Tuesday, July 28, 2015

Poster Session: 9:30 AM - 5:00 PM

Cancer/Tumor Markers

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Development of magnetic bead-based multiplex immunoassays to evaluate serum biomarkers for the early detection of pancreatic cancer

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Background: Pancreatic cancer is the 4th leading cause of cancer death in the United States. The majority of patients present with unresectable disease leading a median survival of 6 months and an overall 5-year survival of < 5%. The early detection of this disease is critical because surgery at an early stage is the most promising therapy that could greatly improve the prognosis of patients. The current existing serum markers such as CA19-9 lack the necessary sensitivity and specificity. Multiplex immunoassay simultaneously measuring multiple analytes in the same sample using minimum volume allows us to evaluate serum biomarker panels that can potentially complement CA19-9 in early detection of pancreatic cancer.

Objective: To develop magnetic bead-based multiplex immunoassays to evaluate serum biomarkers for the early detection of pancreatic cancer.

Method: Curated results from PUBMED database search using a combination of terms “pancreatic cancer, pancreatic neoplasm, PANIN, pancreatic adenocarcinoma, sensitivity, and fold change” were analyzed. Candidate biomarkers were selected using a weighted scoring system based on 1) fold changes and number of publications, or 2) sensitivity/specificity and study sample sizes. Magnetic bead-based multiplex immunoassays were developed for the selected candidate serum biomarkers using a Bio-Plex 200 suspension array system (Bio-Rad). Briefly, monoplex assays of individual candidates were first developed, cross-reactivity checked, and multiplex assays validated and optimized. All of these proteins plus HE4 (Roche) and CA19-9 (Tosoh) were analyzed in sera of patients diagnosed with pancreatic ductal adenocarcinoma (PDAC: IB/IIA/IIB, n=10; IV, n=10), benign pancreatic conditions including intraductal papillary mucinous neoplasm (IPMN, n=10) and chronic pancreatitis (n=10), and healthy controls (n=19). The performances of these candidate markers were evaluated individually or in combination on their capacity to complement CA19-9 in early detection of pancreatic cancer.

Results: The biomarkers evaluated included 1) a 5-plex assay of OPN, CEACAM-1, MIC-1, MIA, and SPON1; 2) a 2-plex assay of POSTN and HSP27; and 3) a monoplex assay of LGALS3BP. These assays were all in-house developed with negligible cross-reactivity, recovery of 75-119%, and intra-assay or inter-assay precision of 0.3-9.6% or 0-18%, respectively. LOD or LLOQ was 0.179 ng/mL or 0.181 ng/mL (OPN), 0.101 ng/mL or 0.213 ng/mL (CEACAM-1), 0.001 ng/mL or 0.046 ng/mL (MIC-1), 0.009 ng/mL or 0.016 ng/mL (MIA), 0.041 ng/mL or 0.191 ng/mL (SPON1), 0.094 ng/mL or 0.767 ng/mL (POSTN), 0.005 ng/mL or 0.062 ng/mL (HSP27), and 0.035 ng/mL or 0.289 ng/mL (LGALS3BP). Individually, the best biomarkers (AUC in ROC analysis, 95% CI) to separate PDAC from benign pancreatic conditions were CA19-9 (0.9425, [0.85-1.00]), CEACAM (0.845, [0.71-0.98]), MIC (0.79, [0.65-0.93]), and SPON1 (0.68, [0.51-0.85]). However, stepwise backward logistic regression selected a three marker panel of CA19-9, HSP27, and MIA (p-values: <3E-9, <0.03, <0.01, respectively) with an AUC=0.99 [0.97-1.00]. Probably due to the small sample size, the improvement over CA19-9 alone is not statistically significantly.

Conclusion: The multiplex immunoassay workflow provides sufficient analytical performance to evaluate serum biomarker panels that complement CA19-9 in early detection of pancreatic cancer. The biomarker panels identified in this study warrant further validation with a larger number of patient samples.

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Validity of serum Eotaxin-1 in diagnosis of prostate cancer

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Backgrounds: Prostate cancer (PCa) is the second most common male cancer worldwide and ranked the sixth male cancers in most Arab world. Eotaxin-1/CCL11 is a member of chemokines, which are a superfamily of small proteins that bind to G protein-coupled receptors on target cells. Chemokines and chemokine receptors are shown to play an important role in regulation of tumor growth, migration, and invasion of different types of cancers. Although, the best and most sensitive screening test available for PCa is prostate specific antigen (PSA) there is a large overlap between PCa and benign prostatic hyperplasia (BPH) especially in patients with moderately elevated PSA levels, which reinforced the critical need to develop, validate and determine the utility of other diagnostic biomarkers for PCa. Eotaxin-1 offer a hope to overcome these drawbacks by virtue of its cancer specific expression. Objective: This study aimed to explore the diagnostic and prognostic value of serum Eotaxin-1 as non-invasive biomarker for PCa as well as to validate its combination with PSA to improve the overall value of sensitivity, specificity and diagnostic accuracy of PCa patients. Patients and Methods: This study included 62 newly diagnosed PCa patients (preoperative), 84 BPH patients and 70 healthy male subjects of matched age. Full history and clinical data were recorded for all subjects. PCa patients were undergo digital rectal examination (DRE), trans-rectal ultrasonography (TRUS) guided biopsy, computed tomography (CT) scan of the pelvis and histopathological examination, accordingly TNM stages were confirmed and PCa patients grouped as early PCa (T I/II) in 69.4% (n=43) and advanced (T III/IV) in 30.6% (n=19). Blood samples were withdrawn from all patients after at least one-week gap following DRE and prior to any prostate biopsy. Serum levels of Eotaxin-1, PSA and free/total PSA were measured as well as possible association between parameters were assessed. The validity (sensitivity and specificity) were evaluated by ROC curve analysis. Results: Serum PSA levels were significantly higher in PCa than BPH and control groups ($p<0.05$) and attained sensitivity of 85% at 83% specificity with a diagnostic accuracy of 84%. f/tPSA ratio had a sensitivity, specificity and accuracy of 82%, 84% and 86% respectively. Serum eotaxin-1 levels differentiated significantly among PCa, BPH and control groups (p0.05), its ratio with PSA differentiated significantly between advanced and early PCa stages ($p<0.05$) and provide a sensitivity, specificity and diagnostic accuracy of 90%, 84% and 87% respectively for diagnosis of PCa. Combination of serum eotaxin-1 and f/tPSA ration seems to improve the overall value of sensitivity, specificity and diagnostic accuracy (92, 85 and 89% respectively). Conclusion: Serum eotaxin-1 may provide a useful diagnostic tool to help distinguish between BPH and PCa. Combination of this chemokine with standard marker PSA can improve the overall value of sensitivity, specificity and diagnostic accuracy of patients with PCa eventually sparing unnecessary prostate biopsies. However, larger prospective studies are warranted to validate the diagnostic value of serum eotaxin-1 level in PCa.

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CCCTC-binding factor inhibits breast cancer cell proliferation and migration via inactivation of nuclear factor-kappaB pathway

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Background: CCCTC-binding factor (CTCF) is an important epigenetic regulator and an evolutionarily conserved and ubiquitously expressed zinc finger protein. CTCF regulates a wide range of genes associated with tumor development, in particular genes involved in growth, proliferation, differentiation, and apoptosis. In this study, we aimed to observe the expression of CTCF in breast cancer cell lines, tumor tissue and serum of breast cancer patients, and investigate the effect of CTCF on proliferation and migration of breast cancer cells. Methods: Western blot was used to detect CTCF expression in human breast cancer cell lines MCF7, SKBR3, MDA-MB-231 and normal breast cells MCF-10A. Real-time quantitative PCR and immunohistochemistry were applied to detect the mRNA and protein levels of CTCF in invasive ductal carcinoma (n=23), peritumoral tissue (n = 10) and fibroadenoma (n = 10). Enzyme-linked immunosorbent assay (ELISA) was used to detect the concentration of CTCF protein in serum. In addition, CTCF was overexpressed and knock-down in MCF7 cells by virus packaging and infection, and then cell viability and proliferation was detected using MTT assay. Furthermore, cell migration and invasion were measured in vitro by scratch/wound healing and transwell migration assays. Last, Affymetrix U133 Plus 2.0 microarray was performed to screen the

possible target genes and pathways that CTCF regulated. Results: CTCF expression in MCF-10A was the highest, and decreased gradually in MCF7, SKBR3 and MDA-MB-231. CTCF expression in breast carcinoma tissue were significantly lower than that in peritumoral tissue and benign lesions (P <0.01). Moreover, CTCF expression in poorly differentiated breast cancer was significantly lower than that in well differentiated breast carcinoma. CTCF protein concentration in serum of breast cancer patients were also significantly lower than that of healthy control (P <0.01). In addition, CTCF could inhibit the proliferation and migration of MCF7 cells. By transcriptomic analysis and further experimental confirmation, we identified the HIPK2 as a target gene of CTCF and found that CTCF could inhibit the activation of nuclear factor-kappaB pathway. Conclusions: In summary, our findings demonstrate that CTCF expression were declined significantly in invasive breast cancer, compared with the non-invasive breast cancer and normal controls, suggesting CTCF may be a potential marker for breast cancer. CTCF could inhibit the proliferation and migration of breast cancer cells via inactivation of nuclear factor-kappaB pathway.

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Better mutation discrimination for a multiplexed KRAS assay using PASS MNAzyme qPCR.

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Background: Many clinical diagnostic assays are now detecting somatic mutations such as gene insertions, deletions and point mutations. Due to the complicated design of these assays they are often in a singleplex format and/or lack sensitivty and specificity. We have developed a novel method to improve sensitivity and specificity of detecting mutations in a multiplex format referred to as PASS MNAzyme qPCR. In this study we apply this method to detect 7 KRAS codon 12 and 13 mutations in characterised colon cancer and melanoma FFPE samples and we compare mutant cross reactivity to the Qiagen Therascreen assay.

Material and Methods: PASS primers consist of target specific regions separated by a unique insert sequence (INS) which is not complementary to the target region. A long 5' target-specific region anchors the primer and a short 3' region, targeting the variant base/s, directs specific binding and extension. After amplification, the INS sequence is incorporated into the amplicon and acts as a 'barcode' to identify the amplicon and/or genetic variation when coupled with MNAzyme® qPCR. MNAzymes (Multi-component Nucleic Acid enzymes) composed of DNA oligonucleotide "partzymes" can be used to mediate a unique real-time detection technology. Active MNAzymes form when partzymes bind to target amplicons, and catalyse cleavage of fluorescently labelled probes thus generating a real-time signal. Superior multiplexing capacity is enabled by coupling PASS with MNAzyme® qPCR. Each multiplexed target is given a distinct INS and this 'barcode' is specifically detected by the MNAzyme. Previously (1), a multiplexed KRAS PASS MNAzyme qPCR assay, which detects 7 mutations in 3 wells, was evaluated using colon cancer and melanoma FFPE samples and compared to analysis from Sequenom MassARRAY and Illumina MiSeq. In this study, the cross-reactivity between individual mutations at each codon was evaluated and compared to Qiagen Therascreen assay.

Results: Previous results demonstrated the performance of the assay against characterised colon cancer and melanoma FFPE samples demonstrating >96% concordance with Sequenom MassARRAY and Illumina MiSeq. PASS MNAzyme® qPCR demonstrated sensitivities of <1% of mutant alleles (down to 0.01% in well optimised assays) and had excellent specificity with minimal cross-reactivity for all individual targets.

In the current study, this multiplex format showed delta Cq values >8 between mutants and >12 between mutant and WT, in comparison to the singleplex Qiagen therascreen product sheets which states delta Cq values of >3 between mutants.

Conclusion: Since PASS MNAzyme® qPCR affords greater multiplex capacity, along with high specificity and sensitivity, it provides a superior tool for ascertaining mutations from tumour tissues. Its ability to detect low levels of mutant alleles makes it particularly well suited for use with liquid biopsies.

(1)Vandenbroucke et al. Cancer Res October 1, 2014 74; 1502

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Determination of Reference Intervals for Catecholamine and Metanephrine Excretion Using Archived Patient Data

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Objective: Fractionated urinary metanephrines and catecholamines are measured for diagnosing and monitoring treatment of pheochromocytoma tumors and used infrequently to investigate dysautonomias. We sought to establish reference intervals for excretion (ug/day) in pediatric and adult populations using archived patient values.

Methods: A data set of ~122,000 archived urine metanephrine and catecholamine results obtained by LC-MS/MS assay were filtered based on criteria including 24-hour collection (20-28 h), creatinine concentration (>25 mg/dL), and total urine volume (400-3000 mL). Duplicate patient results were removed and Chauvenet's criterion was used to identify and exclude outliers. Age and gender partitions were identified visually by plotting excretion per day against patient age, then confirmed statistically by Student's T-test (gender) or one-way ANOVA analysis (age) and by evaluating the clinical significance of each partition, based on research literature. Reference intervals (central 95%) for dopamine, epinephrine, norepinephrine, metanephrine, and normetanephrine were determined by linear regression analysis of the percent cumulative frequency (Hoffmann method, Am J Clin Pathol. 2010; 133: 180-186) with a 5% error allowance. Graphs depicting excretion per day reference intervals as a function of age range for each analyte (in essence, a two-dimensional Forest plot) were used as a visual comparison between these derived reference intervals and published 24-hour excretion reference intervals established by conventional methods.

Results: Excretion per day (ug/day) reference intervals are summarized in the table.

Catecholamines	Age	RI	**Metanephrines**	Age	Gender	RI
	(y)	(ug/d)		(y)		(ug/d)
Dopamine	4-6	95 - 221	**Metanephrine**	7-17	F	42 - 135
	7-12	76 - 371		≥18		39 - 143
	13-17	137 - 393		7-12	M	45 - 179
	18-69	77 - 324		13-17		61 - 202
	≥70	56 - 272		≥18		62 - 207
Epinephrine	4-17	1 - 9	**Normetanephrine**	7-12	F	52 - 247
	18-69	1 - 7		13-17		73 - 266
	≥70	1 - 5		≥18		109 - 393
Norepinephrine	4-12	6 - 45		7-12	M	70 - 273
	13-17	15 - 57		13-17		92 - 312
	18-69	16 - 71		18-29		95 - 379
	≥70	11 - 60		≥30		125 - 510

Discussion: Catecholamine and metanephrine excretion is age-related while partitioning by gender is necessary only for metanephrines. Excretion per day (ug/day) provides greater clinical sensitivity while reducing interindividual variation when compared to random urine collections that are corrected to creatinine concentrations. Reference intervals derived from linear regression analysis align well with intervals determined by conventional methods. Minor discrepancies appear to be related to the timing of urine preservation and analytical methods employed in the studies.

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Prognostic biomarker of the combination of TROY and LGR5 in patients with colorectal cancer

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Background:

Wnt signaling plays an important role not only in the regeneration of colon mucosa but also in the development of colorectal cancer (CRC). Recent studies have shown an association of two Wnt target molecules with CRC in vitro. One is LGR5 (leucine-rich-repeat-containing G-protein-coupled receptor 5), and the other is a Type I transmembrane receptor member of tumor necrosis receptor superfamily 19 called TROY. LGR5 marks stem cells in multiple adult tissues and cancers including CRC, and TROY is produced in fast-cycling stem cells of the small intestine. Although LGR5 and TROY may be involved in the development and progression of CRC in vivo, in clinical samples, and in vitro, such relationships have not been investigated. To clarify these matters, we performed this study using CRC cell lines and surgical specimens.

Methods:

We used 7 CRC cell lines, 106 primary CRC tissues, and 36 non-tumor tissues. The expression levels of LGR5 and TROY were measured by quantitative real time-PCR.

Results:

Expressions of LGR5 and/or TROY mRNA were detected in 5 of the 7 CRC cell lines, of which 4 cell lines revealed overexpression of both genes. In the clinical samples, the expression levels of LGR5 correlated statistically with TROY ($r = 0.485$, $p < 0.001$, two-tailed Spearman's test). Expression levels of LGR5 and TROY were significantly higher in the CRC specimens of stages I to IV than in the non-tumor tissues ($p < 0.0001$). In addition, the duration of disease-free survival was shorter in the patients with high levels of both LGR5 and TROY than in those without these molecules ($p = 0.0376$ by Kaplan-Meier analysis).

Conclusion:

This study suggests that co-overexpression of LGR5 and TROY may play an important role in CRC progression, and these two genes may be possible biomarkers for the prediction of CRC prognosis.

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Serum human epididymis protein 4 (HE4) is a better tumor marker in the early lung cancer diagnosing

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Abstract

Background:HE4 had been shown to be a novel tumor marker in lung cancer. However, there were few reports about the comparison of serum HE4 with conventional tumor markers. This study aimed to explore the diagnostic value of serum HE4 as a tumor marker in early pulmonary cancer and compared it with CEA,NSE,Cyfra21-1 and proGRP .

Methods: We collected blood from 172 individuals, 112 with lung cancer who had indications for surgery and the postoperative pathology showed Ior II stage according to TNM classification, 60 health examination people as healthy controls in Shandong Tumor Hospital from May 2014. The levels of traditional biomarkers and HE4 were measured through electrochemiluminescence assays (Roche E601 MODULAR Immunoassay Analyzer).

Results: Serum HE4 could accurately distinguish between lung cancer and healthy controls (p<0.0001). Using the cut-off value of 66.8 pmol/l, HE4 had a sensitivity of 43.80% and specificity of 95.00% with a receiver operating curve (ROC) of 0.822 in early lung cancer. In terms of histological types, serum HE4 had better diagnostic value than CEA in lung adenocarcinoma (ROC-AUC: 0.859 vs 0.663; sensitivity: 43.50% vs 26.10%; specificity was both 95.00%). HE4 showed a higher sensitivity than Cyfra21-1 in patients with pulmonary squamous cancer (ROC-AUC: 0.87 vs 0.834; sensitivity: 57.10% vs 37.10%;specificity was both 95.00%); However, ProGRP was the more sensitive biomarker than HE4 in diagnosing SCLC. Furthermore, combination HE4 with traditional biomarkers further improved the sensitivity in diagnosing lung cancer.

Conclusion: Serum HE4 could serve as a biomarker to diagnose early lung cancer and the diagnostic value of it was better than traditional tumor markers.

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CA 19.9 and CA 125 for diagnosis of mucinous ovarian cancer

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Background: Mucinous ovarian cancer (MOC) is an epithelial ovarian cancer that contains cysts and glands lined by mucin-rich cells and constitute 5-20% of ovarian carcinomas. The aim of this study was to determine the accuracy of carcinoembryonic antigen (CEA), cancer antigen 15.3 (CA 15.3), cancer antigen 19.9 (CA 19.9) and cancer antigen 125 (CA 125) for diagnosis of MOC in patients with mucinous ovarian tumors.

Methods: Samples were collected preoperatively from patients with mucinous ovarian tumor. We measured the serum concentrations of the tumor markers by electrochemiluminescence immunoassay (ECLIA) in MODULAR E-170 (ROCHE DIAGNOSTIC®). The reference ranges are: CEA (0-3.4 ng/mL), CA 15.3 (0-30 U/mL), CA 19.9 (0-37 U/mL) and CA 125 (0-35 U/mL). After surgery, histology and stage were determined according to FIGO-classification. Patients were classified into two groups according to the diagnosis of ovarian biopsy: NOT MOC (mucinous ovarian cystadenomas and mucinous ovarian borderline tumor) and MOC. All variables were included in a multivariate regression analysis to identify variables independently associated with MOC.

Results: We studied 94 patients with ages between 15 and 80 years old (median = 43). Eighty-two patients were NOT MOC (68 mucinous ovarian cystadenomas and 14 mucinous borderline ovarian tumor) and 12 were MOC. All MOC patients were in FIGO I or II stages. No statistically significant differences were found between MOC and NOT MOC patients according to CEA and CA 15.3 ($p>0.05$). All MOC patients had abnormal serum CA 19.9 and/or CA 125 levels. Using CA 19.9 and CA 125, we performed a linear regression formula CA 19.9+125 = 0.00102 x CA 19.9 + 0.00057 x CA 125. AUCs values were 0.862 ($p=0.0002$), 0.829 ($p=0.0021$) and 0.911 ($p=0.0001$) for CA 19.9, CA 125 and CA 19.9+125 respectively. CA 19.9+125 exhibited 95.1 % specificity and 66.7% sensitivity, increased by 16.7% sensitivity compared with using only CA 19.9 or CA 125.

Conclusions: Preoperative CA 19.9 and CA 125 levels showed high diagnosis efficacy to predict whether a mucinous ovarian tumour is benign or malignant. Using both markers simultaneously increases the sensitivity for diagnosis of MOC.

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Stability and inter-laboratory performance for the determination of the 4Kscore for prostate cancer

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Background: Panels of prostate-specific biomarkers have been reported to improve diagnostic accuracy of blood tests for prostate cancer. The 4Kscore Test, based on a panel of four biomarkers (total prostate specific antigen(tPSA), free PSA(fPSA), intact PSA(iPSA), and human kallikrein 2(hK2)) has been clinically demonstrated to provide a personalized, accurate risk of aggressive prostate cancer (Gleason score ≥7). Clinical validation in multiple European retrospective cohorts and more recently in a prospective study of 1,012 American patients scheduled for prostate biopsy (Parekh et al. 2014) showed excellent discrimination between men harboring clinically relevant cancer and those with indolent tumors or no cancer (AUC 0.82). In clinical practice, the test improves on the current limitations of detection of prostate cancer. The reliable determination of the four biomarkers in routine laboratory practice is an essential requirement to achieve and reproduce the attractive performance characteristics of the test. This work focuses on thorough characterization of the biomarkers' stability profiles and confirms key assay performance characteristics across three laboratories, necessary for the widespread clinical usage of the test.**Methods:**Marker stability in whole blood, plasma, and serum was characterized along with possible recovery bias in frozen specimens using matched clinical samples (single blood collection per patient). iPSA and hK2 custom assays were performed on an AutoDELFIA utilizing time-resolved fluorescence while tPSA and fPSA assays were performed on the Roche Cobas instrument or the AutoDELFIA. Real-time analyte stability studies compared recoveries measured under various handling conditions to that in EDTA plasma frozen at harvest or recovery at Day 0. Recoveries of all analytes were assayed for up to nine days following collection, with up to three days' storage as blood. Analyte recoveries in approximately 400 freshly assayed plasma were evaluated versus frozen plasma. These recoveries were also evaluated in 400 frozen sera versus matched plasma. The analytical performance of the iPSA and hK2 assays, including analytical sensitivity, precision, and accuracy, was evaluated in EDTA plasma across three independent laboratories using CLSI protocols.**Results:**95% of tPSA, fPSA, iPSA, and hK2 recoveries remained within 5% of control values in EDTA plasma refrigerated seven days; recoveries were also stable in specimens stored as EDTA whole blood 60 hours refrigerated and 24 hours at room temperature. Conversely, iPSA and hK2 recoveries decreased rapidly in stored serum. tPSA, fPSA, and hK2 recoveries in frozen plasma were equivalent to those in frozen serum (slope=0.95-1.05,r2>0.98) while iPSA recoveries in the two matrices are linearly related (slope=1.11,intercept =0.017,r2=0.98). Recoveries of all four analytes in fresh plasma were equivalent to those in frozen plasma (slope=0.95-1.05,r2>0.99). A complete characterization of analytical performance on three instruments confirmed robust reproducibility and accuracy at clinically relevant ranges of iPSA and hK2 in EDTA plasma, with the limit of quantitation for the iPSA assay at 13.9 pg/mL and the hK2 assay at 22.0 pg/mL.**Conclusion:**All four biomarkers used to calculate the risk of aggressive prostate cancer are stable in both EDTA plasma, and in EDTA-anticoagulated whole blood. Inter-laboratory performances demonstrate excellent overall analytical performance for iPSA and hK2 custom assay.

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Value of dFLC data in monitoring response to therapy in a patient with Primary Amyloidosis

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Background: Recent evidence have shown that serum free light chains (sFLC) and, particularly, the difference between involved and uninvolved sFLC (dFLC) are a well-established method for identifying and monitoring the response to therapy in patients with Primary Amyloidosis (AL).

Methods: A 46 years old woman was diagnosed with AL presenting renal and cardiac damage. In the initial study, the patient presented a creatinine of 5.9 mg/dL, total proteins=5,8 g/dL, normal serum protein electrophoresis (SPE), negative serum immunofixation (IFE), Beta-2-microglobulin=17.8 mg/L, albumin=3.19 g/dL, pro-BNP=6233 ng/L, Bence Jones Proteinuria (BJP) positive for free kappa (16.8 mg/dL), 7% of plasma cells in bone marrow, renal biopsy positive for kappa amyloid material and the echocardiography study showed septal hypertrophy. The patient began treatment with Melphalan and Prednisone (MP). The sFLC were measured by turbidimetry (Freelite, The Binding Site).

Results: The sFLC were expressed as dFLC (free kappa / free lambda / ratio). At diagnosis (day 0), dFLC was 71.6 mg/L (94.8 mg/L / 23.2 mg/L / 4.08) and PBJ was 16.8 mg/dL. After two cycles of MP (day +61), the patient achieved a status of partial response (PR) with dFLC value of 25.9 mg/L (52.8 mg/L / 2.03 mg/L / 3.12) and the reduction in dFLC was of 64%. PBJ was 18 mg/dL. After fourth, fifth and sixth cycles of MP, the dFLC increases with value of 28.5 mg/L (41.7 mg/L / 13.5 mg/L / 3.08) at day +120; 39.6 mg/L (53.8 mg/L / 14.2 mg/L / 3.78) at day +150 and 43 mg/L (58 mg/L / 15 mg/L / 3.86) at day +181. PBJ values were 10.0, 13.9 and 12.5 mg/dL, respectively. The increase of dFLC was the only parameter that showed us the existence of biological progression of the disease and the hematologist decided to change the treatment to Bortezomib/Cyclophosphamide/Dexamethasone (VCD). The patient presented good tolerance at this new treatment with dFLC of 28 mg/L (38.2 mg/L / 10.3 mg/L / 3.7) at day +221 (after first cycle of VCD) and 8.32 mg/L (18.1 mg/L / 9.78 mg/L / 1.85) at day +255 (after second cycle of VCD). The BJP were 8.26 and 14.21 mg/dL, respectively. Serum SPE and IFE were negative during the two lines of treatment of the patient.

Conclusion: The quantification of sFLC was the only assay that allows us the monitoring of the response to the treatment in this patient. dFLC was an effective tool that helps the hematologist to evaluate the response to the treatment and the presence of biological progression. PBJ was positive but ineffective to evaluate the response to the treatments. Periodic monitoring of dFLC allows us to predict whether the patient responds correctly to chemotherapy or, conversely, has a relapse and need to change treatment.

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Verification and Validation of LUMIPULSE G CA125II Assay under Development

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BACKGROUND: Serum CA125 values are useful for monitoring the course of disease in patients with invasive epithelial ovarian cancer (OC) (NIH Consensus Conference, JAMA, 1995). The current study was to evaluate the analytical and clinical performance of the LUMIPULSE ***G*** CA125II assay (LUMIPULSE CA125II assay) under development. The LUMIPULSE CA125II assay has not been cleared by the FDA.

METHODS: LUMIPULSE CA125II assay is a second-generation CA125 immunoassay using monoclonal antibody (MAb) OC125 as the solid phase antibody and the MAb M11 as the labeled antibody. Serum or plasma is incubated with the MAb OC125-linked magnetic particles followed by washing and rinsing steps. Alkaline phosphatase-labeled MAb M11 is then incubated with the CA125-bound particles followed by washing and rinsing steps. Substrate AMPPD (3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy)phenyl-1, 2-dioxetane disodium salt) is then added to generate luminescence signals reflecting the amount of CA125 in the samples.

RESULTS: In the monitoring study of serial serum samples collected in SST tubes from 59 patients with OC, a total of 289 pairs of observations were undertaken. A positive change of CA125 values was defined as an increase if the change was at least 20% greater than the previous value. 67% of 52 samples with a positive change correlated with the disease progression of OC while 76% of the 237 samples with no significant change correlated with no progression of OC. The total concordance was 74%. A reference range study showed CA125 ≤ 35.0 U/mL in 99.2% of 240 healthy female subjects, 80% of 40 females with pregnancy, 90.4% of 260 females with benign gynecological disease, 92.5% of 40 females with a non-gynecological benign disease, 80.0% of 40 females with congestive heart failure, and 85.0% of 40 females with hypertension, respectively. Analytically, the assay's LoQ (Limit of Quantitation) was ≤ 2.0 U/mL, and linearity ranged 2.5 - 1000 U/mL. The imprecision studies showed a total CV ≤ 6.4% in a 20-day CLSI EP5-A2 study (8 sera, n = 80), inter-site study with 3 sites and inter-lot study using 3 lots of reagents. Spike recovery ranged from 96% - 115%. The average levels of CA125 in the individual interferent-spiked samples (9 endogenous substances including human anti-mouse antibodies and rheumatoid factor, and 16 therapeutic drugs) were within -5% to 3% difference of that in the unspiked samples. No high dose hook effect was observed for samples with up to 200,000 U/mL of CA125. Method comparison to ADVIA Centaur CA125II generated a Passing Bablok correlation as [LUMIPULSE CA125II] = 0.753 + 0.991 [ADVIA Centaur CA125II] within the range of 3.4 - 17755.2 U/mL (n = 120, r = 0.9998). Specimens collected in SST, K2-EDTA, sodium heparin, and lithium heparin tubes correlated with that in serum tubes with r ≥ 0.9 and a 95% confidence interval of slope ranging 0.9 - 1.1.

CONCLUSION: The LUMIPULSE ***G*** CA125II assay under development demonstrates to be an accurate, precise, sensitive and robust assay for measuring CA125 in human serum and plasma.

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Validation of Fluorescence in Situ Hybridization assay for detection of TP53 gene deletion

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Background: Fluorescence in Situ Hybridization (FISH) provides an important adjunct to conventional cytogenetic in the evaluation of chromosomal abnormalities associated with hematologic and other neoplasias. FISH analysis offers a sensitive, specific, and reliable strategy for identifying acquired genetic abnormalities such as loss of a chromosomal region associated with hematologic disorders. Hemizygous deletion of TP53 gene has been identified in the Chronic Lymphocytic Leukemia, Acute Myeloid Leukemia, Multiple Myeloma and others.The presence of TP53 deletion is associated with adverse prognosis. Although the performance of most FISH probes has been evaluated by the manufacturer prior to marketing, it also must be validated prior to implementation of assay for clinical use. Clinical laboratories must independently adopt protocols in order to verify the performance of the assay.

Objective: To validate FISH assay for detection of TP53 gene deletion following recommendations from the American College of Medical Genetics (ACMG).

Methods: We used the P53 deletion probe manufactured by Cytocell®. During the familiarization phase of probe validation the analysts should become familiar with the concepts underlying the probe labeling and testing strategy. Metaphases cells obtained from 5 karyotypically normal male blood samples were used to localize the probe and determine its sensivity and specificity. To establish a reference range (normal cutoff) were estimate the false positive rate from 10 uncultured bone marrow samples and 10 uncultured peripheral blood samples that would be unlikely to harbor P53 deletion. Two analysts score 500 interphase cells (250 per analyst). All P53 probe signal patterns were recorded. The normal cutoff value for the P53 FISH assay was calculated using the beta inverse function (BETAINV) available in Microsoft Excel.

Results:The TP53 deletion probe presents two differentially labeled probes: a probe covering the TP53 gene labeled in red and a control probe labeled in green. A typical result of using this probe should present two green signals and two red signals (2G2R). In the analysis of 10 bone marrow samples were identified six atypical signals patterns: 2G1R, 1G1R, 3G2R, 2G3R, 3G3R, 4G4R. Analyzing 10 peripheral blood samples we observed four atypical signal patterns: 2G1R, 1G1R, 3G2R, 2G3R. The cutoffs obtained with BETAINV function was validated for counting 200 cells. The signal patterns and respective normal cutoffs for bone marrow samples are 2G1R (5,67%), 1G1R (2,34%), 3G2R(3,78%), 2G3R(2,34%), 3G3R(2,34%), 4G4R(2,34%) and for peripheral blood samples are 2G1R(3,78%),1G1R(2,34%), 3G2R(2,34%), 2G3R(2,34%).The clinical validation of FISH showed presence of the TP3 deletion, in agreement with conventional cytogenetic. The probe demonstrated 100% specificity and sensitivity higher than the recommended by ACMG.

Conclusion:The assay FISH for detection of the TP53 gene deletion was considered approved since it showed excellent reproducibility and high quality in different hybridizations, specificity and sensitivity higher than those guaranteed by the manufacturer of the probe.

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A familial adenomatous polyposis case with a novel (c.4091delG; p.Ser1364Metfs*51) mutation

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Familial adenomatous polyposis (FAP) is an autosomal dominant inherited colon cancer syndromecaused by mutations in theadenomatous polyposis coli (APC) gene (OMIM#611731). APC is a tumor suppressor gene that encodes a 2,843 amino acid protein involved in several cellular processes, including the control of β-catenin turnover in the Wnt pathway.Inactivation of the APC gene plays a significant role in FAP and more than1315 variants are described for APC in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), with most of the FAP related mutations being nonsense or small insertions/deletions that lead to a truncated protein. This report describes a 32-year-old male with a familial adenomatous polyposis (father, brother and aunts affected) and a case of an aunt diagnosed with Gardner syndrome, a variant of FAP. PCR and Sanger sequencing of all APC exons and 50bp flanking intronic regions identified a novel case of pathogenic APC mutation in exon 15: a G deletion at cDNA position 4091 (c.4091delG; p.Ser1364Metfs*51 - NM_000038.5). This mutation results in a frame shift and stop signal 51 codons downstream, causing a premature truncation of the APC protein at amino acid position 1415. ClinVar, Ensembl,LOVD, 1000 Genomes Project, ESP6500 and dbSNP141 databases searches revealed this mutation has never been described before. Online prediction program Mutation Taster (http://doro.charite.de/MutationTaster/index.html) classified this mutation as disease causing with a probability value of 1. Genetic investigation of this mutation in at risk family members should be performed to allow presymptomatic diagnosis and prophylactic interventions when necessary, as well as to increase the knowledge about genotype-phenotype correlations of this novel mutation.

CONCLUSION: We validated the test using Sanger sequencing methodology and demonstrated that this technique is a reliable and useful tool for the detection of the Jewish founder mutations in *BRCA1* and *BRCA2* genes. Based on the relatively high frequency of the founder mutations we reinforce the importance of this test for initial screening of *BRCA* mutations in the Ashkenazi population.

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Gene panel NGS testing for hereditary breast cancer in Brazilian patients: Hermes Pardini Institute case report

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Breast cancer is the most common cancer in women worldwide and alsothe main cause of death from cancer among women.Around 5-10% of these tumors present a strong hereditary component due to mutations in highly penetrant genes. BRCA1 and BRCA2 are the two most frequently mutated genes related to Hereditary Breast and Ovarian Cancer Syndrome (HBOC) and account for up to 45% hereditary breast cancers. The remaining 55% of severely affected families that do not carry mutations in BRCA genes represent a diagnostic challenge from the genetic and clinical perspective, and are less benefited from screening and prevention measurements. Recent advances in sequencing technologies have enabled the discovery of several novel genes related to breast cancer increased risk, such as BRIP1 and PALB2. Additionally, the development of the Next Generation Sequencing (NGS) technologies allowed the clinical use of targeted gene panels that screen multiple susceptibility genes in parallel, increasing the mutation detection rates and reducing results turnaround time. Hermes Pardini Institute, one of the most important diagnostic laboratories in Brazil, developed a NGS gene panel - named as Breast Cancer Panel 2 (BCP2) - for screening 15 breast cancer related genes in BRCA mutation negative patients.This panel includes 15 genes: ATM, BARD1, BRIP1, CHEK2, CDH1, MRE11A, NBN, PTEN, PALB2, RAD50, RAD51C, RAD51D, STK11, TP53 and XRCC2. Since January 2014, 12 female patients were tested with BCP2 panel. From these, no significantly clinical mutation was detected in four patients and 8 patients presented variants of unknown clinical significance (VUS) in one or more genes. We considered as VUS undescribed missense mutations or described variants with minor allele frequency (MAF) <0.02. ATM mutations were identified in four patients (p.F858L and p.P1054R in the same patient, p.F858L, p.P604S, p.K1454N); two NBN mutations (p.F263S and p.P593A) were identified inone patient; MER11A (p.T303I), BARD1 (p.K130T), CDH1 (p.A617T); BRIP1 (p.V193I) and CHEK2 (p.D438Y) mutations were identified in one patient each. One PALB2 (p.G998E) mutation was identified in three patients. Although reported dbSNPMAF for this variant is 0.008, novel studies reported a frequency of 2% in the studied population, reducing the probability of this being a pathogenic mutation. Our study demonstrates the utility of using NGS panel testing to investigate mutations in breast cancer susceptibility genes, establishing it as a cost effective and sensible test for clinical diagnosis. The main challenge for the scientific and clinical communities now resides in increasing the available information on phenotypes and genotypes of these novel susceptibility genes, allowing a correct classification of these genes VUS, as already occurs for BRCA1 and BRCA2 genes.

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Pleural fluid homocysteine levels for diagnosis of malignant pleural effusion

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Background: Malignant pleural effusions (MPE) are a common clinical problem in patients with cancer. A cytologic test is the standard basis for diagnosis of MPE, but positivity rate is only 60%. Pleural biopsy can greatly improve diagnostic sensitivity (90%), however it has a high cost and is associated with injury and other complications and thus is limited in its clinical application. Efforts have been made to find markers that would improve the positivity rate in MPE, including tumor markers. Homocysteine (HCYS) levels in serum are used as tumor marker in colorectal and breast cancer. We have not found any paper published of HCYS levels in pleural fluid. The aim of this study was to measure the accuracy of HCYS in pleural fluid for diagnosis of MPE.

Methods: We studied pleural fluids obtained by thoracocentesis in patients with pleural effusion. HCYS in pleural fluid were measured by nephelometry in BNII (SIEMENS®). Patients were classified into two groups according to the aetiology of pleural effusion: benign pleural effusions (BPE) and MPE. Pleural effusion was categorized as MPE if malignant cells were demonstrated in pleural fluid or pleural biopsy. Statistical analysis was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC) using the software MEDCALC®.

Results: We studied 60 patients with ages between 1 and 89 years old (median = 61.2), 23 women and 37 men. Half of the patients were MPE, 3 mesotheliomas, 13 lung cancers, 4 breast cancers and 10 other tumors. The BPE were: 11 transudates, 9 parapneumonics, 2 tuberculosis and 8 other benign aetiologies. The median HCYS value in pleural fluid was significantly higher in MPE 13.95 µmol/L (CI 95%: 12.31-18.37) vs. 8.22 µmol/L (CI 95%: 7.08-12.74) in BPE patients. The AUC of HCYS in pleural fluid for diagnosis of MPE was 0.806 (CI 95%: 0.683-0.896) ($p<0.0001$). The patients with HCYS in pleural fluid 16.40 µmol/L were MPE.

Conclusions: HCYS levels in pleural fluid showed high diagnosis efficacy to predict whether a pleural effusion is benign or malignant.

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Measurement of Human Epididymis Protein 4 (HE4) may be Closely Associated with Breast Cancer Progression

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Background: Human epididymis protein 4 (HE4) is described as a useful new biomarker in ovarian cancer however, it can be elevated in a variety of benign and malignant diseases and therefore it is neither tumor nor organ specific. For example approximately 10% of ovarian cancers are familial and relate to mutations of BRCA1, BRCA2, and mismatch repair genes. In this study, we have investigated the occurrence of this protein in female with breast cancer to monitor any diagnostic utility with the progression of the disease after therapy in combination with CA153.

Methods: HE4 was measured in the sera of 20 healthy individuals, and 30 patients with breast cancer under treatment. HE4, CA153 and CA125 were measured by immunoassay. The statistical analysis were also performed and p-value was calculated. All clinical and histological data were reviewed.

Results: The sensitivity and specificity of HE4 in patients with breast cancer were found to be 47% and 93% respectively .The median, mean, and standard deviation (SD) for HE4 were found to be 35.6, 38.0 and 9.5 in healthy women respectively. For patients with breast cancer but normal CA153 and CA125, the median, mean, and SD of HE4 were found to be 51.9, 54.1 and 18.2 respectively and 89.6, 117.0 and 78.8

respectively for those with abnormal CA153. There was a significant difference in the mean of HE4 among patients with breast cancer of those with normal CA153 versus those with abnormal CA153 ($p< 0.01$). Similarly both groups with either normal or abnormal CA153 values have shown a significant difference in the mean of HE4 against the healthy and breast cancer patients ($p<0.0001$). Among 30 cases of breast cancer, 28 were negatively free from any ovarian or pelvic cancer. One case confirmed with ovarian cancer and the other with endometrium cancer.

Conclusion: HE4 may have a role in monitoring breast cancer patients, however, due to some limitations and low sample size, further studies may be needed to obtain a solid conclusion for the utility of this marker in breast cancer patients.

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Prognostic value of onconeural antibodies in patients with paraneoplastic neurological syndromes

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Background: Onconeural antibodies (OA) are strongly associated with cancer and paraneoplastic neurological syndromes (PNS). PNS can be defined as remote effects of cancer and are seen <1% of patients with cancer. Most of these antibodies are well-characterized (antibodies against Hu, Yo, Ri, CRMP5, amphiphysin, Ma-2 and Tr) and are in common use for the diagnosis of definite PNS. The aim of our study is to determine the percentage of OA detected in our Laboratory of Autoimmunity during last two years (2013-2014) and the possible association with PNS and tumor pathology.

Methods:OA were studied on 421 patients with neurological symptoms during a period of two years. OA were identified in serum sample by indirect immunofluorescence (IIF, Euroimmun AG) and recombinant immunoblot assay (Ravo Diagnostika) that detects Hu, Yo, Ri, CV-2, Ma-1, Ma-2 and amphiphysin autoantibodies. One result is considered positive when it is confirmed by the two techniques.

Results:OA were positive in 7 patients only (2%). The OA detected were: anti-Hu in 5 samples (72%), anti-amphiphysin in one sample (14%) and anti-Ma-2 in one sample (14%). Three positive results of anti-Hu corresponded to the same patient with multiple sclerosis and suspected of tumor pathology in which the OA were measured periodically during the two years of the study without finding associated neoplastic pathology. The PNS and tumor associated to the other four positive results are shown in the table.

Conclusion: In our study the percentage of OA detected is very low (2%). Except one patient with multiple sclerosis, positive anti-Hu antibodies and absence of tumor pathology, the rest of the OA were associated with tumors and poor prognostic outcome.

Paraneoplastic neurological syndromes, onconeoural antibodies and tumors associared

Onconeural antiobodies	Gender	Age (years)	Paraneoplastic neurological syndromes	Tumor associated	Survival (months)
anti-Hu	Male	67	Paraneoplastic encephalitis	Lung adenocarcinoma	19 months (still alive)
anti-Hu	Female	50	Paraneoplastic encephalitis	Small cell lung cancer	7 months (exitus)
anti-Ma-2	Female	65	Acute cognitive impairment	Breast cancer	2 months (exitus)
anti-amphiphysin	Male	79	Limbic encephalitis	Squamous cell lung carcinoma	2 months (exitus)

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A quick protocol for the identification of Multiple Myeloma in patients attending Emergency Services with severe bone pain

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Background: Lytic bone metastases are due to a variety of primary tumors that include Multiple Myeloma (MM) and solid tumors like lung, breast, thyroid or prostate cancer. Its effects result in pain refractory to conventional analgesic treatments and osteolysis leading to spinal cord compression and pathological fractures. In 70% of all cases, the pain is the most common symptom of the bone metastases. Sometimes, patients with age over 50 years and intense bone pain are treated with analgesics without assessing the possibility of a MM at Emergency Services. After several visits to the emergency service because of the progressive increase pain and evidence of bone damage as pathological fractures the patient is admitted to study a possible MM. MM is one of the cancers with greater delay in diagnosis. Early study of the presence of pathological bone lesions is crucial for a correct diagnosis and increase the survival time of patients. The combination of quantification of serum free light chains (FLC) and serum protein electrophoresis (SPE) enables sensitive quantification of monoclonal component in the study of MM. This protocol (SPE+FLC) can help us to detect a MM in patients with incidental clinical findings without diagnosis at Emergency Service of the Hospital.

Methods: During a period of 12 months, we studied 44 patients with age>50 years old, intense bone pain and recurrent visits to Emergency Service where imaging methods (X-Rays, CT scan and MRI) showed osteolytic lesions, vertebrae collapse and pathological fractures that may be associated a MM or metastasis from a primary tumor of unknown origin (TU). The protocol (SPE+FLC) was applied to every patient to study a possible MM and the determination of tumor markers to discard a TU with bone metastasis.

Results: The diagnosis was: MM in 16 patients (36%), TU with bone metastasis in 14 patients (32%) and 14 patients without tumoral pathology (32%). In MM patients, the median age was 68 years (range 58-75) and the median time from symptoms to diagnosis was 5 months (range 2-7) with a median number of visits to Emergency Service of 3. The diagnosis was intact immunoglobulin MM in 13 patients and Bence-Jones MM in 3 patients. According to ISS system for MM; there were 2 patients in stage 1 (12%), 4 patients in stage 2 (25%) and 10 patients in stage 3 (63%). During the study there were 3 MM related deaths. The protocol "SPE+FLC" had a sensitivity of 100%, specificity of 97%, PPV of 94% and PNV of 100%.

Conclusion: In patients with age>50 years, intense bone pain with pathological bone lesions, the application of the protocol "SPE+FLC" allow us to detect a possible MM in order to apply an early treatment and increase the survival time of the patient.

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QClamp a rapid and highly sensitive assay for detection of tumor FGFR3 mutations

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Background: Biological fluid-based noninvasive biomarker assays for monitoring and diagnosing disease are clinically powerful. A major technical hurdle for developing these assays is the requirement of high analytical sensitivity so that biomarkers present at very low levels can be consistently detected. In the case of biological fluid-based cancer diagnostic assays, sensitivities similar to those of tissue-based assays are difficult to achieve with DNA markers due to the high abundance of normal DNA background present in the sample.Somatic mutations in the FGFR3 gene have been associated with several cancers, most notably bladder cancer. Activating mutations in FGFR3 occur in approximately 50% of all bladder cancers and at higher frequencies in tumors of low-grade and low stage (approximately 60%-70%).There are nine common FGFR3 mutations associated with bladder cancer that are located in three exons, i.e, exons 7, 10, and 15, with the exon 7 (S249C) mutation being the most prevalent (about 62%).

Methods:Here we describe a new urine-based assay that uses a xeno-nucleic acid (XNA) clamping technology to detect low frequency somatic mutations of fibroblast growth factor receptor 3 (FGFR3) DNA that are indicative of bladder cancer. Detection of FGFR3 mutations in urine will provide clinicians with a noninvasive means of diagnosing early-stage bladder cancer.

Results:The XNA enrichment real-time qPCR assay (QClamp) detects activating oncogenic point mutations in Exon 7 (R248C and S249C) and Exon 10 (G370C and Y373C) of FGFR3 tumor derived DNA when present at an allelic frequency as low as 0.1% in a background of wild-type DNA.

Conclusion:We have developed a highly sensitive real-time PCR assay for detection of activating mutations in the FGFR3 gene that can be used to monitor the cancer recurrences and the development of tumor resistance to targeted therapies. The technology is rapid, noninvasive and can be used to detect mutations in tumor DNA found in the urine of bladder cancer patients, presenting a patient-friendly diagnostic alternative to standard-of-care cystoscopy.

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Utilization of specific intact human chorionic gonadotropin analysis for pregnancy screening significantly reduces false positive results in cancer patients with the potential to improve patient care and institutional efficiency

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Background: It is standard protocol at our cancer center that women of childbearing age undergo pregnancy screening prior to any major medical procedure. False positive (FP) results are an issue as they cause delays to procedures, undue stress on the patient, scheduling conflicts, and delays for other patients. These FP results occur because a number of epithelial neoplasia can produce beta-human chorionic gonadotropin (bHCG). This subunit of human chorionic gonadotropin (HCG) is detected by the majority of HCG quantitative pregnancy assays available in the United States as they all target the beta subunit without differentiating between intact HCG (iHCG) and bHCG.

Objective: Since it is reported that iHCG predominates in early pregnancy, we believe that a reagent specific for iHCG would be useful in cancer patients. We will show that such an assay can potentially eliminate FP pregnancy tests due to tumor-derived bHCG.

Methods: From 10/21/14 – 01/20/15, patient serum samples tested on the Tosoh AIA 2000 by immunoenzymometric assay were identified having total HCG (tHCG) values ≥5 mIU/mL. This method uses enzyme labeled monoclonal antibody specific to the beta subunit to quantify the total amount of HCG. These samples were retested using a quantitative assay (Tosoh) specific for iHCG on the Tosoh AIA 2000 analyzer. Patient specimens with tHCG >14 mIU/mL, our institutional cutoff, were considered compatible with pregnancy. This unique use of 14 mIU/mL as the upper limit of normal was validated in our patient population because the cutoff of 5 mIU/mL resulted in an unacceptable amount of FP results. Results were compared with clinical history as the gold standard.

Results: Samples from 57 female cancer patients of childbearing age were attained among 2241 specimens analyzed for tHCG during the time period. Of them, 32 had tHCG >14 mIU/mL and 25 between 5-14 mIU/mL. Of the 32 with >14 mIU/mL tHCG, eleven had iHCG below 14 mIU/mL attributable to tumor-derived bHCG yielding FP results. There were an additional two non-pregnant patients with elevated iHCG possibly due to their cancer. Together this makes up 13 FP cases due to HCG secreting tumors, of which 11 (85%) would be eliminated via iHCG analysis. The remaining 19 cases were due to pregnancy and exogenous HCG from oocyte harvesting procedures. Of the patients with results between 5-14 mIU/mL, two showed low amounts of iHCG with comparatively higher tHCG (iHCG of 0.7 and <0.5, and tHCG of 13.5 and 7.6 mIU/ml, respectively) consistent with tumor origin. The remaining 23 of these patients had similar values for both iHCG and tHCG suggesting that iHCG is more commonly elevated above 5 mIU/mL in cancer patients.

Conclusion: Pregnancy screening within the cancer population should utilize iHCG specific analysis. Based on our preliminary data, this method definitively rules out pregnancy and markedly reduces the FP rate in this setting at a cutoff at14 mIU/mL. In turn, interruptions to patient care are decreased, patients experience less undue stress, and efficiency in scheduling and delivering of medical procedures and treatments would be improved. Additional studies are recommended to further support these conclusions.

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Validation of the Abbott Architect alpha-fetoprotein and total beta-human chorionic gonadotropin assays in cerebrospinal fluid for the management of central nervous system germ cell tumors

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Background: Central nervous system (CNS) germ cell tumors are a heterogeneous group of malignant brain tumors formed from primitive germ cells that primarily afflict children and young adults. The measurement of alpha-fetoprotein (AFP) and human chorionic gonadotropin (hCG) in cerebrospinal fluid (CSF) is of clinical utility for diagnosis, assessment of treatment efficacy and monitoring for recurrence. However, commercial assays for AFP and hCG are validated for use in serum or plasma but not for CSF. Therefore, the objective of this evaluation is to validate the analytical performance of the Abbott Architect alpha-fetoprotein (AFP) and total beta-human chorionic gonadotropin (beta-hCG) assays in cerebrospinal fluid (CSF) for their use in the management of CNS germ cell tumors. **Methods:** The evaluation was performed using a pool of nineteen AFP and beta-hCG-negative CSF pediatric samples to which known concentrations of AFP or beta-hCG from serum samples were added. Potential matrix effects were assessed in recovery experiments comparing serum versus CSF. Functional sensitivity was determined by dilutions to obtain the lowest concentration within 20% of the expected value having a coefficient of variation (CV) less than 20%. Linearity was evaluated with dilutions spanning the lower end of the measuring range. For imprecision, pooled samples at three different concentrations were measured in duplicate over 10 days. To determine values in the absence of disease, beta-hCG and AFP were measured in 30 pediatric CSF samples from individuals free from pregnancy, trophoblastic disease or malignancy. **Results:** Recovery values ranged from 81% to 90% for beta-hCG and 87-101% for AFP in CSF compared to serum, suggesting no significant matrix effects. Functional sensitivity was determined to be 1.59 IU/L for total beta-hCG and 0.26 ug/L for AFP, below conventionally used diagnostic cut-off values for CNS germ cell tumors of 5 IU/L and 10 ug/L. Linearity was confirmed from 2.8 to 27.8 IU/L for beta-hCG and 2.4 to 45.2 ug/L for AFP, both spanning the lower ends of their measuring ranges where the cut-off values lie. Between-day imprecision was low, with CV values from 4.1-7.7% for beta-hCG and 1.8-2.6% for AFP. Measurements in control samples were all below the measuring range of 1.2 IU/L for beta-hCG and ranged from 0-0.05 ug/L for AFP, confirming that both these markers are normally absent in CSF. **Conclusions:** The Abbott Architect assays for total beta-hCG and AFP can be used to detect beta-hCG and AFP in CSF at the low end of the analytical measuring range without significant matrix effects and with good precision, linearity and functional sensitivity. Measurements of control samples indicate that, normally, beta-hCG and AFP are essentially absent in CSF. Thus, these assays provide a suitable means to assist in the detection of malignancy for the management of CNS germ cell tumors.

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Serum tumor markers in lung cancer patients

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Background: Lung cancer (LC) is the most common malignancy in the world and a leading cause of cancer deaths. The aim of this study was to determine the accuracy of serum tumor markers: carcinoembryonic antigen (CEA), cancer antigen 15.3 (CA 15.3), cancer antigen 19.9 (CA 19.9), cancer antigen 125 (CA 125), neuron-specific enolase (NSE) and cytokeratin 19 fragment (CYFRA 21-1) for diagnosis of LC.

Methods: We studied patients with clinical suspicion of LC (tos, hemoptysis, chest pain, pulmonary node, pleural effusion). We measured serum tumor markers (CEA, CA 15.3, CA 19.9, CA 125, NSE and CYFRA 21-1) by electrochemiluminescence immunoassay (ECLIA) in MODULAR E-170 (ROCHE DIAGNOSTIC®). Patients were classified into two groups according to the diagnosis of bronchial cytology or lung biopsy: NOT LC and LC. The accuracy of serum tumor markers for diagnosis of LC were determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC). The optimal cuts off value of tumor markers for diagnosis of LC were considered with a high specificity (>90%), to avoid detection of numerous false positives, because of the low prevalence of cancer. The statistical software used was MEDCALC®.

Results: We studied 191 patients with ages between 33 and 86 years old (median = 65 years old). Ninety patients were LC (9 small cell lung cancer, 78 non small cell lung cancer and 3 cancer metastasis), and 101 were NOT LC. No statistically significant differences were found between LC and NOT LC patients according to CA 19.9 ($p>0.05$). AUC values were 0.732 ($p<0.0001$), 0.665 ($p<0.0001$), 0.666 ($p<0.0001$), 0.645 ($p=0.0005$) and 0.768 ($p<0.0001$) for CEA, CA 15.3, CA 125, NSE and CYFRA 21-1 respectively. The optimal cuts off value were 8.4 ng/mL (37.7% sensitivity and 90.7% specificity), 30 U/ml (34.1% sensitivity and 90.7% specificity), 55.3 U/ml (34.9% sensitivity and 91.8% specificity), 18.1 ng/mL (25.9% sensitivity and 90.5% specificity) and 2.9 ng/mL (51.3% sensitivity and 90.5% specificity) for CEA, CA 15.3, CA 125, NSE and CYFRA 21-1 respectively.

Conclusion: CYFRA 21-1 is the serum tumor marker that shows a higher accuracy for diagnosis of LC, exhibited higher sensitivity than CEA, CEA 15.3, CA 19.9, CA 125 and NSE.

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Tube type selection to support next generation sequencing of single cells

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Introduction: The ability to analyze a single cell using next generation sequencing (NGS) is of great value when analyzing rare cells like circulating tumor cells (CTCs) and capabilities are rapidly evolving. Sequencing a single cell is technically challenging due to many factors including cellular stability, therefore specialized tubes have been developed and optimized to retain cellular stability. Because the NGS process is labor intensive and expensive, the use of quality control checks to monitor both quality and quantity are necessary throughout the workflow. To select the most appropriate blood collection tube type when single cells will be analyzed by NGS, we chose to use one such quality control method (QuantiMIZE, Qiagen) due to its quantitative capabilities. Here, we report DNA quality from 12 single cells that were incubated in one of two blood collection tubes, K2EDTA or Streck Cell-Free DNA BCT.

Objective: The purpose of this study was to determine the most appropriate blood collection tube type to use when single cells are collected from patient samples for NGS.

Methods: We used a QC check (QuantiMIZE) that is a component of our NGS workflow to determine DNA quality of single cells incubated in 2 different tube types. Breast cancer cells (SKBR-3) were incubated for 2 hours in either K3EDTA or Streck Cell-free DNA blood collection tubes and were then sorted by DEPArray technology or hand sorted into single cell samples. A single cell whole genomic amplification (RepliG, Qiagen) was performed on each cell followed by a QC check for quality and quantity. The QC check uses 2 pools of 20 primers and probes that are either 100 or 200 base pairs long to analyse each sample as well as provided controls. Each sample and control is interrogated by 6 reactions: 3 (triplicate) reactions using both pools. Each sample is compared to the controls to determine ΔCts and a ratio of ΔCts between the 200 and 100 group is calculated. Ratios <0.04 (values are often negative) are indicative of high quality DNA. If Ct values are not reported (as is often the case for single cells), then the ratio is indeterminate and the quality is reported as poor.

Results/Validation: Out of the 6 single cells incubated in K2EDTA tubes, 4 cells (66%) were found to be of high quality with QC ratios of -.045, -.0129, -.084 and -.0154. The 2 cells determined to be poor quality had indeterminate values. Out of the 6 single cells incubated in Streck DNA tubes, only 2 (33%) were found to be of high quality with reported QC ratios of -.023 and -.051. Of the 4 cells determined to have low quality scores, the QC ratios were reported as 0.056 or indeterminate.

Conclusion: Although Streck tubes were designed to stabilize cells, this pilot found that single cells incubated in K2EDTA tubes resulted in overall higher quality DNA than cells incubated in Streck tubes. Further optimization of patient sample processing may be needed to fully maximize the stability of cells when collected in Streck tubes.

A-372

The Role of Circulating Angiogenesis-Associated Factors in Early-Stage Non-Small Cell Lung Cancer: Implications for Mechanistic Insights and Improved Methods for Prognostication

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Background:

Non-small Cell Lung Cancer (NSCLC) is the most common cause of mortality from cancer disease worldwide. The overall 5-year survival rates for NSCLC are less than 15%, given only a small portion of the cases being diagnosed at stage I where surgical resection being the most curative option. However, even in stage I, survival rates may still be as low as 50% due to occult diseases, mainly in nodal metastasis, leading to a high risk of recurrence. Angiogenesis being one of the hallmarks of cancer is believed to play a main role in cancer metastasis. Tie -2, an angiopoietin receptor, one of the angiogenesis related molecules expressed by vascular endothelium, yet to be elucidated in the relation to tumor progression. The objective of this study is to develop a panel of serum biomarkers based on circulating angiogenesis factors and to measure the levels of Tie-2 to improve prognostication of disease recurrence in stage I patients.

Method:

Serum levels of commercially available distinct angiogenesis biomarkers (EMD Millipore®, Billerica, MA) were evaluated against a cohort of 279 cases of NSCLC using quantitative multiplex bead based immunoassays (Luminex FlexMAP 3D) with concentrations calculated based on a five-parametric fit algorithm. Cohort was comprised of 188 cases of stage I ($T_{1\text{-}2}N_0M_0$) disease, compared to a cohort of 91 cases of lymph node positive ($T_{1\text{-}4}N_{1\text{-}3,\,x}M_0$) disease. In addition, a novel immunobead based assay for Tie-2 was validated in its performance both analytically and clinically against a subset of cases from previous cohorts of stage I and node positive serum cases. Statistical significance was determined using Mann-Whitney Rank Sum test (two-tailed) and Log-Rank to identify associations of biomarkers with nodal status or recurrence. Finally, the Random Forest algorithm was used to identify the optimal combination of biomarkers for prognosticating disease recurrence.

Results:

Significant associations were identified between nodal status and clinical outcome parameters including progression free and overall survival (r=0.331 and 0.336, respectively with significance of p<0.001 for both). TNF-RI, HB-EGF, pg130 and EGFR were found to be an optimal combination of biomarkers capable to predict the recurrence in stage I cases with good performance parameters (specificity = 89.5%; sensitivity = 24.3%; and a negative predictive value =75.3 %). Tie-2 was found to have a significant correlation in tumor differentiation (r= 0 .296, p=0.018). Further, significant increases in Tie-2 levels between well and poor differentiation (p=0.048) and between poor and moderate differentiation (p=0.018) were also identified.

Conclusion:

Precise identification of nodal status is crucial to develop an accurate treatment plan for patients with stage I NSCLC. In this study we evaluated a series of angiogenesis biomarkers and developed a panel that significantly can be utilized to predict recurrence in stage I. Combined with improved early detection methods, this panel can be a platform for developing a clinical laboratory diagnostic tool that could help further improve long term survival in this dreaded disease.

A-374

Use of Prostate-Specific Antigen Test in National Samples of Privately-Insured, Medicare and Medicaid Patients, 2007-2012

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Background: Prostate-specific antigen (PSA) is currently the most commonly used cancer screening test. The U.S. Preventive Services Task Force (USPSTF) recommended against PSA-based screening for prostate cancer in men older than 74 and younger than 50 years of age in 2008, and against PSA screening of all men in 2012. However, other organizations, such as American Cancer Society and American Urological Association recommend that PSA screening be provided to men aged 50-74 years only after provision of information on the screening's known harms and potential benefits. The objective of this study was to evaluate the use of PSA screening in different age groups from 2007 through 2012 by using claims data from convenience samples of privately insured, Medicare and Medicaid populations, hence providing baseline information to inform interventions needed to implement evidence-based recommendations. Methods: Outpatient claims data were collected using Truven Health Analytics' MarketScan databases for 3 enrollee population samples: commercial claims and encounters (CCAE), Medicaid, and Medicare supplemental for the years 2007 through 2012. These populations constituted an average of ~30% of privately insured US population (range, 14.0-26.0 million), as well as ~10% of Medicaid (range, 2.2-3.5 million) and Medicare (range, 1.0-2.2 million) enrollees. In order to consider PSA testing for screening only, all encounters with any one or more of 62 prostate or urinary conditions implying use of PSA for purposes other than screening were deleted. Annual claims rates per 10,000 enrollees were evaluated for up to 14 age cohorts ranging from <20 to ≥90 years. Annual claims rates for all Medicare Part B enrollees obtained from Centers for Medicare & Medicaid Services were also determined for each year. Results: Annual claims for PSA-based screening tests per 10,000 enrollees for the 3 enrollee samples in 2007-2012 ranged from 0.3-2.3 in 0-19-year age cohort to 484-3,715 in 55-59-year age group. There were 12-36 screening tests performed per 10,000 enrollees in 20-29-year age group, increasing to 428-1,726 in men 45-49 years of age. There were 188-2,158 screening tests per 10,000 enrollees in 75-79-year age group, decreasing to 69-878 in the ≥ 90-year age cohort. While there were no significant temporal trends for privately ensured (P = 0.13) populations, both Medicaid and Medicare enrollees showed significant downward trends (P = 0.02 and P = 0.04, respectively) while Medicare supplemental enrollees showed a significant upward trend (P < 0.0001). Conclusion: Prostate cancer screening with PSA continues to be done in men younger than 50 years of age and those older than 74 years old, contrary to all recommendations. In view of the current

USPSTF recommendations, these results can serve as the baseline for future studies beyond 2012, and can be further extended to also evaluate insights into geographic variability in PSA screening rates. Furthermore, these findings call for the design and evaluation of intervention studies to dissuade the use of PSA screening, particularly in men younger than 50 and older than 74 years of age.

A-375

Evaluation of Chromogranin A assay on the automated Kryptor Compact Plus analyzer and comparison with current manual Elisa method

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Background: To evaluate Chromogranin A assay on the BRAHMS KRYPTOR COMPACT PLUS analyser and compare it with the current manual ELISA assay.

Methods: We evaluated the Chromogranin A assay based on its performance in the imprecision, sensitivity,linearity, carryover and correlation studies. For imprecision studies (total & within run) Brahms CGA quality control materials were used. For correlation studies between the two methods patient's serum specimens were used. The 'Analyse It' software was used for computing the evaluation statistics

Results: Onthe BRAHMS KRYPTOR COMPACT PLUS, we obtained a within-run imprecision of <1.2% CV and a total imprecision of <2.3% CV. Linearity studies showed good recovery (100 %-110 %). The lower limit of detection for the assay was10.7µg/L. Comparison between the two methods showed 91.5 % concordance for test results.

Conclusion: The performance of the automated Chromogranin A assay on the BRAHMS KRYPTOR COMPACT PLUS is comparable and acceptable to that of the current manual ELISA assay.Automated assay is better than manual assay in terms of precision and accuracy and faster turnaround time.

A-376

Results from National Colorectal Cancer Screening Program by fecal occult blood test in South Korea: analysis using the Korean National Health Insurance Corporation databases of recent 8 years (2006-2013)

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Background: There have been controversies over the clinical utility of fecal occult blood test (FOBT) as a screening tool for colorectal cancer (CRC) in the general population. Many countries, including United Kingdom, France, Italy, Croatia, and Finland, have published the results of national colorectal cancer screening by various screening strategies such as FOBT, colonoscopy and sigmoidoscopy. Since Korea is one of Asian countries with the most rapidly increasing CRC prevalence and mortality, the Korean government implemented national CRC screening in 2006, which obligated all Korean individuals who are the national health insurance subscribers and aged over 50 years to take FOBT annually. Herein, we report the results of the Korea national CRC screening by FOBT in 2006-2013, which is the largest data ever, and evaluate implementation of the program as well as diagnostic significance of FOBT for CRC.

Methods: With database from the national cancer screening program, the results of participants of CRC screening from 2006 to 2013 were analyzed. After eliminating erroneous results, the results of a total of 20,609,909 individuals who participated in the CRC screening program were included in the study. For the participants with a positive result in FOBT, colonoscopy or double-contrast barium enema test was conducted as a further additional screening test. When colonoscopy found abnormal findings, biopsy was performed. Results of FOBT, colonoscopy or double-contrast barium enema, and biopsy were analyzed by medical statistics specialists.

Results: Considering the uptake rate, the proportion of actual examinees divided by eligible target population ranged from 27.1 % to 36.8 % in 2008-2013. Continuous increasing pattern of uptake rate was observed from 2008 to 2011. Consistently, female participated more thoroughly than male in all years. Positivity rate of FOBT ranged from 5.0 % to 9.1 % in 2006-2013, which did not show significant differences by gender, age, year, or type of FOBT (quantitative or qualitative). Even though sensitivity and specificity were unavailable to be calculated due to lack of the control group, positive predictive values (PPVs) of FOBT for cancer, adenoma, and polyp were 6.3 %, 60.9 %, and 32.3 %, respectively. Male gender and older age were associated with higher PPVs of FOBT for cancer throughout study years. Complications of colonoscopy as further evaluation for CRC after positive FOBT result included bleeding, bowel perforation, and infection, all of which occurred in less than 0.5 %. Follow-up colonoscopy uptake accounted for 57.5 % of all FOBT tests performed in CRC national screening program during 8 years. **Conclusion:** These results suggest a need for intervention strategies that include organizational changes and educational activities to improve awareness of CRC screening usefulness and increase participation rates. Even though false positive rates of FOBT were still high as criticized by previous researchers, implementation of FOBT as a screening modality for Korean national CRC screening could be assessed as a success based on key performance indicators. More studies are needed to establish the value of FOBT with feasibility of scaling-up organized CRC screening by satisfactory process measures.

A-377

Comparison of pre and post radiotherapy serum butyrylcholinesterase levels in oral cancer

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Background: Oral squamous cell carcinoma (OSCC) is one of the most common malignancies recognized nowadays, and represents a public health problem. The clinical and histological features alone cannot always accurately predict whether potentially malignant disorders of the oral mucosa remain stable, regress or progress to malignancy. Identification of molecular markers which can predict disease progression is necessary for better management of these disorders. Studies have shown correlation of butyrylcholinesterase with tumorigenesis, cell proliferation and cell differentiation. So, we sought to estimate and compare serum butyrylcholinesterase levels among healthy controls and biopsy proven oral cancer patients before and after radiotherapy.

Methods: Institutional Ethics Committee gave us the permission conduct this study. After obtaining consent from biopsy proven oral cancer patients (n= 39) 2 ml of blood was taken twice once before onset of any definitive treatment and again one day after completion of radiotherapy. Simultaneously, same amount of blood was taken form age and sex matched healthy controls (n = 20). After clot formation samples were centrifuged and serum was collected for estimation of butyrylcholinesterase.

Results : Median values of pretreatment serum butyrylcholinesterase levels were significantly elevated ($p \leq 0.0001$) in oral cancer patients [6956 IU/l] as compared to that of controls [1725.5 IU/L]. There was a significant increase in median values of pretreatment serum BChE levels with advancement of oral cancer. The median values of post treatment BChE levels of cancer patients in different stages were significantly decreased as compared to their respective pretreatment levels. Conclusion: Thus, there could be a role for butyrylcholinesterase in the management of oral cancer.

A-378

Tumor markers and diabetic patients

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Background: Carcinoembryonic antigen (CEA), cancer antigen (CA) 15.3 and CA 125 are glycoproteins, and CA 19.9 is high molecular weight glycolipid. They all have been widely used as tumor biomarkers. Our aim was to investigate values of these tumor markers in diabetic patients and compare them with non-diabetic patients.

Methods: Two groups of subjects were included in the study: patients without history of diabetic (NDP) and diabetic patients (DP). Patients with cancer or other pathology that increase the serum tumor markers levels were excluded. All tumor markers were determined by electrochemiluminescence immunoassay (ECLIA) in MODULAR E-170 (ROCHE DIAGNOSTIC®). Statistical analysis was performed using the software SPSS®.

Results: A total of 1718 patients enrolled in the study, 562 (32.7%) were NDP and 1156 (67.3%) were DP. The medians of serum tumor markers levels in NDP and DP were: CEA: 1.93 ng/ml vs. 2.53 ng/ml; CA 15.3: 9.29 U/mL vs. 12.65 U/mL; CA 19.9: 15.87 U/mL vs.18.73 U/mL and CA 125: 12.37 U/mL vs. 12.47 U/mL. No statistically significant differences were found between NDP and DP according to the CA 125 levels ($p>0.05$). Serum CEA, CA15.3 and CA19.9 levels were significant higher in DP (all $p<0.0001$).

Conclusions: Serum CEA, CA15.3 and Ca19.9 levels are increased in diabetic regarding non-diabetic patients.

A-379

FREND-LC Fluorescence Immunoassay for Quantitation of SAA and Haptoglobin in Lung Cancer

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Background: The FREND™ System is a portable FREND™ cartridge reader which is based on immunoassay technology capable of quantifying single or multiple analytes by measuring laser-induced fluorescence in a single-use disposable reagent cartridge. Acute-phase proteins (APPs) have been reported in many literatures that altered levels of various APPs are correlated with different types of cancers. Our study focused on the measurement of two highly abundant serum proteins – haptoglobin(HP) and serum amyloid A(SAA) - from APP class, which were previously reported to be altered in serum from patients with lung cancer. We developed a fluorescence immunoassay (FREND™-LC*) to measure the levels of SAA and HP on the FREND™ system.

Objective: The objective of this study was to evaluate the analytical and clinical performances of the FREND™-LC assay.

Methods: The imprecision, linearity, method comparison and detection limit of FREND-SAA/FREND-HP were evaluated according to CLSI guidelines EP05-A2, EP 06-A, EP 09-A2IR and EP 17-A2. The FREND-SAA and FREND-HP assays are standardized against NIBSC 92/680 and ERM®-DA470k/IFCC respectively. For the method comparison, aliquots of serum samples over the measuring ranges were measured with FREND-SAA/FREND-HP assays on NanoEnTek FREND™ system. The comparative assays were N-latex SAA kit and N Haptoglobin assay on Siemens BNII system. For the clinical performance evaluation, the retrospective cohort study was designed, where enrolled 120 lung cancer patients and 120 apparently healthy subjects under the oversight of Kyungbook National University Hospital Institutional Review Board. Plasma specimens from the enrolled subjects were procured to NanoEnTek Research Laboratory and run on the FREND™-LC assays, and Receiver-Operating Characteristic (ROC) curves analysis was conducted. Reference Interval for each analyte was investigated using 120 serum samples from apparently healthy subjects according to CLSI guideline C28-A.

Results: The imprecision for SAA produced coefficient of variation (CV) of <10% (range 5.8-9.3%) at concentrations of 29.71, 60.85 and 119.4 mg/L. The imprecision for HP also produced CV of <10% (range 5.7- 8.0%) at concentrations of 78.67, 158.47 and 310.14 mg/dL. The AMR of the assays were 20 ~200 mg/L(SAA) and 30~400 mg/dL(HP) with Passing-Bablock regression fits of y = 0.912x+7.040 (r^2=0.987) (SAA) and y = 1.013x -3.205 (r^2=0.995) (HP), respectively. LoDs were determined to be 3.3 mg/L(SAA) and 11.3 mg/dL(HP). In the method comparison studies with SIEMENS BNII N-Latex SAA and N Haptoglobin assays, the spearman correlation coefficients were 0.9789 (SAA, N=40) and 0.9247 (HP, N=43), and the slopes /intercepts were 0.9908/-0.0204 (SAA) and 0.9395/10.3(HP) by Passing-Bablock regression fit. The area under the curve (AUC) was 0.745 when SAA and HP assay values were combined, where the sensitivity and specificity were 70.6% and 75.0%. Reference Intervals were established 0.9~9.8 mg/L (SAA) and 1.2~184.6 mg/dL(HP), respectively.

Conclusion: Data indicates that the newly developed FREND™-LC assay exhibits reliable analytical performance and can be useful as an easy-to-use lung cancer screening kit.

This work was supported by the R&D program of MKE/KEIT (10040393, Development and commercialization of molecular diagnostic technologies for lung cancer through clinical validation)

*Assay currently under development and not for clinical use

A-380

Dermcidin isoform-2 induced abolition of the effect of insulin in breast cancer being the causal pathophysiology behind the development of CAD through the nitric oxide inhibition.

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Background: Insulin plays an important role as antithrombotic humeral factor for the prevention of CAD. We have also found that dermcidin isoform 2 (DCN-2), small 11Kda stress protein, was responsible for the hypertension and diabetes and predisposing them to atherosclerosis. It has been also found that levels of estrogen and progesterone were decreased in breast cancer patients. Lower amount of estrogen cannot produce enough nitric oxide (NO) to take part in physiological function. It has been also claimed that dermcidin is the potent inhibitor of all forms of nitric oxide synthases. In fact, insulin resistance has a greater effect on breast cancer patients.

Methods: Blood was collected from the breast cancer patients and equal numbers of age and sex matched normal volunteers. The insulin resistance was measured by HOMA score analysis [HOMA is found from glucose (mmol/L) and insulin level (micro Unit/L)]. Triglycerol, HDL, HbA1c% and CRP were determined for the validity of the experiment. DCN2 level was measured by enzyme linked immunosorbant assay by using dermcidin antibody and amount of NO was assayed by methomoglobin method (Zia et al)through spectral changes.

Results: Some new interesting data were found from the experiment. Insulin resistance (IR) was confirmed by HOMA score analysis which was found to elevate in these patients than healthy normal volunteers. Triglycerol, HDL, HbA1c% and CRP levels were found to increase in this case. DCN2 protein which was elevated in acute myocardial infarction was also found to increase in the breast cancer patients and the decreased level of nitric oxide was also obtained from the experiment.

Conclusion: From the above experiment it can be concluded that insulin resistance plays the key role in pathophysiology of breast cancer development. Here we point out the role of DCN-2 protein in resistance of insulin and also examined the correlation of Triglycerol, HDL, CRP with insulin and as well as DCN-2 protein. The above experiment also demonstrates that nitric oxide is the important factor for the activity of insulin and its resistance. Therefore, insulin might have the mechanism in the development of breast cancer.

A-381

TMCO1 is a novel marker in cancer metastasis

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Objective: Discovered that transmembrane and coiled-coil domains 1 (TMCO1) could be as a novel prognostic marker in cancer therapy.

Clinical Relevance: Transmembrane and coiled-coil domains 1 (TMCO1) is highly conserved in amino acid sequence among species and ubiquitously expressed in all human tissues. Homozygous frameshift mutation in TMCO1 causes distinctive craniofacial dysmorphism, skeletal anomalies, and mental retardation. However, its physiological functions, particularly in cancer biology, are largely unknown.

Method and Results: In this study, we have found that knock down of TMCO1 in HeLa cells, a human cervical cancer cell line, and U2OS cells, an osteosarcoma cell line, remarkably inhibited their migratory capability; TMCO1 was highly expressed in the cells of the invasive front of high grade lung cancer and metastatic cancer cells in the clinical specimens, and lung cancer cells at the metastatic bone site in our animal model; Immunohistostaining revealed that TMCO1 was co-localized with microtubules and was able to be co-sedimentated with microtubules in the presence of paclitaxel and GTP; and deficiency of TMCO1 in cells dramatically increased acetylation of tubulin. In this study, other investigation demonstrated that TMCO1 impacted microtubule dynamics, which is closely correlated with cancer metastasis, TBA drug response and therapeutic prognosis.

Conclusion: In summary, our findings provide new mechanistic insights into cancer metastasis and demonstrate that TMCO1 can be as a novel prognostic marker in cancer therapy.

A-382

Expression of Endoglin (CD105) in Experimental Colorectal Cancer

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Background:

Endoglin (CD105) is a receptor for the TGF-β1 molecule with crucial role in tumor angiogenesis. It has been shown to be a more useful marker to identify proliferating endothelium involved in tumor angiogenesis especially in cancer patients at risk of developing metastases. The aim of our study was to investigate the efficacy of COX-2 inhibitors on tumor development incidence and endoglin expression in Sprague Dawley rats in which an experimental model of colorectal cancer (CRC) was created.

Methods:

Rats were divided into 4 groups. Control group received 1 mM EDTA saline (sc, weekly) for 12 weeks and DMSO (po, daily) throughout experiment (25 weeks). DMH group received 25 mg/kg DMH in 1 mM EDTA-saline (sc, weekly) for 12 weeks and

DMSO (po, daily) for 25 weeks. The groups received 8 mg/kg diclofenac and 6 mg/kg celecoxib in DMSO (po, daily) simultaneously with DMH throughout experiment were identified as treatment groups. The rats were sacrificed by decapitation at the end of experiment. Quantitative assessment of endoglin protein expression was performed on each colorectal tissue specimen by Western blot analysis.

Results:

In histopathological evaluations, no pathological change was observed in control rats, while adenocarcinoma (62.5%), dysplasia (31.25%) and inflammation (6.25%) were detected in DMH group. In treatment groups, a marked decrease was observed in adenocarcinoma rate. Expression of endoglin protein was significantly elevated in DMH group compared with the controls ($p< 0.001$). When compared to DMH group, a decrease was detected in endoglin expression in celecoxib-treated groups but no statistically significance. However, no differences in endoglin expression were observed between diclofenac-treated group and controls.

Conclusion:

In conclusion, it was confirmed by histopathological and western blotting results that COX-2 inhibitors, particularly celecoxib, decrease rate of disease and slow down progression of existing disease in CRCs. These data show that endoglin expression may have an important role in tumor angiogenesis and predict of tumor invasion.

A-383

Comparison of K2EDTA Tubes and a Specialized Tube to Stabilize RNA for Rare Single Cell Gene Expression Analysis

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Background:

Messenger RNA (mRNA) within a cell reveals a picture of real time activity and is highly sensitive to changes in the cellular environment. Therefore, to gain a true assessment of a native cellular state, collection methods must maintain the state of the cell at time of collection. This is especially important in RNA expression profiling of circulating tumor cells (CTCs), which may yield useful information in biomarker discovery for disease diagnosis and prognosis. Therefore, specialized blood collection tubes containing preservatives have been developed (i.e. Streck Cell-Free RNA BCT™ (BCT)). While these tubes have been validated for cell-free RNA they have also been shown to work with intracellular RNA and have been used to enumerate CTCs. But as diagnostic applications of rare, single cells in blood, (i.e. CTCs) continues to increase, BCT tubes must be shown to be compatible with gene expression assays demonstrating comparable or better results than traditional blood collection tubes (i.e. K2EDTA).

Methods:

Millions of SKBR3 cells, a breast cancer cell line, were spiked into either K2EDTA or BCT tubes. At time points 1 and 24 hours, cells were isolated as either ten or single cell aliquots (n=3). Additionally, cells at time point 0 were also isolated and aliquoted as described above but without being spiked into a tube for baseline comparison. Cell aliquots were then lysed, reversed transcribed and preamplified using the single cell-to-Ct kit (Life Technologies). Following cell lysis, xenoRNA a synthetic RNA transcript was spiked into each lysate to serve as an internal control. Finally, real-time PCR (qPCR) was performed and Ct values of two housekeeping genes (i.e. Actin and GAPDH) accessed to determine quality of the RNA.

Results:

Successful amplification was obtained in each ten and single SKBR3 cell aliquots at all time points in both tubes as determined by the presence of a Ct value for both housekeeping genes. For actin, the mean Ct value at time point 1 hour for a single cell was 33.35±0.69 in K2EDTA and 33.96±1.45 in BCT. At time point 24 hours, it was 33.55±4.12 in K2EDTA and 35.42±0.70 in BCT, suggesting bad RNA quality. The same Ct trends were seen in the GAPDP expression and for a single cell versus ten cells. Additionally, for single cells at time point 0, cells not spiked into either tube showed no statistical difference compared to cells in either K2EDTA ($p>0.23$) or BCT ($p>0.25$) tubes at 1 hour or K2EDTA tubes at 24 hours ($p>0.71$). However, there was a statistical difference seen for 10 cells in BCT tubes at 24 hours compared to 10 cells in K2EDTA tubes ($p<0.01$), suggesting K2EDTA superior to BCT at higher cell concentrations. Finally, to ensure validity of our methods, we demonstrated precision between run was within ±2SDI of the mean Ct value for xenoRNA expression (28.58±1.69).

Conclusion:

We have shown that BCT tubes are promising as an alternative to standard K2EDTA tubes for use in single cell gene expression assays. However, further refinement of RNA extraction and amplification methods after cell removal from BCT tubes is still needed.

A-384

Waldenstrom's Macroglobulinemia Express B-Cell Maturation Antigen and Serum Levels Correlate with Disease Status and Conventional M-Protein and IgM Levels

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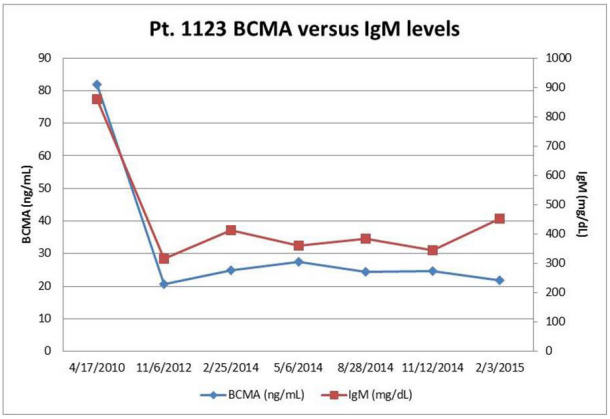
Waldenström's macroglobulinemia (WM) is an incurable B-cell lymphoplasmacytic lymphoma. B-cell maturation antigen (BCMA) is expressed on malignant B cells and we have previously shown that serum BCMA levels are elevated in multiple myeloma (MM) patients and correlate with disease status in multiple myeloma (MM) patients. Our objective was to determine whether BCMA is present in WM; and, furthermore, whether its serum levels also correlate with disease status and track with conventional tumor markers for patients with WM. Data was obtained on 20 WM patients who received treatment in a single clinic specializing in monoclonal gammopathies that was established 10 years ago. Mann-Whitney analysis was used to measure statistical significance (p partial response (n=7) contained significantly lower levels of BCMA than samples from patients with stable disease (n=4) or progressive disease (n=7; p=0.003 and p=0.0003, respectively). Untreated WM patients (n=9) also had significantly higher BCMA levels than healthy individuals (n=14; p<0.0001). Additionally, the BCMA levels of seven WM patients were correlated with the serum M-protein and IgM levels of these patients during the course of their therapy. Changes in BCMA levels corresponded with changes in serum M-protein as well as changes in IgM levels. These results indicate that BCMA is present on the malignant cells from WM patients and serum levels of this protein can be used as a potential marker for tracking the course of their disease.



A-385

Identification of the Long Interspersed Nuclear Element-1 (L1) Product in Human Plasma as an Epigenetic Biomarker for Environmentally-Induced Diseases

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Waldenström's macroglobulinemia (WM) is an incurable B-cell lymphoplasmacytic lymphoma. B-cell maturation antigen (BCMA) is expressed on malignant B cells and we have previously shown that serum BCMA levels are elevated in multiple myeloma (MM) patients and correlate with disease status in multiple myeloma (MM) patients. Our objective was to determine whether BCMA is present in WM; and, furthermore, whether its serum levels also correlate with disease status and track with conventional tumor markers for patients with WM. Data was obtained on 20 WM patients who received treatment in a single clinic specializing in monoclonal gammopathies that was established 10 years ago. Mann-Whitney analysis was used to

measure statistical significance (p partial response (n=7) contained significantly lower levels of BCMA than samples from patients with stable disease (n=4) or progressive disease (n=7; p=0.003 and p=0.0003, respectively). Untreated WM patients (n=9) also had significantly higher BCMA levels than healthy individuals (n=14; p<0.0001). Additionally, the BCMA levels of seven WM patients were correlated with the serum M-protein and IgM levels of these patients during the course of their therapy. Changes in BCMA levels corresponded with changes in serum M-protein as well as changes in IgM levels. These results indicate that BCMA is present on the malignant cells from WM patients and serum levels of this protein can be used as a potential marker for tracking the course of their disease.