymphatic ganglia were affected metastatically. Conclusions: Through our first clinical experience, cabazitaxel demonstrated good effect in the IIInd line of the treatment in case of patients with GS>7 as well as patients with a good long-term response to the docetaxel treatment in the IInd line of CRPC.

**PP-53. Cancer specific profiles of epigenetically altered circulating nucleosomes measured by simple ELISA detect and differentiate colorectal and prostate cancer**

Herzog M, Chapelier M, Cuvelier S, Scoubeau K, Josseaux E, Eccleston M, Micallef J (j.micallef@volitionrx.com)

Belgian Volition SA, Rue du Seminaire 20A, BE-5000 Belgium

BACKGROUND: Genome wide epigenic signals are altered in cancer cells. In addition, circulating nucleosome bound DNA fragments contain the same mutations as cancer tissue samples taken from the same subjects suggesting a tumour origin for at least some circulating nucleosomes. We have developed ELISA tests for circulating nucleosomes (NuQ®) that contain specific epigenetic signals and used these to investigate global epigenetic profiles in the serum and plasma of colorectal (CRC) and prostate (PCA) cancer patients. METHODS: Serum and plasma samples taken from healthy subjects and subjects with newly diagnosed PCA (n=19) or subjects referred for colonoscopy (n=39), were assayed for total nucleosomes as well as nucleosomes containing methylated DNA, and histone modifications H3K9(Me)3 and H4K20(Me)3 using NuQ® ELISA kits. RESULTS: A reduced level of circulating nucleosomes containing methylated DNA was observed in both cancers. The results of two NuQ® assays for circulating nucleosomes containing methylated DNA or H3K9(Me)3 and H4K20(Me)3 using NuQ® ELISA kits. RESULTS: A reduced level of circulating nucleosomes containing methylated DNA was observed in both cancers. The results of two NuQ® assays for circulating nucleosomes containing methylated DNA or H3K9(Me)3 and H4K20(Me)3 using NuQ® ELISA kits. RESULTS: A reduced level of circulating nucleosomes containing methylated DNA was observed in both cancers. The results of two NuQ® assays for circulating nucleosomes containing methylated DNA or H3K9(Me)3 and H4K20(Me)3 using NuQ® ELISA kits. RESULTS: A reduced level of circulating nucleosomes containing methylated DNA was observed in both cancers. The results of two NuQ® assays for circulating nucleosomes containing methylated DNA or H3K9(Me)3 and H4K20(Me)3 using NuQ® ELISA kits. RESULTS: A reduced level of circulating nucleosomes containing methylated DNA was observed in both cancers. The results of two NuQ® assays for circulating nucleosomes containing methylated DNA or H3K9(Me)3 and H4K20(Me)3 using NuQ® ELISA kits. RESULTS: A reduced level of circulating nucleosomes containing methylated DNA was observed in both cancers. The results of two NuQ® assays for circulating nucleosomes containing methylated DNA or H3K9(Me)3
PP-54. Clinical utility of cytokine biomarkers in a lung cancer patient treated with tociluzumab

Nechushtan H, Appelbaum L, Kalichman I, Peretz T and Barak V

Background: Recently, we were able to demonstrate that oral vit.D suppl. With 1000 to 20 000 IU per day allows a long time normalization of serum 25(OH)D even in patients with exocrine insufficiency due to non-malignant and malignant pancreatic diseases (Tumoriobiology, 20012, 33 Suppl. 1, p.61).

Aims: We now report the results in 22 patients compared to 4 controls (people without upper-gastrointestinal-diseases) supplemented with oral vit.D for 12 to 47 months (median 35 months), looking for potential side effects of daily 1000 IU to 20.000 IU vit.D supplementation on vitamin D metabolites in the serum and some functional bone parameters.

Methods: In 4 controls (C) and 22 pancreatic patients (n= 5 chronic pancreatitis (CP), n= 8 pancreatic cancer (PC) and n= 9 after curative resective surgery (PR)) we measured the serum concentrations of 25(OH)D3 (ng/ml), 25(OH)D2 (ng/ml), 3-c-epi25(OH)D3 (ng/ml), 24,25(OH)2D3 (ng/ml), 1,25 DHCC (ng/l), as well as serum calcium, PO4, alkaline phosphatase (AP), creatinin, plasma PTH and urine crosslinks in the urine. The median vit.D supplementation was 40.000IU/week (7.000 – 140.000 IE/week) in the pancreatic patients.

Results: Serum 25(OH)D3 increased significantly in all pancreatic patients and controls resp. into the normal range during oral vit.D supplementation: 12,5+/-8,9 (med. 10,4) to 44,7+/-11,7 (med. 43,9) in the pancreatic patients and 12,8 (med. 12,2) to 41,2 (med.40,0) in the controls resp. The vit.D metabolites showed an expected behaviour like a significant increase of 1,25DHCC from 41,0+/-15,6 (med. 40,50) to 70,0+/-18,6 (med. 74,8). Serum calcium showed a slight increase (med. 2,26 to 2,39 mmol/l) (s.), 24 h calcium in the urine remained in the normal range (med. 2,4 mmol/die), serum creatinin and serum phosphor remained unchanged, plasma PTH and urinary crosslinks showed a decrease of the medians from 110,0 to 35,7 pg/ml (s.) and 67,0 to 54,0 (ug/gCreatinine)(s.). Median serum AP only showed a trend to slightly decreased levels (n.s.) although, in single patients, a significant decrease was observed, partly combined with significant decreases of plasma PTH.

Conclusions: Oral vit.D supplementation allows a long time normalization of serum 25(OH)D3 in patients suffering from pancreatic insufficiency due to non-malignant/malignant pancreatic diseases without or after pancreatic resection, even after total pancreatectomy (n=5). Oral supplementation seems to be a safe procedure in the case of dose adaption to serum 25(OH)D3 levels, and a necessary procedure in order to normalize pathological bone parameters, induced by malsorption/malnutrition. These data might be of interest also with respect to recent discussions suggesting relevance of vit.D metabolism for immunologic diseases, cancer prevention and/or treatment of diabetes mellitus.

PP-55. Long time oral vitamin D supplementation in patients suffering non-malignant/malignant pancreatic disease – effects on vitamin D metabolites and functional bone parameters

Klapdor S1,A, Bahlo M1, Roth P3, Klapdor R2,A

1Compound and Care, Hamburg. 2ZeTDT GmbH, Hamburg, 3MVZ Labor Limbach, Heidelberg, 4Internal Unit, Hamburg, Germany

Background: Recently, we were able to demonstrate that oral vit.D suppl. With 1000 to 20 000 IU per day allows a long time normalization of serum 25(OH)D even in patients with exocrine insufficiency due to non-malignant and malignant pancreatic diseases (Tumoriobiology, 20012, 33 Suppl. 1, p.61).

Aims: We now report the results in 22 patients compared to 4 controls (people without upper-gastrointestinal-diseases) supplemented with oral vit.D for 12 to 47 months (median 35 months), looking for potential side effects of daily 1000 IU to 20.000 IU vit.D supplementation on vitamin D metabolites in the serum and some functional bone parameters.

Methods: In 4 controls (C) and 22 pancreatic patients (n= 5 chronic pancreatitis (CP), n= 8 pancreatic cancer (PC) and n= 9 after curative resective surgery (PR)) we measured the serum concentrations of 25(OH)D3 (ng/ml), 25(OH)D2 (ng/ml), 3-c-epi25(OH)D3 (ng/ml), 24,25(OH)2D3 (ng/ml), 1,25 DHCC (ng/l), as well as serum calcium, PO4, alkaline phosphatase (AP), creatinin, plasma PTH and urine crosslinks in the urine. The median vit.D supplementation was 40.000IU/week (7.000 – 140.000 IE/week) in the pancreatic patients.

Results: Serum 25(OH)D3 increased significantly in all pancreatic patients and controls resp. into the normal range during oral vit.D supplementation: 12,5+/-8,9 (med. 10,4) to 44,7+/-11,7 (med. 43,9) in the pancreatic patients and 12,8 (med. 12,2) to 41,2 (med.40,0) in the controls resp. The vit.D metabolites showed an expected behaviour like a significant increase of 1,25DHCC from 41,0+/-15,6 (med. 40,50) to 70,0+/-18,6 (med. 74,8). Serum calcium showed a slight increase (med. 2,26 to 2,39 mmol/l) (s.), 24 h calcium in the urine remained in the normal range (med. 2,4 mmol/die), serum creatinin and serum phosphor remained unchanged, plasma PTH and urinary crosslinks showed a decrease of the medians from 110,0 to 35,7 pg/ml (s.) and 67,0 to 54,0 (ug/gCreatinine)(s.). Median serum AP only showed a trend to slightly decreased levels (n.s.) although, in single patients, a significant decrease was observed, partly combined with significant decreases of plasma PTH.

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PP-56. Progastrin-releasing peptide (proGRP) in patients operated for pulmonary carcinoid tumors

Broughton MN, Bolstad N, Brustugun OT, Helland A, Liadal T and Paus E

Department of Medical Biochemistry and Department of Oncology, Oslo University Hospital, Radiumhospitalet, Oslo, Norway

Study Aim: We wanted to investigate if the novel tumor marker proGRP could be a useful marker in patients operated for lung carcinoids, compared to more established markers such as chromogranin A and NSE.

Material and Methods: ProGRP, NSE, chromogranin A, CEA and Cyfra 21-1 were measured in biobanked pre-operative serum samples from 143 patients surgically treated for early stage lung cancer. There were 40 adeno-carcinomas (AC), 30 large cell carcinomas (LCLC), 40 squamous cell carcinomas (SCC), 15 small cell lung cancers (SCLC) and 18 carcinoids.

Results and Discussion: Of 18 patients with carcinoids, proGRP was elevated in 11 (61.1 %), five having values > 1000 pmol/L, whereas NSE and chromogranin A were only elevated in 1 (5.6 %) and 4 (22.2 %), respectively. As expected, both NSE and proGRP were elevated in the majority of patients with SCLC (66.7 % and 73.3 %, respectively). ProGRP was elevated in 20/110 (18 %) samples from patients with LCLC, SCC and AC combined. This could suggest that some of these tumors have neuroendocrine features. However, most of the elevated results in this group were only marginally above upper normal limit. CEA and Cyfra 21-1 were only elevated in 17 % and 32 % of the patients in this study, respectively, probably reflecting the limited disease in this patient cohort.

Conclusions: ProGRP may be a useful marker for carcinoid tumors of the lung, with markedly improved sensitivity over chromogranin A and NSE. Further studies are needed to evaluate proGRP in the post-operative follow-up of patients treated for lung carcinoids, where perhaps the greatest clinical benefit can be found. The potential value of this marker in the diagnosis and follow-up of gastrointestinal carcinoids should also be investigated.

PP-57. Verification of bone magnetic panel

Windrichova J, Fuchsova R, Topolcan O

Bone markers are not only important in osteoporosis or bone diseases but their necessity is strongly increasing in research and personalized treatment of tumour disease spread into bones. Nowadays the novel magnetic fomat of multiplex xMAP assays increases the applicability of multiplex assays into clinical routine, but after verification from analytical and clinical point of view. In our study we have compared the performance and values obtained by magnetic and nonmagnetic kit for bone markers in 30 serum samples from patients with solid tumour disease. The comparison was run for osteopontin, osteoprotegerin, osteocalcin, leptin and parathormone. Furthermore we have run in magnetic fomat FGF23, insulin and SOST assays.

Methods: The blood was collected by venipuncture using Vacuette collection tubes (Greiner Bio-One, Austria). The blood samples were centrifuged at 640 g for 10 min. Serum aliquots were stored until analysed in a -75 °C freezer at -75°C. The assays were run using commercially available multiplex kits MILLIPLEX® MAP Human Bone Bead Panel in magnetic and non-magnetic version (Merck Millipore, USA) according to
manufacturer’s instructions for use. The analysis was performed on Luminex 100 IS instrument and data analysis on Luminex 100 IS software version 2.3 for nonmagnetic kit and MagPix instrument and Biorad Software Bioplex Manager 6.1 for magnetic kit. The assays were run in doublets. For recovery two samples were spiked using standards and two samples were used for serial dilutions.

Statistical interpretation: There were counted coefficients of variations as relative standard deviations for all samples. The comparison between magnetic and non-magnetic results were performed by Passing-Bablok analyses.

Results: The performance was for both formats of kit acceptable, all CV% below 8%, with slightly lower levels of CV% for magnetic version compare to nonmagnetic.

When comparing the values obtained by magnetic and non-magnetic format, we have found very closely related results for osteoprotegerin, parathormone and osteocalcin. Surprisingly, we have found quite large differences between magnetic and non-magnetic results for osteopontin and leptin. Complete results will be displayed on poster. For FGF23 the most of the samples were out of the calibration ranges.

Conclusion: The magnetic format of xMAP bone panel possesses acceptable analytical performance for clinical routine but it could produce slightly different values than older nonmagnetic versions.

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